

Cobalt oxide nanostructure-modified glassy carbon electrode as a highly sensitive flow injection amperometric sensor for the picomolar detection of insulin

Abdollah Salimi · Rahman Hallaj

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Abstract Glassy carbon electrode modified with electro-deposited cobalt oxide nanostructure shows an excellent electrocatalytic activity toward insulin oxidation at a wide pH range. Cyclic voltammetry, hydrodynamic amperometry, and flow injection analysis (FIA) were used for insulin determination at a picomolar and higher-concentration range. Amperometric determination of insulin at this modified electrode yielded a calibration curve with the following characteristics; linear range, 100 pM–15 nM; sensitivity of 83.9 nA nM⁻¹ and detection limit 10 pM. FIA yielded the calibration curve with sensitivity and detection limit of 2.0 nA nM⁻¹ and 25 pM, respectively. Furthermore, the RSD of repetitive FIA for 200 pM insulin ($n=13$) is 2%. In addition, the interference effect of electroactive existing species (lactic acid, cholesterol, ascorbic acid, uric acid, and glucose) was eliminated by covering the surface of the modified electrode with nafion film. Fast response time, signal stability, high sensitivity, low cost, and ease of preparation are the advantages of the proposed insulin sensor.

Keywords Cobalt oxide · Nanostructures · Electrode · Insulin · Amperometry · Flow injection

Introduction

Metal oxide, oxyhydroxide, and their relevant metal alloys are extensively used in many different areas such as corrosion protective coating, electrochemical capacitors, electronic industry, magnetic devices, photochemical energy conversion, lithium ion batteries and ion storage materials, and display technology [1–6]. In addition, due to the low production cost, high stability, good electrical properties, low resistivity, and remarkable redox properties of metal oxide particles and nanoparticles, they are suitable for application in gas sensors [7–9]. Metal oxide or electron transfer mediators immobilized onto metal oxide films are suitable materials for sensors fabrication and electrode modification technologies [10–16]. Over the recent years, an increasing number of studies have focused on the production of novel; typically nanometer-scale forms of metal oxide include: ZnO [17], NiOx [18, 19], CuO [20], MnOx [21], IrOx [22], cobalt oxide [23, 24], and many others.

Cobalt oxide particles or nanoparticles are of particular interest due to their uses in heterogeneous catalysis for the oxidation of different molecules and biomolecules. The electrochemical sensor based on cobalt oxide material and nanomaterials for the detection of glucose [25], methanol [26], hydrogen peroxide [23], arsenic [27], guanine [24], and hydroquinone had been fabricated [28]. Furthermore, due to the biocompatibility of cobalt oxide nanostructures, they have been used on the immobilization of biomolecules such as FAD and hemoglobin [17, 29]. Based on the high stability, antifouling properties, and excellent electrocatalytic activity of cobalt oxide redox couples, these

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A. Salimi · R. Hallaj
Department of Chemistry, University of Kurdistan,
P.O. Box 416, Sanandaj, Iran

A. Salimi (✉)
Research Center for Nanotechnology, University of Kurdistan,
P.O. Box 416, Sanandaj, Iran
e-mail: absalimi@uok.ac.ir
e-mail: absalimi@yahoo.com

nanomaterial-based modified electrodes are promising as a sensor for the detection of different molecules and biomolecules.

Insulin is a peptide hormone that controls the glucose levels in blood, and its determination is greatly important for the clinical diagnosis of diabetes. The fasting blood insulin concentration in normal condition is around 50 pM and decreases significantly for patients with type 1 diabetes [30]. Higher fasting plasma insulin levels is often indicating type 2 diabetes which is independent of insulin resistance [31]. Therefore, the methods for measuring insulin concentration at picomolar levels are required for discriminating diabetic patients. Immunoassay [32], bioassay, [33] and chromatographic methods [34] are three main methods for the determination of insulin. The bioassay was not used for routine clinical analysis due to relative imprecision, length, and insensitivity. Cross-reaction and non-specific binding with coexistent biomolecules are the major interferences for insulin detection based on the immunoassay procedure [35]. High-performance liquid chromatography with UV-visible and fluorescence detectors [36, 37] and mass spectrometry with isotope dilution assays [38] have been used for insulin sensing. Chromatographic methods suffer from several drawbacks because they require a time-consuming work and expensive instruments.

The insulin molecule contains one intrachain and two interchain disulfide bonds [39]. The oxidation of disulfide bonds at different modified electrodes was reported [40, 41]. Direct electrooxidation of insulin is important for the development of fast and sensitive amperometric detectors coupled to flow systems or chromatographic instruments for this hormone. Low sensitivity, stability and reproducibility, slow kinetics, surface fouling, and high overpotential at which the insulin oxidation process occurred are the limitations of unmodified electrodes. Various electron transfer mediators have been used for the electrooxidation and determination of insulin [41–56]. Although the modified electrodes have been successfully employed for monitoring insulin, they usually have many disadvantages such as reduced stability and activity under a physiological condition, high detection limit, poor long-term stability, and complicated multi-step preparation methods. In the present study, the glassy carbon electrode modified with cobalt oxide nanostructures is used for the electrocatalytic oxidation of insulin at wide pH range. Cyclic voltammetry and amperometry techniques were used for insulin detection with this modified electrode. Finally, in terms of the analytical performance of a modified electrode, it was described as an amperometric detector for the picomolar detection of insulin in a flow injection system. By covering the modified surface with nafion film, the interfering effects of different electroactive compounds was diminished.

Experimental

Chemicals and apparatus

Bovine insulin (27 USP IU mg⁻¹) was obtained from Sigma and used as received. Phosphate buffer solution (PBS) was prepared from analytical reagent-grade chemicals without further purification using double-distilled water. Nafion (5% in ethanol), CoCl₂, and other used reagents were of analytical grade. Electrochemical experiments were performed with a computer-controlled μ -Autolab modular electrochemical system (Eco Chemie Ultecht, The Netherlands), driven with GPES software (Eco Chemie). A conventional three-electrode cell was used with an Ag/AgCl (sat., 3 M KCl) (Methrom, The Netherlands) as a reference electrode and a Pt wire as a counter-electrode. The working electrode was GC electrode ($\Phi=2$ mm) modified with CoOx nanostructures. All measurements were conducted in a thermostated temperature of 20±1 °C.

Modification of GC electrode with electrodeposited CoOx nanostructures

CoOx nanomaterials were electrodeposited in a conventional method as reported previously [23]. GC electrode (2 mm in diameter) was carefully polished with alumina on polishing cloth. The electrode was placed in ethanol container and a bath ultrasonic cleaner was used in order to remove the adsorbed particles. Repetitive potential cycling (30 cycles at a scan rate of 100 mV s⁻¹ in a potential range between 1.2 and -1.1 V) in phosphate buffer solution, pH 7, containing 1 mM cobalt chloride was used for the electrodeposition of cobalt oxyhydroxide film on the surface of GC electrode. Scanning electron microscopy (SEM), atomic force microscopy (AFM), and XRD spectroscopy are used in order to examine the crystal structure and surface morphology.

Flow injection assembly set-up

Amperometric measurement was done using a single-line flow injection manifold with a three-electrode electrochemical cell of the wall jet type [57]. An Ag/AgCl reference electrode was present in a circular chamber filled with 3 M KCl supplied with an external syringe. A separate chamber containing the working and a Pt auxiliary electrode (encircling the chamber) was connected to the chamber containing the reference electrode through four holes concentrically surrounding the inlet. The working electrode was a glassy carbon electrode modified with cobalt oxide nanoparticles. The distance between the inlet nozzle and the working electrode was about 2 mm. A Waltson Marlow peristaltic pump model 205CA (Delden, The Netherlands)

was equipped with silicon tubing (0.76 mm, i.d.) propelled the carrier, phosphate buffer solution 0.1 M, pH 9, with a flow rate of 0.8 ml min^{-1} into the flow line (Supelco Teflon tubing, 0.5 mm i.d.). A working potential of 0.7 V was applied to the electrode. The sample solution (100 μl) was injected into a carrier stream via a Rheodyne six-way Teflon rotary valve type 50 and the output signal was transferred to a Pentium IV 400 MHz computer at 0.1-s intervals via a RS-232 port using a 16-bit data acquisition module (Axiomtek 1–7018, Taiwan) operated with a graphical Lab VIEW program under Windows 2000.

Results and discussions

Electrochemical properties and morphology investigation of GC electrode modified with CoOx nanostructures

Cyclic voltammetry, AFM, and SEM techniques were used for the characterization and morphology investigation of electrodeposited metal oxides. The cyclic voltammogram, SEM, and AFM images of the electrodeposited CoOx nanoparticles are shown in Fig. 1 of “[Electronic supplementary material](#)”. SEM and AFM images show that the electrodeposited film exhibits a uniform and highly porous cobalt oxide nanostructure containing nanowires with an average diameter ranging from 50 to 80 nm. The XRD pattern of CoOx thin film onto GC electrode was recorded (not shown). No peaks corresponding to cobalt oxide were observed, indicating that the CoOx film showed an amorphous nature [29]. The formation of CoOx layer on the surface of the electrode was also checked by recording cyclic voltammograms of the modified electrode in alkaline solution (pH 12) without cobalt ions (Fig. 1 of “[Electronic supplementary material](#)”). The observed cyclic voltammogram of the proposed modified electrode is similar to the cyclic voltammograms of cobalt oxide-based modified electrodes which have been reported in literature [58–61]. The electrochemical properties, pH dependence, and stability of the modified electrode were reported in our previous study [23].

Electrocatalytic oxidation of insulin at CoOx nanoparticle-modified GC electrode

The oxidation of insulin at GC and CoOx nanoparticles/GC electrode was investigated at pH 9 phosphate buffer solution. Figure 1 shows the recorded cyclic voltammograms in the absence and presence of 70 μM of insulin at modified and unmodified electrodes dipped in PBS, pH 9, at a scan rate of 20 mV s^{-1} . As can be seen, no electrochemical responses at GC were observed in the absence and presence of insulin at a potential range of 0.1

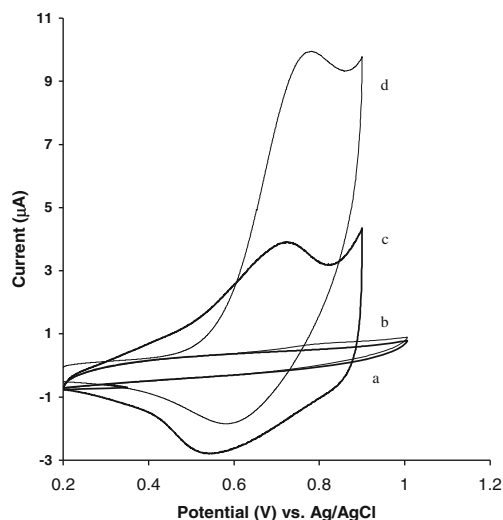


Fig. 1 Cyclic voltammograms of GC electrode modified with cobalt oxide nanostructures in buffer solution, pH 9, at a scan rate of 20 mV s^{-1} in the absence (c) and presence of 70 μM insulin (d). a and b are the same results for bare GC electrode

to 0.8 V. However, in the same conditions at GC/CoOx, the oxidation current of cobalt oxide film was greatly increased due to the catalytic oxidation of insulin, while the reduction peak has largely disappeared. The decreasing overvoltage and increasing oxidation peak of insulin confirm that cobalt oxide nanoparticles have a high catalytic ability for insulin oxidation. Therefore, CoOx nanoparticles are suitable as mediators to shuttle electron between insulin and working electrode and facilitate electrochemical regeneration following electron exchange with insulin. The effect of pH on the electrocatalytic activity of modified electrode toward insulin oxidation was investigated. Figure 2 illustrated the cyclic voltammograms of modified electrode in different pH solutions in the presence of 70 μM of insulin. As

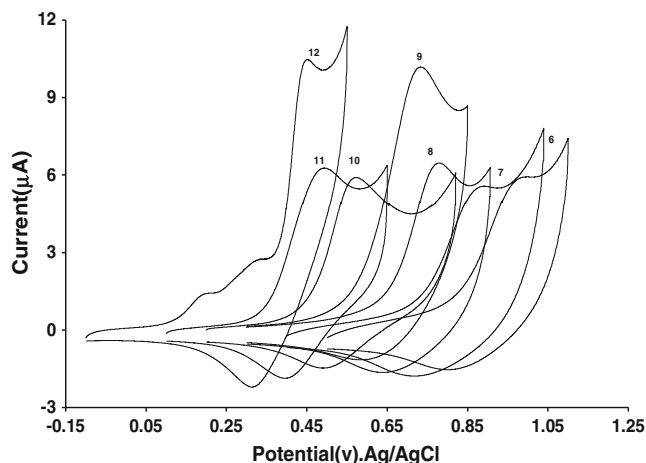


Fig. 2 Cyclic voltammograms of cobalt oxide nanomaterial-modified GC electrode in different pH solutions in the presence of 70 μM of insulin at a scan rate of 20 mV s^{-1}

shown, the modified electrode has excellent electrocatalytic activity toward insulin oxidation at a wide pH range. Both the peak current and peak potentials were dependent on the solution pH values. The anodic peak potential shifted negatively along with the increase of pH. This is a consequence of the deprotonation involved in the oxidation process, which is facilitated at higher pH values. With the change of pH from 5 to 12, the anodic peak currents increase and reach a maximum around pH 9 and pH 12. Since pH 9 is closer to the physiological pH, it was selected as an optimal value for insulin measurements.

Figure 3 shows the cyclic voltammograms at CoOx nanoparticles/GC electrode in PBS, pH 9, containing an increasing concentration of insulin. It can be noted that the peak currents increase with the increasing concentration of insulin, indicating that insulin can be detected at the proposed modified electrode. As can be illustrated by the inset of Fig. 3, the good linear relationship between the current response and insulin concentration (correlation coefficient 0.9962) is exhibited in the range up to 50 μM , with sensitivity and detection limit at 11.7 $\mu\text{A } \mu\text{M}^{-1}$ and 0.5 μM , respectively.

The cyclic voltammograms of 50 μM insulin at different scan rates were recorded (not shown). The peak current for the anodic oxidation of insulin is proportional to the square root of the scan rate, suggesting that the process is

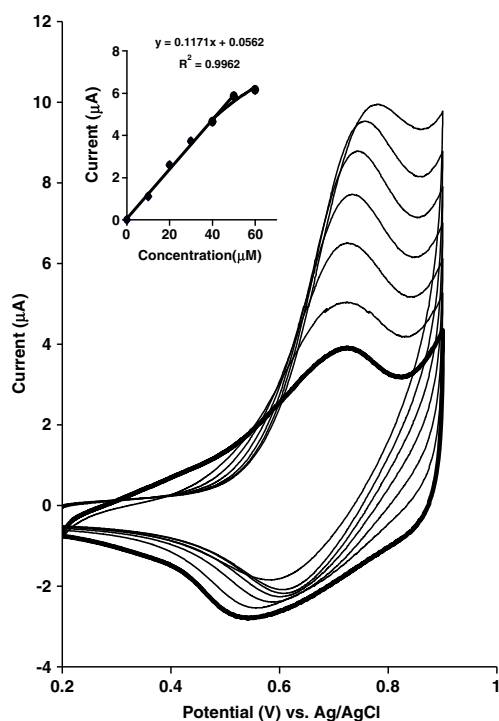
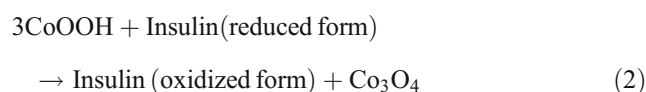


Fig. 3 Cyclic voltammograms of cobalt oxide nanoparticle-modified GC electrode in pH 9 buffer solution at scan rate 20 mV s^{-1} with increasing insulin concentration (from inner to outer): 0.0, 10, 20, 30, 40, 50, and 60 μM . *Inset*, plot of peak current vs. insulin concentrations

controlled by a diffusion of analyte as expected for a catalytic system. It can also be noted that, by increasing the scan rate, the peak potentials shift to positive values and the plot of peak current vs. square rate of scan rate deviates from linearity (at $\nu > 200 \text{ mV s}^{-1}$), suggesting a kinetic limitation in the reaction between the redox sites of adsorbed cobalt oxide and insulin. Also, a plot of the scan rate-normalized current ($I_p/\nu^{1/2}$) vs. scan rate exhibited the characteristic shape of a typical EC' catalytic process [62]. Based on the observed results, the following catalytic scheme describes the reaction sequence in the oxidation of insulin by cobalt oxide nanoparticles.



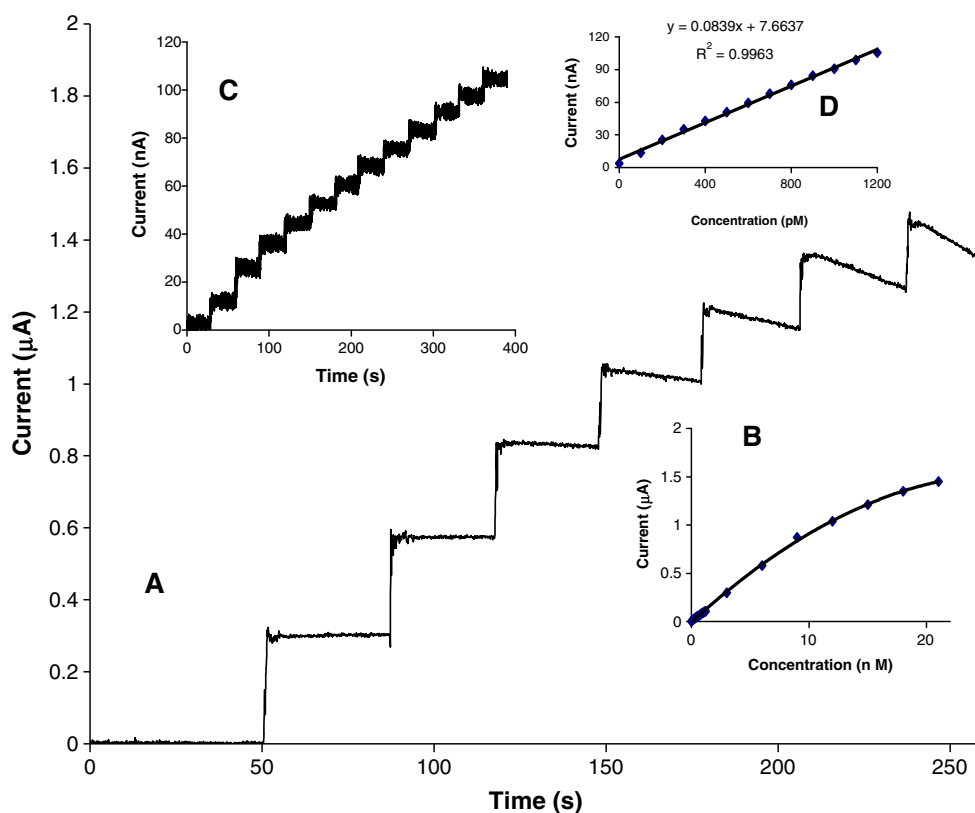
Amperometric detection of insulin at CoOx nanoparticles-modified GC electrode

Hydrodynamic amperometric method was used to examine the sensitivity of a modified electrode towards the detection of insulin. Figure 4 shows the amperometric $i-t$ curve obtained for insulin at CoOx-modified electrode in a homogeneously stirred PBS at an applied potential of 0.7 V. It is clear that, during the successive additions of 100 pM and 3 nM of insulin, a well-defined response was observed and which quickly reached a stable value within 3 s. Furthermore, the amperometric current response was increased linearly with increasing insulin concentration in the range 100 pM to 15 nM with a correlation coefficient of 0.9963. Detection limit and sensitivity were found to be 10 pM and 83.9 nA nM^{-1} , respectively. The linear dynamic range and the low detection limit of the modified electrode toward insulin oxidation were compared with the recently reported modified electrodes (Table 1).

The electrode response toward interference species

The influence of the common interfering substances of ascorbic acid, cholesterol, lactic acid, glucose, and uric acid on the determination of insulin was investigated. Ascorbic acid and uric acid are the main interferences toward insulin detection using a proposed modified electrode (not shown). For elimination of the interference effects of the proposed compounds, the glassy carbon electrode was modified with cobalt oxide nanoparticles covered with a thin film of nafion. In comparison to GC/CoOx nanoparticles, in the electrode for GC/CoOx nanoparticles/nafion electrode the electrochemical response toward insulin oxidation slightly

Fig. 4 Amperometric response at rotating modified GC electrode (rotation speed 2,000 rpm) held at 0.70 V in buffer solution (pH 9) for the successive addition of **a** 3 nM and **c** 100 pM of insulin. **b, d** Plots of chronoamperometric currents vs. insulin concentrations



decreased (10%). Figure 5 shows the recorded chronoamperograms of modified electrode during the injection of 2 nM of insulin and 200 μ M of ascorbic acid, glucose, and uric acid to the buffer solution (pH 9). As illustrated in Fig. 5, glucose, ascorbic acid, and uric acid did not show any interference to the insulin detection in phosphate buffer solution, pH 9, with the applied potential of 0.7 V. The interfering effects of lactic acid and cholesterol to the insulin signal were also negligible. These results indicate that the selectivity of this electrode in the detection of insulin is acceptable.

Flow injection analysis of insulin at CoOx nanomaterials modified GC electrode

Flow injection analysis is used for assessing the temporal response and overall analytical performance of the sensor toward insulin detection. The accelerated insulin oxidation associated with the low potential operation at CoOx nanomaterial-modified electrode results in effective amperometric detection. The subsequent flow injection experiments were performed using phosphate buffer, pH 9.0, as a carrier solution and 0.7 V as a sensing potential. Figure 6 compares the amperometric response of the sensor to successive additions of 100 pM insulin. Alternative injections produce reproducible amperometric response signals, indicating that there were no memory effect and

surface fouling that occurred during the successive measurements of insulin. As indicated from the resulting calibration plot (inset), the response of the modified electrode is linear over the entire 100–800 pM concentration range with a slope of 2.0 nA pM^{-1} and a correlation coefficient of 0.9905. The favorable signal-to-noise characteristics of these data indicates a detection limit of 25 pM (based on $S/N=3$). Table 1 compares the detection limit, sensitivity, response time, and linear concentration range of the proposed sensor for insulin determination with other modified electrodes. These results indicate that these analytical parameters are the best reported values for insulin determination with a simple, cheap, and reproducible modified electrode without using any specific reagent.

Stability and reproducibility of the modified electrode toward insulin detection

The stability and repeatability of the modified electrode were investigated. When the modified electrode was stored in the atmosphere, the current response decreased 6% after 2 months. Furthermore, the current response of the modified electrode in buffer solution, pH 9, after 100 rounds of cyclic scanning was almost unchanged. It proved the good stability of the modified electrode. To check the inter-electrode reproducibility of the modified electrode, five modified electrodes were tested simultaneously by

Table 1 Analytical parameters for different electrochemical insulin sensors

Electrode	Method	Dynamic range	Limit of detection	Sensitivity	pH
GC electrode modified with nickel oxide nanoparticles and guanine [12]	Amperometry	100 pM–4 μ M	22 pM	100.9 pA/pM	7.4
GC electrode modified with Ru-containing inorganic polymer film [41]	FIA	8.2 to 81.6 ng in 7.5 μ L	–	0.42 nA/ng	2.0
CCE modified with [Ru(bpy)(tpy)Cl]PF ₆ [42]	Amperometry	0.5–850 nM	0.4 nM	7,600 nA/ μ M	7.0
Carbon fiber microelectrode modified with a polynuclear RuO/RuCN film [43]	Amperometry	–	500 nM	0.441 nA/ μ M	7.4
Carbon electrodes modified with Ru-DRMs [44]	FIA	6–400 nM	2 nM	225 nA/ μ M	7.0
CP microelectrode modified with RuOx [45]	FIA	100–1,000 nM	50 nM	0.875 nA/ μ M	7.4
GC electrode modified with CNT [46]	FIA	100–1,000 nM	14 nM	48 nA/ μ M	7.4
GC electrode modified with chitosan and MWCNTs [47]	Amperometry	nM 3,000–100	30 nM	135 nA/ μ M	7.4
CCE modified with Ni powder [48]	Amperometry FIA	0.1–700 nM 15–100 pM	40 pM 2.6 pM	29.8 pA/pM 271.9 pA/pM	13
GC-modified IrOx [49]	Amperometry	50–500 nM	20 nM	35.2 nA/ μ M	7.4
RuOx-CNT-modified carbon electrode [50]	FIA	10–80 nM	1 nM	541 nA/ μ M	7.4
CCE modified with K ₄ [Mo(CN) ₈]+Ni powder [51]	Amperometry FIA	0.5–500 nM 100–500 pM	0.45 nM 40 pM	6140 nA/ μ M 8.1 nA/nM	7.4
Silicon carbide nanoparticle-modified GC electrode [52]	FIA DPV	100–600 pM 100–1,000 nM	3.3 pM 20 nM	101 pA/pM 864 nA/pM	7.4
(RuOx) on carbon fiber microelectrode [53]	FIA	100–1,000 nM	23 nM	0.072 nA/ μ M	7.4
Enzyme immunoassay based on H ₂ O ₂ detection at GC electrode modified with Os(II)-complex/HRP /polymer film [54]	Coulometry	Up to 4 nM	2 pM	-	7.0
CNT-modified electrode with insulin proteolysis assay [55]	CV	3.4 to 70 nM	1.34 nM	-	7.4
Silica gel-modified carbon paste [56]	Amperometry	0.09–1.4 nM	36 pM	0.107 nA/pM	7
Cobalt oxide nanomaterials modified GC electrode— This work	FIA Amperometry	100 pM–2 nM 100 pM–15 nM	25 pM 10 pM	2 nA/pM 83.9 nA/nM	9

GC glassy carbon, CCE carbon ceramic electrode, Ru-DRMs ruthenium metallodendrimer multilayers, CP carbon paste, CNT carbon nanotubes, MWCNTs multiwalled carbon nanotubes, FIA flow injection analysis, DPV differential pulse voltammetry, CV cyclic voltammetry

recording cyclic voltammograms in buffer solution, pH 9, containing 50 nM of insulin at a scan rate of 20 mV s⁻¹.

The average catalytic peak current is 6.5 μ A with a relative standard deviation of 3%. The GC electrode modified with

Fig. 5 Amperometric response of GC electrode modified with cobalt oxide nanostructures (rotation speed 2,000 rpm) at an applied potential of 0.70 V in buffer solution (pH 9) for the successive addition of 2 nM of insulin and 200 μ M of ascorbic acid, uric acid, and glucose

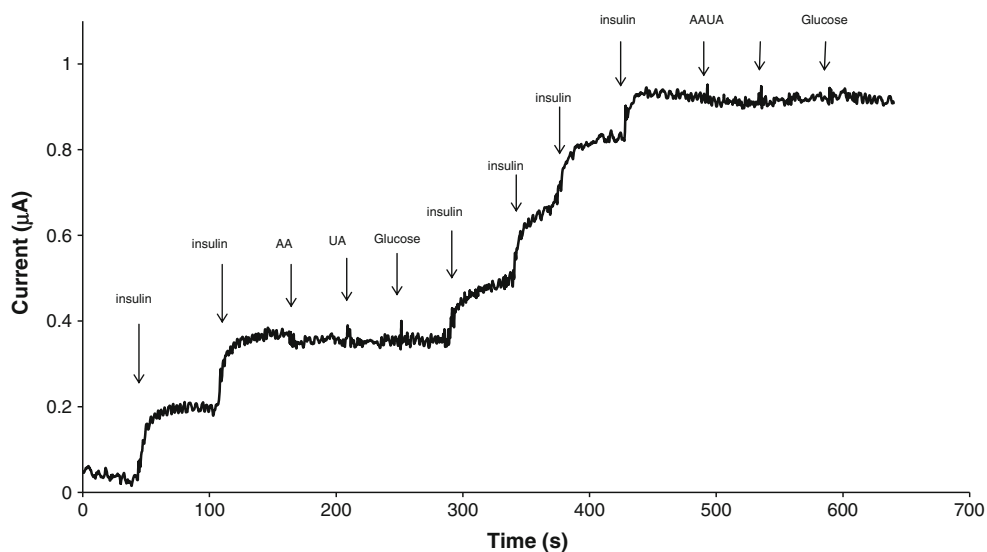
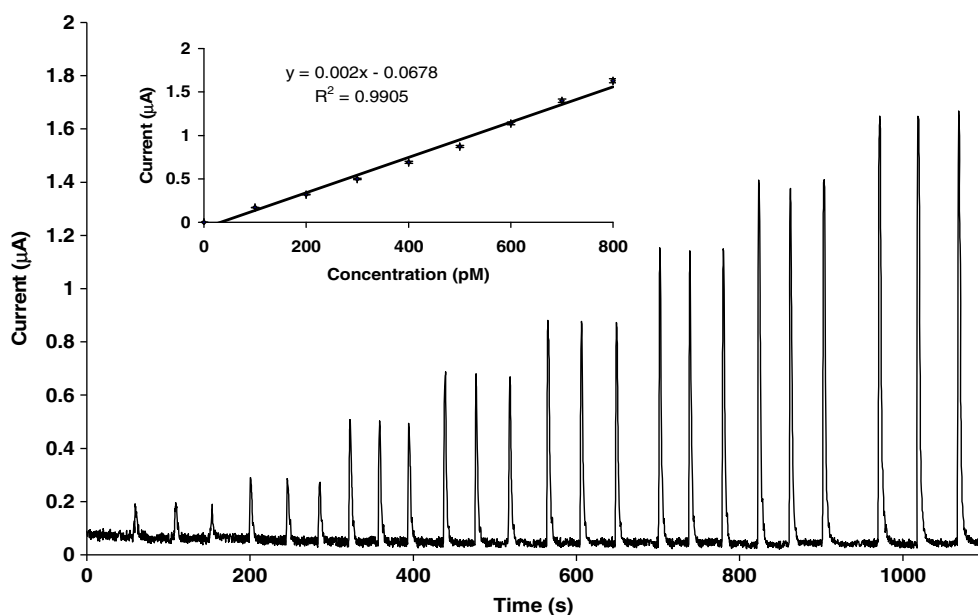


Fig. 6 Flow injection amperometric responses of the modified electrode to 100 to 800 pM of insulin, operation potential 0.7 V. *Inset*, plot of amperometric response vs. insulin concentration

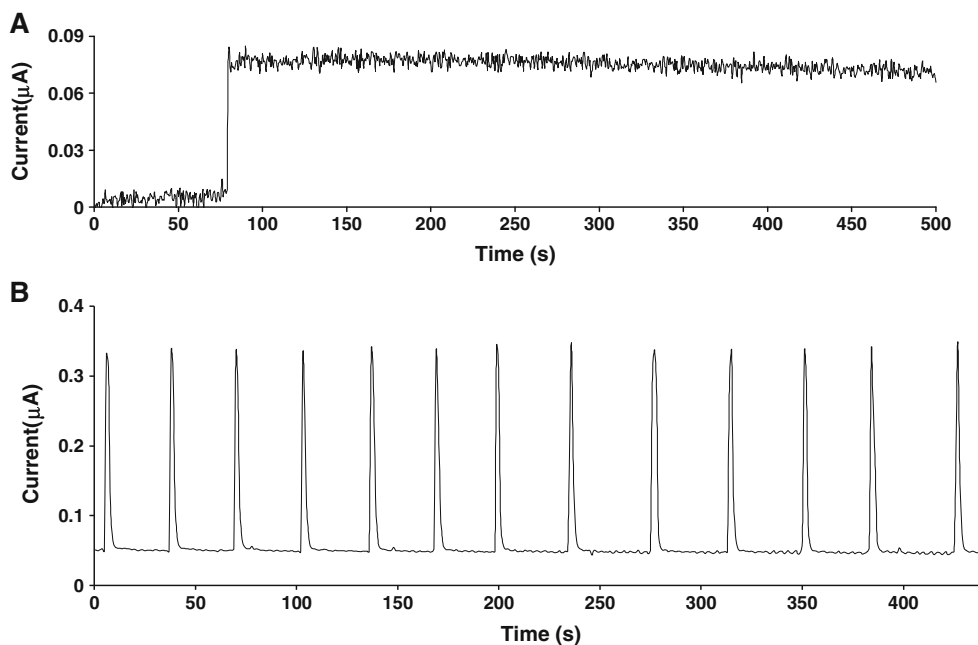


cobalt oxide nanoparticles imparts a higher stability onto amperometric measurements of insulin. The stability of the modified electrode toward insulin detection was also tested (Fig. 7a). It has been noted that the response of the rotated modified electrode toward 1 nM of insulin is extremely stable throughout the entire experiment with only 4% of loss during 500 s. Figure 7b displays the stability of the response for 13 repetitive flow injection measurements of 200 pM of insulin. As shown well, a stable and reproducible current peak for insulin oxidation was obtained (RSD 2%). These results indicate that a CoOx nanoparticle-modified GC electrode is a stable and efficient electrochemical sensor for insulin analysis.

Conclusion

In summary, we have demonstrated a novel, yet simple, electrochemical sensor for the detection of insulin at a wide pH range. The sensor was fabricated by immobilizing cobalt oxide nanomaterials onto a glassy carbon electrode. The presence of cobalt oxide nanoparticles improved the performance of the electrode and increased the sensitivity of the sensor for detecting insulin. This sensor has adequate sensitivity and precision, with a limit of detection at 10 pM and an acceptable dynamic range of three orders of magnitude. The sensitivity, linear dynamic range, and limit of detection of the proposed sensing system are comparable

Fig. 7 a The recorded chronoamperogram for 1.0 nM of insulin during 500 s; other conditions as in Fig. 6. **b** Repetitive flow injection analysis for 20 pM of insulin



to those of previously reported electrochemical sensors. By covering the modified surface with a nafion film, the interference effect of ascorbic and uric acid is eliminated. Considering the high sensitivity, as well as the cost-effectiveness and portable features of electrochemical techniques, we expect that the CoOx nanostructure-based electrochemical sensor will be a promising candidate for the selective detection of insulin at nanomolar and picomolar concentration ranges.

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