Cu₂O hollow microspheres as electrode materials for non-enzymatic electrochemical detection of glucose

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An enzyme-free glucose electrochemical sensor with high sensitivity and selectivity is highly attractive. In this work, an enzyme-free glucose sensor with Cu₂O hollow microspheres (Cu₂O HMs) was prepared via hydrothermal method. The glucose sensor constructed by the prepared Cu₂O HM structure exhibited a low detection limit of 0.13 μM, a high sensitivity of 277.1 μA/mM cm², and the wide linear ranges of 0.05–1.5 and 1.5–6.0 mM. Furthermore, anti-interference tests indicated that the prepared sensor exhibited good selectivity for glucose and practical application in human serum. The Cu₂O sensor has great application potential in the development of enzyme-free glucose sensor.

1. Introduction: Blood glucose level is a physical health standard for measuring metabolic capacity and clinical diagnosis of diabetes. Therefore, effective measurement of glucose plays an important role in the diagnosis, treatment, and rehabilitation of human health. The rapid and accurate detection of glucose has been widely needed in many fields such as diabetes, medical, food, biotechnology, and so on [1, 2]. At present, a lot of research has been done on the detection of glucose, and a variety of methods for detecting glucose have been developed, mainly including chromatography, spectroscopy, biological methods, and electrochemical methods. Since the sensitivity of electrochemical detection of glucose is greatly improved compared with other methods, it has received great attention in the field of glucose detection and has also been greatly developed. Electrochemical sensors as an effective method for detecting glucose have been a research hotspot. Electrochemical detections of glucose are divided into enzymatic and non-enzymatic sensors [3]. Enzyme sensors utilise the enzyme's high efficiency, specificity, and mild reaction conditions (near physiological pH) for the substrate, and have satisfactory sensitivity, excellent selectivity, and mild measurement conditions. However, the activity of the enzyme in the enzyme sensor is susceptible to the external environment, which limits the application of the enzyme sensor [4–6]. An enzyme-free glucose sensor can overcome the above disadvantages. The key to the improvement of enzyme-free glucose sensor performance (such as response time, service life, sensitivity, selectivity etc.) is to develop a new electrocatalytic material with simple preparation process, moderate cost, and high electrocatalytic activity for glucose oxidation.

Precious metals (Pt, Au), transition metals, and transition metal oxides with unique photoelectric properties, good stability, can overcome the self-toxicity of catalytic glucose, and the electrode surface exhibits excellent mediating ability, so it is often used for no enzyme electrochemical sensor [7]. The precious metals have disadvantages of high cost and low abundance. Transition metal oxides exhibit good catalytic effects under neutral and alkaline conditions and have low cost. Therefore, enzyme-free glucose sensors prepared from transition metal oxides have received more and more attention and have good application prospect. Cu₂O is an important p-type semiconductor with a bandgap of 2.17 eV, and has attracted much attention due to its ultra-low potential for electron transfer, higher exciton binding energy, non-toxicity, and low price [8, 9]. Therefore, varieties of Cu₂O nanostructure, including hollow nanocubes [10], nanowires [11], nanospheres [12], polyhedrons [13] were synthesised and investigated to improve the electrochemical activities in glucose sensing. It is noted that the hollow or porous Cu₂O has more exposed sites and more channels to facilitate glucose diffusion and electron transfer [8]. The sensors built by the type of material will have high sensitivity and wide linear ranges with low detection limits. For example, Gao *et al.* [10] synthesised Cu₂O hollow nanocubes to detect glucose. The sensor showed a sensitivity of 52.5 μA mM⁻¹ in the range of 0.001–1.7 mM, indicating a low detection limit of 0.87 μM. Ma *et al.* [12] prepared Cu₂O mesoporous nanospheres to detect glucose. The sensor showed a sensitivity of 2116.9 μA mM⁻¹ in the range of 0.0003–7.8 mM, indicating a low detection limit of 0.42 μM.

Generally, Cu₂O hollow structures are fabricated by hard template method or soft template method. For example, Feng *et al.* [14] prepared hollow Cu₂O octahedral and spherical nanocrystals by employing ethylenediamine as a molecular template. Ma *et al.* [12] synthesised Cu₂O mesoporous nanospheres with copolymer as soft template followed by an etching treatment. These synthesis processes are always complex, time consuming and the templates used often bring in contamination to the products. Hence, developing a simple non-templated method to fabricate the Cu₂O hollow nanostructures is crucial.

In our work, Cu₂O HMs are prepared via hydrothermal method with Cu(OAc)₂ and sodium citrate as reactants. The self-templated method for fabricating Cu₂O hollow structure could avoid the products' impurities brought by the subsequent treatment. The obtained hollow products have porous nature which facilitates the diffusion of glucose. The constructed glucose sensor shows excellent electrochemical performance with high selectivity and good stability. All the results show it is promising to be used as an enzyme-free glucose sensor. The progress in fabrication hollow structure of Cu₂O will surely promote the electrochemical applications of this type of material.

2. Experimental section

2.1. Materials: All the chemicals containing Cu(CH₃COO)₂·H₂O, sodium hydroxide (NaOH), sodium citrate (Na₃C₆H₅O₇), dopamine (DA), ascorbic acid (AA), uric acid (UA), and Nafion were purchased from Aladdin Co., Ltd.

2.2. Fabrication of Cu₂O HMs: Cu₂O HMs were synthesised by a conventional hydrothermal reaction according to the previous report [10]. In a typical procedure, 70 mM Cu(CH₃COO)₂·H₂O and 20 mM Na₃C₆H₅O₇ were dissolved in 40 ml distilled water and stirred vigorously for 30 min. Then the solution was poured

into a Teflon-lined stainless autoclave and heated at 180° for 6 h. After the reaction ended, the brick-red precipitates were formed. Then the products were centrifuged, washed with water and alcohol, dried in an electric oven at 60°C for 3 h.

2.3. Characterisation: The structure of the products was characterised by X-ray diffraction (XRD, PANalytical) with Cu Kα radiation. The surface morphology of materials was characterised by scanning electron microscopy (SEM, JSM-6390LA). The surface components were characterised by X-ray photoelectron spectroscopy (XPS, Thermo Scientific K-Alpha+). The porosity and surface area of the materials were studied by N_2 adsorption–desorption measurements on analyser (ASAP 2010).
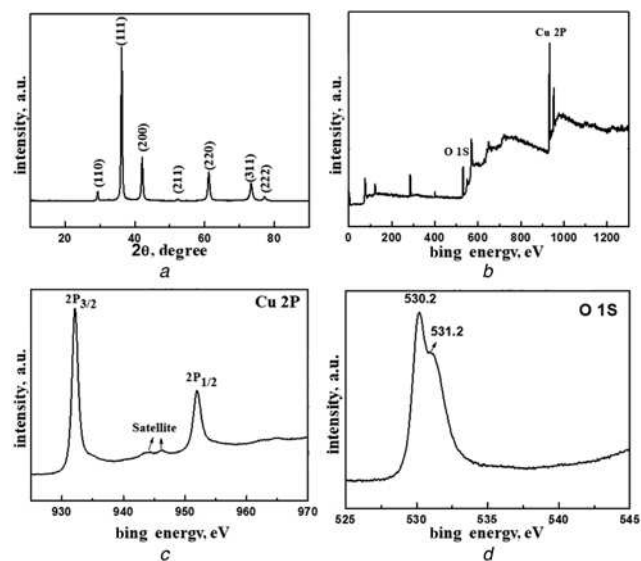
2.4. Electrochemical measurements: All of the electrochemical measurements were performed on a electrochemical workstation (CHI660E) in the three electrode cell. Usually, 5 μl of active materials solution (2.0 mg ml^{-1}) was dropped on a glass carbon electrode (diameter: 3 mm, area: 0.07 cm^2) serving as a working electrode, and dried at room temperature. Then 5 μl of 0.1% (diluted with ethanol) Nafion solution was dropped onto the electrode. The prepared electrode is referred to as Cu_2O HMs sensor or Cu_2O sensor. Electrochemical testing was carried out in deoxygenated 0.1 M NaOH solution using a saturated calomel electrode as the reference electrode and the Pt wire as the counter electrode.

3. Results and discussion: In this work, the formation mechanism of the resulted Cu_2O products was depicted as follows: when $Cu(CH_3COO)_2 \cdot H_2O$ were added in distilled water, Cu^{2+} and OAc^- ions were generated from $Cu(CH_3COO)_2$. As time goes by, OAc^- might combine with water, which could generate CH_3COOH and OH^{-1}. $Cu(OH)_2$ could be produced between OH^{-1} and Cu^{2+} and changed to CuO at high temperature [15]. Then $Na_3C_6H_5O_7$ as a reducing agent could reduce CuO to Cu_2O small crystallites. During the growth process of the Cu_2O small crystallites, the Cu_2O hollow structures might rely on inside-out Ostwald ripening [16]. Hence, we conclude the Cu_2O HMs were prepared via self-templated manner [17].
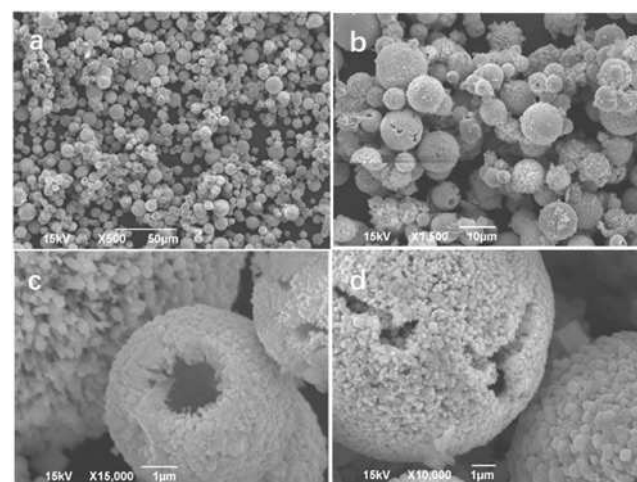
The XRD pattern of Cu_2O is shown in Fig. 1a. The diffraction peaks at 29.6°, 36.5°, 42.4°, 52.6°, 61.5°, 73.7°, 77.6° can be attributed to the (110), (111), (200), (211), (220), (311), and (222) plane of Cu_2O (JCPDS. 65-3288), respectively. No other impurity peaks appear, which proves that the method can produce pure Cu_2O. To study the surface element state of Cu_2O, XPS test was performed. The scan survey spectrum indicates the existence of Cu, and O element in the surface of Cu_2O (Fig. 1b). Cu 2p spectrum of high resolution is shown in Fig. 1c. The two main peaks around 953 and 933 eV could be indexed to the Cu 2p$_{1/2}$ and Cu 2p$_{3/2}$ of copper(I) oxide [18]. The satellite peaks at 944.1 and 946.0 eV between the Cu 2p$_{1/2}$ and Cu 2p$_{3/2}$ are ascribed to the Cu^{2+} [19]. The peak around 531 eV can be attributed to O^{2-} in the Cu_2O product, and the peak at 531.2 eV can be indexed to the oxygen adsorbing on the surface of the product (Fig. 1d) [20]. XPS and XRD illustrate the high purity of synthesised Cu_2O.

The morphology of Cu_2O sample was observed by SEM. SEM images in Figs. 2a and b indicate that the sample is composed of microspheres having the diameter from 2 to 15 μm. It is found from Fig. 2c that these microspheres are hollow nature and have cavities in them. Moreover, observed from Fig. 2d, the nanoparticles constitute a hollow outer shell, and the pores between the particles form a hollow structure. The high porosity of the Cu_2O HMs is also proved by the N_2 adsorption–desorption isotherm in Fig. 3a. The S_{BET} and pore volume are 3.1 m^2/g and 0.026 cm^3/g, respectively. The pore size distribution in Fig. 3b shows the presence of many mesopores with the diameter from 1.5 to 160 nm. It is speculated that this porous structure could contribute to the ample contacting sites between the glucose and our catalysts.

Fig. 2 *The morphologies of the Cu_2O sample*
a-b Low-resolution SEM images of the Cu_2O HMs
c-d High-resolution SEM images of the Cu_2O HMs

Fig. 1 *The structure characterisations of the products*
a XRD patterns for Cu_2O
b XPS survey spectrum of Cu_2O
c High-resolution XPS spectra of Cu 2p region
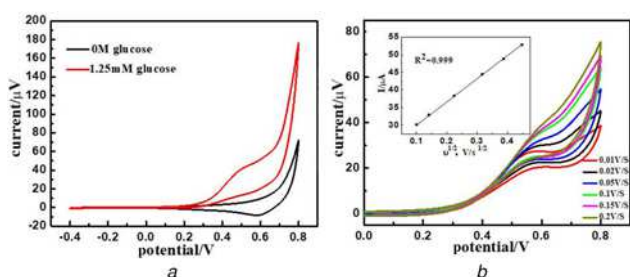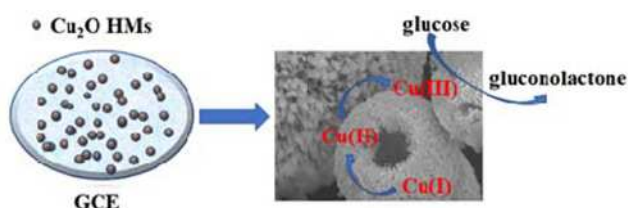*d* High-resolution XPS spectra of O 1s region
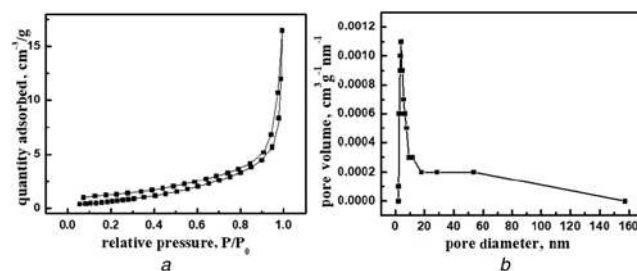
Fig. 3 *The surface characterisation of the Cu_2O HMs*
a Nitrogen adsorption–desorption isotherm curve of the products
b Corresponding pore size distribution curve

The electrochemical performances of the Cu₂O HMs based non-enzymatic glucose sensor are tested through cyclic voltammetry (CV) method in 0.1 M NaOH under nitrogen atmosphere. As shown in Fig. 4a, in the absence of glucose, the Cu₂O HMs sensor did not have a significant redox peak. In the presence of 1.25 mM glucose, the oxidation current value is significantly greater than in the absence of glucose, and the cathodic peak current decreases correspondingly (red curve). The behaviours are typical electrocatalytical process. It is believed that the oxidation behaviour of glucose originated from the Cu(II)/Cu(III) redox couple, and Cu (III) acts as an active carrier in the oxidation behaviour of glucose [21]. The principle of electrochemical oxidation of glucose is shown in Fig. 5: Cu(I) is firstly turned into Cu(OH)₂ (Cu(II)) and then changed into CuO. Secondly, CuO (Cu(II)) is turned into CuOOH (Cu(III)), and CuOOH can catalyse the oxidation of glucose to be gluconolactone [22]. As shown in Fig. 4b, the anode peak current intensities increase with increasing scan rate. The oxidation peak current has a good linear relationship with the square root of the scanning rate ($R^2 = 0.999$) (Fig. 4b inset), which indicates that the electrochemical process of the electrode is a surface process [23].
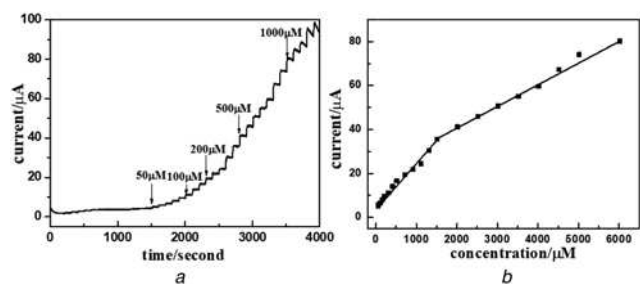
Fig. 6a is the amperometric responses for the Cu₂O sensor in response to glucose. When the potential is 0.5 V, glucose ranging from 50 to 1000 µM is continuously added in the 0.1 M NaOH solution. It can be seen from the figure that the response current increases stepwise as the glucose concentration increases. Fig. 6b shows a linear plot of glucose concentration versus response current. The linear relationship in the glucose concentration from 0.05 to 1.5 mM is superior. The linear equation is I (µA) $= 4.9216 + 0.0194C$ (µM) ($R^2 = 0.9878$). The sensitivity of the sensor is 277.1 µA/mM cm², and the detection limit can be calculated to 0.13 µM ($S/N = 3$). For the glucose ranging from 1.5 to 6.0 mM, the linear equation is I (µA) $= 20.0818 + 0.0102C$ (µM) ($R^2 = 0.9877$) and the sensitivity can be calculated to 145.7 µA/mM cm². The multiple linear relationship between the current and glucose concentration is similar with the former reports [24, 25]. As shown in Table 1, the Cu₂O sensor was compared with the previous works. The results suggest that the Cu₂O sensor obtains large linear range with low detection limit and a higher sensitivity.

In actual tests, DA, UA, and AA often coexist with glucose in the human body. Although the concentration of these substances is lower than that of glucose, it may cause some interference to the detection of glucose. In order to study the anti-interference of Cu₂O HMs sensor, 0.5 mM glucose, 0.05 mM DA, 0.05 mM UA, and 0.05 mM AA were added to 0.1 M NaOH solution at a voltage of 0.5 V as shown in Fig. 7. It can be seen that when DA, UA, and AA are added, the current response is much smaller than that of the glucose solution, which proves that the Cu₂O HMs sensor has better anti-interference ability to DA, UA, and AA. The stability and reproducibility of Cu₂O sensor were examined by amperometric responses to 2 mM glucose solution. As shown in Fig. 8a, with ten continuous measurement, the current values showed the relative standard deviation (RSD) of 4.27%. This suggests the good stability of the sensor. In order to evaluate the reproducibility of the sensor, we measured the current responses of five sensor prepared under the same condition. As displayed in Fig. 8b, the RSD is 2.12%, revealing that the sensor is highly reproducible.

Fig. 4 *The electrochemical measurements of the Cu₂O HMs sensor*
a CVs of the Cu₂O HMs electrode in the presence (red curve) and absence (black curve) of 1.25 mM glucose in 0.1 M NaOH
b CVs of the Cu₂O HMs electrode at various scan rates of 10, 20, 50, 100, 150, 200 mV/s in 2 mM glucose solution, inset: the curve of square root of scan rate versus the anodic response at 0.5 V

**Fig. 5** *Mechanism graph for the glucose oxidation process*

Fig. 6 *The electrochemical measurements of the Cu₂O HMs sensor*
a Amperometric current–time response of the Cu₂O HMs electrode with successive addition of glucose in 0.1 M NaOH
b Corresponding calibration plot of the electrode

Table 1 Performance comparison of various non-enzymatic glucose sensors

Electrode materials	Linear range, µM	Sensitivity, µA/mM cm²	Detection limit, µM	Reference
Cu₂O nanoparticles	50–1100	190	47.2	[26]
Cu₂O nanocubes	0–500	38	121.7	[27]
porous Cu₂O microcubes	1.5–500	1002	0.8	[9]
Cu₂O nanospheres	1.25–37.5	2038	0.41	[8]
Graphene-wrapped Cu₂O	300–3300	285	3.3	[28]
Cu₂O HMs	50–6000	277.1/154.7.7	0.13	this work
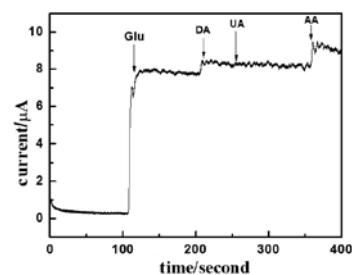
Fig. 7 *Amperometric responses of the sensor at 0.5 V upon successive addition of 0.5 mM glucose, 0.05 mM DA, 0.05 mM UA, 0.05 mM AA in 0.1 M NaOH solution*
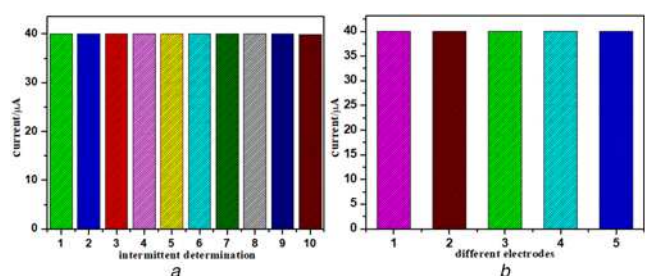
Fig. 8 *The electrochemical measurements of the Cu$_2$O HMs sensor*
a Stability measurements of the Cu$_2$O sensor
b Reproducibility measurements of the Cu$_2$O sensor

Table 2 Detection results of glucose in human serum samples

Added, mM	Founded, mM	Recovery, %	Relative average deviation, %	Average recovery, %
0.9	0.92	102.2	4.16	102.1
2.0	1.98	99.0	3.24	
4.0	4.2	105.0	3.85	

To investigate the practical application of the designed Cu$_2$O sensor, the recovery rate of the sensor was measured by standard addition methods in human serum samples. Human serum is mixed with 0.1 M NaOH with a ratio of 1:20. The results of the recovery were shown in Table 1. The recoveries are ranging from 99.0 to 105.0%. The determination of the glucose in human serum samples by the sensor was precise and could be used for glucose in actual measurement (Table 2).

4. Conclusions: In summary, Cu$_2$O HMs were prepared by self-templated hydrothermal method through the reduction reaction between Cu(OAc)$_2$ and sodium citrate. Hollow structure exists in the surface of Cu$_2$O, which is convenient to the diffusion of glucose and has high electron transfer efficiency. The Cu$_2$O sensor has an excellent performance in the glucose detection with a linear concentration from 0.05 to 1.5 mM and 1.5 to 6.0 mM, the high sensitivity of 277.1 µA/mM cm^2, and good anti-interference ability. The sensor is simple to manufacture and has high stability. It can be used for the determination of glucose in human serum samples with high accuracy and precision.

6 References

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