Short communication

Ultrasensitive electrochemical sensing of the anticancer drug tirapazamine using an ordered mesoporous carbon modified pyrolytic graphite electrode

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A new ordered mesoporous carbon (OMC) modified pyrolytic graphite electrode (PGE) was prepared to investigate electrochemical behavior of the anticancer drug tirapazamine (TPZ). Compared to the bare PGE, the modified electrode showed an excellent electrochemical response to TPZ. The anodic peak current (Ipa) of TPZ at the OMC/PGE is 180-fold higher than that of the bare PGE. The Ipa is proportional with TPZ concentration in the range of 5.0 × 10⁻¹¹ to 1.5 × 10⁻⁸ mol L⁻¹. The linear regression equations are Ipa (μA) = 0.0000044 + 16.928CTPZ (μmol L⁻¹), with a detection limit (S/N = 3) of 2.0 × 10⁻¹¹ mol L⁻¹. This proposed method can be potentially used for ultrasensitive electrochemical sensing of TPZ in physiological condition.

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1. Introduction

Recently, tirapazamine (TPZ) has been introduced as a novel antitumor drug for treatment of cervical cancer, which is one of the most common cancers in women and is one of the leading risk factors for death in adult women. Every year, over 250,000 women die from malignant cervical tumors (National Cancer Institute, 2007). TPZ is unique in that it is selectively activated by the low oxygen environment in solid tumors, and is therefore widely used in conjunction with anti-nausea medicines for actinotherapy during the treatment of terminal cervical cancer (Craighead et al., 2000). However, TPZ is not without adverse side effects (Adam et al., 2006) and can be rapidly metabolized by the body tissues, with a low concentration of it in human blood (commonly lower than 10⁻⁶ mol L⁻¹, Hogan and Lunte, 1994). Therefore, it is important to have rapid and sensitive TPZ detection methods that are also sufficiently cost-effective to be used in both developed and developing countries. Traditionally, TPZ detection has consisted of UV (Walton and Workman, 1988), photodiode-array detection at 462 nm (Robin et al., 1995) or dual-electrode amperometric detection (Liang et al., 1996) following separation by high performance liquid chromatography (HPLC) or capillary electrophoresis (McLaughlin et al., 2000). However, these methods involve the use of expensive equipment and complicated procedures, which make them unsatisfactory for routine or cost-effective screening.

As an electroactive molecule, it should be possible to detect and measure TPZ with electrochemical methods (Tocher and Edwards, 1994; Tocher, 2001). However, most conventional solid electrodes currently available show only weak responses to TPZ. To date, there are few reports regarding rapid and low-cost electrochemical detection methods for accurate determination of TPZ.

Since its discovery in 1999, ordered mesoporous carbon (OMC) has attracted the interest of chemists, due to its well-ordered pore structure, highly specific surface area and chemical inertness (Joo et al., 2001). OMC was an excellent electrode material, even superior to carbon nanotubes, and can be used to prepare electrochemical sensors as well as enzyme-based biosensors (Zhou et al., 2007a,b, 2008a,b,c). Some electrodes modified with OMC have been proposed for determination of a number of biomolecules. For example, a glassy carbon electrode modified with OMC functionalized with ferroceneacarboxylic acid was developed for the electrochemical determination of uric acid (Ndamanisha and Guo, 2008).

In the present paper, a simple OMC-modified pyrolytic graphite electrode (OMC/PGE) was fabricated for investigation of the electrochemical behavior of the anticancer drug, TPZ. The proposed electrode showed a strong response to TPZ. This electrode can potentially be used for ultrasensitive TPZ determinations under physiological conditions.

2. Experimental

2.1. Chemicals

Tirapazamine was purchased from Sigma (analytical purity). Other reagents were of analytical grade and were used as received.
A 0.1 mol L\(^{-1}\) phosphate buffer solution (PBS) at pH 7.0 was used as the supporting electrolyte, unless it is expressly stated.

2.2. Preparation of OMC/PGE

Synthesis of OMC was done at China University of Geosciences according to the previously published method (Jun et al., 2000). Prior to modification, the PGE was polished with 1.0, 0.3 and 0.05 µm alumina powder, rinsed thoroughly with redistilled water between each polishing step, washed successively in an ultrasonic bath with 50% nitric acid (v/v), acetone, and redistilled water, then dried in air. For OMC suspension preparation, 10 mg OMC was added to 10 mL N,N-dimethylformamide (DMF) and ultrasonicated for 15 min. To fabricate the OMC/PGE, 10 µL of OMC/DMF suspension was dropped onto the pretreated PGE surface and allowed to dry under an infrared lamp to evaporate the DMF solvent.

2.3. Measurements

Electrochemical experiments were performed using a CHI 660C electrochemical analyzer (CH Instruments, Shanghai) with a conventional three-electrode cell consisting of a PGE working electrode (4 mm in diameter), a platinum wire counter electrode and a saturated calomel electrode (SCE). All solutions were prepared with redistilled water and deoxygenated by bubbling with nitrogen for 15 min before each voltammetric experiment. Scanning electron microscope (SEM, JSM-6701F, JEOL, Japan) and transmission electron microscope (TEM, JEM-1010, JEOL, Japan) images were obtained for characterization of the OMC. Nitrogen adsorption and desorption isotherms were measured using an ASAP-2010 analyzer (Micrometrics, USA).

3. Results and discussion

3.1. Characterization of OMC

OMC was characterized by SEM images, TEM images and N\(_2\)-adsorption isotherms in supplementary file (SFig. 1). The SEM image in SFig. 1A shows that high quality bamboo-shaped OMCs with diameters in the range of 500–1500 nm were synthesized. The TEM image (see SFig. 1A inset in supplemental materials) confirms that the mesoporous carbon was highly ordered and possessed a pore diameter in the mesopore range, at 4–6 nm. N\(_2\)-adsorption isotherms (see SFig. 1B in supplemental materials) show

![Figure 1](image-url)

Fig. 1. (A) CV curves of (black line) PGE in the presence of 1.0 \times 10^{-5} mol L\(^{-1}\) TPZ, OMC/PGE in the (green line) absence and (red line) presence of 1.0 \times 10^{-5} mol L\(^{-1}\) TPZ, scan rate: 100 mV s\(^{-1}\), 0.1 mol L\(^{-1}\) PBS at pH 7.0, scan rate: 100 mV s\(^{-1}\), rest time: 2 s; (B) CV curves of 1.0 \times 10^{-5} mol L\(^{-1}\) TPZ at different scan rates: (a→j) 20, 60, 100, 150, 200, 250, 300, 350, 400, 500 mV s\(^{-1}\); (C) CV curves of 1.0 \times 10^{-5} mol L\(^{-1}\) TPZ at different pHs from 5 to 9: (a→g) 5.0, 6.0, 6.5, 7.0, 7.5, 8.0, 9.0, scan rate: 100 mV s\(^{-1}\), rest time: 2 s; (D) effects of solution pH on the \(E_{pa}\) (red triangle) and \(I_{pa}\) (green roundity). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)
that the OMC exhibited capillary condensation steps on the nitrogen adsorption isotherm and consequently narrow mesopore size distributions ([Sfig. 1B inset in supplemental materials]). The average pore diameter of OMC was calculated to be 4.4 nm, which was consistent with the TEM result.

### 3.2. Electrochemical behavior of TPZ

The electrochemical behavior of TPZ was investigated with cyclic voltammetry (CV) using the OMC/PGE. Bare PGE showed a weak electrochemical response to TPZ (shown as the black line in Fig. 1A). With OMC/PGE without TPZ, a pair of broad redox peaks was observed (Fig. 1A, green line), which originated from protonation and deprotonation of surface oxygen-containing functional groups ([Jia et al., 2007]). Upon addition of 1.0 mol L\(^{-1}\) TPZ, another pair of well-defined reversible redox peaks appeared, with a peak-to-peak separation of 0.044 V (Fig. 1A, red line), demonstrating that these peaks corresponded to the electrochemical redox reaction of TPZ. The anodic peak current \(I_{\text{pa}}\) of TPZ for the modified electrode was 180-fold that of the bare PGE, indicating a good electrochemical response to TPZ. The probable reason is that the large surface area of OMC and the oxygen-containing functional groups on the electrode surface accelerated the electron transfer between TPZ and the electrode surface.

Fig. 1B depicts a series of CV curves of 1.0 \(\times\) 10\(^{-5}\) mol L\(^{-1}\) TPZ at different scan rates ranging from 20 to 500 mV s\(^{-1}\). The redox currents were linear at scan rates ranging from 20 to 200 mV s\(^{-1}\) (see Sfig. 2A in supplemental materials), showing that this modified electrode had thin-layer electrochemical characteristics. When the scan rate higher than 200 mV s\(^{-1}\), the redox currents were proportional to the square root of the scan rate ranging from 200 to 1000 mV s\(^{-1}\) (see Sfig. 2B in supplemental materials), indicating diffusion behavior in charge transport ([Mamantov et al., 1965]). Based on the adsorption of TPZ at OMC/PGE, the effect of accumulation time was investigated. The data showed the anodic current of TPZ increased with accumulation time in the first 150 s, and then became constant afterward. So 180 s was chosen as the optimized accumulation time in this study.

Anodic peak potential \(E_{\text{pa}}\) shifted negatively with an increase in solution pH (Fig. 1C). The \(E_{\text{pa}}\) of TPZ was proportional to the solution pH in the range of 5.0–9.0, with a slope of \(-69\) mV s\(^{-1}\) (Fig. 1D). The electrode process would therefore appear to be an equal proton–electron transfer. For a reversible electrochemical reaction ([Bard and Faulkner, 2001]): \(|E_p - E_{\text{pa}}| = 2.3RT/nF\), the electron transfer number \(n\) was calculated to be approximately 2 in this study. A probable reaction mechanism is described in Fig. 2 ([Tocher et al., 1990]).

The stability of OMC/GCE was also examined. The CV experiments were also carried out using the modified electrode once a day under the same operation conditions. In 1 month the anodic peak current of TPZ scarcely changed, and after 4 weeks the anodic current of TPZ at OMC/PGE reached 92.5% of the current initial value, showing that the modified electrode is of good stability.

### 3.3. Electrochemical determination of TPZ

Differential pulse voltammetry is one of the most sensitive electrochemical detection methods for drug molecule determination. Fig. 3A depicts the DPV curves of different concentrations of TPZ using the OMC/PGE. The \(I_{\text{pa}}\) of TPZ is proportional to its concentration in the range of 5.0 \(\times\) 10\(^{-11}\) to 1.5 \(\times\) 10\(^{-5}\) mol L\(^{-1}\). The linear regression equations are \(I_{\text{pa}}\) (\(\mu\)A) = 0.0000044 + 16.928C\(_{\text{TPZ}}\) (\(\mu\)mol L\(^{-1}\)), with a correlation coefficient of 0.996. The detection limit \((S/N = 3)\) is 2.0 \(\times\) 10\(^{-11}\) mol L\(^{-1}\). The relative standard deviation (RSD) for 10 measurements of 1.0 \(\times\) 10\(^{-5}\) mol L\(^{-1}\) is 2.35%, showing a good reproducibility of the OMC/PGE for TPZ determination.

As uric acid (UA) and ascorbic acid (AA) are also electroactive molecules that would be expected to coexist in a biological sam-

![Fig. 2. Redox mechanism of TPZ at OMC/PGE.](image)

![Fig. 3. DPV curves of TPZ at OMC/PGE in 0.1 mol L\(^{-1}\) PBS (pH 7.0) in different concentrations of CD: (a–f) 0, 1, 10, 100, 1000, 3000 nmol L\(^{-1}\); (inset): DPV curves of TPZ in its different consistency: (g–j) 0, 0.02, 0.05, 0.1 nmol L\(^{-1}\); (B) DPV curves of OMC/PGE (black line) by successively adding 1.5 \(\times\) 10\(^{-6}\) mol L\(^{-1}\) TPZ (red line), 2.0 \(\times\) 10\(^{-5}\) mol L\(^{-1}\) UA (green line) and 1.0 \(\times\) 10\(^{-4}\) mol L\(^{-1}\) AA (blue line). Increasing potential: 4 mV, amplitude: 50 mV, pulse width: 50 mV, sampling width: 0.0167, pulse period: 0.2 s, rest time: 2 s. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)](image)
ple (such as blood or urine), it is also important to investigate the interference of these types of compounds in TPZ determination under physiological conditions. Fig. 3B shows the CV curves of $1.5 \times 10^{-6}$ mol L$^{-1}$ TPZ after successive additions of UA and AA. The peak-to-peak separation between TPZ and AA (or UA) was over 0.3 V, while the anodic peak current for TPZ showed no change, illustrating that UA and AA did not interfere with TPZ determination. Compounds with higher oxidation potentials than AA, such as catecholamines (e.g., dopamine, epinephrine and noradrenaline) and serotonin, also did not affect the accurate detection of TPZ. The influence of a number of inorganic ions on the TPZ peak current was also investigated. For $1.0 \times 10^{-6}$ mol L$^{-1}$ TPZ, no interference was found to occur with the following substances: 1000-fold Na$^+$, K$^+$, NH$_4^+$, Mg$^{2+}$, Ca$^{2+}$, SO$_4^{2-}$, Cl$^-$, or Br$^-$, with a RSD below 5%.

The fabricated electrode was also used for TPZ determination in human serum. A volume of human serum (100 μL) was dissolved in a 10 mL 0.1 mol L$^{-1}$ PBS at pH 7.0. A standard addition was used for TPZ concentration determination. The recoveries were from 97% to 104% and the RSD was lower than 2.5%.

4. Conclusion

An ordered mesoporous carbon modified pyrolytic graphite electrode (OMC/PGE) was fabricated for investigating the electrochemical behavior of tirapazamine. The proposed electrode exhibits a strong electrochemical response to TPZ. The experimental results indicate the feasibility of using the proposed electrode for selective electrochemical sensing of TPZ in human serum. Uric acid, serotonin, catecholamines and ascorbic acid had no effect on the accuracy of TPZ determination. Taken together, the data from this study indicate that this proposed electrode could have potential use in the selective determination of TPZ in a clinical setting.

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Appendix A. Supplementary data


References