Mainstream testing methods and non-enzyme electrochemical biosensors for glucose detection

Zhang Xiao *
Department of Northwestern Polytechnical University, Xi’an, China
* Corresponding author: jp2020304022@qmul.ac.uk

Abstract. Glucose is an indispensable substance in human’s body. A normal and stable blood glucose rate plays a significant role in leading a healthy living condition. Thus, an effective method to monitor glucose is what scientists are continuously studying to find. This work introduces the currently mainstream testing methods of glucose and a cutting-edge detecting equipment in this field. The mainstream methods include chromatography and biosensor methods. The biosensors are divided into two branches, optical and electrochemical biosensors. Colorimetric, fluorescence and chemiluminescence are three main principle of optical biosensor which already have a long-term development and are mutual for application. While for the electrochemical biosensor, enzyme-based biosensors are widely known and have already been introduced to the public. The non-enzyme, however, is the brand-new field of electrochemical biosensors, which have an ultra-high sensitivity and selectivity for glucose in blood. Carbon-based composites, noble metal-based composites, copper-based composites, and other metal-based materials can be the main function material of electrochemical non-enzyme biosensors. Here, carbon-based composites, noble metal-based composites, copper-based composites, and other metal-based materials biosensors are presented as the example to discuss the advanced aspects compared to other methods and the significance and feasibility of researching and applying this approach.

Keywords: Glucose, biosensor, chromatography, optical, colorimetric, fluorescence chemiluminescence, electrochemical enzyme-based non-enzyme.

1. Introduction

Based on statistical data, the global diabetic population is approaching nearly 500 million individuals. Projections indicate an anticipated 25% surge of patients over the coming decade [1]. Glucose, one important component in blood, undoubtedly. Glucose level in blood is a significant factor to diagnose diabetes 1&2 [2].

Furthermore, either too high or too low of the blood sugar level will also become a serious issue. When its level remains high for long time, individual will soon become unconscious caused by the ketones [2]. This symptom is regard as diabetic ketoacidosis. While the blood sugar is to low (hypoglycaemic), a diabetic coma will possibly develop [3]. Thus, finding an available method to monitor glucose level has become an urgent need.

2. Glucose Detection Method

2.1. Chromatography Method

Chromatography is a rapid, non-invasive, enzyme-free means of detecting blood glucose concentration. An analytical method is realized by adapting the non-invasive collection of condensation coupled with ion chromatography [4] to measure glucose content in exhaled breath of humans. Here, we apply ion chromatography in a pulsed amperometric instrument to analyse glucose in exhaled breath condensate from humans. When proportion of glucose changes, temperature of the exhaled breath condensate changes. Investigations reveal that they correlate linearly well [4]. So, we can just check the condensation temperature to find out the glucose level of the human breath, and so on to the blood sugar level.
Though this method has a relative competitive method for glucose analysis of exhaled breath in humans, the glucose level of breath may still not exactly equal to that of blood, causing an unignorable deviation.

2.2. Biosensor Method

2.2.1. Optical biosensor

Optical biosensor is a series of testing methods containing colorimetric, fluorescence and chemiluminescence [5], which are widely welcomed for their point-of-care testing ability.

Colorimetric biosensor: the theory it works on adding the analyte to cause the aggregation of nanoparticles, which will lead to colour change [5]. As an illustration, Jang et al. [6] produced PVP-AuAg spheres through the application of a polyvinylpyrrolidone (PVP) coating on the surface of a gold-silver alloy. This enables nanoparticles to form stable colloid in blood environment and can be used directly for colorimetric testing.

Fluorescence biosensor: the primary advantages of this kind of detection method are its high sensibility sensitivity and exceptional selectivity [5]. Special materials, whose fluorescence would be quenched when particular substance exists (glucose), are applied to achieve the design of fluorescence probe. According to the electron transferring mechanism, fluorescence will be quenched by glucose. In solution, as the concentration of glucose rises, the fluorescence signals decreases. The fluorescence signal noise emitted by other substance can be ignored at the excited wavelength [5]. The GOx-Eu3þ@UMOF [6] is an example of the metal-enzyme composite used as the fluorescence detector. At room temperature, it performs an extraordinary sensitivity and selectivity for glucose in serum and urine samples.

Although this approach for glucose testing is relatively sensitive and selective, it usually requires extra ultraviolet to excite so that the fluorescence signal can be detected in the solution. Moreover, the procedure of preparing the biosensor is complex.

Chemiluminescence biosensor: Luminol is a chemiluminescent reagent with broad applications. Characterized by minimal toxic potential, robust steadfastness, and heightened oxidation-reduction capability, this compound demonstrates the capability to maintain its steadfastness across an extensive spectrum of pH levels and temperature variations [5]. Luminol has widespread application in the field of biology and medical research. A novel detecting system which applied indigo carmine (IC)/glucose/hemin/H2O2 to test for serum and urinary glucose as an innovative form of chemiluminescence which was designed by Fereja et al. [7]. The reduction of IC by glucose occurs with catalytic oxidation of hemin and H2O2. This process is initiated subsequently by generating intermediates that emit chemiluminescent signals. Moreover, glucose exhibits the ability to interact with dissolved oxygen and H2O2, consequently intensifying the chemiluminescent signals, leading to a remarkable limit of detection (LOD) of 15.0 nM [5].

Chemiluminescence biosensors also have some shortcomings. First of all, chemiluminescence has a relatively poor selectivity, which has a series of components to react with, instead of a single compound. Moreover, various environmental factors may also affect the intensity of chemiluminescence output. Besides, the chemiluminescence signal is an instant message which requires to read immediately.

2.2.2. Electrochemical biosensor

Enzyme-based: Glucose oxidate which usually abbreviated as GOx is an enzyme that is widely used to be the building block of the majority of enzymes [8] for its high glucose selectivity coupled with substantial resilience against alterations in pH and temperature. Furthermore, the application of glucose dehydrogenase (GDH). It catalyses the oxidation of glucose that contains flavin adenine dinucleotide, nicotinamide adenine dinucleotide, or pyrroloquinoline quinone in the presence of cofactors whose abbreviations are FAD, NAD, PQQ respectively, in this way, it is able to catalyse glucose oxidation.
The enzyme-based glucose electrochemical biosensors exhibit a drawback in terms of temperature sensitivity, susceptibility to pH variations, and the utilization of potentially hazardous chemicals. Additionally, the process of enzyme immobilization contributes to a decline in the enzyme's long-term stability and catalytic efficiency.

Enzyme-free: the advances of nanotechnology, metal oxide and various nano structures (like the mixture of metal and carbon nano materials) have significantly affect the design and inspiration of enzyme-free electrochemical biosensors. The majority of enzyme-free glucose detecting equipment has an ultra-high sensitivity when it compares to enzyme-based biosensors. Currently, the enzyme-based biosensors have become the main detecting method.

3. Electrochemical Biosensor

3.1. Non-Enzymatic Electrochemical Biosensor

The non-enzymatic electrochemical biosensors to detect glucose currently have divided into carbon-based composites, noble metal-based composites, copper-based composites, and other metal-based materials.

3.2. Mechanism of Non-Enzyme Electrochemical Biosensor

Glucose electrochemical biosensors are a type of equipment capable of transferring the conversion of glucose into a measurable electrical signal, which is often a current of electricity or voltage. The electrochemical glucose biosensors contain biological identification element, electrochemical converter and system for processing and presenting signals (Fig.1) [9]. The main component among these parts is the molecular identification element, which has the high selectivity and sensitivity to catalyse the electro-oxidation of glucose. Glucose biosensors were first introduced to the market in 1962 by Leland C. Clark [9]. GOx-modified platinum electrodes as biosensors. GOx, on the other hand, is much more site-specific towards the oxidation of glucose (occurring approximately 5 × 10^3 times a second during approximately half of the electrochemically relevant transactions). The basic operating principle of the modified GOx glucose Pt sensor designed by Clark is shown in Fig. 1b, which is based on the following reactions:

![Figure 1](image-url)
Glucose + O$_2$ $\xrightarrow{GOx}$ Gluconic acid + H$_2$O$_2$

H$_2$O$_2$ $\xrightarrow{R}$ 2H$^+$ + O$_2$ + 2e$^-$

GOx catalyses the oxidation of glucose during the reaction although molecular O$_2$ and generates both gluconic and H$_2$O$_2$. After H$_2$O$_2$ is generated, the H$_2$O$_2$ is oxidized at the platinum electrode and the electron flux produced by the oxidation is in proportion to the glucose content of a given specimen [9].

3.3. SWCNTs-Mesoporous Silicon Nanocomposite

A newly prepared biosensor based on SWCNTs-PSi modified GCE whose full name is glassy carbon electrode could detect various concentrations of glucose in phosphate buffer solution (PBS) (0.5 to 28.5 mM) with a sensitivity of 0.0614 $\mu$A mM$^{-1}$ cm$^{-2}$ and a limit of detection of 9600 ± 100 nM [10], whereas the conventional level of the concentrations of glucose in human serum is 3.9–7.1 mM. The biosensor shows brilliant selectivity when looking for common interferents that could affect human blood. This new SWCNTs-PSi/GCE biosensor shows remarkable repeatability, reproducibility, and long-term stability.

This selective challenge is a major concern in non-enzymatic glucose biosensors as it could compromise the electrode’s ability to detect. We have checked the selectivity of the electrode against current interferents. Amperometric response assays were performed at +0.50 V (vs Ag/AgCl) [10], showing that the electrode is indeed responsive to glucose (20 mM), but has a minimal response to interfering chemicals (except for certain concentrations of dopamine, ascorbic acid, and D (+) ribose). The SWCNTs-PSi/GCE electrode demonstrates excellent repeatability and stability shows 20 cycles of CV response measurement in glucose samples using the SWCNTs-PSi/GCE). Due to its unique properties, SWCNTs-PSi nanocomposites contribute to increased sensor performance, including a wide linear range, remarkable repeatability, and stability, which makes it a promising biosensor for glucose.

The presence of a porous surface in SWCNTs-PSi nanocrystals creates a favourable nanoscale environment conducive to accurate glucose detection, thereby contributing to improved sensor performance. The proposed biosensors show brilliant performance such as an incredibly large linear dynamic range, remarkable repeatability, reproducibility, and stability. Table 1 shows that the overall performance for the SWCNTs-PSi/GCE biosensor is quite favourable in comparison to the existing glucose sensors.

Table 1. Contrast among non-enzymatic glucose biosensors utilizing diverse electrodes composed of varying nanocomposites or nanomaterials

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Techniques</th>
<th>LDR/mM</th>
<th>LOD/ $\mu$M</th>
<th>Sensitivity/ $\mu$A M$^{-1}$ cm$^{-2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnO-CeO2/ GCE</td>
<td>CV &amp; DPV</td>
<td>5×10$^{-4}$–0.3</td>
<td>0.224</td>
<td>–</td>
</tr>
<tr>
<td>PDDA/CuO- C-dot on SPCE</td>
<td>Amperometry</td>
<td>0.5–2, 2–5</td>
<td>200</td>
<td>110, 62.3</td>
</tr>
<tr>
<td>Ag-PANI/ rGO</td>
<td>Amperometry</td>
<td>5×10$^{-4}$–0.05</td>
<td>0.79</td>
<td>–</td>
</tr>
<tr>
<td>h-CuS NCs/ Naftion/ GCE</td>
<td>Amperometry</td>
<td>0.1–10.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Co@MoS2/CNTs</td>
<td>Amperometry</td>
<td>0–5.2</td>
<td>0.08</td>
<td>131.69</td>
</tr>
<tr>
<td>GOx/ SBP/ GCE</td>
<td>DPV</td>
<td>5×10$^{-4}$–4.0</td>
<td>0.27</td>
<td>–</td>
</tr>
<tr>
<td>NiO-TiO2/ GCE</td>
<td>Amperometry</td>
<td>2×10$^{-3}$–2.0</td>
<td>0.7</td>
<td>25.85</td>
</tr>
<tr>
<td>Ni3N NA'/ GCE</td>
<td>Amperometry</td>
<td>2×10$^{-3}$–7.5</td>
<td>0.48</td>
<td>–</td>
</tr>
<tr>
<td>N-GR - CNTs/ AuNPs</td>
<td>Amperometry</td>
<td>2×10$^{-3}$–19.6</td>
<td>0.5</td>
<td>0.9824</td>
</tr>
<tr>
<td>NiO HPA/ GCE</td>
<td>Amperometry</td>
<td>2.5 × 10$^{-3}$–1.1</td>
<td>0.32</td>
<td>1323</td>
</tr>
<tr>
<td>SWCNTs - PSi/GCE</td>
<td>CV</td>
<td>0.10–18.0</td>
<td>9.1± 0.1</td>
<td>0.0641</td>
</tr>
<tr>
<td></td>
<td>Amperometry</td>
<td>0.50–28.5</td>
<td>9.6± 0.1</td>
<td>0.0614</td>
</tr>
</tbody>
</table>
3.4. 3D Au@Pt Carbon Nanotubes Composite

The non-enzyme electrochemical biosensors usually use the intermediate metal such as copper, nickel and cobalt as well as novel metals like platinum, gold and palladium to detect glucose due to their ability to electro-catalyze glucose oxidation.

Though the process of catalysing the electrochemical oxidation of $H_2O_2$, novel metal nano-catalysts acquire the ultra-high sensitivity. Due to its remarkable catalytic prowess and exceptional steadfastness, platinum is widely applied in electrodes. Enhancing the exceptional attributes of platinum involves extending its surface area to the nanoscale. Through multiple high-voltage oxidation and reduction cycles, the oxide layer is gradually reduced, roughening the platinum surface to create a porous platinum nanowire electrochemical electrode for achievement of heightened accuracy and sensitivity of determining glucose within blood samples.

The construction of a sensing interface for non-enzymatic electrochemical glucose detection involves the fusion of a new carbon composite material with Au@Pt core-shell structured nanoparticles (Au@Pt NPs). Graphene oxide (GO) serves as a surfactant for the dispersion of multi-walled carbon nanotubes (MWCNTs) [11], making it a commonly employed nanotube in the field of graphene. The surface of a glassy carbon electrode (GCE) is modified by decorating Au@Pt nanoparticles (NPs) following the application of a mixture of graphene oxide (GOs) and multi-walled carbon nanotubes (MWCNTs). By consecutively assembling the GO/MWCNT mix and Au@Pt nanoparticles onto the clean surface of the GCE, here, we successfully develop a novel non-enzymatic biosensing interface featuring a minimal oxidation potential, heightened sensitivity, excellent selectivity, an expansive linear range, and a minimal limit of detection. GO dispersion of MWCNTs effectively prevents their aggregation and increases the formation of films on the GCE surface. Complete contact between GOs and MWCNTs increases surface area. Au@Pt nanoparticles, boasting a distinctive core-shell structure and remarkable electrocatalytic efficacy, are meticulously integrated onto the three-dimensional framework of GO/MWCNT. The aforementioned configuration highlights a synergistic and cooperative electrocatalytic performance that has been specifically enhanced for the purpose of glucose detection. Furthermore, constructed GO/MWCNT/Au@Pt/GCE system facilitates precise glucose detection in real samples.

3.5. CuO Microstructures Biosensor

Biosensors based on CuO nanostructures outperform many other metal oxide materials in the detection of glucose because of their superior electrochemical activity and electron-transfer efficiency [12]. In contrast, they have gained immense popularity as prevalent nanomaterials employed in glucose sensors for their cheap prices, easy to synthesis, remarkable electrochemical performance, high sensitivity, good chemical stability, and are a popular choice for use in glucose sensors.

3.5.1. CuO/ZnO Microstructure

A non-enzymatic outstanding durability glucose biosensor has been proposed by researchers, utilizing mixed metal oxides as the foundation. Dumbbell-shaped hollow porous double shell CuO/ZnO microstructures were synthesized by a hydrothermal technique employing Pluronic F-127 as the surfactant [13]. The CuO/ZnO-DSDSHNM structure exhibits notable characteristics such as a substantial surface area and a porous structure, which play a crucial role in augmenting the electrochemical reactivity for glucose oxidation. The CuO/ZnO-DSDSHNM composite was utilized as a surface coating upon a glassy carbon electrode (GCE) to function as the active constituent for experimental analysis. Following this, a layer of Nafion was utilized in the fabrication of the Nafion/CuO/ZnO-DSDSHNM/GCE biosensor.

The sensor exhibits a substantial dynamic range spanning from 500 nanomolar to 100 millimolar, resulting in a sensitivity of 1536.80 microamperes per millimolar per square centimetre [13]. Nevertheless, it is crucial to acknowledge that not all glucose sensors possess these characteristics. The detector also demonstrates enduring stability and commendable reproducibility, favourable consistency, exceptional specificity, and remarkable suitability for glucose analysis in human serum.
samples. Additionally, it exhibits a low limit of detection of 357.5 nM and a rapid response time of 1.60 s. The utilization of high-performance Nafion/CuO/ZnO-DSDSHNM/GCE as a glucose sensing platform exemplifies the promising capabilities of materials synthetic and sensor fabrication techniques, hence offering valuable insights for future research endeavours.

### 3.5.2. Flower-Shaped CuO-Colloid Nanoparticle

The morphology of CuO nanoparticles ranges from flower-like structures to peony-like structures, with alkaline mediums being used during the synthesis and preparation processes. Copper microstructure nanoparticles based on nano-flower structures and precursor Cu$_2$O are synthesized using a simple solution method, and they have been found to be suitable materials for non-enzyme glucose biosensors. Metallic copper is the primary phase generated when synthesizing non-enzyme glucose biosensors at room temperature, and it is related to the electrochemical characteristics. Because of the high sensitivity, rapid response, and repeatability, Cu flower-like particles prepared via a solution-based colloid method show significant potential in glucose biosensors that are not enzymatic and show promise for practical applications. It is possible that the small and medium-sized open pores of the petal-like structures with sharp edges and the unique morphology of the flowers are the origin of the activity of the Cu-colloid particles. This morphology has a large surface area that is conducive to oxygen adsorption chemistry and charge transfer. The purpose of small- and medium-sized pores is to augment the number of active sites and facilitate the electrochemical oxidation of glucose. In comparison to situations involving CuO nanoparticles of lesser or larger sizes, it has been determined that the most favourable particle size for graphene adorned with CuO is 15.75 nm. This particular size exhibits enhanced sensitivity, measuring at 1065.21 μA mmol1μL cm$^{-2}$ [14]. The biocathode treated with CuO-G exhibits negligible interference and demonstrates excellent stability while conducting amperometry current time measurements. This cutting-edge, electrochemical measurements based non-enzymatic glucose biosensor demonstrates significant potential when using nano flower CuO-colloid/graphene nanocomposites.

### 4. Summary

The electrochemical non-enzyme biosensors for glucose can be used without the present of excitation of ultraviolet and fluorescence signal, the preparing methods are also relatively simple. Secondly, it won't be affected by the environmental factors (pH, temperature, and harmful chemical substances) and the noise level is low. Instead of the instant message, it can generate constant electric signal for a long-term detecting. Due to the special structure of these carbon or metal base materials, they acquire the significantly high sensitivity and selectivity for blood glucose, which hold significant research value, boast substantial research prospects, and are poised to potentially replace outdated detection methods in the future, gaining widespread application.

### References


