Microliter Sample Insulin Detection Using a Screen-Printed Electrode Modified by Nickel Hydroxide

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ABSTRACT: The monitoring of insulin, which is the only hormone that helps regulate blood glucose levels in the body, plays a key role in the diagnosis and treatment of diabetes. However, most techniques today involve complicated electrode fabrication and testing processes, which are time-consuming and costly, and require a relatively large volume of sample. To overcome these drawbacks, we present here a low-cost insulin detection method based on a screen-printed electrode (SPE) modified by nickel hydroxide (Ni(OH)₂). This novel method only requires 300 μL of insulin sample, and the time it takes for electrode preparation is about 12 times shorter than traditional electrode fabrication methods such as coating and sol-gel methods. The electrochemical behaviors of the Ni(OH)₂-coated SPE (NSPE) sensing area in insulin aqueous solutions are studied using cyclic voltammetry, amperometric i-t curves, and electrochemical impedance spectroscopy. The results demonstrate that the NSPE sensing surface has excellent detection properties, such as a high sensitivity of 15.3 μA·μM⁻¹ and a low detection limit of 138 nM. It takes a short time (~10 min) to prepare the NSPE sensing surface, and only two drops (~300 μL) of insulin samples are required in the detection process. Moreover, the selectivity of this method for insulin detection is verified by detecting mixtures of insulin and ascorbic acid or bovine hemoglobin. Finally, we discuss the potential clinical applications of this method by detecting various concentrations of insulin in human serum.

1. INTRODUCTION

Insulin, a critical polypeptide hormone produced by the pancreas, is the only hormone that can regulate the blood glucose levels and keep them in a narrow concentration range. The hyposecretion or dysfunction of insulin in humans can cause diabetes mellitus, a severe illness that may lead to chronic damage or dysfunction of various tissues such as eyes, kidneys, heart, blood vessels, and nerves and even death. Compared to glucose, insulin is a stronger predictor of the condition of the pancreas and plays a more significant role in diabetes diagnosis and typing. As a result, fast and straightforward methods for high-accuracy insulin detection have become a popular topic among researchers around the world. Besides insulin, the detection of other kinds of proteins directly from human serum samples also plays a key role in illness diagnosis, drug development, and medical technology.

Typical insulin detection methods include enzyme-linked immunosorbent assay, radioimmunoassay, and high-performance liquid chromatography. However, they are time-consuming, cumbersome, and costly while requiring extra complex materials and unique instruments. By contrast, the direct electrochemical approach has become a preferred choice because of its advantages such as simplicity, low cost, high efficiency, and high sensitivity.

An insulin molecule is composed of two polypeptide chains referred to as the A and B chains. The A chain consists of 21 amino acids and the B chain consists of 30 amino acids. Figure 1 shows an insulin structure with three disulfide bonds and four tyrosine residues. Some functional groups of an insulin molecule are reported to be electrically active, and the redox sites are usually deemed hydroxyl and disulfide bonds. However, most research studies today have focused on three disulfide bonds. The one formed in the A chain is exposed to the surface of the insulin’s three-dimensional structure and proved to be electrically active. The other two link the A and B chains together. Of these two disulfide bonds, one is
buried deep in the molecule and can hardly react with the other matter; the other one is covered up and reacts very slowly. Additionally, the electrochemically active tyrosine residues are usually buried deep in the three-dimensional structure to form hydrophobic centers. This characteristic makes it difficult for the tyrosine residues to take part in the electrochemical oxidation process on the electrode surface. Therefore, the uncovered disulfide bond in the A chain is usually used for the catalytic oxidation of insulin. However, this direct electrooxidation method has shortcomings such as slow oxidation kinetics and surface contamination, which can be addressed by surface modification.

Unmodified electrodes offer limited sensitivity and stability when they are directly used for the electrochemical detection of insulin. Their surface may be easily polluted by the high potential produced during insulin oxidation. A variety of functionalized electrodes have been employed to facilitate insulin oxidation and detection. Examples include screen-printed electrodes (SPEs) modified by carbon nanotube–nickel–cobalt oxide, carbon electrodes modified by nickel nanoparticles, carbon paste electrodes modified by silicon carbide nanoparticles and silica gel, glassy carbon electrodes modified by nickel oxide nanoparticle-multiwalled carbon nanotubes (MWCNs), and nickel nanoparticle-modified electrodes. In particular, Lin et al. demonstrated a method that uses a graphene nanometer light sensor for real-time detection of insulin. The concentration of insulin is reflected by the conductivity changes during the combination of electrodes and insulin.

In addition, electrokinetics has emerged as a promising method to enhance the sensitivity and applicability of modified electrode-based detection methods for electrochemical applications. It has been demonstrated that electrokinetics enables in situ improvement of the performance of electrochemical

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**Figure 1.** NSPE reaction process and the molecular structure of insulin.

**Figure 2.** (A,B) SEM images of the SPE. (C,D) SEM images of the SPE after modification. (E) EDX image of the modified electrode.
biosensors in pathogen and cytokine detection. Wong et al. enhanced the detection results of *Escherichia coli* by using an AC electrothermal flow technique. Furthermore, this kind of electrodynamic enhancement also brings improved detection limits for electrochemical sensors.

Metal oxides and semiconductor nanomaterials (e.g., Ag, Au, and Ni(OH)₂) have been widely applied in fabricating functionalized electrodes. Various forms of Ni and Ni(OH)₂ materials have been used in sensors because they are nontoxic, low cost, and easy to prepare and store. Nickel, as a modifier, has a variety of oxidation states and is therefore a suitable candidate for fast electron transfer. Ni(OH)₂/NiOOH exhibits good electrocatalytic effects on insulin. Because of their respective advantages, these materials have drawn considerable attention from the research community. Lin et al. proposed a Ni(OH)₂−GN/GC electrode synthesized via a one-pot method. Martinez-Periñán et al. presented a Ni(OH)₂ NPs/Nafion-MWCNTs/GC electrode by means of electrodeposition. Jia et al. reported a stable ultrathin film of Ni(OH)₂ nanoparticles at the gas/liquid interface.

In this work, we functionalized a SPE with Ni(OH)₂ through a simple and fast electrodeposition process in which only a milliliter scale of electrodeposition solutions (3 mL) was used. We chose the SPE because of its small size and low cost. The fabricated Ni(OH)₂-coated SPE (NSPE) exhibits satisfactory electrocatalytic activity, stability, high sensitivity, and low detection limits, as well as quick response times for insulin detection.

### 2. RESULTS AND DISCUSSION

#### 2.1. Characterization of the NSPE

The morphology and element composition of the NSPE were investigated utilizing scanning electron microscopy (SEM) and energy-dispersive X-ray (EDX). In the experiment, a layer of platinum film was sprayed on the surface of the SPE to ensure better conductivity. The SEM images of the SPE before and after deposition and the EDX images of the decorated SPE are shown in Figure 2. The surface of the coated electrode is flat, while after deposition, the surface of the modified electrode has different flakes of particles and large bulges (Figure 2C). At 100k times magnification (Figure 2D), differences can also be observed in surface morphologies of the bare electrode and the modified electrode, which indicates that the target material was attached to the bare electrode. Furthermore, the elements present on the coated SPE surface were analyzed using EDX. As shown in Figure 2E, Ni and O elements are present on the surface of the carbon electrode. Because the current energy spectrometer cannot detect elements before the fifth element, the H element is not shown in the EDX result. Therefore, the above results demonstrated that the SPE was successfully modified by nickel hydroxide.

### 2.2. Electrochemical Characterization of the NSPE via Electrochemical Impedance Spectroscopy

Electrochemical impedance spectroscopy (EIS) is a powerful and informative technique. It can provide characteristics of the electrode surface to help determine whether a functionalization process is successful. Figure 3A shows the Nyquist plots of the bare (black dots) and functionalized electrodes (red dots) with the same voltage as applied in the open-circuit condition and a frequency range of 1 × 10⁻² to 1 × 10⁶ Hz. It is composed of two typical parts: the semicircular part that corresponds to the electron-transfer restriction process and the linear part that represents the diffusion restriction process. The semicircle’s diameter represents the electron-transfer resistance on the electrode surface. The nickel hydroxide-modified electrode has a 10.87% smaller electron-transfer resistance on its surface than the bare electrode.
As shown in Figure 3A, the low-frequency part of the NSPE ($R^2 = 0.994$) is much closer to a straight line than that of the SPE ($R^2 = 0.813$). It demonstrates that modifying the electrode surface with nickel hydroxide can dramatically improve the conductivity and electron-transfer process. In other words, the functionalization process was successful, as evidenced by the changes in the electrode surface's characteristics.

2.3. Electro catalytic Activity of the SPE. The electrocatalytic activity of the NSPE was investigated using cyclic voltammetry (CV) in a potential window of 0.0 to 1.0 V. The NSPE and SPE were placed in 0.1 M NaOH solution with and without insulin, respectively. In Figure 3B, curves a and b show the CV of the SPE in the absence and presence of 1 mM insulin in 0.1 M NaOH solution, respectively. No peak is observed, indicating that almost no electrochemical reaction took place on the bare electrode surface. Curves c and d, in which oxidation peaks are observed, represent the CV of the NSPE in the absence and presence of 1 mM insulin in 0.1 M NaOH solution.

As shown in Figure 3B, the oxidation peak of curve d is larger than that of curve c. This means that when insulin is present, the oxidation current of the NSPE is larger than when it is absent, indicating that insulation was involved in the redox process. Oxidation peaks were observed under the voltammetry of the NSPE in the absence and presence of 1 mM insulin in 0.1 M NaOH solution. As the concentration of insulin increased from 1 mM to 10 mM, the oxidation peak current also increased, indicating that the oxidation peak is concentration-dependent. A voltage range from 0 to 1 V and a constant scan rate of 100 mV s$^{-1}$ were used throughout the experiment. As indicated in curve c, the oxidation current peak occurred in the oxidation of Ni(II) to Ni(III) (as shown in eq 1). A reduction peak attributed to the conversion from $\beta$-NiOOH to $\beta$-Ni(OH)$_2$ (as shown in eq 2) can also be observed in the direction of negative potential scan.

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\begin{align*}
ni(OH)_2 + OH^- & \rightarrow NiOOH + H_2O + e^- \\
\beta-NiOOH + H^+ + e^- & \rightarrow \beta-Ni(OH)_2
\end{align*}
\]

These results indicate that the NSPE has excellent electrocatalytic activity for insulin oxidation and can be further applied for accurate insulin determination.

2.4. Effects of Scan Rate and Insulin Concentration. How the scan rate affects the oxidation current was studied using CV, where the NSPE was immersed in 0.1 M NaOH solution in the presence of 0.1 mM insulin. As shown in Figure 3D,E, the oxidation current increases with the scan rate. This indicates that the electrode surface was undergoing redox reaction with insulin, which is believed to be a surface-controlled electrochemical process where electron transfer takes place. The potential of the oxidation peak gradually increases as the scan rate increases from 50 to 200 mV s$^{-1}$ with uneven steps. This implies that the redox reaction between the modified electrode and insulin is a typical surface-controlled electrochemical process.

According to the conclusion drawn by Yu et al.,$^{13}$ $i-t$ curve is another sensitive measurement technique for detecting low concentrations of insulin. Figure 3C shows how varying concentrations of insulin affect the oxidation current through amperometric $i-t$ curves. The $i-t$ curve moves up with the increase in insulin concentration. Surface control plays an important role in the whole oxidation process, which is catalyzed by the modified electrode as the insulin concentration increases. In our work, the NSPE and the analysis of amperometric $i-t$ curves were further applied for insulin detection.

2.5. Electrochemical Determination of Insulin Based on the NSPE and Amperometry. Insulin detection was...
μ concentration. Table 1 provides a comparison between our results and that of Zhang et al. detected insulin using an EDA-CNF-NiO/GCE electrode.39 The results demonstrated that the influence of a small amount of UA, AA, or glucose on insulin detection was negligible. Based on the same principle, Zhang et al. detected insulin using an EDA-CNF-NiO/GCE electrode.39 They examined the roles of γ-globulins, myoglobin (Myb), and pepsin as interferents in biological liquids and found that these compounds had less influence on insulin detection. A more detailed comparison is shown in Table 2. In order to study the selectivity on insulin detection, the NSPE was also tested with two interferents: 0.1 mmol/L AA and 1 mmol/L bovine hemoglobin (BHB). Figure 4C shows that the NSPE works well on detecting human insulin (10 pmol/L), regardless of the presence of AA and BHB, which demonstrates that the NSPE is a highly selective method for detecting insulin.

To examine the detection capability of the NSPE in a more realistic working environment, the NSPE was used to detect the content of insulin in human whole serum (purchased from Shanghai Qiaoxing Biology). In a normal human body, the fasting plasma insulin content is 35–143.5 pmol/L.40 The half-life of insulin is less than 90 min.41–43 This means that the insulin originally contained in that serum can be ignored. Therefore, different concentrations of human insulin were added to the serum to simulate the blood of diabetics with different secretion levels of insulin. The current was studied by amperometric i–t curves. Figure 4D shows the relationship between the concentration of insulin and the detecting current based on the NSPE. Because the serum environment is different from the calibration environment, that is, 0.1 M NaOH solution, used in the experiment in Section 2.5, the sensitivity and detection limit of the NSPE in detecting whole serum vary slightly from those obtained from our experiment. Finally, we demonstrated that the NSPE is capable of detecting insulin originally contained in that serum.

Table 1. Analytical Parameters for the Detection of Insulin by Several Modified Electrodes

| electrode | fabrication method | time consumed | electrode size | detection limit (nM) | sensitivity (μA·μM−1) | reference |
|-----------|--------------------|---------------|---------------|----------------------|------------------------|-----------|
| Ni(OH)2/GN/GCE | amperometry | | | 200 | | 31 |
| SiO2 NPS-Nafion/GCE | CV | | | 30 | | 35 |
| NiONPs/Nafion-MWCNTs/SPE | amperometry | D = 3 mm | | 6.1 | | 27 |
| NiCoO2/CNT/SPE | amperometry | D = 3 mm | | 184.88 | | 14 |
| Ru-o/NC-Ru film | amperometry | D = 0.15 mm, L = 10 cm | | 500 | | 36 |
| NSPE | amperometry | D = 2.5 mm, L = 3 cm | | 138 | | this work |

“D represents the diameter and L represents the length; the unit conversion of ref 14 used for the molecular mass of insulin was 5733.59.

Table 2. Comparison of Several Modified Electrodes

| electrode material | fabrication method | time consumed | electrode size | fabrication material | interference | reference |
|--------------------|--------------------|---------------|---------------|----------------------|-------------|-----------|
| NiONPs/ITO | ion implantation | more than 0.5 h | D = 5.47 mm | ethanol, nitrogen gas, nickel ions | AA, UA | 13 |
| Ni(OH)2/NPs-Nafion-MWCNTs/GCE | cast electrodeposition | more than 0.5 h | D = 3 mm | NiCo2,2Ni(OH)2·4H2O, ethanol, carboxylated multivalved carbon nanotubes, chloroform, NaOH | AA, UA, glucose, acetoaminophen | 1,46 |
| Ni(OH)2/GN/GCE | one-pot | | | graphite powders, NiC6H6·H2O, NaOH, ethanol | | |
| silica gel/chitosan/Ni(OH)2 paste | electrolytic, drops of paint | more than 8 h | D = 3 mm | silice gel, sodium citrate solution, solid paraffin, Ni | AA, UA, glucose | 47 |
| EDA-CNFs/NiO/GCE | drops of paint, ultrasonic | more than 24 h | D = 3 mm | H2SO4, HNO3, A-CNPs, ethylenediamine anhydrous, NiO·6H2O, A-CNPs, NH4HCO3 | γ-globulins, Myb, pepsin | 39 |
| NSPE | electrodeposition | ~0.16 h | D = 2.5 mm, L = 30 mm | Ni(NO3)2·6H2O | AA, BHB | this work |

“Multivalved carbon nanotubes. Electromagnetic molecularly imprinted polymers. Glassy carbon electrodes. Graphene nanostructures. Screen-printed electrode. Indicates that the electrode has selectivity among these interferents. D represents the diameter and L represents the length. All these methods were built based on the same insulin detection principle: the (Ni(OH)2/NiOOH) redox.

performed based on the amperometric i–t curve. In this experiment, a voltage of 680 mV was applied, and for every 10 s, 5 μL of 1 μM insulin was injected near the NSPE immersed in 0.1 M NaOH solution. The insulin concentration was raised gradually by the step of 1 μM. Figure 4A shows the current response of insulin oxidized on the surface of the NSPE.

As illustrated in Figure 4B, there is a linear relationship (correlation coefficient: 0.9939) between the mean current (every 10 s) after each insulin injection and insulin concentration. Table 1 provides a comparison between our results and that of Zhang et al. detected insulin using an EDA-CNF-NiO/GCE electrode.39 They examined the roles of γ-globulins, myoglobin (Myb), and pepsin as interferents in biological liquids and found that these compounds had less influence on insulin detection. A more detailed comparison is shown in Table 2. In order to study the selectivity on insulin detection, the NSPE was also tested with two interferents: 0.1 mmol/L AA and 1 mmol/L bovine hemoglobin (BHB). Figure 4C shows that the NSPE works well on detecting human insulin (10 pmol/L), regardless of the presence of AA and BHB, which demonstrates that the NSPE is a highly selective method for detecting insulin.

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the concentration of insulin in human body and thereby aiding doctors in the diagnosis of diabetes.

3. CONCLUSIONS

In this paper, we present a novel method that uses a SPE modified by nickel hydroxide for insulin detection. The method allows the fabrication of an insulin detection sensor with a high sensitivity of 15.3 μA·μM⁻¹ and a low detection limit of 138 nM. Both the EIS and CV curves demonstrate that Ni(OH)₂ can be modified on the SPE surface to produce excellent conductivity and catalytic activity for insulin. Experiments with varying CV scan rates and insulin concentrations prove that the NSPE is highly sensitive to insulin. The results from insulin detection based on i−t curves reveal a linear relationship between the response current and insulin concentration, with a correlation coefficient of 0.9939. Furthermore, the selectivity for insulin of this method has been verified by testing the interferents of AA and BHB. In addition, the method’s capability of detecting insulin in human serum has been validated experimentally. To sum up, our work provides a fast, inexpensive, and simple solution to the detection of microliter scale insulin. The proposed NSPE proves to have good catalytic activity and may be used in other electrochemical detection applications in the future.

However, there are still some issues that remain to be addressed. Although the NSPE is easy to fabricate and ready for mass production, further studies are required to miniaturize it for commercialized fingertip blood detection while maintaining high levels of accuracy. Also, other types of insulin such as porcine insulin need to be factored in for comparison. Furthermore, in order to make the Ni(OH)₂ electrode more stable, it is essential to add some organics to modify its properties.

4. MATERIALS AND METHOD

4.1. Chemicals and Materials. The bovine insulin (5733.49, ≥27 UPS units mg⁻¹) was purchased from Sigma-Aldrich (USA) (the action of bovine is very similar to that of human insulin; the detection method for bovine insulin is also applicable to human insulin. An insulin stock solution (2 mM) was prepared daily by dissolving powdery insulin in water with 10.0 μL of HCl (1 M). Then, the stock solution was progressively diluted.

The pristine SPE was acquired from Xenslet Studio (China). The deposition solution was prepared by dissolving 0.992 g of Ni(NO₃)₂ powder in 100 mL of ultrapure water, and its concentration was 0.08 mol/L. 0.1 M NaOH was used as the electrolyte in the experiments given that it allows insulin to have good electrochemical response, and it closely matches the alkaline environment in human blood. AA and BHB were purchased from Sigma-Aldrich (USA). The human insulin (5807.69) was purchased from China Institute of Pharmaceutical and Biological Products (Beijing, China). All the electrochemical experiments were performed at room temperature. All solutions were prepared with ultrapure water. All other chemicals were of analytical grade and used without further purification.

4.2. Instruments. The structure and morphology of the SPE were observed by a scanning electron microscope (Hitachi, S4800, Japan). The electrochemical experiments were carried out in the CHI660e electrochemical workstation (Shanghai Chenhua Co., China). A conventional three-electrode setup was used with a bare or modified carbon electrode as the working electrode, Ag as the reference electrode, and a carbon electrode as the counter electrode. Both the EIS and CV experiments were carried out in 0.5 mL centrifuge tubes.

4.3. Preparation of the SPE. The SPE was immersed in the deposition solution for 600 s for the electrodeposition of Ni(NO₃)₂ under a voltage of 0.7 mV as provided by the electrochemical workstation. Then, the voltage was removed, and the treated SPE was left in the deposition solution for 30 s or longer to stabilize the modified layer. The SPE was then taken out and air-dried at room temperature. After these operations, the SPE surface was successfully coated by Ni(OH)₂ and ready for catalyzing the redox reaction of insulin. For convenience, we refer to the SPE coated with Ni(OH)₂ as the NSPE in this paper.

To identify the best potential for a complete Ni(II) to Ni(III) conversion, the NSPE was placed in 0.1 M NaOH solution for CV at different potentials, and a scan rate of 100 mV s⁻¹ was used. The portion of the SPE serving as the sensing element was originally in black (as shown in Figure 5A). When the SPE was submerged in the electrodeposition solution, bubbles would appear near the sensing area (as illustrated in Figure 5B). After the electrodeposition process, the surface of the sensing area would turn green, that is, coated with green Ni(II). During the detection of insulin by the NSPE, the green Ni(II) was oxidized to black Ni(III), and then the black Ni(III) would be reduced back to green Ni(II) (i.e., “grayish green”, as not all Ni(III) were reduced back to Ni(II). The entire process is shown in Figure 5.

Table 2 provides a comparison between our electrode and several other modified ones from the perspectives of electrode material, functionalization method, time consumed, electrode size, interference, etc.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (grant no. 61873307 and 61503322), the Scientific Research Project of Colleges and Universities in Hebei Province (grant no. ZD20190304), the Autonomous Research Program of Yanshan University (grant no. 14LGB011), the Hong Kong Research Grants Council (project no. 11204918), and the Shenzhen Municipality Science and Technology Innovation Commission (grant no. SGDX201908162312172S; “Nano Scale Imaging Microscopy System”, CityU).

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