Sarcosine is a newly discovered effective biomarker for prostate cancer. However, the low concentration of sarcosine in tissue cells, plasma or urine blocks the development of sarcosine biosensors. In this manuscript, porous zeolitic imidazolate framework-8 (ZIF8) was synthesized and was used as carriers to load nano platinum (Pt@ZIF8). The porous structure of ZIF8 helped to stabilize the nano platinum and keep its high catalytic activity, and lead to high sensitivity of the prepared sarcosine biosensor.

**Experimental**

**Chemicals and reagents.**—Sarcosine oxidase (SOx) was purchased from J&K scientific company, the activity was 37 units mg\(^{-1}\). Sarcosine was purchased from Acros organic company. Poly (3,4-ethylenedioxythiophene)-poly(styrenesulfonate) (PEDOT:PSS, 1.3wt% dispersion in H\(_2\)O) was purchased from Sigma-Aldrich. \(\text{H}_2\text{PtCl}_6\cdot 6\text{H}_2\text{O}\) (0.2g, 20ml) was purchased from Sigma-Aldrich. \(\text{H}_2\text{PtCl}_6\cdot 6\text{H}_2\text{O}\) (0.2g, 20ml) was purchased from Sigma-Aldrich. 

**Instrumentation and measurements.**—The scanning electron microscopy (SEM) photographs were measured on a Hitachi SU-70 electron microscopy, the accelerating voltage of electron beam was 5 kV. Mapping analysis was performed by energy dispersive spectrometer (EDS) with 15 kV accelerating voltage. Samples for SEM and EDS measurements were prepared by adhering a tiny bit of the powder on carbon conductive tape. The crystal structure of the obtained microstructure were verified by X-Ray diffraction (XRD, D8 ADVANCE). Cyclic voltammetry (CV) and amperometric measurements were carried out by using a CHI 660E electrochemical workstation (Shanghai Chen Hua Instrument Co. Ltd). All experiments were proceeded in a three electrode cell. The counter electrode was platinum electrode and the reference electrode was saturated calomel electrode (SCE). All experiments were conducted at room temperature (about 298 K).

**The synthesis of ZIF8 and Pt@ZIF8.**—The synthetic method of the ZIF8 is according to the steps reported by Venna et al.\(^{20}\) Brieﬂy, a methyl alcohol solution of 2-methylimidazole (3.3g, 70ml) was added into a methyl alcohol solution of Zinc nitrate hexahydrate (1.5g, 70ml), and the mixture was stirred at room temperature for 24 hours to obtain a milky solution. The milky solution was centrifuged to obtain powder samples of ZIF8. The pristine ZIF8 powders was washed 3 times with 50 mL of methanol, and was dried at 353 K for 12 h. Pt nanoparticles which loaded on the ZIF8 (Pt@ZIF8) were synthesized by using the method of in situ reduction. Firstly, 0.1 g ZIF8 was added into the ethanol solution of \(\text{H}_2\text{PtCl}_6\cdot 6\text{H}_2\text{O}\) (0.2g, 20ml) and was stirred for 24 h. Then an ethanol solution of sodium borohydride was added into the solution with stirring for 60 min. The obtained black suspension (Pt@ZIF8) was centrifuged for 10 min at 10000 rpm (room temperature) and was washed 3 times with ethanol. The as prepared Pt@ZIF8 powder was heated at 353 K for 12 h under Ar atmosphere.

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Nano Pt@ZIF8 Modified Electrode and Its Application to Detect Sarcosine

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Prostate cancer (PCa) is one of the major diseases that influence the health and life expectancy of men over 50 years’ old.\(^1\) However, the prognosis is excellent if early prostate cancer (EPCa) is caught early. The 5-year survival rate for EPCa is almost 100%. So an obvious question arises: can we identify PCa early enough? Yes, the clinical evidence shows that early detection is a key to improving the overall survival rate. The most widely used method for directly determining the concentration of sarcosine in urine or plasma or tissue cells, it is easy to distinguish healthy individuals, benign prostate disease, clinically localized prostate cancer and metastatic prostate cancer. Furthermore, sarcosine can be detected non-invasively in urine. This report immediately caused a worldwide research upsurge. More and more results show that sarcosine in urine is a more effective biomarker for prostate cancer.\(^5\)-\(^8\)

Although sarcosine has been recognized as an effective biomarker for PCa, it is still very hard to be detected due to its very low concentration and complicated composition.\(^9\) The concentration of sarcosine in urine ranges from several micromolar per liter to several dozen micromolar per liter.\(^4\) With such a low concentration, the analytical methods available for direct determination of sarcosine in urine often include chromatography and tandem mass spectrometry.\(^9\) The major disadvantages of these methods include high instrumentation costs, complicated sample preparation and experienced operator requirements. So they are difficult to be applied as a wide range of routine analysis.\(^10\),\(^11\)

Due to the high cure rate of early PCa, the accurate measurement methods for trace amount of sarcosine with properties of simple, fast and low cost is especially important. Some efforts have been made towards the low-cost bioanalytical tools for sarcosine diagnostics.\(^3\),\(^10\),\(^12\) On the other hand, amperometric biosensor has the advantages of simple, rapid and low cost, and has played an important role in disease diagnosis.\(^13\)-\(^16\) The most widely used amperometric biosensor is portable blood glucose meter, which has greatly facilitated the patients and medical staff. However, the study of amperometric sarcosine biosensor is very limited.\(^13\)-\(^17\),\(^19\) Here we report a sarcosine biosensor with excellent performance. Zeolitic imidazolate framework-8 (ZIF8), a kind of metal organic framework (MOF) materials, was synthesized successfully and was used as carriers to load nano platinum (Pt@ZIF8). The porous structure of ZIF8 helped to stabilize the nano platinum and keep its high catalytic activity, and lead to high sensitivity of the prepared sarcosine biosensor.
Results and Discussion

Fabrication of sarcosine biosensor.—Glassy carbon (GC) electrode with a diameter of 3 mm were polished with 1.0 μm alpha alumina powder and 0.5 μm alpha alumina powder on a polish cloth. The polished glassy carbon electrode was modified by dripping 5 μL suspension solution of Pt@ZIF8 powder. The suspension solution was made by blending 5.0 mg of Pt@ZIF8 powder, 0.4 mL of water and 0.1 μl of PEDOT:PSS. A 5 μL drop of sarcosine oxidase (SOx) solution (1 μL/μL) was covered on the dried Pt@ZIF8 modified glassy carbon electrode and dried in room temperature for 1 h. Then 5 μL of glutaraldehyde solution (0.5%) and Nafion solution (0.5%) was dropped on the electrode to immobilize the sarcosine oxidase.

Electrochemical characterization of Pt@ZIF8 modified electrode.—In cyclic voltammetry (CV) measurements, the potential of oxidation and reduction peak in reversible process was an important factor to characterize the electrochemical properties of the electrode. To characterize the electrochemical property of the Pt@ZIF8 modified electrode, CV measurements were done in Fe(CN)6 4−/3− solution. In this work, the CV curves of the bare glassy carbon electrode and modified electrodes were done in 5 mM of potassium ferricyanide solution (adding 0.01646 g K3[Fe(CN)6] to 10 ml phosphate buffer solution (PBS) with a pH value of 7.0), and were presented in Fig. 3. The glassy carbon electrode was polished with Al2O3 powders and ultrasonicated in alcohol and water solution before use. The CV curve of the bare glassy carbon electrode in PBS solution shows only the non-Faraday current. In Fe(CN)6 4−/3− solution, the oxidation peak of the bare glassy carbon electrode caused by the Fe3+/Fe2+ redox couples occurs at 0.25 V (vs. SCE) and the reduction peak appears at 0.11 V (vs. SCE), the ΔE is 0.14 V. However, the oxidation peak of the Pt@ZIF8 modified electrode caused by the Fe3+/Fe2+ redox couples occurs at 0.25 V (vs. SCE) and the reduction peak appears at 0.14 V (vs. SCE), the ΔE is 0.11 V. The ΔE reduced from 0.14 V to 0.11 V. Though the ΔE of ZIF8 modified electrode is smaller than the Pt@ZIF8 modified electrode, it has smaller peak current. The peak current of Pt@ZIF8 modified electrode is much larger than the bare glassy carbon electrode and ZIF8 modified electrode, this is mainly caused by the large surface area of Pt nanoparticles dispersed on the modifying layer. According to the X-ray powder diffractometer (XRD) patterns of ZIF8 and Pt@ZIF8, the as-prepared ZIF8 is consistent with the Reference 20. The XRD pattern of the as-prepared Pt@ZIF8 consistent with Pt, and also consistent with ZIF8. The XRD patterns verify the successful preparation of ZIF8 and Pt@ZIF8.

Fig. 2 presents the X-ray powder diffractometer (XRD) patterns of the prepared ZIF8 and Pt@ZIF8. The XRD pattern of the as-prepared ZIF8 is consistent with the Reference 20. The XRD pattern of the as-prepared Pt@ZIF8 consistent with Pt, and also consistent with ZIF8. The XRD patterns verify the successful preparation of ZIF8 and Pt@ZIF8.
to Randles-Sevcik equation\textsuperscript{21}

\[ I_p = 2.69 \times 10^5 A D^{1/2} n^{3/2} v^{1/2} c \]

where \( A \) is the electroactive area of the electrode (cm\(^2\)); \( D \) is the diffusion coefficient of the molecules in solution, and is 6.70 ± 0.02 \times 10^{-6} \text{ cm}^2 \text{s}^{-1}; \( n \) is the number of electrons participating in the reaction, and usually is equal to 1; \( c \) is the concentration of the probe molecule in the solution (5.0 mM), and \( \nu \) is the scan rate of the CV measurement (0.1 \text{ V s}^{-1}). Thus the electroactive area of the Pt@ZIF8 modified electrode is estimated as 35.8 mm\(^2\), which is much higher than the electroactive area of the ZIF8 modified electrode (26.7 mm\(^2\)) and the bare glassy carbon electrode (22.3 mm\(^2\)). This showed that the Pt@ZIF8 nanoparticles has excellent electrocatalytic activity.

**Amperometric detection of sarcosine with Pt@ZIF8 modified biosensor.—**The Pt@ZIF8 modified biosensor was fabricated by casting a layer of Pt@ZIF8 over the surfaces of glassy carbon electrode and immobilizing sarcosine oxidase (SOx) on the electrode with glutaraldehyde solution (0.5\%) and Nafion solution (0.5\%). All chronoamperometric experiments were proceed in 10.0 ml of PBS solutions with stirring.

At an applied potential of 0.4 \text{ V}, with the successive injections of 5 or 10 \mu M sarcosine into PBS solution, the oxidation current change is presented in the inset of Fig. 4. It shows that the as prepared biosensor could work linearly from 5 to 30 \mu M. The corresponding regression equation of the linear plot is: \( i/nA = 5.36 + 4.05 c/\mu M, R = 0.996 \). The sensitivity is thus estimated as 4.05 nA \mu M\(^{-1}\). The detection limit is estimated to be 1.06 \mu M (S/N = 3) according to the calibration curve.

The linear range, limit of detection (LOD) and applied potential (V vs. SCE) in some typical amperometric sarcosine biosensors are shown in Table I. The above parameters at this work are comparable with those mentioned sarcosine biosensors. On the other hand, the concentration of sarcosine in human urine ranges from several micromolar per liter to several dozen micromolar per liter. Very few of the reported results is consisted with this range. More efforts are needed to provide better amperometric sarcosine biosensors.

Possible interferents such as ascorbic acid, uric acid, L-cysteine, glycine, fructose, maltose, lactic acid, citric acid, sucrose, calcium chloride are added into 50 \mu M sarcosine solution to measure the response signal. All other possible interferents do not substantially change the response signal except the ascorbic acid and L-cysteine. In 50 \mu M of sarcosine solution, the response signals of 0.1 mM ascorbic acid and 0.1 mM L-cysteine are comparable to the signal from sarcosine. This is a question for low concentration substrate test in real uric or serum samples. We will pay close attention to this question in the future studies.

The longevity activities of the Pt@ZIF8 modified sarcosine biosensor were tested in two weeks. It shows that the sarcosine biosensor could work in 3 days (only lost 15\% of its initial activities). But in two weeks, it lost almost 50\% of its initial activities. Compared with the long lifetime of glucose biosensor (almost 2 months), its believed that the immobilized SOx has very low stability. Great efforts should be done to prolong the lifetime of sarcosine biosensor in the future studies.

**Conclusions**

This work presents an amperometric sarcosine biosensor consisting of nano Pt@ZIF8 and sarcosine oxidase. Due to the unique 3D network structure of ZIF8, homogeneous platinum nanoparticles are obtained and the high electrocatalytic activity of platinum nanoparticles is maintained. The Pt@ZIF8 nanoparticles modified biosensor exhibits excellent sensitivity and could detect sarcosine in the concentration scope of micro molar. The linear range of the sensor is from 5–30 \mu M, which is consisted with the concentration range of sarcosine in human blood plasma or urine (from several micromolar per liter to several dozen micromolar per liter). This novel nanostructure provides excellent electrode materials for the development of high performance biosensors and other bioelectrochemical devices.

| Electrode matrix | Linear range (\mu M) | Limit of Detection (\mu M) | Applied potential (V vs. SCE) | Ref. |
|------------------|---------------------|---------------------------|-----------------------------|-----|
| SOx/carbon screen printed electrodes | 0.01–0.1 | 0.016 | 0.6 | 1 |
| SOx/polyaniline film | 100–1000 | not mentioned | 0.4 | 17 |
| SOx/polypryrole film | 100–1000 | not mentioned | 0.4 | 18 |
| SOx/PTA-Au-pph/TEOS | 0.5–7.5 mM | 0.5 mM | 0.55 | 19 |
| SOx/Pt@ZIF8/Nafion | 5–30 | 1.06 | 0.4 | Present work |

**Figure 3.** The CV curves of three different types of electrodes: (a) The bare glassy carbon electrode in PBS solution (pH 7.0); The bare glassy carbon electrode (b), the ZIF8 modified electrode (c), and the Pt@ZIF8 modified electrode (d) in K\(_3\)[Fe(CN)_6] solution (5 mM). The scan rate is 100 mV s\(^{-1}\).**

**Figure 4.** The response of the Pt@ZIF8 modified electrode to successive injections of 5 or 10 \mu M sarcosine into PBS (pH 7.0) solution at 0.4 V vs. SCE. The inset shows the corresponding i-c relationship.
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