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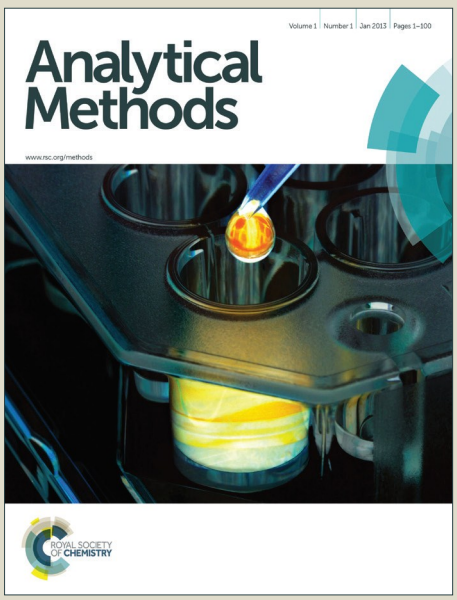

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Coaxial carbon fiber/ZnO nanorod as electrodes for electrochemical determination of dopamine

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Abstract: In this paper, coaxial carbon fiber/ZnO nanorod fibers (CF/ZnONR) was synthesized by plasma-assisted strategy with carbon fibers templates and characterized by scanning electron microscopy, which revealed that ZnO were attached on the surface of the carbon nanofibers. The as-synthesized fibers were used to fabricate microelectrode and exhibited a strong electrocatalytic activity toward the oxidation of Dopamine (DA). The sensor performed a sensitivity 9.84 μA/μM, and detection limit 0.07 μM. Moreover, the mechanism of catalytic oxidation DA on ZnONR surface is discussed. This biosensor demonstrates that the biomolecule such as DA can be detected by a simple and quickly method, and without assistant of specific function biomolecule such as enzyme. Finally, the method was applied to the selective and precise analysis of DA in commercial injection and serum samples.

Key words: Dopamine;coaxial;core-sheath; carbon/ZnO

1. Introduction

Recently, increasing interest has been given to the carbon/ZnO core-sheath fibers because of their specific morphologies and functions, such as electricity properties,[1,2] optical properties.[3] For example, the ZnO nanowires grown on carbon cloth showed extremely high field enhancement factor due to the combined effect of high aspect ratio and geometry of woven carbon cloth,[4] and ZnO nanowires grown on carbon microfibers also showed good potential for application as flexible gas sensor.[5] It can be concluded that good biological compatibility of ZnO and carbon is the underlying reason for the application in biosensor.[6,7] So, it is expected that coaxial carbon/ZnO core-sheath fibers would has good performance in biosensor.

DA is crucially important neurotransmitters in the mammalian central nervous system. It affects many aspects of brain circuitry, neuronal plasticity, organization and control of stress responses, the cardiovascular and renal systems [8–10] and a variety of motivated behavior such as perceiving rewards and pleasure. It is also of crucial importance in the attention span, learning, and memory.[11–14] Low levels or practically complete depletion of DA in the central nervous system is implicated as a major cause of several neurological diseases, such as schizophrenia, Parkinson's disease, and ADHD/ADD.[15,16] Furthermore, all drugs of abuse affect the dopaminergic pathways. Thus, given the wide

range of physiological and pathophysiological implications, the development of a sensor for measurement of DA [17, 18] could make a great contribution to disease diagnosis. Most recently published works have been devoted to detection of dopamine, such as grapheme sensor,[19,20] PAMAM/Pt composites electrode sensor,[21] nanochain assembled ZnO flowers sensor,[22] etc. However, a precious metal composite material is generally associated with these sensor, such as Pd, Pt, Au nanoparticles,[23] poly/Pd cluster[24] etc. The main problem in practical applications is their non-economical, especially heavy-metal toxicity. Thus, the development of a low-cost and biological compatibility sensor for DA detection is significant and necessary.

In this work, carbon fiber is covered with ZnO nanoparticles as a seed layer through magnetron sputtering, then ZnONR is grown on the treated CF *via* hydrothermal process. And then, the prepared coaxial carbon/ZnO core-sheath fibers is used as electrode for DA detection without other constituent auxiliary. The results demonstrated the sensor is sensitivity, quick, and stable.

2 Experimental

2.1 Chemicals

Dopamine, $Zn(CH_3COO)_2 \cdot 2H_2O$,hexamethylenetetramine (HMTA), Na_2HPO_4, KH_2PO_4 purchased from Sigma-Alderich (USA) and Carbon fiber, purchased from Beyondmaterials Co. Ltd (China). Dopamine hydrochloride injection (specified content of DA is 20 mg/mL, Shanxi Jingxi Pharmaceutical Co. Ltd.). Carbon fiber-6k were purchased from Shanxi institute of coal chemistry, Chinese academy of sciences and used without further purification. All the other reagents were used without further purification and all of the aqueous solutions were prepared with doubly distilled water. Phosphate buffered saline (PBS) is prepared by admixture of 0.1M Na_2HPO_4 solution and 0.1 M KH_2PO_4 solution to adjust the pH=7.0. Human blood samples were provided by 3A maternity and child hospital of Nantong. The blood treated with ethylene diamine tetraacetic acid as an anticoagulant and centrifuged at 3000 rpm. After 30 min, three phases appeared. The upper phase (blood plasma) was separated and fractionated by further centrifugation at 9000 rpm for 30 min. The supernatant blood plasma was used to determine the DA concentration.

2.2 Cover the ZnO seed on CF surface

Sputter ZnO seed on the surface of carbon fibers using the reactive RF magnetron sputtering technique (MSP-300C). Simply, ZnO (5N, 7.5 cm diameter) metal targets were used for depositing ZnO layer onto carbon fiber substrates (the length and diameter is 3 cm and 2 mm, respectively.). A gas mixture of Ar (10%) and O_2 (1%) at a total pressure of 1.0 Pa was used as the sputtering gas. 5 minutes, 3.3 Pa, 50 sccm Ar and 10 sccm N_2: Spraying was carried out for all the samples under the above conditions.

2.3 Grown ZnONR on CF surface

80 mL solution containing 0.01 M $Zn(CH_3COO)_2$ and 0.01 M HMTA is prepared and poured into a teflon inner tank. Put the CF that covered ZnO seed layer into the solution and seal the inner tank in a steal reactor. Reaction at 95 ℃ for ~ 5 h, then the CF is took out and rinsed with distilled water. Before used, the CF grown with ZnONR is storage in pH=7.0 PBS.

2.4 Measurements and detections

The morphology and structural of the prepared materials were examined by field emission scanning electron microscopy (SEM, JEOLJSM–7401F, EDS, Energy Dispersive Spectrometer).Fourier transform infrared spectroscopy (FTIR) spectra were recorded with a FTIR spectrometer (Thermo Nicolet). All the electrochemical measurements were carried out in a conventional three-electrode cell with a CHI-660E electrochemical station. The CF/ZnONR fiber (~0.5 cm long) working electrode was fabricated by tightening CF/ZnONR and copper wire (~1.5 cm long, ~0.5 mm in diameter) with line. Then, moderate conductive gel was coated to increase the conductivity of working electrodes. And Ag/AgCl (saturated with KCl) electrode and a Pt wire were used as reference and counter electrode. The apparent area of the CF/ZnONR electrode was 0.4 cm^2.The parameters applied for differential pulse voltammetry (DPV) method as follows: 50 mV amplitude, 50 ms pulse width, 4.0 mV step, and 0.20 s pulse period. The scan rate setting for cyclic voltammetry (CV) is 50 mVs^{-1}.

3. Results and discussion

3.1 Electrode morphological properties and mechanism for DA detection

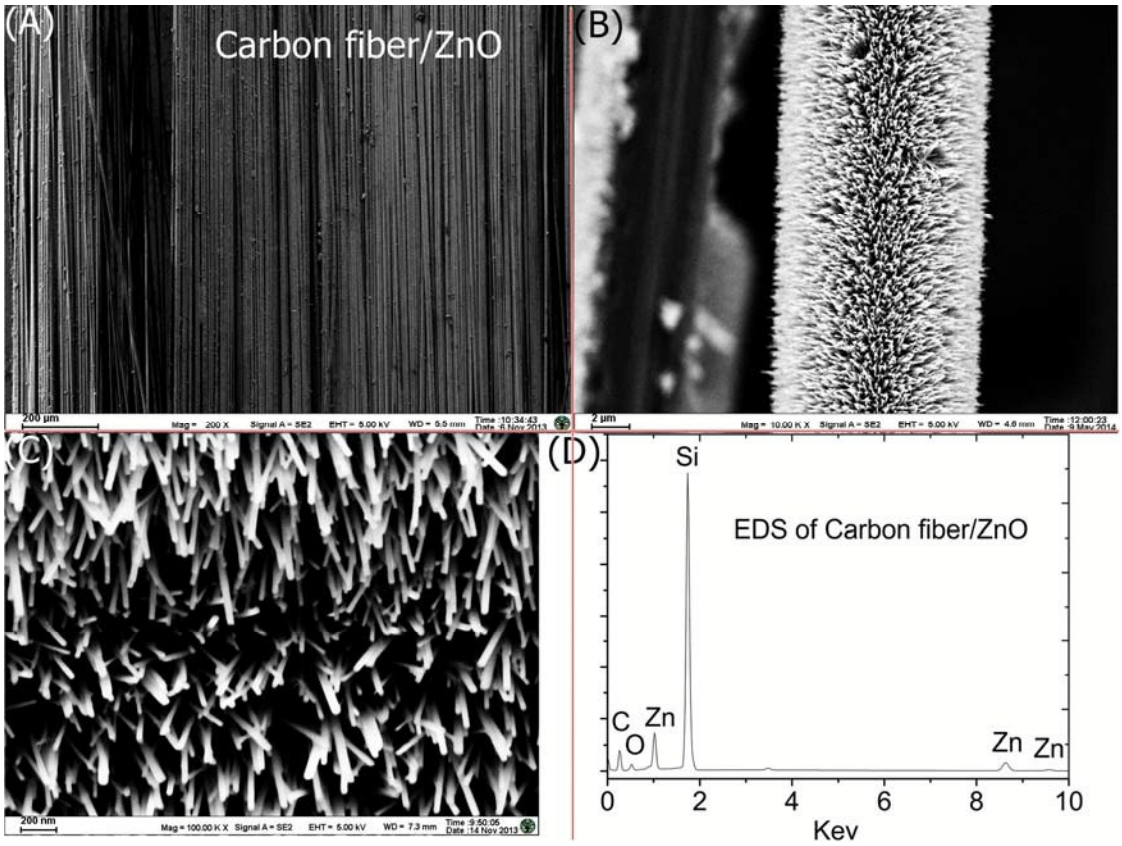


Fig. 1. Electrode morphology image, panorama of CF covered locality with ZnONR (A), SEM images of the carbon fiber substrates with ZnO, (B-C). SEM images of CF/ZnONR at different magnifications and (D) EDS spectra of CF/ZnONR.

In order to investigate the morphologies and crystal phase of the obtained CF/ZnONR fibers, SEM and EDS were conducted. The SEM images of bare CF and the obtained CF/ZnONR fibers are shown in Fig. 1A-C. All overview of CF/ZnONR fibers is presented at various magnification levels. As depicted in Fig. 1A, all of the CF/ZnONR fibers exhibited long and continuous cylindrical morphologies, having an average diameter of 5 μm. The ZnO nanowires were grown radically on the surface of CF templates and dense ZnO nanowires uniformly and compactly cover on the CF. From the magnified SEM image in Fig. 1B, the ZnO nanowires appear to have a relative uniform and hexagonal prismatic in shape. In the more magnified view (Fig. 1C), the hierarchically structured ZnO wires were aligned like a tree with the diameter of ZnONR is ~80 nm. The theoretical calculations showed that the area of single CF surface is

~4.157×10^{-3} μm^2 , and the CF/ZnONR have 230 times more surface area than without ZnONR(from Fig. 1B).The EDS of CF/ZnONR fibers show characteristic peak positions of ZnO and carbon fibers. This indicates that ZnO coated on the surface of CF were successful. The results further confirmed by the XRD of the samples, which were shown in Fig. 2A. Major peaks at 2θ = 26.5 and 44° were attributed to the (002) and (100) of the carbon structure in CF and clearly observed ZnO diffraction peaks which could be indicated as hexagonal wurtzite structure ZnO perfect (JCPDS 36-1451).Moreover, no peak of any other phase is detected, indicating that high purity ZnO nanostructures were produced. Furthermore, the surface condition is changes from nonpolar of CF to polar of ZnONR. All of these factors are propitious to adsorption of DA on CF-ZnONR electrode.

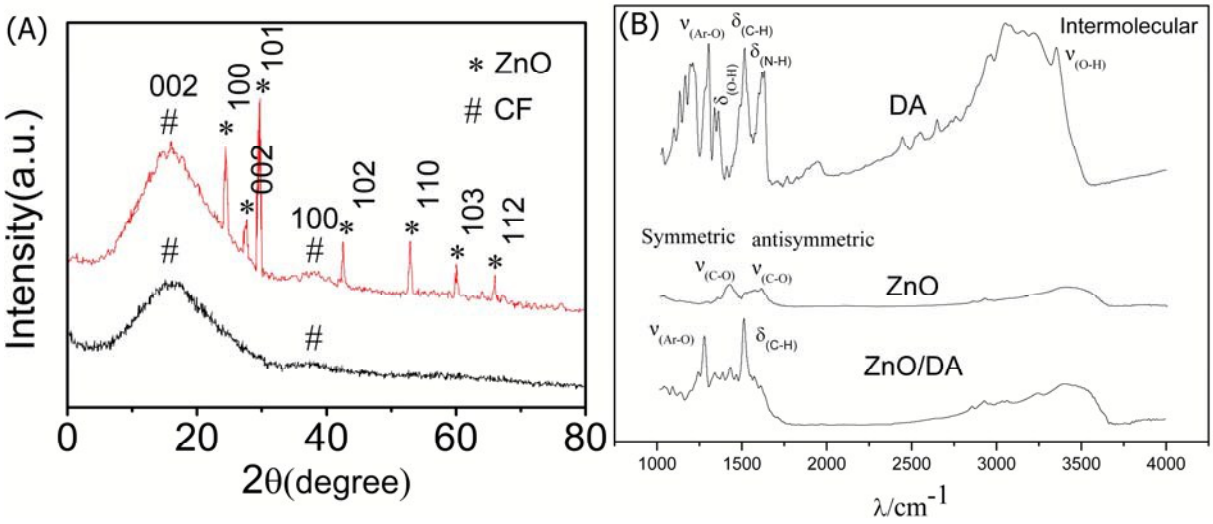
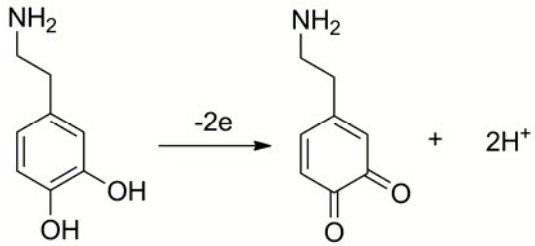


Fig. 2. (A) XRD patterns of the CF and CF/ZnONR.(B) FTIR spectra of DA, ZnO and their composites.

Due to the positively charged surface states of ZnONR crystal,[25] it is can be supposed that DA molecule that adsorbed on ZnONR surface is oxidized during electrochemical detection, which are illustrated below .



The valence electron orbit of Zn atom in ZnONR is sp^3 hybridized.[26] Because Zn atom has two valence electrons, so there are two “empty” orbits, which called unoccupied

orbital. When the unoccupied orbital formed bonds with O atom, the electrons in the covalent bond between Zn atom and O atom all come from O atom. In the outmost layer of ZnO, most of Zn atoms are exposed, and each Zn atom has one or two free unoccupied orbital. In other word, there are plenty of unoccupied orbital on ZnONR surface. Meanwhile, the valence electron orbit of O atom in DA is sp^2 hybridized. When O atom formed a bond with C atom and formed a bond with H atom, others two orbits that each "fill" with two electrons are remaindered, called unshared pair electrons.

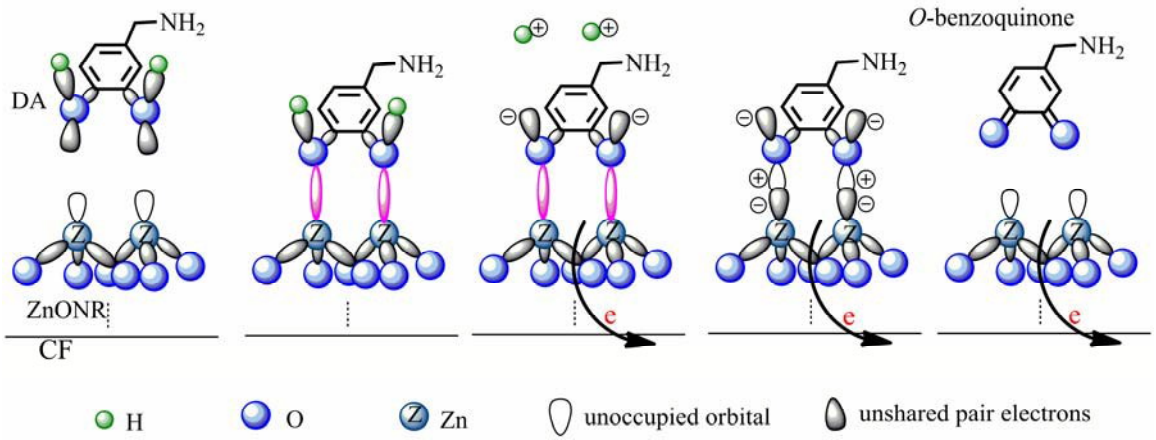


Fig. 3. The diagrammatic sketch of DA adsorption and oxidization on CF/ZnONR surface.

The process of DA oxidation mechanism is showed in Fig. 3, which is completed as following steps: (1) DA is adsorbed on ZnONR surface and the unshared pair electrons of O atom closed to unoccupied orbital of Zn atom. (2) One O atom of H_2O_2 molecule provides two electrons that in one sp^2 orbital and not bound with other atoms to share with the unoccupied orbital of Zn atom. Thus, a "like coordinate bond" is built between O and Zn. (3) because the electrons of O atom are shared with Zn atom, so the electron density of O atom is reduced. Then the polarity of "O-H" is increased, which means that the pair electrons between O atom and H atom is more closer to O atom, so that the H^+ can act as the free positive ions and leave DA molecule. Proof of dopamine covalently chelated ZnO composites were provided by FTIR spectra shown in Fig. 2B. Such evidence is demonstrated in the FTIR spectrum by the presence of the bending vibration of CH groups of dopamine at 1500 cm^{-1} as well as aryl oxygen stretching at 1246 cm^{-1}, the disappearance of the stretching vibration at 3345 cm^{-1} for the intermolecular hydrogen-bonded hydroxyl groups, and bending vibrations at 1342 cm^{-1} of dopamine OH groups. [27] (4) During detection, when potential is added at CF/ZnONR electrode,

electrons will be transferred from ZnONR to CF. The pair electrons in "like coordinate bond" between O atom and Zn atom will more closer to Zn atom, the connection of O atom and Zn atom will more like a electrovalent bond, so that O atom loses its electrons, and like a free ion leaves Zn atom. (5) Thus, in DA molecule, the energy of O atom is very high because its two orbital that not bound with other atom. Due to total energy requirements decreased, the big π bound of benzene ring is broken and new π bound is formed between O atom and C atom, and the new conjugated system is established. Thus the DA molecule is conversion to *o*-benzoquinone molecule during the electrochemical process. At the same time, with the electrons leave the electrode, Zn atom regained the unoccupied orbital. Thus, DA molecule could be oxidized to an *o*-benzoquinone on the ZnO surface. In fact, the adsorption mechanism of solute in solution is complex, and there is still no acknowledged dividing standard for application now. Usually, the Freundlich adsorption isotherm equation is applied.[26]

$$\lg \alpha = \frac{1}{n} \lg c + \lg k$$

In this equation, a is the solute amount that adsorbed on solid surface; c is the concentration of the solution; n and k are constants at a stationary temperature.

This equation suggests that the relationship of lg$c$ and lg$a$ is linear at a stationary temperature. This indicated that the amount of solute adsorption is increased followed by the increasing of solute concentration. Correspondingly, the signal would be enhanced when the solute concentration increasing. In this study, the solute is DA.

3.2 Electrochemical detection

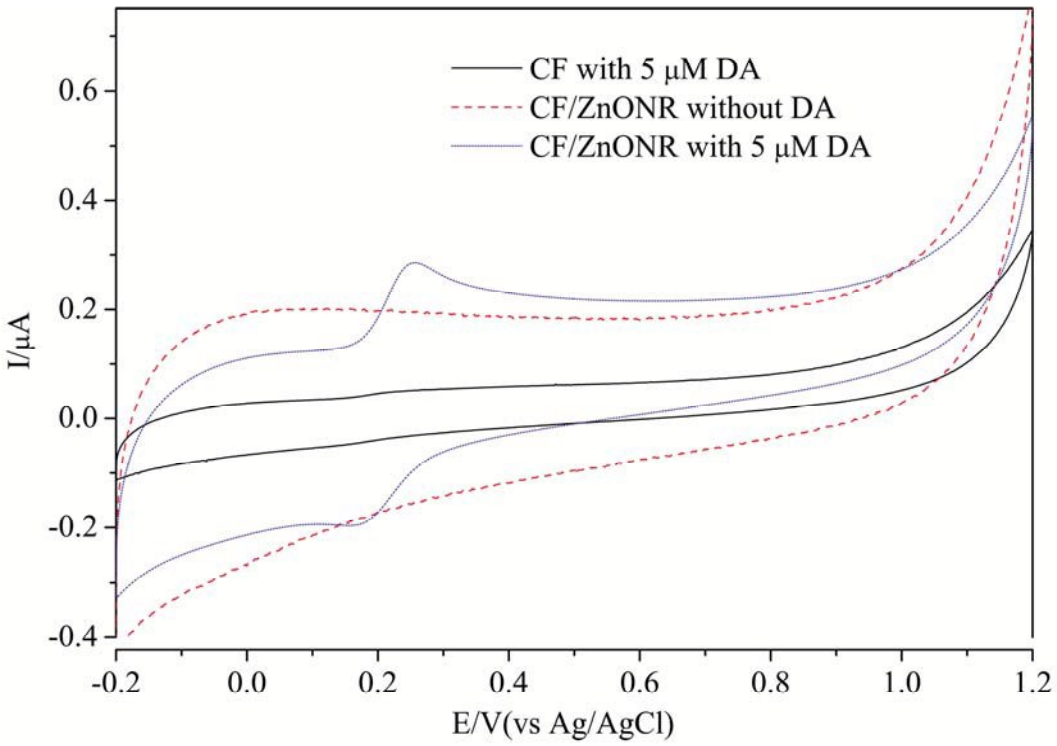


Fig. 4. CV curves of CF electrode, CF/ZnONR electrode in pH 7.0 PBS with and without 5 μM DA.

To verify the performance of CF electrode and CF/ZnONR electrode for DA detection, CV curves is detected in pH 7.0 PBS with and without 5 μM DA. Scan rate is 50 mVS^{-1}. As shown in Fig. 4, no obvious peak is observed in the CV of CF electrode with 5 μM DA. It illustrate that no catalytic reaction is occurred on the electrode surface. Meanwhile, no obvious peak is also observed at CF/ZnONR electrode without DA, but the background current is increasing obviously. This is ascribed to the growth of ZnONR. The area of CF/ZnONR electrode surface is larger than CF electrode, and the electrical conductivity is increased. When 5 μM DA is added, the marked anodic peak and cathodic peak are observed at 0.25 V and 0.18 V respectively. This suggests that DA is catalyzed oxidation on CF/ZnONR surface, and the catalyzed current is detected.

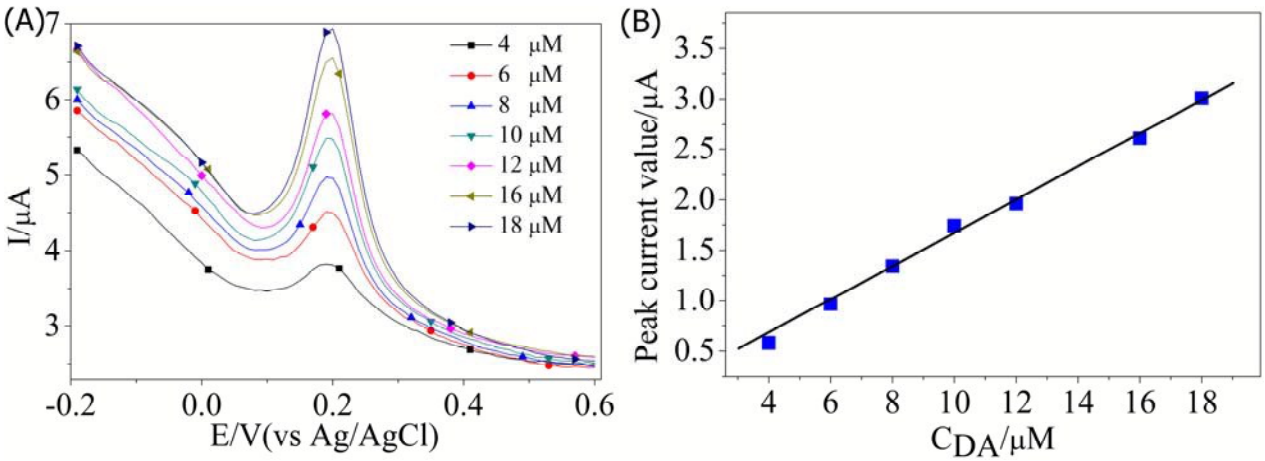


Fig. 5. DPV curves of CF/ZnONR electrode for different concentration of DA (a), and the liner relation of DPV peak value vs DA concentration (b).

To detect DA standard solution, differential pulse voltammetry (DPV) method is used for CF/ZnONR sensor. The DPV curves with different DA concentration are shown in Fig. 5. The results show that peak current increases with the rising level of dopamine. The linear relationship between peak current and concentration of DA was I_{pa}=4.0824C-0.7718 (R^2=0.9956).

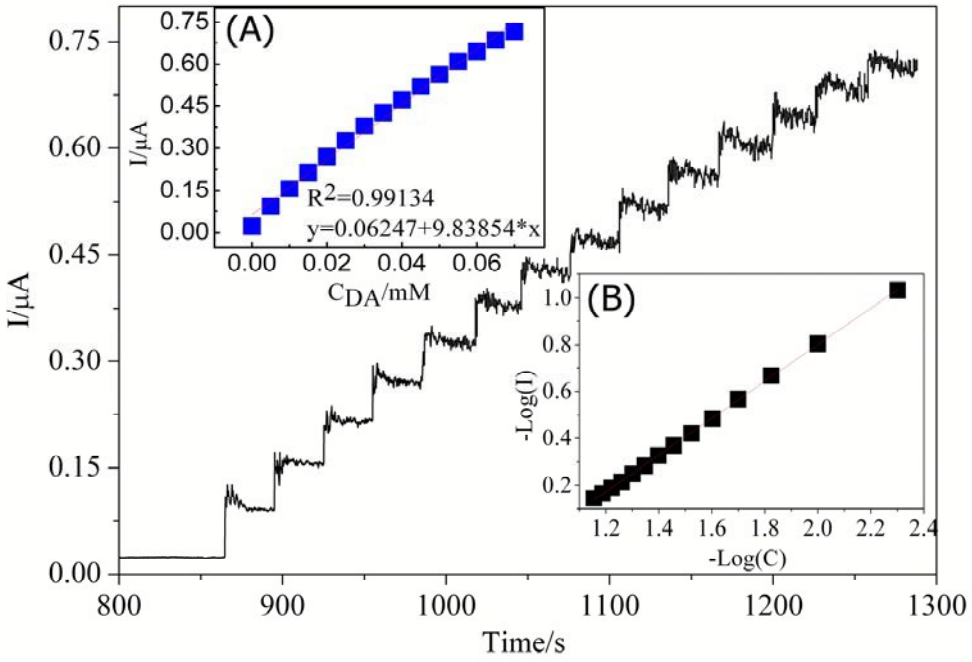


Fig. 6. The current-time response of CF/ZnONR ,inserted with the calibrated current-DA concentration relationship (A), and the calibrated negative logarithm of current-the negative logarithm of DA concentration relationship(B).

DA behaves an electrocatalytical oxidation peak at ~0.18V (from the CV electrocatalysis, as seen in Fig.4). Meanwhile, the selectivity of the sensor for the detection of DA was showed that the oxidation of interference did not occur at potential of ~0.18V (from the selectivity of the sensor, as seen in Fig.7). Therefore, for fulfilling both the selectivity and sensitivity of the present biosensor, ~0.18 V was selected as the working potential. The typical current-time response of the CF/ZnONR sensor is obtained at a working potential of 0.18 V by successively adding 5 μM DA in pH 7.0 PBS. As shown in Fig. 6, which shows the calibration plots of the current versus the concentration of DA. The results showed that although the sensor exhibits a broad response range from 5 μM to 70 μM with a good liner (R^2= 0.9913), the liner of response is a bit not perfect. For further logarithmic relationship of the current and DA, the corresponding linear relationship have changed for the better (R^2 =0.9999, Fig. 6B). This is a good liner relationship accorded with Freundlich adsorption isotherm equation that mentioned above. In other word, it is supposed that the adsorption of DA onto CF/ZnONR surface is accorded with Freundlich adsorption isotherm equation, and is the rate-determining step. When DA molecule is adsorbed on electrode surface, the catalytic reaction is rapid and easily. So, in this work, the surface area of electrode is the decisive factor for the sensor performance.

According to Fig. 6A, the sensitivity of CF/ZnONR for DA detection is 9.84 μA/μM, and the detection limit at a signal-to-noise ratio of 3 is 0.07 μM. The response time is less than 5 s when the current reached 95% of the steady-state value. The sensitivity is higher and the detection limit is lower than the other report [17-24].

3.3 The stability, anti-interference ability, repeatability of CF/ZnONR sensor for DA detection.

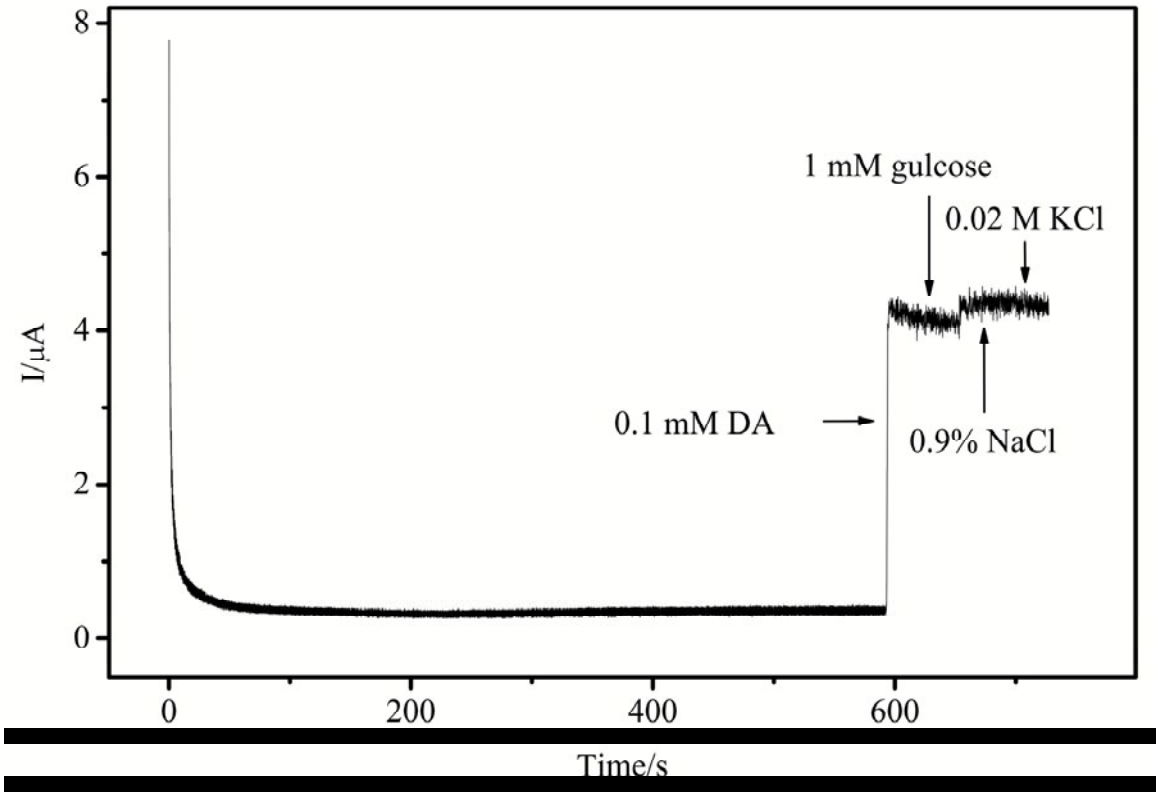


Fig. 7. The anti-interference of CF/ZnONR sensor for DA detection.

As a non-enzymatic sensors, the mainly advantages are its stability, simply constructed and convenient for detection. To test the anti-interference of CF/ZnONR sensor, the current-time response of CF/ZnONR sensor for 0.1 mM DA, 1 mM glucose, 0.9% NaCl and 20 mM KCl are took and the results are shown in Fig. 7.The response of interference reagents such as 0.9% NaCl, 20 mM KCl and 1 mM glucose is insignificant compared with 0.1 mM DA at initial potential 0.18 V. The slightly increasing of current after 0.9% NaCl and 20 mM KCl addition is supposed as the increasing of electrical conductivity of solution. Glucose, NaCl and KCl are the common influencing factors in the real sample. However, anodic current of ascorbic acid (AA) and uric acid (UA) were obtained (data not shown), indicating that the AA and UA interferes with the response of the sensor to DA. Fortunately, they have different oxidation potential and can be distinguished from the oxidation potential of DA. It illustrated that although as a non-enzymatic sensor, CF/ZnONR sensor for DA detection has good anti-interference ability. As the inherent properties of materials, the stability and repeatability are the advantages

of CF/ZnONR sensor. In experiment condition, after 20 days stayed at room temperature, CF/ZnONR sensor for DA detection almost remains the initialization activity. Using the one CF/ZnONR sensor to detect DA 20 times, the results almost reproduce the same value.

Table 1. Results of determination of DA in spike samples.

Samples	Original(μM)	Added(μM)	Found(μM)	RSD[a](%)	Recovery(%)
Dopamine hydrochloride injection	6	0	6.03	1.35	100.5
	6	5	10.91	1.68	99.2
	6	11	17.22	1.45	101.3
Serum	0	5	5.12	1.3	101.2
	0	10	10.78	1.7	99.2
	0	15	15.07	0.8	100.6

[a]Relative standard deviation(RSD)of 5 measurements.

3.4 Real sample determination

To evaluate the practical applicability of constructed DA sensors, the detection of DA in human urine samples was performed by using CF/ZnONR. In this study, the determination of DA was performed in quintuplicate, using the standard addition method. 10 μL aliquots of serum samples were added to the electrochemical cell containing PBS buffer solution (pH 7). No DA was detected, which could be explained either by the absence of DA in the serum sample or by the fact that any DA present were below the detection limit of the electrode. After the serum samples were spiked with various concentrations of DA standard solution and their DPVs were obtained. The calibration curve was used to determine the concentration of each solution; the recovery and RSD are shown in Table 1. This clearly indicates that the proposed method is reliable and effective for DA determination.

4. Conclusion

In summary, a rapid and convenient method was employed to synthesis coaxial carbon/ZnO core-sheath fibers. These ZnO fibers were produced from carbon fibers template through coupling the technique of plasma sputtering and hydrothermal routine. Simply prepared by this method makes it extremely easy to reproduce. The obtained fiber showed excellent electrocatalytic properties towards DA. The linear detection range for DA detection is 5 μM to 70 μM at initial potential 0.18 V. The detection limit is 0.07 μM and the sensitivity is 9.84 μA/μM. Furthermore, CF/ZnONR sensor shows good anti-interference ability, reproducibility and stability for the detection of DA. Moreover, the mechanism of catalytic oxidation DA on ZnONR surface is discussed. Furthermore, this method has been proven to be applicable in human serum samples.

Acknowledgements

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References

[1] I.Shakir, M.Shahid, U.A .Rana, I.M. Al Nashef, R. Hussain, Electroch Acta. 129(2014) 28.

[2] C.S. Huang, C.Y.. Yeh, Y.H. Chang, Y.M. Hsieh, C.Y. Ku, Q.T. Lai, Diam. Relat. Mater. 18 (2009) 452.

[3] P.Sahendra , C.K.Sharma, J.M. Ting, Thin Solid Films, 2(2014) 343.

[4] S.H. Jo, D. Banerjee, Z.F. Ren, Appl. Phys. Lett. 85 (2004) 1407.

[5] M. Tonezzera, R.G. Lacerda, Phys. E. 44 (2012) 1098.

[6] J .Wang, Y. Lin, Trends Anal Chem 27(2008)619.

[10] F .Mora, G .Segovia, A. del Arco, M .de Blas, P .Garrido,Brain Res..12(2012) 049

[11] D.L. Robinson, B.J. Venton, M.L.A.V. Heien, R.M .Wightman ,Clin Chem 49(2003) 1763.

[12] V. Hefco, K. Yamada, A. Hefco, L. Hrticu, A .Tiron, T. Nabeshima ,Eur J Pharmacol 475(2003)55.

[13] B.E.K. Swamy, B.J. Venton , Analyst 132(2007)876.

[14] K.A. Conway, J.C. Rochet, R.M. Bieganski, P.T. Lansbury, Science, 294(2001)1346̃.

[15] J.W. Dalley, J.P. Roiser , 215(2012) 42.

[16] M .Shou, C.R .Ferrario, K.N. Schultz, T.E. Robinson, R.T. Kennedy , Anal Chem 78(2006) 6717.

[17] D .Wang, W .Zhu, Y .An, J. Zheng, W. Zhang, L .Jin, H .Gao, L .Lin ,Chromatographia 67(2008)369.

[18] L .Wu, L .Feng, J Ren, X. Qu Biosens Bioelectron 34(2012)57.

[19] S. Hon, M.L. Kasner, S. Su, K. Patel, R. Cuellari , J Phys Chem C 114(2010)14915.

[20] M.G. Garcia, G.M.E .Armendariz, L.A. Godinez, J .Torres, S .Sepulveda- Guzman, E .Bustos, Electrochim Acta 56 (2011)7712.

[21] C .Xia, W. Ning, W. Long, G .Lin, Sens Actuators B 147 (2010) 629.

[22] M.C. Henstridge, E.J.F. Dickinson, M.Aslanoglu, C. Batchelor-McAuley, R.G. Compton, 145 (2010)417.

[23] N.F. Atta, M.F. El-Kady, A. GalalAnal Biochem 400(2010)78.

[24] Z. L. Wang, Mater Sci Eng R, 64(2009)33.

[25] L.S. Labo, C.A. Bernardo, J. Chem. Educ. 51(1974)723.

[26] Y. Zhao, S.H. Li, J.Chu, Y.P. Chen, W.W. Li, H.Q. Yu, G. Liu, Y.C. Tian, Y. Xiong, Biosens Bioelectron 35(2012)115.

[27] T. Rajh, L.X. Chen, K. Lukas, T. Liu, M.C. Thurnauer, D.M.Tiede, J. Phys. Chem. B 106(2002)10543

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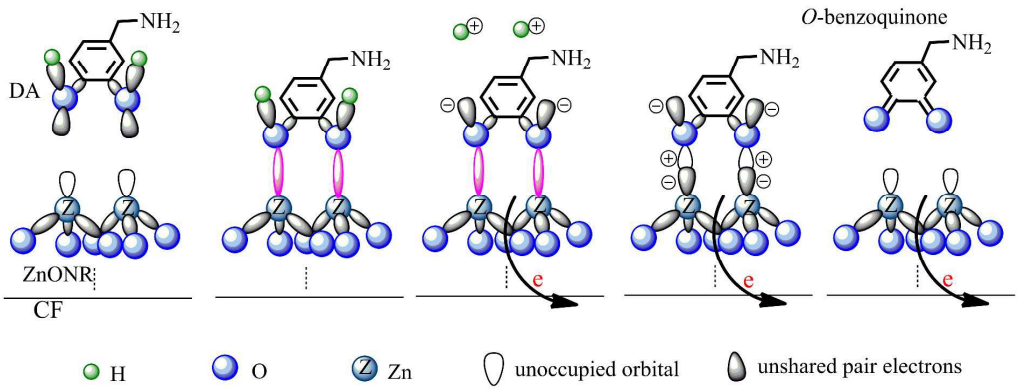

The adsorption and oxidization mechanism of dopamine on the surface of carbon fiber/ZnO nanorod.