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Nickel nanoparticle-modified electrode for ultra-sensitive electrochemical detection of insulin

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ABSTRACT

An ultra-sensitive electrochemical sensor for the detection of insulin was fabricated, using low-cost and environmentally friendly nickel nanoparticles (NiNPs) by ion implantation. The morphology and structure of the NiNPs are characterized by scanning electron microscopy (SEM), revealing diameters ranging from 4 to 8 nm. The insulin assay performances were evaluated by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and chronoamperometry (I–t). The NiNPs-modified indium tin oxide electrode (NiNPs/ITO) showed excellent analytical features, including ultra-high sensitivity (2140 μA μM^{-1}) for detecting low concentrations of insulin, an incredibly low detection limit (10 pM) and a wide dynamic range (100 pM to 2400 pM and 1 nM to 125 nM). In addition, the NiNPs/ITO electrode was also used to analyze the insulin concentration in bovine insulin injections. The NiNPs/ITO electrode is expected to be used as a potential biosensor for insulin.

1. Introduction

The insulin molecule consists of two polypeptide chains linked by two disulfide bridges, an A chain of 21 amino acid residues and a B chain of 30 amino acid residues (Arvinte et al., 2010). The dysfunction of insulin hormone production in humans can cause diabetes mellitus, which is a severe illness in humans. Insulin controls the glucose levels in blood within a narrow concentration range. Therefore, diabetes patients are reliant on intake of adequate and accurate amounts of insulin to regulate blood glucose levels (Schirhagl et al., 2012). The detection of insulin is of great importance for clinical diagnostics, because it serves as a predictor of diabetes, insulinoma and trauma (Melloul et al., 2002; Tannuri et al., 1993). The common methods for insulin determination include enzyme-linked immunosorbent assays (Kumada et al., 2007), radioimmunoassay (Murayama et al., 2006) and high performance liquid chromatography (HPLC) (Mercolini et al., 2008). However, these current technologies for the detection of insulin are slow, cumbersome, and costly and require special instruments as well as derivatization materials. Direct and efficient electrochemical detection of insulin is important for the development of fast, sensitive insulin detectors. As a result, a variety of modified electrodes have been suggested for promoting the oxidation and detection of insulin. A number of reports for insulin determination are based on electrode modifications using cobalt oxide nanostructures (Salimi and Hallaj 2012), silica nanoparticles-nafion modified glassy carbon electrodes (Amini et al., 2014), carbon nanotubes (Wang and Musameh 2004; Zhang et al., 2005), nanocarbon black electrode surface (Guo et al., 2012), carbon nanotube-nickel-cobalt oxide modified electrode (Arvinte et al., 2010), nickel nanoparticles (Salimi et al., 2006, 2007), guanine/nickel oxide nanoparticles (Salimi et al., 2008) and nickel oxide nanoparticles-multiwalled carbon nanotubes (Rafiee and Fakhari 2013). However, some of these electrodes suffer from poor long-term stability, or they require complicated and time-consuming methods for preparing the synthetic modifiers.

The usage of nickel nanoparticles (NiNPs) as the modification material is of interest bacause it is not expensive compared with Ag, Pd or Pt, but it possesses some of the same characteristics as the noble metal nanoparticles. Meanwhile, Ni-based nanomaterials exhibit remarkable catalysis capabilities for insulin, which originating from the redox couple of $Ni(OH)_2$/NiOOH at the electrode surface in an alkaline medium (Lin et al., 2014). Several approaches have been employed to assemble NiNPs on the surface of electrodes such as electrochemical deposition, seed-mediated growth and magnetron co-sputtering deposition (Jin et al., 2007; Prieto et al., 2012; Yang et al., 2009). Ion implantation has been used by our group to fabricate several nanoparticles modified electrodes with good application prospect. In this work, a nickel nanoparticle-modified electrode was prepared by ion implantation at different injection conditions. It is a material surface

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modification providing practical and excellent electrode materials with long-term stabilities. This technique implants the nanoparticles on the surface of the substrate, and the size of the nanoparticles can be controlled just by the implantation conditions. In addition, the process is facile, low cost and eco-friendly without the use of any other chemicals. In our previous reports, Pt, Au, Ag, and Ni ion implanted indium tin oxide (ITO) electrodes were successfully applied to investigate the electrochemical behaviors of H_2O_2 and glucose (Jia et al., 2012; Liang et al., 2012; Tian et al., 2013).

Herein, the ion-implantation method has been applied to fabricate NiNPs-modified ITO electrodes (NiNPs/ITO). ITO was chosen as the electrode substrate due to its wide electrochemical working window and stable electrochemical properties. The NiNPs/ITO electrode exhibited enhanced electrocatalytic activity, ultra-high sensitivity, very low detection limits, wide dynamic range and good stability as well as a quick response for insulin detection, which allows for successful application for determining the concentration of insulin.

2. Materials and methods

2.1. Chemicals and materials

Bovine insulin (5800, $\geq$27 UPS units mg^{-1}) were purchased from Sigma. A stock solution of insulin (0.875 mM) was prepared daily by dissolving powdered insulin in water and adding 100 μL of 1.0 M HCl to dissolve the powder. Insulin solutions were prepared by diluting aliquots of the insulin stock solutions. ITO glass was purchased from Beijing Tsinghua Engineering Research Center of Liquid Crystal Technology. All chemicals were of analytical grade and used as purchased without further purification. All measurements were performed at room temperature. All solutions were prepared with triple distilled water.

2.2. Apparatus

The structure and morphology of the electrode were characterized by scanning electron microscope (SEM) (Hitachi X650, Japan). The SEM was also equipped with an energy dispersive X-ray (EDX) detector for elemental analysis. Electrochemical experiments were carried out in a CHI660 electrochemical work station (CH Instrument Inc., USA) with a conventional three-electrode setup, with the bare or modified ITO as the working electrode, Ag/AgCl (saturated KCl) as the reference electrode and platinum wire as the counter electrode. Amperometric measurements were carried out by the successive injection of a certain concentration of insulin into a stirring 0.1 M NaOH solution. All of the measurements were performed at a controlled temperature of 25.0 $\pm$ 1.0 °C.

2.3. Preparation of the NiNPs/ITO electrode

For preparation of the NiNPs/ITO Electrode, please see the Supporting information.

3. Results and discussion

3.1. Characterization of NiNPs/ITO electrode

The film morphology and size distribution were confirmed by SEM (Fig. 1). There is a dramatic change in the surface morphology after ion implantation. Some compact arrangements of spherical-shaped grains are observed on the bare ITO electrode surface (Fig. 1A). As shown in Fig. 1B, after implantation, most of the spherical faces of the bare ITO disappear, at the same time NiNPs are clearly observe. The NiNPs have a diameter ranging from 4 to 8 nm in the area examined and are evenly distributed, which may provide a large surface area for the electrocatalytic reaction.

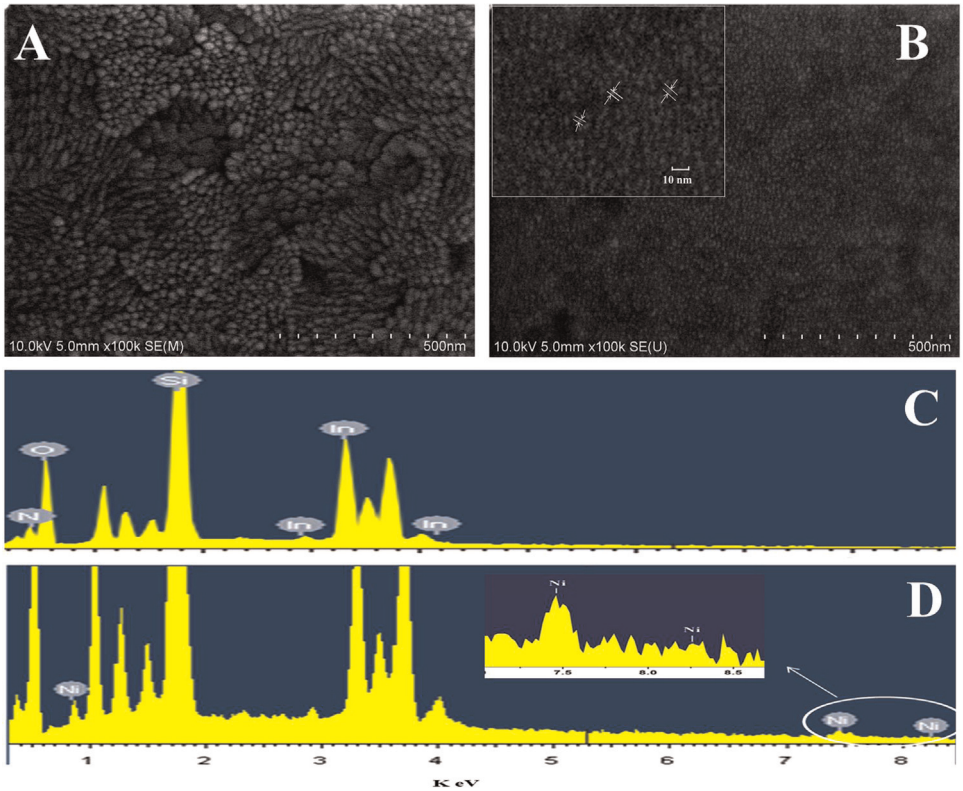

Fig. 1. SEM images of the bare ITO (A) and the NiNPs/ITO (B). EDX image of bare ITO (C) and NiNPs/ITO (D).Inset is a zoom of the area.

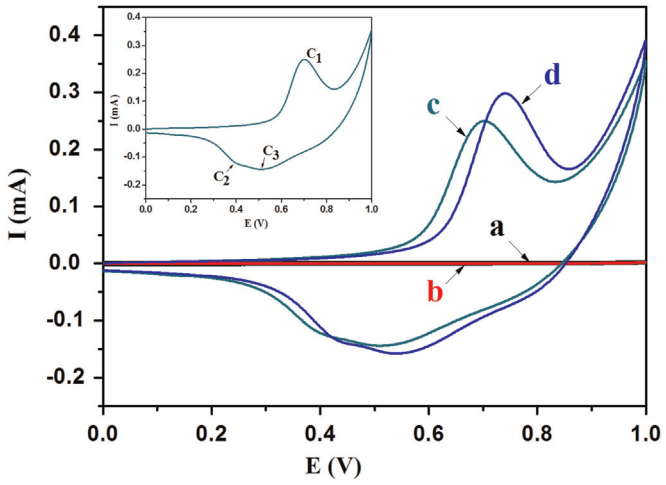

Fig. 2. Cyclic voltammetry of the bare ITO (a) and NiNPs/ITO (c) in the absence of insulin, and the bare ITO (b) and NiNPs/ITO (d) in the presence of 5 μM in 0.1 M NaOH. Inset: cyclic voltammetry of the NiNPs/ITO in 0.1 M NaOH without insulin. Scan rate: 100 mV s^{-1}.

Furthermore, Ni peaks are clearly detected by EDX (Fig. 1C), indicating the successful implantation of NiNPs onto the ITO film. The above results are in good agreement with the SEM finding.

3.2. Direct electrochemical detection of insulin

The electrochemical behavior of the NiNPs/ITO electrode was investigated using cyclic voltammetry (CV) in a potential window of (0.0–1.0 V). In Fig. 2, curves a and b show the CV of the bare ITO in the absence and the presence of 5 μM insulin in 0.1 M NaOH, respectively. No peak is found for the bare ITO. As seen from curve c, the anodic and cathodic peaks are detected, which are formed over 30 cycles in 0.1 M NaOH in a scanning ranging from −1.0 to 1.0 V. The bell shaped peak is indicative of a surface confined electrochemical reaction. This oxidative current peak is ascribed to the oxidation of Ni(II) to Ni(III) (Asgari et al., 2011; Rafiee and Fakhari, 2013; Vuković, 1994), as shown in Eqs. (1) and (2).

$$Ni + 2OH^- \rightarrow Ni(OH)_2 + 2e^- \quad (1)$$

$$Ni(OH)_2 + OH^- \rightleftharpoons NiOOH + H_2O + e^- \quad (2)$$

A pair of reductive current peaks is observed during the negative potential scan direction. The reductive current peak observed at approximately 0.509 V (peak C_3) has been ascribed to the cathodic peak current of β-NiOOH to β-$Ni(OH)_2$, while the weak peak observed at approximately 0.385 V (peak C_2) has been ascribed to the other cathodic peak current of γ-NiOOH to α-$Ni(OH)_2$ (Pissinis et al., 2012, 2014).

Curve d shows the CV responses of NiNPs/ITO electrode with 5 μM insulin. This clearly shows that the applied modifier in this process participates directly in the electrocatalytic oxidation of insulin. The increase in the oxidation current is attributed to the production of $Ni(OH)_2$ through the reaction between NiO(OH) and insulin; the $Ni(OH)_2$ produced is further oxidized to NiO(OH) at the electrode surface, indicating that the NiNPs/ITO electrode exhibits excellent electrocatalytic activity for the oxidation of insulin (Rafiee and Fakhari, 2013; Zhang et al., 2015). However, the anodic peak potential shifts to a higher potential, which could be due to the diffusion limitation of insulin at the electrode surface. The catalytic effect of the Ni(II)/Ni(III) redox couple for the oxidation of insulin occurs according to the following reactions shown in Eqs. (3) and (4):

$$Ni(OH)_2 + OH^- \rightarrow NiOOH + H_2O + e^- \quad (3)$$

$$NiOOH + insulin \rightarrow Ni(OH)_2 + Product \quad (4)$$

Meanwhile, It is observed that the cathodic peak current of β-NiOOH to β-$Ni(OH)_2$ increases and the cathodic peak current of γ-NiOOH to α-$Ni(OH)_2$ decrease. It is speculated that the existence of insulin promotes the $Ni(OH)_2$ in the formation of more β-NiOOH in an alkaline solution. Therefore, the cathodic peak current of β-NiOOH to β-$Ni(OH)_2$ increases during the negative potential scan direction.

3.3. Effect of scan rate and varying concentrations of insulin

Fig. 3 (A) shows the CV results for the NiNPs/ITO electrode in the presence of 0.1 μM insulin+0.1 M NaOH at different scan rates. The results demonstrate that the anodic peak current has a linear relationship with the scan rate (inset of Fig. 3A), with a correlation coefficient of 0.9958. This result suggests that the electron transfer between the redox sites of the NiNPs/ITO and the insulin is a typical surface-controlled electrochemical process. The effect of varying the insulin concentration added to the system was investigated in the range of 1–10 μM (Fig. 3B). A linear dependence of the catalytic oxidation currents versus the concentration of insulin can be fitted in the equation: (Ip (mA)=2.79+3.39 $C_{insulin}$ (μM), R^2= 0.9974). In addition, NiNPs exhibit superior catalytic activity for insulin oxidation.

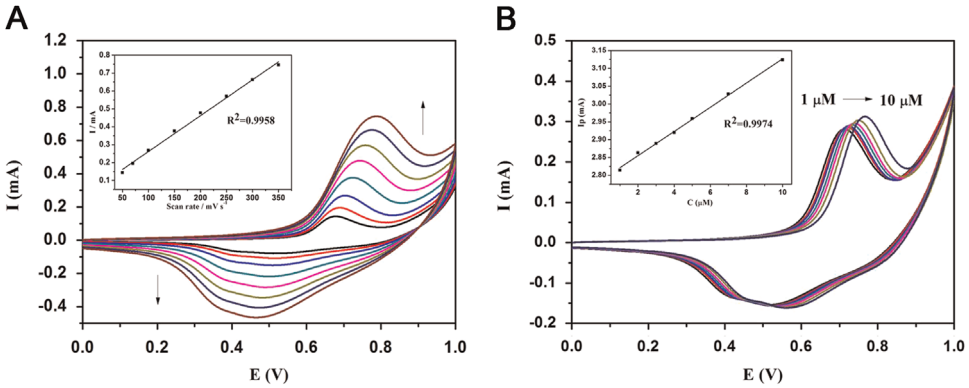

Fig. 3. (A) Cyclic voltammograms of the NiNPs/ITO electrode in 0.1 μM insulin+0.1 M NaOH at the following scan rates: 50, 70, 100, 150, 200, 250, 300, and 350 mV s^{-1}(from inner to outer). Inset: the plot of peak currents vs. scan rates. (B) Cyclic voltammograms of the NiNPs/ITO electrode in 0.1 M NaOH with different concentrations of insulin ranging from 1 to 10 μM. Scan rate: 100 mV s^{-1}. Inset: calibration curve of response current versus insulin concentration.

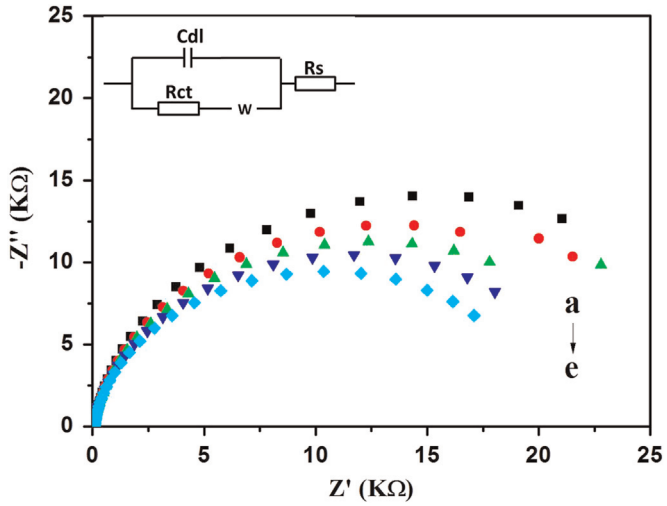

Fig. 4. Electrochemical impedance plots of the NiNPs/ITO electrode in the presence of 1 (a), 2 (b), 3 (c), 5 (d), and 7 (e) μM of insulin in 0.1 M NaOH. Inset: equivalent circuit used for data fitting.

3.4. Electrochemical impedance spectroscopy

Electrochemical impedance spectroscopy (EIS) analysis was further used to discover the catalytic procedures of insulin electrooxidation at the NiNPs modified ITO electrode. Fig. 4 shows the Nyquist plots of the NiNPs/ITO electrode recorded at 0.75 V in the frequency range of 1×10^{-2}–1×10^{6} Hz for selected concentrations of insulin in 0.1 M NaOH. With increasing insulin concentrations from 1 to 7 μM, a steady decrease in the diameter of the depressed semicircle was observed. An equivalent circuit compatible with the results is designed (the inset of Fig. 4). In this electrical equivalent circuit, R_s, C_{dl} and R_{ct} are the solution resistance, the constant phase element corresponding to the double-layer capacitance and the charge-transfer resistance associated with the oxidation of the insulin, respectively (Rafiee and Fakhari, 2013; Zhang et al., 2005). In this equivalent circuit, the double layer, capacitance, C_{dl}, is a constant phase element, and the charge transfer resistant, R_{ct}, decreases with increasing insulin concentration. This may be attributed to the insulin reacting with NiO (OH) to produce $Ni(OH)_2$ at the electrode surface, thus, leading to the target analytes concentration dependency on the charge transfer R_{ct}.

3.5. Amperometric response of the NiNPs/ITO electrode to insulin

Because cyclic voltammetry is not particularly sensitive for low concentrations, the amperometric measurements are carried out at the NiNPs/ITO electrode by successive injections of certain concentrations of insulin at a constant potential of 0.74 V. The response to low insulin concentrations is shown in Fig. 5. As seen from Fig. 5A, the current response is linear with insulin concentrations from 100 to 2500 pM, with a correlation coefficient of 0.9986 (Fig. 5A inset a). The sensitivity is 2140 μA μM^{-1}, which is much higher than that of other similar insulin detecting constructions. The NiNPs/ITO electrode showed ultra-high sensitivity in detecting low concentrations of insulin. Fig. 5A, inset b shows the detection limit of 10 pM at signal to noise ratio of 3. As seen in Fig. 5B, the sensor also displays a narrow linear response to insulin at high concentrations ranging from 1 nM to 15 nM (the correlation coefficient is 0.9940) and from 15 nM to 125 nM (the correlation coefficient is 0.9947). The NiNPs/ITO electrode offers many advantages, including a much lower detection limit, wider linear range, and ultra-sensitivity compared to other modified

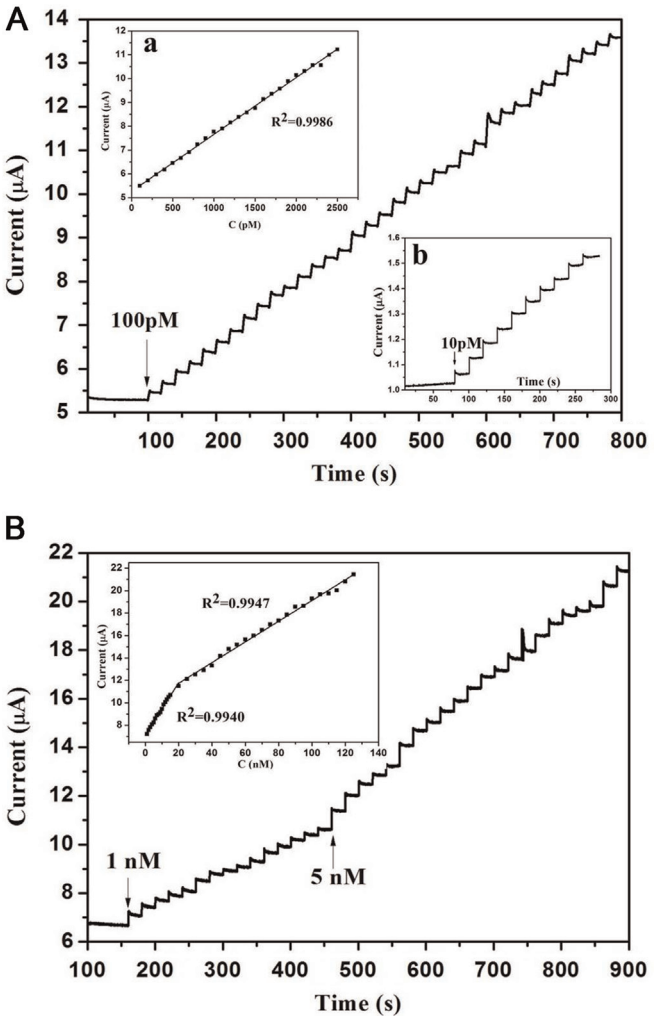

Fig. 5. (A) Chronoamperometry (I–t) responses of NiNPs/ITO for the successive addition of 100 pM and 10 pM (Inset b) insulin into 0.1 M NaOH. Inset a: calibration curves of the response current versus the insulin concentration. (B) Chronoamperometry responses of NiNPs/ITO for successive addition of 1 nM and 5 nM insulin into 0.1 M NaOH. Inset: calibration curves of the response current versus the insulin concentration. Applied potential: 0.74 V.

electrodes, as summarized in Table 1. All of these are attributed to the NiNPs/ITO electrodes with high specific surface area and high electron conductivity. The excellent performance of the NiNPs/ITO shown here indicates the potential of this material to detect insulin.

3.6. Interference and stability of the NiNPs/ITO electrode

Anti-interference is an important factor for sensors. Because the easily oxidative species such as ascorbic acid (AA), uric acid (UA) and glucose usually co-exist with insulin in human blood, the electrochemical response of the interfering species was also examined at the NiNPs/ITO. As the concentration of insulin in human blood is only a few picomoles (Groen et al., 1952; Berson and Yalow, 1966; Tiwari and Prasad, 2014), the interference of AA, UA and glucose to insulin is studied at picomole scale (Fig. S1A). A well-defined insulin response was obtained, while insignificant responses were observed for these interfering species. However, when 5 μM insulin is combined with 500 μM glucose, 250 μM AA and 250 μM UA (Fig. S1B), Strong interferences are observed, indicating that the modified electrode has strong electrocatalytic activity to the excess amount of AA, UA and glucose. This is a common issue (Zhang et al., 2015; Amini et al., 2014; Arvinte et al.,

Table 1
Comparison of the response characteristics of different modified electrodes for insulin detection.

Electrode	Linear range	Detection limit	Sensitivity ($\mu A\ \mu M^{-1}$)	Reference
$K_4[Mo(CN)_8]$+Ni/CCE[a]	0.5–500 nM 100–500 pM	0.45 nM ± 0.05 40 pM	6.14 8.10	Salimi et al. (2006)
NiONPs/Nafion-MWCNTs[b]/SPE[c]	20–260 nM	6.1 nM	1.83	Rafiee and Fakhari (2013)
$Ni(OH)_2$–GN[d]/GC[e]	800–6400 nM	200 nM	–	Lin et al. (2014)
Guanine/NiOxNPs/GC	100–1000 nM	22 pM	100.9	Salimi et al. (2008)
EDA[f]–CNFs[g]–NiO	20–1020 nM	12.1 nM	1.55	Zhang et al. (2015)
NiNPs/ITO	100–2500 pM 1–125 nM	10 pM	2140	This work

[a] carbon ceramic electrode.
[b] multiwalled carbon nanotubes.
[c] screen printed electrode.
[d] graphene nanocomposites.
[e] glassy carbon electrode.
[f] ethylenediamine.
[g] carbon nanofibers.

2010; Salimi et al., 2009) in the electrocatalytic determination of insulin and further study is in progress for a better diminution of oxidizable interfering compounds, for example by coupling with flow system.

Stability tests were carried out for the NiNPs implanted-modified ITO electrode. Fig. S2 shows the chronoamperometric curves of the NiNPs/ITO electrode in a 0.1 M NaOH solution containing 5 μM insulin at a constant potential of 0.74 V versus Ag/AgCl for 600 s. The current remains relatively stable after 100 s. This result suggests that the NiNPs/ITO electrode has good short-term stability for the electrochemical detection of insulin. The long-term stability of the modified electrode was also investigated by storing it at room temperature for 4 weeks. Only a small decrease in the current (approximately 7%) for 5 μM insulin was observed (not shown).

3.7. Analytical applications

To check its practical application, the proposed sensor was used to determine insulin from a commercial injection (Table S1) using the recommended procedure. The insulin injection sample was purchased from a drug company without any further treatment. It is an asepsis liquor of insulin, containing glycerol, phenol (0.25 mg/100 mL) and sodium hydrogen phosphate. There are no possible interferences (such as glucose) in these commercial injection samples. In addition, a certain amount of the standard solution of insulin was added into the corresponding samples to test the recovery. The precision and excellent recovery reported in Table S1 clearly indicats the reliability of the proposed NiNPs/ITO electrochemical sensor for practical application

4. Conclusions

An ultra-sensitive electrochemical sensor composed of implanted NiNPs and ITO electrode was fabricated for insulin detection. SEM results indicate that the NiNPs were immobilized on the ITO electrode surface with sizes of ranging from 4 to 8 nm. The small size of the NiNPs greatly improved the specific surface area of the modified electrode. The NiNPs on the electrode greatly enhanced its electrocatalytic properties of insulin oxidation and detection. A linear relationship was observed in a wide insulin concentration range of 100–2400 pM and 1–125 nM. The proposed electrode exhibited ultra-high sensitivity (2140 $\mu A\ \mu M^{-1}$) and a very low detection limit (10 pM). Moreover, these modified electrodes showed good selectivity and stability. The NiNPs/ITO electrode is expected to be applied as a potential biosensor for insulin.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2015.09.036.

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