In this study, the application of bovine serum albumin (BSA) as a carrier to glucose-sensitive materials for the detection of glucose was proposed for the first time. Au-CuO bimetallic nanoclusters (Au-CuO/BSA) were prepared using BSA as a template, the new sensing material (Au-CuO/BSA/MWCNTs) was synthesized by mixing with multi-walled carbon nanotubes (MWCNT) and applied to non-enzymatic electrochemical sensors to detect glucose stably and effectively under neutral conditions. The scanning electron microscopy was used to investigate the morphology of the synthesized nanocomposite. The electrochemical properties of the sensor were studied by cyclic voltammetry. Glucose detection experiments showed that Au-CuO/BSA/MWCNTs/Au electrode had good glucose detection ability, stability, accuracy, repeatability, and high selectivity in neutral environment. Unlike existing glucose-sensitive materials, due to the use of BSA, the composite material is firmly fixed to the electrode surface without a Nafion solution, which reduces the current blocking effect on the modified electrode. The composite materials can be effectively preserved for extremely long periods, higher than 80% activity was maintained at room temperature in a closed environment for 3 to 4 months, due to the special effects of BSA. In addition, the feasibility of using BSA in glucose-sensitive materials was confirmed.

Key words: Bovine serum albumin, Non-enzymatic sensor, Glucose detection, Bimetallic nanoclusters, Neutral condition

I. INTRODUCTION

As a chronic disease, diabetes has been widely distributed around the world. In recent years, diabetes has become a common disease, with hundreds of millions of people worldwide suffering from various degrees of diabetes and related diseases, such as heart disease, kidney failure and retinal disease [1]. Especially in low-income areas, the incidence of diabetes is increasing rapidly due to inadequate medical care [2]. Low-cost sensors that can effectively and quickly detect glucose levels have become the key to current research.

There are two kinds of electrochemical glucose sensors, enzymatic glucose sensor, and non-enzymatic glucose sensor. Enzymatic detection of glucose was first reported in 1962 [3] and is now widely used. However, enzymatic detection of glucose has many limitations because the sensor relies on enzyme activity. In recent years, non-enzymatic glucose detection has made great progress. Non-enzymatic glucose sensors have many advantages such as low cost, fast response, high sensitivity, and strong anti-interference ability. Meanwhile, the use of non-enzymatic glucose sensors is not restricted by oxygen [4]. Therefore, this method is widely regarded as a promising alternative to the existing glucose sensors in the future. Non-enzymatic glucose sensors still have some deficiencies, such as stability and detection activity, and many of them require an alkaline working environment.

Nanomaterials have attracted great attention due to their large surface area, excellent catalytic activity, and anti-toxicity. Many kinds of metal nanoparticles have been used to construct a variety of non-enzymatic glucose sensors [5, 23, 24]. Among them, Cu is widely used as an excessive metal material. Whether it is used alone [6], made of alloy [7], or combined with carbon material [8, 9], the materials show various excellent properties. The biggest problem for copper-based materials is the need for an alkaline detection environment, which is related to the electrochemical properties of carbohydrates on Cu electrodes [10]. In recent years, the electrochemical detection of Cu-based materials under neutral conditions has been realized by preparing Cu into special nanostructures [11]. Electrochemical detection of glucose under neutral conditions has always been a focus of attention. At present, in addition to precious metals with an electrochemical detection capability of glucose under neutral conditions, only the special nanostructures published in a small number of papers can realize
electrochemical detection of glucose under neutral conditions, such as core-shell structures [12, 13]. Nevertheless, these copper and gold alloys used in neutral environments are structurally unstable and have complex preparation methods. We are trying to find a simple and reliable structure for glucose detection in neutral environment based on the existing work.

Recently, biomaterials have developed rapidly in sensor applications, and a lot of work with protein templating has been done [14, 15]. In principle, bovine serum albumin (BSA) has the potential of biological degradation and the detection results may be affected by immune interactions. As a template, the BSA has many problems to be solved, however, it has many attractive characteristics, and the non-enzymatic sensors based on BSA have the potential to be superior to the enzymatic sensors. Among templates, BSA-protected nanoparticles have some fascinating features, including easiness of synthesis, excellent biocompatibility, stability, and surface functionality [17]. Also, the BSA has good biocompatibility and considerable cost advantages besides [17]. Proteins, though, have all sorts of attractive biological properties [16], its conductivity hinders the development of electrochemistry, and the existing protein-related works are mainly targeted at colorimetric detection [14, 15].

Alloying metals with a noble metal has been documented to be an effective work to influence the electronic structure and enhanced electron transfer. Among them, Cu alloy is a system with great promise and attracts wide attention. Up to now, alloy catalysts are mostly based on particles a few nanometers in size and loaded on carbon materials to increase the utilization efficiency. Such tiny particles tend to aggregate and separate from the carbon material. Besides, the amount of noble metal in an alloy catalyst should be lowered to reduce the cost. Therefore, we chose BSA as the supporting material to in situ prepare Au-CuO alloy. With BSA as a carrier, Au-CuO bimetallic nanoclusters on BSA were synthesized and used for electrochemical detection of glucose under neutral conditions. In this process, we found that BSA has a function similar to gel [17], which can guarantee the stability of the prepared materials. Despite the low cost and availability of the BSA, the problem of its electrical conductivity still remains, and to solve it, carbon materials are used [18]. According to previous studies, N-doped carbon nanotubes (CNT) have enhanced conductivity [19, 20].

In this work, a novel Au-CuO/BSA/MWCNTs composite material was reported, which was synthesized using a simple and economical strategy, used for the electrochemical detection of glucose in a neutral environment. The sensor has good sensitivity, stability, and anti-interference, and can detect glucose effectively after storage for 3 to 4 months. In addition, we found that the combination of the composite material on the electrode surface was very reliable after adding BSA. In this work, the electrochemical properties of the electrode modified by different composite materials were studied, which proved that the application of BSA improved the electrocatalytic performance of the composite materials. We also studied the electrochemical properties of Au-CuO/BSA without carbon materials, and confirmed that the addition of MWCNT can improve the electrical conductivity with BSA as an electrochemical sensing material.

II. EXPERIMENTS

A. Reagents and chemicals

N-MWCNT was provided by Jiangsu XFNANO Materials Tech Co., Ltd. BSA, chloroauric acid (HAuCl₄), cupric chloride dihydrate (CuCl₂·2H₂O), sodium citrate, sodium hydroxide (NaOH), phosphatic buffer solution (PBS), sodium chloride (NaCl), uric acid (UA), dopamine hydrochloride (DA), ascorbic acid (AA), D- (+)-galactose, lactose, sucrose, etc. were all supplied by Shanghai Aladdin Technology L+++-imited liability company. All the other chemicals used in this work were analytical grade and used without further purification. All solutions were prepared in deionized water.

B. Equipment

Scanning electron microscopy (SEM) graphs were taken using a JSM-IT300 (JEOL, Ltd., Japan). Transmission electron microscopy (TEM) graphs were taken using a JEOL 2010 TEM. Electrochemical tests adopted an electrochemical workstation (CS2350, Corrtest Instruments Corp., Ltd., Wuhan, China).

C. Preparation of Au-CuO/BSA/MWCNTs

Au-CuO bimetallic nanoclusters were synthesized with the molar ratio of gold to copper 1:1, and BSA was used as a template and carrier. In the first step, 10 mL of 10 mmol/L HAuCl₄ solution and 10 mL of 10.0 mmol/L CuCl₂ solution were added to 5 mL of 50 mg/mL BSA solution. In the second step, the previous mixture was stirred continuously at room temperature; after 10 min, 2 mL of 2% sodium citrate solution was added immediately, and then stirred at 70 °C for 15 min. In the third step, 1 mL of 0.1 mol/L NaOH solution was added to the mixture and stirred at 70 °C for 15 min. In the final step, the solution is cooled to room temperature, centrifuged, and washed until the pH is neutral to obtain pure Au-CuO/BSA. Then, Au-CuO/BSA bimetallic nanoclusters were freeze-dried to obtain solid powder for characterization and study. Other modification materials, including CuO/BSA and Au/BSA single-metal nanoclusters, are synthesized in the same way, without the addition of HAuCl₄ and CuCl₂, respectively.
The prepared Au-CuO/BSA powder was mixed with MWCNT at a mass ratio of 1:1 and dispersed into water for 30 min under sonication, then the Au-CuO/BSA/MWCNTs were obtained. After being freeze-dried, solid powder was obtained for reserve.

D. Preparation of modified electrode

Firstly, 10 mg of the prepared Au-CuO/BSA/MWCNTs powder was added to 5 mL of absolute ethyl alcohol in a glass beaker, then the beaker with the mixed solution was put into an ultrasonic cleaner, and sonicate for 20 min to obtain the Au-CuO/BSA/MWCNTs dispersion (2 mg/mL). Prior to modification, the Au electrode was polished, washed several times with deionized water and absolute ethyl alcohol under sonication, and dried in a nitrogen stream. Then, 5 μL of 2 mg/mL Au-CuO/BSA/MWCNTs ethanol suspension was dropped on the Au electrode and dried naturally to obtain Au-CuO/BSA/MWCNTs/Au. The electrode is stored at 4°C for further use. Other electrodes, including bare Au, BSA/Au, CuO/BSA/Au, Au/BSA/Au, Au-CuO/BSA/Au, were prepared using the same method.

E. Electrochemical measurement

The traditional three-electrode system, including bare Au working electrode (BSA/Au working electrode, CuO/BSA/Au working electrode, Au/BSA/Au working electrode, Au-CuO/BSA/Au working electrode, Au-CuO/BSA/MWCNTs/Au working electrode), saturated calomel electrode (SCE) reference electrode, and platinum wire counter electrode, was used for electrochemical measurement in the CS series Electrochemical Workstation of Wuhan Cortest Instruments Corp., Ltd. Under continuous agitation, I-t curves were recorded at a certain applied potential.

III. RESULTS AND DISCUSSION

A. Characterization of composites materials

To study the microstructure of the synthesized Au-CuO/BSA/MWCNTs nanocomposites, the composites were observed by SEM. Meanwhile, the Au-CuO/BSA composites without MWCNT were also analyzed, the results are shown in FIG. 1. In FIG. 1(A), it can be observed that Au-CuO bimetallic nanoclusters are uniformly distributed on the surface of BSA. These Au-CuO nanoparticles uniformly dispersed on the surface of the BSA have a considerable specific surface area. In FIG. 1(C), you can see that MWCNTs are evenly dispersed on the BSA surface or embedded in the BSA. TEM was further used to characterize the Au-CuO/BSA and Au-CuO/BSA/MWCNTs composite. The diameter of Au-CuO bimetallic nanoclusters is in the range of 10–25 nm, as shown in FIG. 1(E). Predictably, this unique structure provides a large number of active sites and significantly promotes glucose-sensing activity and electron transfer in BSA materials.

B. Electrochemical characteristics of composite materials

Firstly, the electrochemical behavior of the modified electrode in 0.5 mol/L H₂SO₄ solution was studied by cyclic voltammetry (CV) in order to mea-
FIG. 2 (A) CV curves of bare Au, BSA/Au, Au/BSA/Au, CuO/BSA/Au, Au-CuO/BSA/Au, Au-CuO/BSA/MWCNTs/Au in 0.5 mol/L H$_2$SO$_4$ solution. Scan rate: 50 mV/s. (B) CV curves of bare Au, BSA/Au, Au/BSA/Au, CuO/BSA/Au, Au-CuO/BSA/Au, Au-CuO/BSA/MWCNTs/Au in PBS with 1 mmol/L glucose. Scan rate: 50 mV/s. (C) CV curves of bare Au in PBS in the absence and presence of 1 mmol/L, 2 mmol/L, 3 mmol/L, 4 mmol/L glucose. Scan rate: 50 mV/s. (D) CV curves of Au-CuO/BSA/MWCNTs/Au in PBS in the absence and presence of 1 mmol/L, 2 mmol/L, 3 mmol/L, 4 mmol/L glucose. Scan rate: 50 mV/s.

sure the electrochemically active surface area of the modified electrode. FIG. 2(A) shows the CV graphs of bare Au, BSA/Au, Au/BSA/Au, CuO/BSA/Au, Au-CuO/BSA/Au, Au-CuO/BSA/MWCNTs/Au in 0.5 mol/L H$_2$SO$_4$ solution. As can be seen from the figure, a wide anode peak appears near 1.3 V, which corresponds to the formation of Au$_2$O$_3$. In reverse cathode scanning, a reduction peak due to the reduction of Au oxide at 1.1 V can be observed [21]. The electrochemical behaviors of the six modified electrodes in 0.5 mol/L H$_2$SO$_4$ solution formed a sharp contrast, the active surface area of the modified electrode increased with the modification of the materials. The peak current of the Au electrode modified with the BSA is higher than that of the bare Au electrode, which can be attributed to the special surface structure of the BSA, which increases the relative surface area of the electrode. Although the peak current in the CV graph of Au/BSA/Au and CuO/BSA/Au electrodes did not change significantly, the peak current of the Au-CuO/BSA/Au electrode increased significantly, because the synergistic effect of bimetallic nanoclusters enhanced the electrochemical activity of the modified electrode. The biggest problem of BSA materials was still the lack of conductivity. Therefore, when MWCNT was added into the composites, the peak current of the CV graph of Au-CuO/BSA/MWCNTs/Au electrode was further significantly increased, and the electrochemical activity of the modified electrode was enhanced again.

Further, in order to show the effect of materials modification on the electrocatalytic activity of glucose on the electrode, as shown in FIG. 2(B), CV graphs of the above six modified electrodes were obtained in PBS solution containing 1 mmol/L glucose. The current values of Au-CuO/BSA/MWCNTs/Au electrode were generally higher, the oxidation and reduction peaks were more obvious. In addition, the second peak current strength is Au-CuO/BSA/Au electrode. These results showed that the prepared Au-CuO bimetallic nanoclusters had good glucose electrocatalytic activity under neutral conditions, and the addition of MWCNT effectively improved the electrical conductivity of BSA materials which further improved the electrochemical performance of non-enzymatic glucose sensing.

The electrocatalytic activity of bare Au electrode (FIG. 2(C)) and Au-CuO/BSA/MWCNTs modified Au electrode (FIG. 2(D)) on glucose oxidation was studied by cyclic voltammetry, the graphs were obtained in PBS solution without glucose, containing 1 mmol/L glucose, 2 mmol/L glucose, 3 mmol/L glucose, 4 mmol/L glucose. Scan rate: 50 mV/s.
TABLE I Performance comparison between Au-CuO/BSA/MWCNTs/Au and other reported glucose sensors.

<table>
<thead>
<tr>
<th>Electrode matrix</th>
<th>Linear range/(mmol/L)</th>
<th>Sensitivity</th>
<th>LOD/(µmol/L)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu@Cu2O NPs/GCE</td>
<td>0.01–5.5</td>
<td>123.8</td>
<td>0.05</td>
<td>[25]</td>
</tr>
<tr>
<td>Cu2O Nanothorn array/Cu foam</td>
<td>N/A</td>
<td>97.9</td>
<td>0.005</td>
<td>[26]</td>
</tr>
<tr>
<td>CuO Nano leaf/ ZnO NR/Cu sheet</td>
<td>0.1–1</td>
<td>408</td>
<td>18</td>
<td>[27]</td>
</tr>
<tr>
<td>Cu@Cu2O NS/ rGO/GCE</td>
<td>0.005–7</td>
<td>145.2</td>
<td>0.5</td>
<td>[28]</td>
</tr>
<tr>
<td>CuO NWs/GCE</td>
<td>0.0005–0.488</td>
<td>64.1</td>
<td>0.045</td>
<td>[29]</td>
</tr>
<tr>
<td></td>
<td>0.988–5.488</td>
<td>488</td>
<td></td>
<td>[29]</td>
</tr>
<tr>
<td>Au@Cu2O/GCE</td>
<td>0.05–2</td>
<td>715</td>
<td>18</td>
<td>[30]</td>
</tr>
<tr>
<td>Cu2O Porous NS/ rGO/GCE</td>
<td>0.01–6</td>
<td>185</td>
<td>0.05</td>
<td>[31]</td>
</tr>
<tr>
<td>Cu2O Hollow NC/GCE</td>
<td>0.001–1.7</td>
<td>52.5</td>
<td>0.87</td>
<td>[32]</td>
</tr>
<tr>
<td>Cu NWs-MOFs/GO/GCE</td>
<td>0.02–26.6</td>
<td>N/A</td>
<td>7</td>
<td>[11]</td>
</tr>
<tr>
<td>Au-CuO/BSA/MWCNTs/Au</td>
<td>0.2–8</td>
<td>54.5</td>
<td>5</td>
<td>This work</td>
</tr>
</tbody>
</table>

a NPs: nanoparticles, GCE: glassy carbon electrode, NR: nanorod, NS: nanostructured, NWs: nanowires, rGO: reduced graphene oxide, NC: nanocubes.
b Sensitivity in unit of µA·(mmol/L)−1·cm−2.

cose. In the absence of glucose, the CV graph of Au shows the characteristics including the anodic oxidation peak from the oxidation of Au to Au2O3 and the cathode peak from the reduction of Au2O3 [22]. After the addition of glucose, the CV response of the electrode changed and a series of complex electrochemical behaviors occurred. It is obvious that the response current increased with the increase of glucose concentration. By comparing FIG. 2(C) and FIG. 2(D), it can be found that the peak of response current of Au-CuO/BSA/MWCNTs/Au is about 6 times that of the bare Au electrode. In conclusion, it is clear that Au-CuO/BSA/MWCNTs bimetallic nanomaterials play an important role in the electrocatalytic oxidation of glucose under neutral conditions.

In order to determine the dependence of electrochemical signals on glucose concentration, the potential was kept at 0.6 V in PBS solution, and the ampere response of Au-CuO/BSA/MWCNTs/Au electrode by continuous glucose injection was studied. The current-time curve (I-t) is shown in FIG. 3(A). The results show that the strength of the current increases with the addition of glucose. The calibration curve is shown in FIG. 3(B) with a linear range of 0.2–8 mmol/L, and the sensitivity of 54.5 µA·(mmol/L)−1·cm−2 obtained from the slope of typical calibration graph divided by surface area of the electrode, the limit of detection (LOD) measured by the experiment was 5 µmol/L.

The Au-CuO/BSA/MWCNTs bimetallic nanomaterial shows good activity in the neutral buffer solution, which indicated that Au-Cu alloy was a promising candidate catalyst for non-enzymatic electrocatalytic glucose oxidation. At the same time, due to the application of BSA in electrode materials, the Nafion solution is not needed to modify the electrode with Au-CuO/BSA/MWCNTs composite materials. Due to the characteristics of BSA, the fixation of the composite materials on the electrode surface is very firm, without the effect of Nafion blocking the current, the sensitivity of the modified electrode is further improved.

The performances of glucose detection of the fabricated electrode and other reported electrodes are shown in Table I [11, 25–32]. The LOD and sensitivity of the prepared Au-CuO/BSA/MWCNTs/Au
FIG. 4 (A) The amperometric response of Au-CuO/BSA/MWCNTs/Au to successive injections of 2 mmol/L glucose and AA (0.2 mmol/L), UA (0.2 mmol/L), DA (0.2 mmol/L), lactosum (0.2 mmol/L), galactose (0.2 mmol/L), sucrose (0.2 mmol/L). (B) The amperometric response of Au-CuO/BSA/MWCNTs/Au for 2 mmol/L glucose during long time (500 s).

each interfering substance). We performed the selective analysis of Au-CuO/BSA/MWCNTs/Au glucose sensors on small molecules commonly found in human serum such as ascorbic acid (AA), uric acid (UA), dopamine (DA), galactose, lactose, and sucrose. The results, as shown in FIG. 4(A), indicated that Au-CuO/BSA/MWCNTs/Au had good selectivity.

In the stability study, the current response remained at 96.8% of the maximum value for a long time (500 s), as shown in FIG. 4(B). It is also one of the characteristics of Au-CuO/BSA/MWCNTs that can be stored for a long time. After being stored in a closed container at room temperature for 19 days, the current response decreased by only 3.5%. After being kept under the same conditions for 100 days, the current response remained at 84%. We attributed the excellent stability of Au-CuO/BSA/MWCNTs composites to the special role of BSA, because the current response of Au-CuO/MWCNTs which lack of BSA in our experiment decreased by 20% after being kept under the same conditions for one month.

In order to evaluate its reproducibility, 5 sensors were prepared by the same method, the relative standard deviation (RSDs) in response to glucose was calculated to be 7.4%. These results indicate that Au-CuO/BSA/MWCNTs/Au electrodes have good glucose sensing properties under neutral conditions.

D. Real sample analysis

The feasibility of the Au-CuO/BSAMWCNTs/Au electrode in the practical application was performed by testing the glucose in human blood serum. The tests were conducted in PBS with 100 L serum added and the sensing results are shown in Table II. Glucose was added to human serum with and without glucose continuously, and the detection recovery was 93.75%—97.50%. Au-CuO/BSAMWCNTs/Au electrode was able to work effectively under neutral conditions with different initial glucose levels in human serum. This indicated that Au-CuO/BSAMWCNTs/Au electrode here reported can be potentially utilized for the real sample analysis.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Original</th>
<th>Added</th>
<th>Found</th>
<th>Recovery/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>0</td>
<td>2</td>
<td>1.89</td>
<td>94.50</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>3.75</td>
<td>93.75</td>
<td></td>
</tr>
<tr>
<td>Sample 2</td>
<td>2</td>
<td>2</td>
<td>3.90</td>
<td>97.50</td>
</tr>
</tbody>
</table>

The composite modified electrode must be able to resist the interference of various substances in human serum to be an effective glucose sensor. In general, glucose levels are much higher (at least 10 times) than those of interfering substances in human blood, so the molar ratio used for this work is 10:1 (glucose for...
IV. CONCLUSION

In this work, considering the high cost and low stability, as well as the short storage and service life of enzyme-based electrochemical sensors, we proposed to apply BSA to the electrochemical detection of glucose under neutral conditions. To solve the problem of insufficient conductivity of BSA, MWCNT was added into the composite materials to enhance the conductivity, thus improving the electrocatalytic activity to the glucose of the composite material. The Au-CuO/BSA/MWCNTs composite has good glucose detection ability under neutral conditions, and it is suitable for long-term preservation. Due to the special role of BSA, the composite material is firmly fixed on the electrode surface without Nafton. We believe that these properties of Au-CuO/BSA/MWCNTs are more suitable for commercial glucose sensor applications than the existing non-enzymatic glucose-sensitive materials. The use of BSA as a template makes the synthesis of Au-CuO bimetallic nanoclusters very easy and stable. The use of BSA as a template makes the synthesis of Au-CuO bimetallic nanoclusters very easy and stable. The new Au-CuO/BSA/MWCNTs composite material has not only remarkable structural characteristics but also good electrochemical properties and stability. In addition, the application of BSA to the electrochemical determination of glucose is economically feasible and useful in electrochemical properties.

We believe that this work opens up new sights for the preparation of electrochemical glucose sensing materials and sets a typical example for the production of high-quality nanomaterials for sensing applications.

V. ACKNOWLEDGMENTS

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