Electrochemical detection of serotonin based on a poly(bromocresol green) film and Fe$_3$O$_4$ nanoparticles in a chitosan matrix†

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A composite film containing poly(bromocresol green), magnetic nanoparticles and multiwalled carbon nanotubes was fabricated for the sensitive determination of serotonin. The poly(bromocresol green) was prepared by electropolymerization on a glassy carbon electrode surface, then the multiwalled carbon nanotubes and magnetic nanoparticles in a chitosan matrix were dropped onto the surface of the poly(bromocresol green) film. The modified electrode displayed an enhanced differential pulse voltammetric response to serotonin in the presence of dopamine. Its peak current (at an oxidation voltage of 0.36 V vs. Ag/AgCl) increased linearly in the serotonin concentration range 0.5–100 μM with a detection limit of 80 nM (at an S/N of 3). The sensor was highly sensitive and stable. It was applied to the direct determination of serotonin spiked into human serum samples. These results show that the nanocomposite films are good modifying materials for use in the fabrication of electrochemical sensors.

1. Introduction

Thin films of conducting polymers have attracted much attention as electrode materials. Conjugated polymers can be used in thin film transistors and biosensors as a result of their electronic and electrochemical properties. Conducting polymers can enhance the electrochemical signals produced by the redox reactions of target compounds. Bromocresol green (BCG) has been used as an electrode material in electrochemical sensors, its high-electron-density hydroxyl groups and good conductivity give poly(BCG) films a negative charge and they exhibit electrocatalytic activity towards many substances. Iron oxide (Fe$_3$O$_4$) forms magnetic nanoparticles (NPs), which have excellent chemical stability, good biocompatibility and a high electron efficiency and have therefore been widely applied in electrochemistry. Multiwalled carbon nanotubes (MWCNTs) have a porous nanostructure and have a high electrical conductivity, large surface area and good chemical stability. When used as modifying materials in electrochemical devices, they can promote electron transfer reactions. Chitosan (CS) has a good permeability, good adhesion and excellent film-forming abilities. These make CS a good matrix for immobilizing molecules.

Serotonin (5-hydroxytryptamine; 5-HT) is an important neurotransmitter and plays a variety of parts in many basic biological processes, including sensory perception and behavior. This has encouraged researchers to look for faster and more sensitive methods to determine 5-HT, including detection using various modified electrodes. Sensitivity is a key factor in the detection of serotonin. We describe here nanocomposites based on magnetic Fe$_3$O$_4$ NPs, MWCNTs and poly(BCG), which have been used to study the electrochemical behaviour of 5-HT. Fe$_3$O$_4$ and MWCNTs are ideal modifying materials due to their high conductivity and good absorbability. The poly(BCG) film modified electrode had a large surface area and good conductivity, which led to conjugation between the target analytes and the electrode interface. We describe the preparation and application of the serotonin sensor. The Fe$_3$O$_4$–MWCNT–poly(BCG) composite films exhibited strong electrocatalytic ability and enhanced the sensitivity towards 5-HT. Compared with a bare glassy carbon electrode (GCE), the peak currents of 5-HT increased significantly at the electrode coated with a Fe$_3$O$_4$–MWCNT–poly(BCG) film. The oxidation peak of 5-HT was not affected by high concentrations of dopamine. 5-HT was detected by the poly(BCG) and nanoparticle films using an electrochemical method. The modified electrode had obvious advantages, such as a wide linear concentration range, low detection limit, good selectivity and reproducibility. The nanocomposite films are thus good materials for the fabrication of electrochemical sensors.

2. Experimental

2.1. Reagents and apparatus

CS, BCG and 5-HT were obtained from Sigma. Dopamine (DA), ascorbic acid (AA), uric acid (UA), adrenaline (AD), tryptophan...
Trp), L-cysteine (L-Cys) and glucose (GLU) were obtained from Alfa Aesar. The MWCNTs were obtained from Nanjing XFNANO Materials Tech. Sulfuric acid, nitric acid, acetic acid, sodium phosphate dibasic and sodium phosphate monobasic were obtained from Sichuan Xilong Chemical. All reagents were of analytical-reagent grade. All solutions used were made with ultrapure water. Phosphate buffers with different pH values were prepared using 0.1 M NaH₂PO₄ and 0.1 M Na₂HPO₄. The serum samples were obtained from the hospital of Chongqing Three Gorges University.

A CHI 660D electrochemical workstation (Shanghai CHI Instruments) was used to collect the electrochemical data. The three-electrode system consisted of a modified working electrode, a platinum electrode as the counter electrode and an Ag/AgCl reference electrode. UV absorption spectra were measured on a Cary-300 spectrophotometer (Varian). An EL20K pH meter (Mettler) was used to measure the pH. The morphologies and microstructures of the samples were characterized by an S-4800 scanning electron microscope and H-7500 transmission electron microscope (Hitachi).

2.2. Preparation of poly(BCG)/GCE

The GCE was polished successively with 0.3 and 0.05 μm alumina powders, rinsing thoroughly with ultrapure water between each polishing step. The electrode was washed in 1 : 1 nitric acid, then ultrasonicated in ultrapure water before drying in high-purity nitrogen. The GCE was placed in a beaker containing pH 7.4 phosphate buffer and 0.5 mM BCG solution and was cyclically swept for 25 cycles between −0.4 and 1.8 V vs. Ag/AgCl at 100 mV s⁻¹ to form the poly(BCG)/GCE (Fig. S1 and S2, ESI†). The anodic peak currents increased with the number of scan cycles, indicating the deposition of BCG on the surface of the GCE by electropolymerization (Fig. 1). The impurities on the electrode surface were removed by ultrapure water. The poly(BCG)/GCE was then stored at room temperature.

2.3. Preparation of GCE with composite Fe₃O₄–MWCNTs–poly(BCG)

The MWCNTs were sonicated with a strong oxidizing agent consisting of a mixture of concentrated sulfuric and nitric acids (3 : 1 v/v) for 10 h and then washed with ultrapure water, filtered and centrifuged. A 1.0 mg mass of MWCNTs and 1.0 mL of 0.5 wt% CS were ultrasonicated to obtain a suspension. Magnetic Fe₃O₄ NPs were prepared by Batalha’s method. Fe₃O₄ (1.0 mg) was dispersed in the suspension to obtain an Fe₃O₄–MWCNT solution. The modified electrode was prepared by dropping 5.0 μL of the modified solution onto the surface of the poly(BCG)/GCE. The modified solution was then dried at room temperature.

3. Results and discussion

3.1. Characterization of Fe₃O₄–MWCNT composite films

The TEM image of the Fe₃O₄ NPs in Fig. 2a shows that the Fe₃O₄ NPs were rounded NPs with a uniform distribution. The SEM images of the MWCNTs and the Fe₃O₄–MWCNT nanocomposite film are shown in Fig. 2b and c. It can be clearly seen that the Fe₃O₄ NPs were attached to the MWCNTs. These spherical particles were confirmed to be agglomerations of Fe₃O₄ NPs.

To further investigate the Fe₃O₄–MWCNT solution, the modified solution was characterized by its UV absorption spectrum. The UV absorption spectra of different samples in phosphate buffer are shown in Fig. 3. An obvious absorption peak appeared at about 220 nm, originating from the structure of the MWCNTs.

Fig. 1 Repeated cyclic voltammograms in phosphate buffer (pH 7.4) and 5 × 10⁻⁴ M BCG. Scan rate: 100 mV s⁻¹; no. of sweep circles, 25.

Fig. 2 (a) TEM image of Fe₃O₄ NPs and SEM images of (b) MWCNTs and (c) Fe₃O₄–MWCNTs.

Fig. 3 UV–vis absorption spectra of (a) MWCNTs, (b) Fe₃O₄–MWCNTs and (c) Fe₃O₄ in 0.1 M phosphate buffer (pH 7.0).
3.2. Electrochemical behaviour of 5-HT at the Fe₃O₄–MWCNT–poly(BCG) films

Fig. 4 shows the cyclic voltammograms of different modified electrodes in pH 7.0 phosphate buffer and 2.0 × 10⁻⁵ M 5-HT solution. No peak was observed at the bare GCE (curve a), but an oxidation peak was observed for the poly(BCG)/GCE (curve b). The oxidation peak potentials occurred at 0.400 V vs. Ag/AgCl. An oxidation peak was also observed for the MWCNT–poly(BCG)/GCE (curve c) and Fe₃O₄–MWCNT/GCE (curve d). Under identical conditions, when the bare GCE was modified with the Fe₃O₄–MWCNT–poly(BCG) film, the oxidation peak observed at the Fe₃O₄–MWCNT–poly(BCG)/GCE (curve e) was much larger than for the other modified electrodes and the oxidation peak potential shifted to 0.360 V vs. Ag/AgCl. It was therefore concluded that the Fe₃O₄–MWCNT–poly(BCG) composite enhanced the electron transfer and peak currents of 5-HT. The sensitivity of 5-HT was improved by this electrocatalytic behaviour.

3.2.1. Optimum amounts of Fe₃O₄ and MWCNTs. The optimum amounts of Fe₃O₄ and MWCNTs were investigated. The total concentration of Fe₃O₄ and MWCNTs was fixed at 6.0 mg mL⁻¹, but we changed the concentration of Fe₃O₄ from 0 to 6.0 mg mL⁻¹. Fig. 5 shows that the peak currents gradually increased with an increase in the concentration of Fe₃O₄ from 0 to 3.0 mg mL⁻¹. When the concentration of Fe₃O₄ reached 3.0 mg mL⁻¹, the peak currents reached the highest value. However, the peak currents gradually decreased with an increase in the concentration of Fe₃O₄ from 3.0 to 6.0 mg mL⁻¹. Therefore the optimum proportion of Fe₃O₄ to MWCNTs was 1:1.

3.2.2. Effects of scan rate and solution pH. The scan rate influenced the electrode reaction of the peak potential (Eₚₐ) and the peak current (Iₚ) (Fig. 6a). In the range 40–180 mV s⁻¹, Iₚ was linearly related to the square root of the scan rate. The linear regression equation was: Iₚ (μA) = 0.081 + 0.699 V mV⁻¹ s⁻¹ (R² = 0.997), meaning that the oxidation process was diffusion controlled. As the scan rate increased, Eₚₐ shifted to a more positive potential, indicating that the electron transfer process was quasi-reversible. When the pH of the phosphate buffer changed, the oxidation Eₚₐ and the Iₚ of 5-HT also changed. When the pH of the phosphate buffer was 7.0, the peak current of 5-HT reached a maximum value at the Fe₃O₄–MWCNT–poly(BCG)/GCE (Fig. 6b) and therefore pH 7.0 was selected as the optimum pH for the determination of 5-HT. By contrast, when the pH increased, the peak potential of 5-HT shifted to a negative potential and Eₚₐ was linear with pH. The linear regression equation was: Eₚₐ (mV) = 654.4 − 38.3 pH (R² = 0.990). The slope of −38.3 mV pH⁻¹ indicates that only one proton was involved in the two-electron oxidation process.

3.3. Electrochemical response of 5-HT in the presence of DA at the modified electrode

Differential pulse voltammetry (DPV) has a much better current sensitivity than cyclic voltammetry, so it was used to determine the concentration of 5-HT in the presence of DA at the Fe₃O₄–MWCNT–poly(BCG)/GCE and to estimate the limit of detection. The anodic peak currents were linearly related to the 5-HT concentration in the range 0.2–100 μM (Fig. 7a). The linear regression equation was: Iₚ (μA) = 0.3088 + 0.5279 C (M) (R² = 0.998) and the detection limit was 60 nM (S/N = 3). The oxidation peak current was linearly related to the concentration of 5-HT in the range 0.5–100 μM in the presence of 10 μM DA.
Fig. 7 (a) DPV for 5-HT at the Fe₃O₄–MWCNT–poly(BCG)/GCE in phosphate buffer (pH 7.0). 5-HT concentrations (from a to h): 0, 0.2, 0.5, 1, 5, 10, 50 and 100 μM. (b) DPV of 5-HT at the Fe₃O₄–MWCNTs–poly(BCG)/GCE in phosphate buffer (pH 7.0) and 1.0 × 10⁻³ M DA. 5-HT concentration (from a to g): 0, 0.5, 1, 5, 10, 50 and 100 μM. The inset shows the relationship between Iₚ and different concentrations of 5-HT.

Table 1. Performance of previously reported modified electrodes in the detection of 5-HT

| Modified electrode       | Linear region (μM) | Detection limit (μM) | Ref. |
|--------------------------|-------------------|---------------------|-----|
| Ru/WGE                   | 0.3–9.0           | 0.1                 | 23  |
| 5-HTP/GCE                | 5.0–35.0          | 1.7                 | 25  |
| ACh/GCE                  | 1.0–30.0          | 0.5                 | 33  |
| Carbon fibre electrode   | 2.5–100.0         | 1.0                 | 34  |
| CNT-IL/GCE               | 5.0–900.0         | 2.0                 | 35  |
| PM-PG/GCE                | 1.0–100.0         | 0.49                | 36  |
| Fe₃O₄–MWCNT–poly(BCG)/GCE| 0.5–100.0         | 0.08                | This work |

(a) Ru: rutin; WGE: paraffin-impregnated graphite electrode; 5-HTP: 5-hydroxytryptophan; GCE: glassy carbon electrode; ACh: acetylcholine; CNT: carbon nanotube; IL: ionic liquids; PM: polymelamine; PG: pyrolytic graphite.

Table 2. Recovery for the determination of 5-HT in serum samples

| Sample Original (μM) | Added (μM) | Found (μM) | Recovery (%) |
|----------------------|------------|------------|--------------|
| 1 ND                 | 2.00       | 1.89       | 94.50        |
| 2 ND                 | 3.00       | 2.79       | 93.00        |
| 3 ND                 | 4.00       | 3.75       | 93.75        |

ᵃ Mean of three determinations. ᵇ Not detected.

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References

1 S. Gunes, H. Neugebauer and N. S. Sariciftci, Chem. Rev., 2007, 107, 1324.
2 D. T. McQuade, A. E. Pullen and T. M. Swager, Chem. Rev., 2000, 100, 2537.
3 G. Inzelt, M. Pineri, J. W. Schultze and M. A. Vorotyntsev, 
*Electrochim. Acta*, 2000, 45, 2403.

4 X. Q. Ouyang, L. Q. Luo, Y. P. Ding, B. D. Liu, D. Xu and 
A. Q. Huang, *J. Electroanal. Chem.*, 2015, 748, 1.

5 J. Y. Hou and S. Y. Ai, *Chem. Res. Chin. Univ.*, 2011, 27, 934.

6 E. Katz and I. Willner, *Angew. Chem., Int. Ed.*, 2005, 44, 4791.

7 S. Yu and G. M. Chow, *J. Mater. Chem.*, 2011, 27, 934.

8 E. Katz and I. Willner, *Chem. Commun.*, 2005, 4089.

9 D. J. Kim, Y. K. Lyu, H. N. Choi, I. H. Min and W. Y. Lee, 
*Chem. Commun.*, 2005, 2966.

10 J. Q. Wan, W. Cai and J. T. Feng, *J. Mater. Chem.*, 2007, 17, 1188.

11 R. H. Baughman, A. A. Zakhidov and W. A. de Heer, *Science*, 2002, 297, 787.

12 Y. P. Sun, K. F. Fu, Y. Lin and W. J. Huang, *Acc. Chem. Res.*, 2002, 35, 1096.

13 H. Boo, R. A. Jeong, S. Park and K. S. Kim, *Anal. Chem.*, 2005, 78, 617.

14 Y. J. Yin, Y. F. Lu, P. Wu and C. X. Cai, *Sensors*, 2005, 5, 220.

15 J. Cruz, M. Kawasaki and W. Gorski, *Anal. Chem.*, 2000, 72, 680.

16 M. G. Zhang, A. Smith and W. Gorski, *Anal. Chem.*, 2004, 76, 5045.

17 H. Zhang, M. Oh, C. Allen and E. Kumacheva, 
*Biomacromolecules*, 2004, 5, 2461.

18 C. Lau and M. J. Cooney, *Langmuir*, 2008, 24, 7004.

19 R. A. Glennon, *J. Med. Chem.*, 2003, 46, 2795.

20 D. E. Nichols and C. D. Nichols, *Chem. Rev.*, 2008, 108, 1614.

21 R. N. Goyal, M. Oyama, V. K. Guptaa and S. P. Singha, *Sens. Actuators, B*, 2008, 134, 816.

22 K. B. Wu, J. J. Fei and S. H. Hu, *Anal. Biochem.*, 2003, 318, 100.

23 G. P. Jin, Q. Z. Chen, Y. F. Ding and J. B. He, *Electrochim. Acta*, 2007, 52, 2535.

24 N. Yusoff, A. Pandikumar, R. Ramaraj, L. H. Ngee and 
N. M. Huang, *Microchim. Acta*, 2015, 182, 2091.

25 Y. X. Li, X. Huang, Y. Chen, L. Wang and X. Lin, *Microchim. Acta*, 2009, 164, 107.

26 A. Babaei and M. Babazadeh, *Electroanalysis*, 2011, 23, 1726.

27 A. Abbaspour and A. Noori, *Biosens. Bioelectron.*, 2011, 26, 4674.

28 I. L. Batalhha, A. Hussainaand and A. C. A. Roquea, *J. Mol. Recognit.*, 2010, 23, 462.

29 C. S. Chen, X. H. Chen, B. Yi and T. G. Liu, *Acta Mater.*, 2006, 54, 5401.

30 J. Y. Qu, Y. Dong, Y. Wang and H. H. Xing, *Sens. Biosensing Res.*, 2015, 3, 74.

31 S. Ogawa, E. A. Decker and D. J. McClements, *J. Agric. Food Chem.*, 2004, 11, 3595.

32 X. H. Jiang and X. Q. Lin, *Anal. Chim. Acta*, 2005, 537, 145.

33 G. P. Jin, X. Q. Lin and J. M. Gong, *J. Electroanal. Chem.*, 2004, 569, 135.

34 K. H. Parker and D. O’Hare, *Anal. Chem.*, 2006, 78, 6990.

35 M. M. Ardakani and A. Khoshroo, *J. Electroanal. Chem.*, 2014, 717, 17.

36 P. Gupta and R. N. Goyal, *Talanta*, 2014, 120, 17.