Selective electrochemical sensing of calcium dobesilate based on an ordered mesoporous carbon-modified pyrolytic graphite electrode

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ABSTRACT

Cyclic voltammetric investigation of calcium dobesilate (CD) in aqueous acid media was carried out by using an ordered mesoporous carbon-modified pyrolytic graphite electrode (OMC/PGE). A pair of well-defined redox peaks of CD was observed at the OMC/PGE, showing its good electrochemical response towards CD. The anodic current is linear with CD concentration in the range of 1.0 × 10⁻⁷ - 1.3 × 10⁻³ mol L⁻¹, with a detection limit of 4.0 × 10⁻⁸ mol L⁻¹. Meanwhile, the proposed electrode can avoid some interference coexisting with CD, such as uric acid, serotonin, and ascorbic acid. The proposed method can be potentially applied for selective electrochemical sensing of CD in physiological condition.

1. Introduction

Diabetes is now the fourth biggest cause of death worldwide, with diabetes related conditions resulting in 3.8 million deaths in 2007. Around the world, the incidence of diabetes is increasing at a significant rate. There are currently 240 million people globally living with diabetes, and this figure is predicted to increase to 3.8 million by 2025—an increase of 58% [1]. Diabetic retinopathy is a highly specific vascular complication of type I and type II diabetes mellitus [2]. As an effective antioxidant, calcium dobesilate (CD) has been broadly applied for the treatment of diabetic retinopathy by improving the blood’s rheological properties [3]. This kind of drug, however results in an increased risk of an adverse effect [4,5], so it is very important to develop sensitive, selective and low-cost CD determination methods widely that can be widely used in the whole world—the developed and undeveloped countries alike.

Traditional CD determination methods are mainly spectrophotometry [6] and chemiluminescence [7], and high performance liquid chromatography (HPLC) [8], which need expensive equipments or complicated and time-consuming treatment procedures. It is a fact that CD is an electroactive molecule, and it can definitely be detected through electrochemical method, which has significant advantages such as rapidness, low cost, and laboratory and clinic analysis in vivo. However, most conventional solid state electrodes show weak electrochemical response towards CD. To improve the response signal of CD at the electrode surface, some chemically modified electrodes have been constructed for CD determination. For instance, Zheng et al. developed an ionic liquid-type carbon paste electrode for determination of CD with a detection limit of 4.0 × 10⁻⁷ mol L⁻¹ [9]. Wang et al. reported the carbon–iron nanoparticle-modified glassy carbon electrode for the electrochemical determination of calcium dobesilate, with a detection limit of 2.0 × 10⁻⁷ mol L⁻¹. However, 50-fold lactic acid, uric acid (UA) and ascorbic acid (AA) interfered seriously with the accurate determination of CD [10]. In this regard, carbon nanotubes are an excellent electrode material because of their good conductivity, large area, and chemical inertness [11]. Zhang et al. [12] reported a poly-o-phenylenediamine and multi-wall carbon nanotube composite modified glassy carbon electrode for the determination of calcium dobesilate, with a serious interference of inorganic ions such as S²⁻ and NO₃⁻.

Currently, ordered mesoporous carbon (OMC) has been attracting many analysts’ interesting because of its well-ordered pore structure, high-specific surface area, large pore volume, and chemical inertness [13]. Zhou et al. [14] proved that OMC showed better properties for the electrochemical sensing of biomolecules than did multi-walled carbon nanotubes. And some electrochemical sensors based on OMC have been applied for the determination of dopamine [15], uric acid [16], and other environmental pollutants, such as hydroquinone [17] and nitrobenzene [18]. To the best of our knowledge, there are few reports on the electrochemical determination of CD using an OMC-modified electrode.

The present work is aimed at fabricating a new chemically modified electrode for the sensitive determination of CD. The modified...
The electrode can avoid interfere of some biomolecules such as UA, AA, and serotonin (5-HT), which have similar oxidation potentials with CD. An OMC-modified pyrolytic graphite electrode (OMC/PGE) was easily fabricated by coating certain OMC suspension onto the PGE surface. The electrochemical behavior of CD was also investigated with cyclic voltammetry (CV). A pair of well-defined redox peaks of CD is observed at the OMC/PGE. The proposed electrode shows excellent properties for CD determination and can avoid the interference of UA, AA, and 5-HT. This proposed method can be successfully applied to CD determination in capsules with satisfactory results.

2. Experimental

2.1. Chemicals and reagents

OMC was prepared according to the former literature [19]. Calcium dopesilate (CD) was purchased from Jujuyfeng Chemical Materials Limited Corporation (Shandong, China) and used as obtained. CD capsules were obtained from Lijun Pharmaceutical Corporation (Xi’an, China). 0.1 mol L\(^{-1}\) H\(_2\)SO\(_4\) was used as the supporting electrolyte, unless otherwise specified. Other reagents were of analytical reagents grade. All solutions were prepared by double distilled water.

2.2. Electrode preparation

Prior to the electrode modification, bare PGE was polished with alumina powder (followed by 1 μm, 0.3 μm, and 0.05 μm), then rinsed thoroughly with redistilled water, ultrasonically agitated successively in 50% nitric acid (v/v), acetone and double distilled water and dried in air. OMC (5 mg) was added to 5 mL N,N-dimethylformamide (DMF) and ultrasonicated for 15 min. Then 10 μL of the suspension was dropped on the pretreated PGE surface and dried under an infrared lamp.

2.3. Measurements

Cyclic voltammetric experiments were carried out by using a CHI 660C electrochemical analyzer (CHI, China) with a three-electrode configuration consisting of a PGE or OMC/PGE (disk, 4 mm diameter) as a working, Pt wire as the counter and saturated calomel electrode (SCE) as the reference. All potentials reported are related to this reference electrode. Prior to each measurement, the oxygen dissolved in solution was removed with purified nitrogen. X-ray diffraction (XRD) pattern of the sample was recorded with a Philips X-Pert Pro Multipurpose X-ray Diffractometer. Transmission electron microscopy (TEM) images were obtained from a JEOL JEM-2100 LaB6 transmission electron microscope.

3. Results and discussion

3.1. Characterization of OMC

XRD measurement confirmed that as-made OMC has an ordered hexagonal mesostructure (p6mm). As shown in Fig. 1A, the low-angle XRD pattern of OMC consists of three diffraction peaks at a 2θ range of 0.5–2°, corresponding to (1 0 0), (1 1 0), and (2 0 0) reflections. A typical TEM image recorded along the (1 1 1) direction (shown in Fig. 1B) showed that OMC consists of a highly ordered 2D hexagonal mesostructure with circle channels, which consistent with the XRD data. Scanning electron microscopy (SEM) image confirmed that the OMC particles are relatively uniform with an average diameter of about 1 μm. OMC possesses a BET surface area of 1230 m\(^2\) g\(^{-1}\), a total pore volume of 1.519 cm\(^3\) g\(^{-1}\) and a BJH pore size of 4.4 nm. All these results are similar to our previous report [20], indicating that highly ordered OMC has been synthesized by hard template method.

3.2. Electrochemical behavior of CD

The electrochemistry behavior of CD was investigated via cyclic voltammetry (CV) in 0.1 mol L\(^{-1}\) H\(_2\)SO\(_4\). Bare PGE shows a weak electrochemical response towards CD (shown as Fig. 2a). A pair of CD's redox peaks with a peak-to-peak separation of 0.453 V (\(E_{pa} = 0.661\) V, \(E_{pc} = 0.208\) V) was observed. Meanwhile, at the OMC/PGE, a pair of broad peaks (\(E_{pa} = 0.219\) V, \(E_{pc} = 0.188\) V) was observed in the blank solution (Fig. 2b), which is originated from the protonation and deprotonation of surface oxygen-containing functional groups. This phenomenon is similar to former reports [15,21]. Upon the addition of 1.0 × 10\(^{-4}\) mol L\(^{-1}\) CD, another pair of well-defined redox peaks was observed at OMC/PGE (Fig. 2c), indicating that this couple of redox peaks correspond to the electrochemical redox of CD. The anodic (\(E_{pa}\)) and cathodic (\(E_{pc}\)) peak potentials are at 0.479 and 0.452 V, respectively, with a peak-to-peak separation of 0.021 V. In addition, the anodic peak current (\(I_{pa}\)) of CD at the OMC-modified electrode is 7.1-fold higher than that at bare PGE. These results indicate that OMC/PGE shows good electrochemical response towards CD. The most probable reason is that the large surface area of OMC and the oxygen-containing functional groups can accelerate the electron transfer between CD and the electrode surface.

The effect between scanning rate and redox current was also investigated. Fig. 3 depicts a series of CV curves of 1.0 × 10\(^{-4}\) mol L\(^{-1}\) CD at OMC/PGE at different scan rates ranging from 0.02 to
that the electrode process was controlled by diffusion. As the scan rate (shown in Fig. 3 inset), and the linear equation is described as
\[
\text{log } (I_p) = 0.998 \cdot \text{log } (C_2) + 0.447, \\
\text{log } (I_a) = 0.999 \cdot \text{log } (C_6) + 6.45v^{1/2}
\]
with coefficients of 0.998 and 0.999, respectively, indicating that the electrode process is controlled by diffusion. The linear progress equations between log (\(I_p\)) and log (\(I_a\)) have a slope of 0.485 for log (\(I_p\)) and 0.447 for log (\(-I_a\)), respectively, which also indicate that the electrode process was controlled by diffusion.

The pH dependence of OMC/PGE was also explored. It is found that peak potential shifts negatively with the increase of solution pH. The redox peak potentials of CD is proportional to the solution pH in the range of 1.0–6.0, with a slope of 0.2 mV s\(^{-1}\). The relative standard deviate (RSD) of 15 times determination for 1.0 \(\times\) 10\(^{-4}\) mol L\(^{-1}\) is 0.85%, showing an excellent reproducibility of the modified electrode. The stability through direct electrochemical detection methods. Fig. 3A depicts the DPV curves of CD at OMC/PGE in 0.1 mol L\(^{-1}\) pH 7.0 PBS in different concentrations of CD (a \(\rightarrow\) c) 0, 1, 5, 10, 30, 50, 100 \(\mu\)mol L\(^{-1}\) (inset) the relation between anodic peak current and CD concentration. (B) CV curves of OMC/PGE by successively adding 2.0 \(\times\) 10\(^{-3}\) mol L\(^{-1}\) CD (black line), UA (red line), 5-HT (green line) and 2.0 \(\times\) 10\(^{-4}\) mol L\(^{-1}\) AA (blue line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

### Scheme 1. Reaction mechanism of CD.

\[
\begin{align*}
\text{HO} & \quad \text{OH} \\
\text{SO}^- & \quad \text{SO}^* \\
\text{CD} & \quad \text{UA} \\
\text{CD} & \quad 5\text{-HT} \\
\text{CD} & \quad \text{AA} \\
\text{UA} & \quad \text{AA} \\
\text{CD} & \quad \text{CD} \\
\text{UA} & \quad \text{UA} \\
\text{5-HT} & \quad \text{5-HT} \\
\text{AA} & \quad \text{AA}
\end{align*}
\]

\[
\begin{align*}
\text{HO} & \quad \text{OH} \\
\text{SO}^- & \quad \text{SO}^* \\
\text{CD} & \quad \text{UA} \\
\text{CD} & \quad 5\text{-HT} \\
\text{CD} & \quad \text{AA} \\
\text{UA} & \quad \text{AA} \\
\text{5-HT} & \quad \text{5-HT} \\
\text{AA} & \quad \text{AA}
\end{align*}
\]

### 3.3. Electrochemical determination of CD

Differential pulse voltammetry (DPV) is one of the most sensitive electrochemical detection methods. Fig. 3A depicts the DPV curves of the different concentration of CD at OMC/PGE. The results indicate that \(I_{pa}\) is proportional with CD concentration in the range of 1.0 \(\times\) 10\(^{-7}\)–1.3 \(\times\) 10\(^{-3}\) mol L\(^{-1}\), the linear equation was obtained through

\[
\text{CD} (\mu\text{A}) = (3.603 \pm 2.003) + (4.63 \pm 0.0423) [\text{CD} (\mu\text{mol L}^{-1})],
\]

with a linear relative coefficient of \(r = 0.998\). The detection limit is 4.0 \(\times\) 10\(^{-8}\) mol L\(^{-1}\) (S/N = 3). The relative standard deviate (RSD) of 15 times determination for 1.0 \(\times\) 10\(^{-4}\) mol L\(^{-1}\) is 0.85%, showing an excellent reproducibility of the modified electrode. The stability is 0.2 mV s\(^{-1}\). The \(I_{pa}\) and \(I_{pc}\) of CD are linear with the square root of the scan rate (shown in Fig. 3 inset), and the linear equation is described as

\[
I_{pa} (\mu\text{A}) = (1.01 \pm 0.823) + (\pm 0.0784) 6.45v^{1/2} [\text{mV/s}]^{1/2} \\
I_{pc} (\mu\text{A}) = (-5.03 \pm 0.300) - (5.36 \pm 0.028) 6.45v^{1/2} [\text{mV/s}]^{1/2}
\]

...
of the OMC/PGE is also investigated by measuring the anodic peak currents in standard CD solution. DPV determinations were also carried out once a day under the same operation conditions. The anodic peak current of CD scarcely changed in one month. After 6 weeks, the anodic current of CD still reached 94% of the current initial value, showing that the proposed electrode provides a good stability towards the electrochemical sensing of CD.

Some electroactive molecules such as AA, UA, and 5-HT commonly coexist with CD in a biological system, and they can be oxidized at the oxidation potential of CD on bare solid electrodes. Therefore, it is considerably important to evaluate the interference of AA, UA, and 5-HT. Fig. 3B shows the CV curves of 2.0 mol L$^{-1}$/C0 CD at OMC/PGE by successively adding AA, 5-HT, and UA, respectively. The peak-to-peak separation between CD and AA (or 5-HT) is more than 0.2 V. The oxidation potential of UA appears at 0.569 V and experiment results show that the 20-fold UA does not interfere with CD determination. As the plasma catecholamine (such as dopamine, epinephrine and noradrenalin) concentration is extremely lower than the detection limit of CD, these substances do not affect the accurate determination of CD. The anodic peak current of NO$\text{2}^-$ is 0.892 V at OMC/PGE, showing a peak-to-peak separation of 0.673 V to CD, indicating that NO$\text{2}^-$ does not affect the CD’s determination. For a 1.0 x 10$^{-5}$ mol L$^{-1}$/C0 CD, 30-fold S$\text{2}^-$ still does not affect its accurate determination. The influence of other substances was also investigated, and no interference was found in the presence of the following: 1000-fold K$^+$, Na$^+$, Cl$^-$, HCO$\text{3}^-$, H$\text{2}$CO$\text{3}$, Mg$^{2+}$, Ca$^{2+}$, SO$\text{4}^{2-}$, Cl$^-$, Br$^-$, with a deviation below 5%.

The proposed electrode was applied for CD capsules analysis in the market. One-thirty of 30 CD capsules was dissolved in 10 mL 0.1 mol L$^{-1}$/C0 H$\text{2}$SO$\text{4}$ and separated with a centrifugal machine. A standard addition was used for CD determination in pharmaceutical capsules (shown as in Table 1). The recoveries were from 97% to 102% and RSD is lower than 1.5%. The proposed method can be successfully applied to the quantitative determination of calcium dobesilate in pharmaceutical capsules.

4. Conclusions

A simple OMC/PGE has been fabricated to investigate the electrochemical behavior of calcium dobesilate. The experiment results indicated that the feasibility of using the proposed electrode for electrochemical sensing of calcium dobesilate in pharmaceutical formation. Serotonin, ascorbic acid and more than 20-fold uric acid do not interfere with the accurate determination CD, illustrating the proposed electrode can potentially be applied to selectively determination of calcium dobesilate in body fluid, which is under investigation.

Acknowledgment

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References


Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>CD detected (μmol L$^{-1}$)</th>
<th>CD added (μmol L$^{-1}$)</th>
<th>Found (μmol L$^{-1}$)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
<th>Bias (%)</th>
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<tbody>
<tr>
<td>1</td>
<td>11.5 ± 0.2</td>
<td>10</td>
<td>9.7 ± 0.5</td>
<td>97</td>
<td>1.17</td>
<td>–3.0 ± 0.5</td>
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<tr>
<td>2</td>
<td>11.6 ± 0.2</td>
<td>20</td>
<td>20.2 ± 0.4</td>
<td>101</td>
<td>0.95</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>11.8 ± 0.3</td>
<td>30</td>
<td>30.6 ± 1.1</td>
<td>102</td>
<td>1.02</td>
<td>2.0 ± 0.4</td>
</tr>
</tbody>
</table>

Table 1

Determination results of CD in capsule (n = 5).