Gold Nanoparticles Deposited Polyaniline–TiO₂ Nanotube for Surface Plasmon Resonance Enhanced Photoelectrochemical Biosensing

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Supporting Information

ABSTRACT: A novel ternary composite composed of TiO₂ nanotubes (TiONTs), polyaniline (PANI), and gold nanoparticles (GNPs) was prepared for photoelectrochemical (PEC) biosensing. PANI was initially coated on TiONTs with an oxidative polymerization method, and 12-phosphotungstic acid was then used as a highly localized photoactive reducing agent to deposit GNP s on TiONT-PANI. The morphology and composition of the composite were characterized by various spectroscopic and microscopic methods. Electrochemical impedance spectroscopy was also conducted to demonstrate the excellent electrical conductivity of the composite. A PEC biosensor was fabricated by immobilizing a mixture of lactate dehydrogenase and the composite onto ITO electrodes, which regenerated nicotinamide adenine dinucleotide (NAD⁺) to complete the enzymatic cycle and led to an improved method for PEC detection of lactate. Because of the surface plasmon resonance enhanced effect of GNP s, the electrochromic performance of PANI, and excellent conductivity and biocompatibility of the composite, this method showed a dynamic range of 0.5–210 μM, sensitivity of 0.0401 μA μM⁻¹, and a detection limit of 0.15 μM.

KEYWORDS: photoelectrochemical biosensing, surface plasmon resonance enhanced effect, nanoparticle composite, TiO₂ nanotubes, 12-phosphotungstic acid, lactate dehydrogenase

INTRODUCTION

Photoelectrochemical (PEC) sensing techniques have recently achieved dramatic progress in the detection of various biological and biochemical targets such as antigens, nucleic acids, enzymes, enzyme substrates, and some other chemicals. By integrating the traditional electrochemical method with an optical technique, the PEC-based sensing techniques are able to maintain the advantages of both methods and minimize their shortcomings, resulting in the improved signal-to-noise ratio and sensitivity compared with the individual techniques. In particular, the PEC biosensors based on photoactive semiconducting nanomaterials have shown superior performance to the conventional PEC sensors due to the reaction between the photogenerated holes from the photoactive semiconductors and the biocatalyzed products. This reaction can retard the recombination of the photogenerated charges and thus enhance the photocurrent at PEC biosensor. For example, a BiOI nanoflakes/TiO₂ nanoparticles composite was prepared as the photoelectrode, on which acetylcholine esterase (AChE) antibody was immobilized for binding AChE to catalyze the conversion of acetyltiocholine to thiocholine. Then, thiocholine acted as a sacrificial electron donor to react with photoexcited holes, which could increase the photocurrents significantly.

TiO₂ nanomaterials are the most promising and effective materials in PEC biosensing, attributing to their excellent...
biocompatibility, chemical and mechanical stability, strong UV−vis absorption, and low cost.9,10 They have been used to prepare different types of PEC sensors or constructs the detection methods for many biochemical and biological analytes including immunoglobulin G,10 H2O2,11 adenosine,12 glucose,13 and antigen.14 However, the application of TiO2 nanomaterials in PEC sensing is always limited by their wide band gap, poor electrical conductivity, and fast recombination of the photogenerated charges, which significantly lower the photoenergy conversion efficiency and the sensitivity of the PEC sensors fabricated with TiO2.15−17 To minimize these handicaps, TiO2 nanomaterials were doped with various metal or nonmetal elements to obtain the composites with improved PEC properties.18−20 For example, hemin and IrO2 nanoparticles have been codeposited on TiO2 nanowires to enhance the light absorption and charge separation, and the PEC sensor based on this composite can sensitively detect glutathione in both buffer and cell extracts.19

As the most extensively studied conducting polymer, polyaniline (PANI) was widely used in the construction of electrochemical sensors because of its tunable electrical conductivity, reversible proton doping, redox reversibility, high environmental stability, and easy preparation.20,21 Recently, PANI has demonstrated its feasibility as an active component in many PEC devices (e.g., solar cells) by exhibiting its unique electrical and optical properties such as charge storage capacity, electron trapping capability, and photoelectric conversion performance.22 For instance, the electrochromic performance of PANI can enhance the light absorption at visible region, hence producing more photogenerated electrons.23 In addition, the conjugation structure of PANI molecules can capture more photoexcited electrons, retarding the recombination between photogenerated charges. Accordingly, modifying TiO2 with PANI is an effective strategy to improve the photocatalytic performance and shift the spectral absorption toward visible region.24 For example, Li and co-workers prepared a PANI/TiO2 nanoparticle composite, which displayed an improved photocatalytic activity than the pristine TiO2.24 Similarly, it is expected that the PEC performance of TiO2 nanomaterials can be obviously enhanced by combining PANI with TiO2. More interestingly, PANI can also facilitate the deposition of GNP on TNTs by attracting negative charged AuCl4− on protonated secondary amines of PANI. PANI can be able to improve the conductivity of TNTs.26

In the past few years, gold nanoparticles (GNPs) have frequently been applied in electrochemical biosensors ascribing to their biocompatibility, large surface area, chemical stability, ease of preparation, and excellent electronic and optical properties etc.27−30 More importantly, noble metal nanoparticles (Au, Pt, and Pd) exhibited a surface plasmon resonance (SPR) effect during the photocatalytic or PEC process, which can substantially increase the light absorption and enhance the charge separation, thus improve the photocatalytic or PEC performance.31−33 Therefore, incorporating noble metal nanoparticles with wide band gap semiconductors such as TiO2 can effectively enhance the PEC conversion efficiency of these semiconductors. For example, a GNP-decorated TiO2 nanowire modified sensor has been developed for the detection of cholera toxin subunit B.34 The SPR enhancement of GNP/TiO2 nanowire composite produces an electrochemical field amplification effect, leading to a 100% increase of photocurrent density.35

Determination of l-lactate in real samples can provide key information in the clinical diagnostics, medicine validation, and food analysis.32,33 Therefore, various electrochemical biosensors have been developed for the accurate and fast detection of lactate.32,33 However, these biosensors relied on the electrochemical oxidation of reduced nicotinamide adenine dinucleotide (NADH) to generate detection signals, which may be negatively affected by the high overpotential and electrode fouling.36 Accordingly, a new method should be designed for the highly efficient conversion of NADH to nicotinamide adenine dinucleotide (NAD+) to improve the lactate determination.

In this work, a novel functional composite composed of TiONTs, PANI, and GNP was synthesized following a procedure described by Scheme 1A. Briefly, aniline was initially polymerized on TiONTs by an oxidative polymerization method. 12-Phosphotungstic acid (PTA; PW12O403−) was then modified on PANI-TiONT surface, and the photochemically reduced PTA acted as a localized reducing agent for deposition of GNP on PANI-TiONT. By immobilizing lactate dehydrogenase (LDH) and NAD+ on the composite-modified ITO electrode, a PEC biosensor for l-lactate was developed. Through integrating the SPR effect of GNP and the excellent PEC performance of PANI, the ternary composite transformed NADH back to NAD+ in a highly efficient way to satisfy the LDH catalytic circle, improving the biosensor performance for detection of lactate.

■ EXPERIMENTAL SECTION

Materials and Reagents. Aniline monomer (99.5% purity) and ammonium persulfate (98%) were purchased from J&K Scientific,
Ltd., Beijing, China. TiO₂ nanoparticles (P25) were obtained from Tianjin Chemical Reagent Co., Ltd., China. LDH, β-NAD⁺ hydrate, NADH, sodium 1-lactate, Nafion, ethylene diamine tetraacetic acid (EDTA), HAuCl₄·3H₂O, and PTA were purchased from Sigma-Aldrich, USA. All other chemicals were of analytical grade and used without further purification. All solutions were prepared in Milli-Q ultrapure water. Potassium phosphate buffer solution (0.1 M, pH 7.4) was prepared and used as a supporting electrolyte.

**Apparatus.** PEC measurements were performed in a quartz glass PEC cell, which was designed by our lab and fabricated by Gaozhiurilan Co., Ltd., Wuhan, China. Philips tubular ultraviolet low-pressure 16 W mercury lamps were purchased from Philips Lighting B.V., The Netherlands. A Xe lamp equipped with visible light monochromator (CEL-HXUV 300, Beijing AULTT, China) was used as the irradiation source. Electrochemical and PEC measurements were performed using a CHI630D electrochemical workstation (Shanghai CH Instruments, China). Indium tin oxide (ITO) electrodes were ordered from Wuhan Lattice Solar Energy Technology, Ltd., China. UV−vis spectra were recorded by a Hitachi U-4100 UV−vis−NIR Spectrophotometer (HITACHI, Japan). Electrochemical impedance spectroscopy was obtained at an IM6ex electrochemical workstation (Zahnier, Germany). The morphology and chemical composition of the nanomaterials were characterized by transmission electron microscopy (TEM, Tecnai G2 20 USA), X-ray diffraction (XRD, Bruker D8 Advance, Germany) with Cu Kα radiation, and Fourier transform infrared spectroscopy (FT-IR, Nicolet 170, USA).

**Preparation of Nanomaterials.** TiONTs were prepared by initially pouring a mixture of 0.6 g of P25 and 120 mL of 10 M NaOH aqueous solution to a Te flask, which was purged with N₂ gas for 15 min. Then, the mixture was shaken in a thermostat oscillator at 4 °C for 4 h, and 50 μL of the mixture was coated on the cleaned ITO electrode. The PEC biosensor was dried in refrigerator at 4 °C overnight.

**PEC Assay Procedure.** PEC test was conducted in a quartz cell with a three-electrode system consisting of an ITO working electrode, a platinum wire auxiliary electrode, and a Ag/AgCl (3.0 M KCl) reference electrode. A Xe lamp equipped with a monochromator that only allows visible light to pass through was used as a light source to irradiate the ITO electrode in a homemade dark box. After the background photocurrent became stable, the PEC response was recorded.

### RESULTS AND DISCUSSION

**Characterization of Composite.** As shown in Figure 1A, TiONT did not exhibit any obvious absorption peak between 300 and 900 nm except the typical absorption peak at ∼283 nm (curve a), which is consistent with the previous report. Curve b displayed two absorption bands centered at ∼360 and 440 nm, ascribed to the π−π* and polaron−π* transitions of ESPANI. TiONT-GNP (curve c) and TiONT-PANI-GNP (curve e) showed the plasmon resonance peak of pure GNPs (curve c) at ∼516 nm despite a minor peak position shift. The SPR peak position shift may be induced by the strong interaction of GNPs with TiONT and PANI. The existence of localized SPR band in both binary (∼524 nm, curve d) and ternary composites (∼530 nm, curve e) indicated that TiONTs or PANI could not interfere with the plasmon resonance of the GNPs. The characteristic peak of TiONTs moved from 285 to 291 nm after deposition of GNPs on TiONTs (curve d). Most importantly, the typical absorption band attributed to the π−π* transition of PANI at ∼360 nm, the characteristic peak of TiONTs at ∼290 nm, and the SPR peak of GNPs at ∼530 nm were all observed on curve e, demonstrating the successful formation of ternary composite.

The corresponding characteristic X-ray diffraction patterns of TiONTs, PANI, TiONT-PANI, and TiONT-PANI-GNP were shown in Figure 1B. Curve a showed the diffraction peaks at 2θ of 9.6, 24.8, 28.4, and 48.7°, which were assigned to the (200), (110), (600), and (020) crystal planes of orthorhombic TiO₂ nanotubes, respectively. TiONT exhibited four typical peaks at approximately 9.3, 15.0, 21.0, and 25.6° (curve b), assigned to the respective (001), (010), (100), and (110) crystal planes of emeraldine salt PANI (ES-PANI). ES-PANI possesses good electrical conductivity, which is a desirable character for the development of electrochemical and PEC biosensors. The XRD peaks of ES-PANI along with the typical peaks of TiONTs were observed in the XRD pattern of TiONT-PANI (curve c), indicating that PANI was coated on TiONT. However, the intensity of the PANI peaks attributed to the (010) and (100) crystal planes declined significantly in TiONT-PANI, and the PANI peak at 25.6° was overlapped with the TiONT peak at 24.8°. It was also noticed that the 2θ of the peaks attributed to the PANI crystal planes had minor shifts after the formation of TiONT-PANI composite, which is probably due to the crystal form change caused by the strong interaction between PANI and TiONTs. In addition to the typical peaks belonging to PANI and TiONT, curve d exhibited some new XRD peaks at ∼38.6, 44.8, 64.9, and 77.8° ascribed to the (110), (200), (220), and (311) reflections of gold nanoparticles, which

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FT-IR spectra of TiONTs, PANI, TiONT-PANI, and TiONT-PANI-GNP were acquired to investigate the structural information of these materials (Figure 1C). The broad absorption of TiONTs centered at 3404 cm⁻¹ (curve a) was ascribed to the Ti−O−H stretching vibration arising from the TiONT surface hydroxyl. In addition, the Ti−O stretching and Ti−O−Ti bridging vibration could be represented by two absorption bands at ∼680 and ∼475 cm⁻¹, respectively, which are characteristic absorptions of TiO₂ nanomaterials. The spectrum of PANI displayed three peaks at 1298, 1128, and 802 cm⁻¹ corresponding to the C−N stretching of the secondary aromatic amine, the N=Q=N (Q represents the quinoid ring) stretching and the aromatic C−H out-of-plane bending vibration, respectively (curve b). The other two peaks ascribing to C=O stretching vibration of quinoid and benzenoid rings were also observed at 1559 and 1471 cm⁻¹. Furthermore, the peak at 1243 cm⁻¹ attributed to C−N** stretching vibration indicated that PANI produced by the oxidation polymerization method was emeraldine salt, which is highly conductive. More importantly, TiONT-PANI exhibited the characteristic FT-IR bands of both PANI (700–1600 cm⁻¹) and TiO₂ nanomaterials (400–700 cm⁻¹) (curve c), demonstrating that the PANI-TiONT was successfully formed. It is also noticed that the characteristic PANI absorption peaks at 1559, 1471, 1298, and 1128 cm⁻¹ shifted to 1567, 1482, 1303, and 1136 cm⁻¹ respectively, which was induced by the strong bonding formed between the nitrogen atoms on PANI and the oxygen atoms on TiONTs. The strong bonding restricted the chain vibration of PANI, which likely moved the vibration of PANI to higher wavenumbers. The deposition of GNPs on TiONT-PANI resulted in further positive movement of the PANI absorption bands because of the strong interaction between GNPs and PANI (curve d). Interestingly, although GNPs did not produce any typical FT-IR absorption, three characteristics bands at 1079, 975, and 890 cm⁻¹ attributable to the chemical bonds between tungsten atoms, oxygen atoms, and phosphorus atom (P−Oa, W−Oa, and W−Ob−W) in PTA were observed. These characteristic bands could be used as the proof of successful adsorption of PTA on TiONTs by mechanical stirring. The PTA molecules on TiONTs were then photoreduced to become the reducing agent for depositing GNPs on PANI-TiONT.

Figure 1. (A) UV−vis spectra of (a) TiONTs, (b) PANI, (c) GNPs, (d) TiONT-GNP, and (e) TiONT-PANI-GNP composite. (B) XRD patterns of (a) TiONTs, (b) PANI, (c) TiONT-PANI, and (d) TiONT-PANI-GNP composite. (C) FT-IR spectra of (a) TiONTs, (b) PANI, (c) TiONT-PANI, and (d) TiONT-PANI-GNP composite.

Figure 2. TEM images of (a) TiONTs, (b) PANI, (c) TiONT-PANI, and (d) TiONT-PANI-GNP composite.
PANI was attributed to the electrostatic attraction between the molecular chains in HCl-doped PANI.\textsuperscript{37} After polymerization of aniline in the presence of TiONTs, semitransparent PANI was coated on TiONTs surface (Figure 2c).\textsuperscript{48} In addition, TiONTs were not separated from each other anymore because PANI polymer bundled the TiO\textsubscript{2} nanotubes together. The TEM photo of TiONT-PANI-GNP showed a lot of dense dark particles on the surface of TiO\textsubscript{2}, indicating the successful deposition of GNPs on TiONT-PANI composite through photoreduced PTA. From the magnified TEM image (inset in Figure 2d), the average particle size of GNPs was estimated to be 10–14 nm. From above TEM photos, intimate contact could be observed among TiONT, PANI, and GNPs, which would facilitate the electron transfer within the ternary composite and improve the separation of photogenerated charges.

The electrical conductivity of the nanomaterials was evaluated with electrochemical impedance spectroscopy (EIS). Impedance measurements was performed in 0.1 M KCl solution containing 5.0 mM [Fe(CN)\textsubscript{6}]\textsuperscript{3−}/[Fe(CN)\textsubscript{6}]\textsuperscript{4−} at a DC potential of 0.21 V. An alternating voltage of 5 mV was superimposed on the applied DC potential. Nyquist plots were recorded over a frequency range from 100 kHz to 0.1 Hz (Figure 3). The quantity of the nanomaterials immobilized on all the electrodes was identical in this test. For each curve, a semicircle was obtained in the higher frequency region and a straight line was observed in the lower frequency region, indicating a mixed electron transfer and diffusion control mechanism for the [Fe(CN)\textsubscript{6}]\textsuperscript{3−}/4− reaction at the electrode surface. The semicircle diameter is proportional to the electron transfer resistance ($R_{eq}$) at surface of the electrode.\textsuperscript{39} As expected, the Nyquist plot of the bare electrode exhibited a straight line with the smallest semicircle in these four curves, implying a nearly complete diffusion control process (curve a). Noticeably, the semicircle diameters for TiONT-, TiONT-PANI-, and TiONT-PANI-GNP-modified electrodes decreased in accordance with the sequence (curves b–d), indicating the decreasing $R_{eq}$. These results strongly supported the idea that both PANI and GNPs could significantly enhance electrical conductivity of the TiONT-PANI-GNP composite, which will benefit the performance of the electrochemical or PEC sensors using the ternary composite as sensor scaffold.

### Figure 3. Nyquist plots of 5.0 mM [Fe(CN)\textsubscript{6}]\textsuperscript{3−}/[Fe(CN)\textsubscript{6}]\textsuperscript{4−} at (a) bare, (b) TiONT, (c) TiONT-PANI, and (d) TiONT-PANI-GNP-modified GCE in 0.1 M KCl at a DC potential of 0.21 V.

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**Voltammetric Characterization of Electrochemical Biosensor.** In the absence of lactate, both TiONT-PANI-GNP- and TiONT-PANI-GNP|PILDH|NAD\textsuperscript{+}-modified GCE showed flat and featureless voltammograms (Figure 4, curves a and c), indicating that the ternary composite did not produce any electrochemical interference. Because the weight of PANI in the composite was less than 10% and TiONT was electrochemically inert, the redox currents of PANI were so small that they were covered by the charging current of the composite-modified electrode. In addition, TiONT-PANI-GNP-modified GCE did not show any obvious electrochemical signal in the presence of 5 mM L-lactate (Figure 4, curve b), meaning that lactate could not be oxidized or reduced electrochemically within a potential range from 0 to 0.8 V. More importantly, a distinct anodic peak (versus Ag/AgCl) was observed at −0.49 V at TiONT-PANI-GNP|PILDH|NAD\textsuperscript{+}-modified GCE in the presence of 5 mM lactate (Figure 4, curve d), which demonstrated the high catalytic activity of LDH in the ternary composite. The catalytic mechanism of LDH to lactate under the aid of coenzyme—NAD\textsuperscript{+}—can be described by the following eqs 1 and 2. Briefly, LDH initially catalyzed the conversion of lactate to pyruvate with the help of NAD\textsuperscript{+}, which was simultaneously reduced to NADH. Owing to the excellent electrochemical properties of TiONT-PANI-GNP, NAD\textsuperscript{+} could be oxidized back to NAD\textsuperscript{3+} at a moderate potential of −0.49 V (versus Ag/AgCl) to provide an electrochemical signal for lactate detection.

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\begin{align*}
\text{Lactate} + \text{NAD}^+ & \xrightarrow{\text{LDH}} \text{Pyruvate} + \text{NADH} + \text{H}^+ \\
\text{NADH} & \xrightarrow{\text{electrochemical oxidation}} \text{NAD}^3 + \text{H}^+
\end{align*}
\]

The voltammetric results in Figure 4 confirmed that the ternary composite was a feasible immobilization material for the development of electrochemical and PEC biosensors. However, the current for NADH oxidation was relatively low at a high concentration of lactate (5 mM), indicating the low sensitivity of the electrochemical biosensor for detecting lactate.\textsuperscript{52} Furthermore, the electrochemical oxidation regeneration of NAD\textsuperscript{3+} could be slow and incomplete, resulting in poor performance of the electrochemical lactate biosensor. Therefore, it is meaningful and highly necessary to develop a new type of biosensor with improved analytical performance.
PEC Characterization of Modified ITO. The PEC performance of different modified ITO electrodes was evaluated by monitoring the photocurrents produced by illuminating the electrodes with intermittent visible incident light (Figure S5A). The photocurrent at TiONT-PANI-modified ITO (curve b) was ~53% larger than that at TiONT-modified ITO (curve a), which could be attributed to the electrochromic performance, electron capture capability, and good conductivity of PANI. Specifically, the electrochromic character of PANI improved the visible light absorption and enhanced the charge separation. In addition, the good electrical conductivity of PANI facilitated the movement of photoexcited electrons from TiO2NTs to ITO electrode, preventing the recombination of the photogenerated charges. Notably, TiONT-PANI-GNP-modified ITO (curve c) showed a ~79% higher photocurrent than PANI-TiONT-modified ITO (curve b) because of the SPR effect of GNP s, which increased the light absorption and enhanced the charge separation, thus improved the PEC conversion efficiency of the semiconductors. In the presence of 100 μM lactate, the photocurrent of TiONT-PANI-GNP-modified ITO did not change (curve d), which demonstrated that the photoexcited holes were not able to oxidize lactate directly. On the contrary, TiONT-PANI-GNP-modified ITO displayed strong oxidation ability toward NADH, which resulted in a significant photocurrent enhancement (curve e). This photocurrent enhancement indicated that the photoexcited holes could efficiently regenerate NAD+ from NADH to complete the enzymatic cycle as shown in eqs 1 and 2, which was an essential requirement for the development of a LDH-based biosensor. Noticeably, TiONT-PANI-GNP-LDH/NADH*-modified ITO (curve f) displayed the lowest photocurrent among all the modified electrodes owing to the steric hindrance of the enzyme molecules on ITO. The steric hindrance of enzymes retarded the movement of photogenerated electrons to ITO and thus increased their recombination with the holes. Even so the PEC currents at TiONT-PANI-GNP-LDH/NADH*-modified ITO (curve f) are still larger than those at TiONT-PANI-LDH/NADH*-modified ITO (curve g), which offered further proof for SPR-enhanced effect of GNP s. The linear scan voltammetric curves of TiONT-PANI-GNP/LDH/NADH* under the illumination further confirmed these results (Figure S1).

SPR-Enhanced PEC Detection of Lactate at TiONT-PANI-GNP/LDH/NADH*-ITO. The PEC reaction mechanism of i-lactate at TiONT-PANI-GNP/LDH/NADH*-modified ITO was displayed in Scheme 1B. During light irradiation, the SPR-excited electrons on GNP s were first injected into the \( \pi^* \) orbit of PANI. At the same time, the electrons at PANI \( \pi \) orbit were also photoexcited and jumped to the \( \pi^* \) orbit through light absorption. Because the \( \pi^* \) orbit position of PANI is higher than the conduction band (CB) and valence band (VB) positions of TiO2, the electrons at the \( \pi^* \) orbit were rapidly transported into the CB of TiO2. Eventually, all the photoexcited electrons were attracted to ITO by the applied potential (0.2 V) to produce enlarged photocurrent. In addition, LDH catalyzed the transformation of lactate to pyruvate with the aid of NAD+, which was converted to NADH simultaneously. Afterward, NADH was oxidized back to NAD+ by the photogenerated holes at PANI \( \pi \) orbit, which were transferred from the VB TiO2 during the illumination. The increase of lactate concentration in the test solution produced more NADH, which would consume more photoexcited holes and therefore generate larger photocurrent for quantitative determination of lactate.

Some important experimental parameters such as pH value of test solution, applied potential, LDH and NAD+ concentrations were optimized before the final performance evaluation. As shown in Figure S2, the optimal concentrations of LDH and NAD+ in TiONT-PANI-GNP dispersion were 7 mg mL−1 and 15 mM, and the optimal pH value was 7.4. Besides, the photocurrent was noticeably enhanced with increasing applied potential up to 0.2 V. To minimize the oxidation of some possible interfering substances and NADH, an applied potential of 0.2 V was used in the subsequent PEC experiments. Under the optimum experimental conditions, the
The photocurrent response of TiONT-PANI-GNP/LDH/NAD⁺-modified ITO was enhanced with increasing lactate concentration (Figure 5B). The calibration curve could be constructed by plotting $-\Delta I$ ($\Delta I = I - I_0$ where $I$ represents the photocurrent obtained in the presence of lactate and $I_0$ is the blank photocurrent) versus lactate concentration (Figure 5C). A dynamic range between 0.5 and 210 μM lactate was obtained from the calibration plot with a correlation coefficient of 0.996. The sensitivity and detection limit were estimated to be 0.0401 μA μM⁻¹ and 0.15 μM respectively. As displayed in Table 1, the analytical performance of TiONT-PANI-GNP/LDH/NAD⁺-modified ITO showed superior behavior over that of most of the electrochemical biosensors, which is attributed to the SPR-enhanced PEC effect and synergistic effect of TiONT-PANI-GNP composite.

**Precision, Stability, Interference Test, and Real Sample Analysis.** The precision, stability, and practical application of the proposed biosensor were included in the Supporting Information. The relative standard deviation (RSD) of the photocurrents at six biosensors was 4.9%, indicating an acceptable fabrication precision. Furthermore, 96.1, 92.5, and 87.3% of the initial photocurrents were retained after the PEC biosensors were stored at 4 °C for 1, 2, and 4 weeks, respectively. The selectivity of TiONT-PANI-GNP/LDH/NAD⁺-modified ITO was evaluated through investigating the influence of some possible interfering substances including citrate, carbonate, fructose, sucrose, phenylalanine, arginine, glucose, ascorbic acid, and uric acid on the determination of 20 μM lactate, respectively. After 50 μM each of the above interfering substances was added into 20 μM lactate solution, the photocurrent at TiONT-PANI-GNP/LDH/NAD⁺-modified ITO fluctuated ~2.1, 2.7, ~1.7, 1.8, 2.9, 1.5, 3.8, 4.4, and 3.1%, respectively, compared to the photocurrents obtained before the addition of the interfering substances. The interference may be ascribed to the oxidation of the interfering substances by the photogenerated holes at the applied potential (0.2 V). However, the interfering signals are negligible compared with the photocurrents produced by the LDH PEC biosensor. The average recoveries for the detection of lactate in serum and milk samples were 98.2 and 96.9%, indicating the potential practical application of the PEC biosensor.

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