An electrochemical sensor based on nitrogen doped carbon material prepared from nitrogen-containing precursors

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Abstract. In this work, a nitrogen doped carbon material (NDC) was prepared by using a copper adenine complex as precursor and applied to electrochemical sensing of Vitamin B2 (VB$_2$). The experimental results show that the nitrogen doped carbon material is obtained after calcination at 650 ℃ under argon atmosphere, afterwards, which were characterized by X-ray diffraction (XRD), transmission electron microscope (TEM), fourier transform infrared (FT-IR), and electrochemical method. According to the XRD data, the product was a carbon material, and infrared data demonstrates that there are two kinds of carbonyl nitrogen combination, respectively, C-N and C = N combination of ways. Importantly, we used NDC to construct electrochemical biosensor to detect VB$_2$ by differential pulse voltammetry (DPV). The linear range was 6×10^{-6} - 3.5×10^{-4} M (R=0.9962), the minimum detection limit was 5.4×10^{-6} M, and the sensitivity is better. Consequently, it has better sensing performance.

1. Introduction

Carbon-nitrogen compound materials, both in terms of theory, material properties and application development have made some achievements so far. After a small amount of nitrogen is introduced into the skeleton of the carbon material, the physical and chemical properties of the material are greatly optimized, and its application is further improved in electrochemistry. When carbon-nitrogen material is used as electrode material, it has gained remarkable achievements in the fuel cell, double-layer capacitors and lithium-ion batteries and other applications [1-3]. According to its different characteristics, it has been widely used in the aviation industry, optoelectronic industry [4] and other field.

In this work, Cu-Ade is used as a precursor to manufacture NDC by high temperature (about 400-1000 ℃) and high pressure. Due to Cu-Ade has high nitrogen content, it does not need an additional nitrogen source. Metal adenine complex compounds containing carbon and nitrogen can be used to prepare carbon - nitrogen materials. But the metal adenine complex compounds which are as a precursor to synthesis carbonaceous materials have not been reported yet. Nitrogen atoms which have the lone pair of electrons can act as carriers, doping into the carbon material, and will increase the charge density of the carbon material, thereby the conductivity of carbon materials will increase [5]. The introduction of nitrogen atoms is bound to increase the defective position of the carbon material, so that results in an increase in activity in electrocatalytic or electrochemical reactions. Therefore, this paper uses carbon-nitrogen materials to catalyze VB$_2$ for the first time.

Vitamin B$_2$ (VB$_2$), also known as riboflavin, promoting metabolism is its main physiological function as a coenzyme. The yellow enzyme which is combined into riboflavin, phosphate and a molecule of protein plays a crucial role in metabolism of the sugar, fat and amino acid [6]. The growth

of many animals and microbes is closely relevant to vitamin B_2. Scientific research found that, chemical carcinogens carcinogenic effect will be enhanced, provided that the lack of vitamin B_2. Therefore, everyone requires supplementing vitamin B_2 to reduce the incidence of cancer in the daily life. The current methods of detecting VB_2 are chemiluminescence [7], high performance liquid chromatography (HPLC) [8], fluorescence [9], ultraviolet spectroscopy [10], mass spectrometry [11] etc. These methods are more expensive, complicated and time consuming. The electrochemical method is simple, fast response, high sensitivity, cheap which has cause widespread attention.

2. Experimental section

2.1. Reagents
$CuSO_4 \cdot 5H_2O$, Adenine, sodium hydroxide, Glucose, Nitric acid were used as purchased without further purification. Riboflavin (VB_2) was obtained from Huixing Biochemical Reagent Co. (China). Ascorbic acid (AA), dopamine (DA) and uric acid (UA) were sigma reagents.

The 0.1 M phosphate buffer solution (PBS pH 7.0), which was made up from NaH_2PO_4, Na_2HPO_4, was employed as a supporting electrolyte, and all solutions were prepared with double distilled water.

2.2. Instruments
X-ray diffraction (XRD) measurements were carried out on a Shimadzu-XD-3AX-ray equipped with a Cu Kα radiation source ($\lambda = 0.15406$ nm), the scan rate is $10°min^{-1}$. Transmission Electron microscope (TEM) images were taken on Hitachi H-7650. Infrared spectrometers were taken on German brook Tensor 27. Tube furnace was purchased in Hefei Branch Crystal Material Technology Co., Ltd. Voltammetry experiments were performed on CHI660E electrochemical workstation (Shanghai Chenhua Instrument Company, China), which consisted of a bare or modified GCE (3.0 mm in diameter) working electrode, a platinum wire auxiliary electrode and a saturated calomel reference electrode (SCE).

2.3. Synthesis of complex
Firstly, Cu-Ade was prepared according to the reference [12]. Next, the precursor Cu-Ade was placed in a tube furnace, under the condition of argon protection, the temperature of the tube furnace was adjusted to 650 ℃, and the calcination time was 6 h, the heating rate was 5 ℃ min^{-1}, and the black powder was obtained after the reaction was accomplished. Afterward, the final black powder was placed in a reaction kettle and sufficient concentrated nitric acid was added (But the volume of acid cannot exceed 60% of the volume of the reactor). After the reaction vessel was placed in an oven at 80 ℃ for 24 h, the copper nitrate solution was removed and the remaining black solid was washed with distilled water, centrifuged, filtered, and dried at room temperature, spared.

2.4. The preparation of NDC /GCE
2.5 mg of NDC was ultrasonically dispersed in 200 uL of deionized water. 6 μL of this suspension was cast onto the surface of a pre-polished glassy carbon electrode (GCE) and dried in the air. Then 2 μL of 1% nafion solution was dropped to cover the electrode surface. After drying in the air, NDC /GCE were obtained.

3. Results and discussion

3.1. Characterization of NDC
The black powder obtained by calcination of Cu_2 (N3, N9-Ade) $_4$ (H_2O) $_2 \cdot 5H_2O$ as precursor was characterized by XRD. As shown in figure 1, (a) is a simulate carbon material XRD pattern, the PDF number of standard card is 41-1487; (b) is the XRD pattern of NDC obtained experimentally. Compared with the two, the diffraction peak which is at 25 ° in the XRD pattern illustrates that the material obtained is a carbon material. The N element electronegativity is larger than C, the atomic

radius is smaller than C. Therefore, the doped N element causes the crystal plane shrinking, and results interplanar spacing reducing, 2θ shifts to the right.
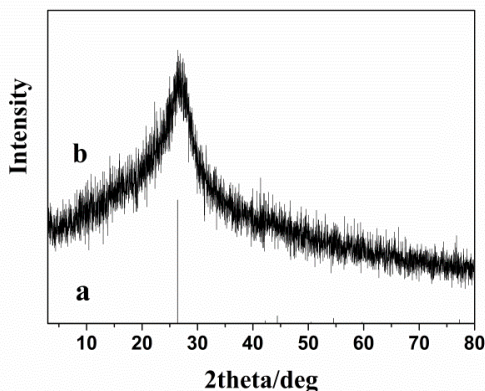

Figure 1. XRD patterns of JCPDS 41-1487 (a), and prepared NDC (b)

The morphology and size of the reaction product were characterized by TEM characterization, figure 2. It was seen from the figure that the NDC exhibits a layered structure. Its size is about 500 nm.
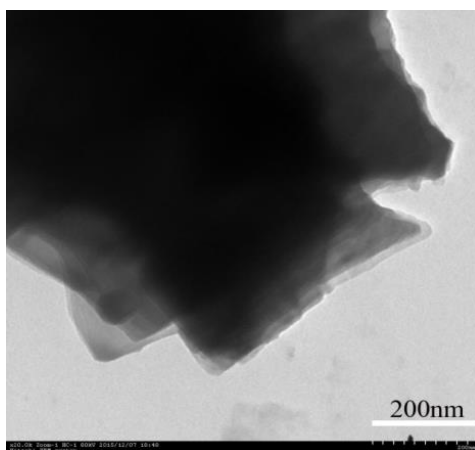

Figure 2. TEM images of the prepared NDC

In the experiment, the infrared peaks of different chemical bonds can be used to determine the type of bonding between different elements. Figure 3 is the infrared spectra of the samples prepared for the experiment. As shown in figure 3, the vibration peaks of C-N and C=N which are at 1378 cm^{-1} and 1623 cm^{-1} respectively were observed according to the reference [13].
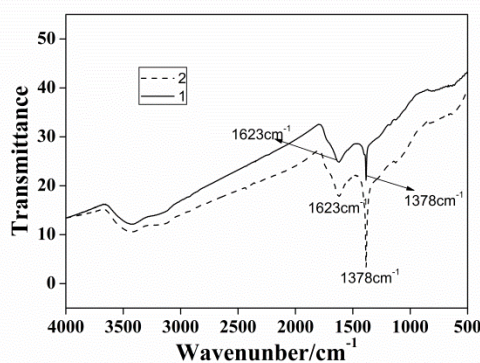

Figure 3. FTIR spectra of the prepared NDC

The elemental composition along with the N/C ratio is 46.4% as measured by XPS, Indicating that a part of nitrogen was lost in the synthetic process. But the content of nitrogen was still high. Clearly, there are three kinds of element which contain nitrogen, carbon, oxygen in the synthesized NDC (figure 4A). The content of C=N and C-N is 54.5% and 45.5% in NDC, respectively (figure 4B).
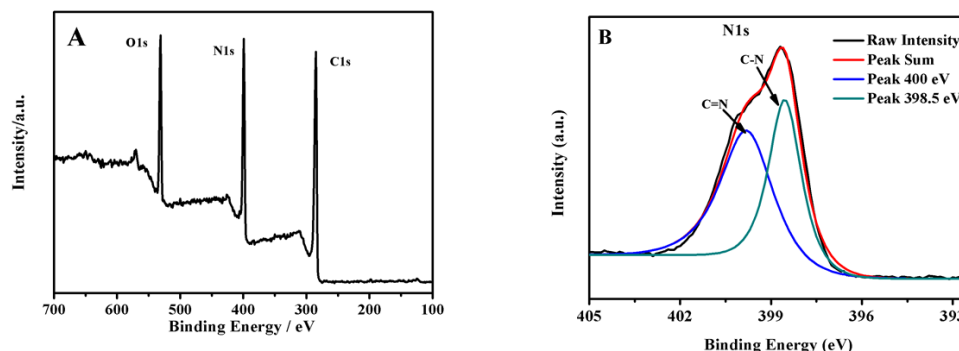

Figure 4. XPS spectrum of the synthesized NDC (A); XPS spectra of the N1s (B)

3.2. The electrochemical properties of NDC and electrochemical sensing of VB$_2$
Differential pulse voltammetry was carried out to investigate the electrochemistry of the NDC/GCE (figure 5). There was no significant redox peak in NDC/ GCE between -1.0 V and 0.2 V. When VB$_2$ was added, a reduction peak appeared at -0.45 V, which was attributed to the reduction peak of VB$_2$. The peak current of the reduction peak increased with the increase of the concentration of VB$_2$. From the DPV diagram can be seen that NDC has electrocatalytic ability towards VB$_2$.
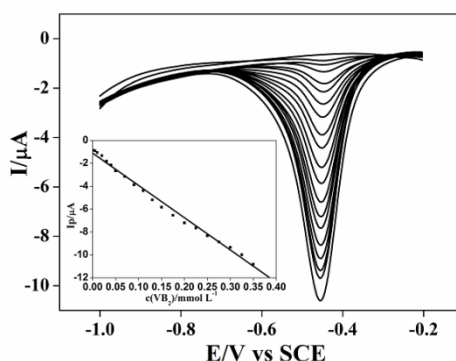

Figure 5. Differential pulse voltammetry for the interaction of VB$_2$ with NDC (from 0 to 0.350 mM) in 4 mL N$_2$-saturated PBS (0.1 mol L^{-1}, pH 7.0) Inset: calibration lot of the reduction peak currents as a function of VB$_2$ concentrations.

Inset in figure 5 is a linear relationship between response current and the concentration of VB$_2$ in the electrolyte. A linear dependence of the VB$_2$ reduction signals was observed for the VB$_2$ concentration in the range of 6×10^{-6} -3.5×10^{-4} M with a high sensitivity of 399 mA M^{-1} cm^{-2} (R=0.9962). Moreover, the detection limits (c_L) for investigated VB$_2$ was built according to the formula recommended by IUPAC: $c_L = 3\ s_b/S$ [14], where s_b is the standard deviation of the current density of the supporting electrolyte solution, S is the slope of the linear i_p vs c_{VB2} dependence. The detection limit is 5.4×10^{-6} M.

We examined the anti-jamming performance of NDC to detect hypoglycemic agents. According to the reference, dopamine (DA), hydrazine (N$_2$H$_4$), uric acid (UA) and sodium nitrite (NaNO$_2$) were selected as interfering substances in the detection of VB2. Figure 6 is the anti-jamming performance of the sensor obtained by adding the same concentration of interferences. The reduction signals are 1.508 μA, 1.529 μA, 1.510 μA, 1.367 μA, 1.538 μA, which are 1.39%, 0.133%, 9.35% and 1.99% of the

current respond signal respectively. It can be seen that the addition of interfering substances has no effect on the detection of vitamin B_2 from the figure (the contribution of the disturbance to the current measurement is less than 10%), indicating that the sensor has good selectivity.
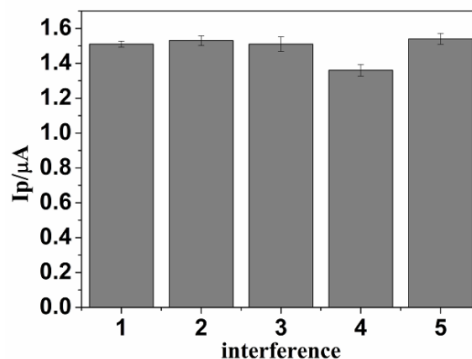

Figure 6. DPV peak currents of biosensor for 2.0×10^{-5} M VB2 in PBS (pH 7.0) before (1) and after adding 2.0×10^{-5} M, (2) DA, (3) N_2H_4, (4) UA and (5) $NaNO_2$

We used the NDC/ GCE sensor to detect the content of VB_2 in the VB_2 tablet (Nanjing Baijingyu Pharmaceutical Co., Ltd.) (table.1). VB2 was used as a standard solution to add into the electrolyte. The tablet was accurately weighed, dissolved in water, and filtered. Then, the filtrate was used as the medicine sample solution. As observed, the recovery of the spiked samples ranged between 93% and 101%, showing that the proposed method could be effectively used for the determination of VB_2 in real samples.

Table 1. Determination of VB_2 in the tablets samples and the recovery

sample	Sample concentration/mM	added/ mM	Found/mM	Recovery (%)	RSD (%)
		0.008	0.01742	93	9.30
1	0.01	0.010	0.02027	103	9.10
		0.015	0.02516	101	5.50

In the experiment, we also examined the reproducibility and stability of the sensor. The relative standard deviation (RSD) for 5 detections resulted from parallel electrodes was 9.2%. The modified electrode was stored at room temperature for 50 days and the current dropped to 90.3%. Reproducibility and stability of the experimental data show that we prepared NDC/GCE sensor has good reproducibility and stability.

4. Conclusions
In this paper, a carbon-nitrogen material was prepared from a copper-adenine complex which is as a precursor and used in electrochemistry to construct a sensor for detecting VB_2. The sensor has a wide linear range, high sensitivity and low detection limit. This work enriches the use of metal adenine complexes as precursors.

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