Supporting Information

Label-free electrochemiluminescent immunosensor for α-fetoprotein: performance of Nafion-carbon nanodots nanocomposite film as antibody carriers

Hong Dai [a]*, Caiping Yang[b], Guifang Xu[a], Xiuling Ma[a], Yanyu Lin[ab], Guonan Chen [b]*

[a] College of Chemistry and Materials Science, Fujian Normal University, Fuzhou, Fujian, 350108,China

[b] MOE Key Laboratory and Fujian Provincial Key Laboratory for Analysis and Detection for Food Safety, and Department of Chemistry, Fuzhou University, Fuzhou, Fujian 350002, China

E-mail: dhong@fjnu.edu.cn; gnchen@fzu.edu.cn

1. Experimental Section

1.1 Chemicals

AFP, α-fetoprotein antibody (anti-AFP, monoclone), AFP ELISA kits and bovine serum albumin (BSA, 96–99%) were purchased from Biss Inc. (Beijing, China). Nafion, L-Ascorbic acid,glycine and luminol was obtained from Sigma (St. Louis, MO, USA) and used without further purification. Other reagents were of analytical regent grade. All solutions were prepared with deionized water by a Milli-Q water purification system (Millipore, Milford, MA, USA).

1.2 Instruments and measurements

ECL intensity versus potential was detected by using a system made in our laboratory, consisting of a BPCL Ultra-Weak Chemiluminescence Analyzer controlled by a personal

computer with BPCL program (Institute of Biophysics, Chinese Academy of Sciences) in conjunction with a CHI 660 electrochemical analyzer (Shanghai Chenghua Instrument Co., China). The electrochemical analyzer was used for controlling waveforms and potentials. The detail description of this system has been given in previous report [1].

A conventional three-electrode system was used for the electrolytic system, including a bare GCE or modified GC electrode as the working electrode, a platinum wire as the counter electrode and Ag/AgCl electrode (sat. KCl) as the reference electrode. A commercial 5 ml cylindroid glass cell was used as ECL cell, and it was placed directly in the front of the photomultiplier tube.

1.3 Synthesis of CNDs through one step approach

CNDs were synthesized according to the previous report [2]. Briefly, 1.1 g L-Ascorbic acid was dissolved in 25 mL deionized water and 25 mL ethanol to form a homogeneous solution. Then, 25 mL as-prepared solution was transferred into autoclave and heated at 180 °C for 4 h and then cooled to room temperature naturally. The dark brown solution was extracted with dichloromethane. The water phase solution was dialyzed by employing dster dialysis membranes for three days to remove all impurity molecules. At last, a yellow CNDs aqueous solution was obtained.

1.4 Preparation of ECL immunsensor

First, a disk glassy carbon with 4 mm diameter as working electrode was polished to a mirror-like surface with define alumina powder, followed it was sonicated thoroughly in 1M HNO_3/1M HCl, 1M NaOH solution, ethanol and deionized water for 5 min, respectively.

The CNDs/Nafion solution was prepared by dispersing 40μL of 5% Nafion and

150μL resultant CNDs solution in 310μL ethanol, the modified electrode was prepared by dipping 4μL the prepared solution on the GC electrode, then dried it in the air. As a comparison, the Nafion/GC electrode was similarly prepared. Followed the electrode was immersed into anti-AFP solution (100μg/ml) for 1.5 h at room temperature, and then incubated into BSA solution (0.1wt.%) for about 1 h at room temperature to block possible active sites, then incubated in different concentration of AFP solution for 1h. The electrochemiluminescent sensing strategy for the detection of AFP was showed in scheme 1. The prepared modified electrode could be stored in refrigerator at 4 □ when not in use.

2. UV-vis adsorption of CNDs

Fig.S1 depicted the UV-vis absorption spectra of the as-prepared CNDs. A strong absorption band at 262.8 nm was observed with a narrow fwhm of 40nm.

3. Optimization of experimental conditions

The amount of Nafion and CNDs in the CNDs-Nafion nanocomposite film greatly affected the ECL response of luminol on the resultant ECL platform. Through simply modulating the Nafion and CNDs amount in the modified solution, the optimal composing of CNDs-Nafion nanocomposite film for luminol ECL system could be achieved. Fig.S1Aa exhibited that, with the increasing volume of CNDs in the modified solution from 50 to 250 μl, the ECL response of this sensor increased firstly, then decreased, and reached maximum at 150 μl. The low concentration of CNDs could lead in homogeneously and finely disperse of CNDs in a certain concentration (0.4%) Nafion solution. Thus it facilitated the electron transfer between electrode matrix and solution,

leading to the increased ECL response. However, too much CNDs in the modified solution resulted in the formation of cluster of CNDs, reducing the area-to-volume ration. Similarly, the ECL emission of luminol at this sensor increased with the increasing concentration of Nafion. While, when the concentration of Nafion beyond 0.4%, the ECL intensity decreased. So, per 500 μl modified solution contained 150 μl CNDs solution and 0.4% Nafion was employed as the optimal composing.

The important parameters that determine the performance of an immunosensor are the amount of immobilized antibody on the sensing interface and the incubation time required for the antigen–antibody immuno-reaction. The robust ECL decreased ratio (referred as described ECL percentage), which means the decreased ECL intensity of the immunosensor after reaction occupys the ratio of this initial ECL response, was employed as the guideline to evaluate these two important parameters. During the procedure of fabricating immunosensor, it took some time for immobilizing antibody onto the sensing interface. Nafion was a well known polyanionic perfluorosulfonated ionomer with rich negative charge. Moreover, by the reason of rich hydroxyl and carboxyl group on the CNDs, thus such CNDs-Nafion nanocomposite film could entrap antibody via the electrostatic adsorption. And compared with other nano-materials, CNDs have huge specific surface area. With the addition of CNDs into Nafion film, it made the nanocomposite film to be more porous. All these facilitated the CNDs-Nafion nanocomposite film being a potential and effective antibody carriers. As seen from Fig.S2Aa, the decreased ECL percentage increased with the increment of incubation time, then leveled off after 60 min. As a result, the optimum immobilized period was set at 60 min for the incubation steps in this study.

To obtain the optimization of time required for completeness of the immunoreactions, anti-AFP/CNDs/Nafion/GCE modified electrode were incubated in 80 ng/mL AFP solution for different time periods. Fig.S2Ba was evidently described that only the incubated time arrived 90 mins or more exhibited a stable response showing the complete interaction between anti-AFP and AFP. So an optimized incubation time of 90min was chose for the incubation steps in this investigation.

4. Real Sample Detection

This ECL immunosensor was utilized to analysis AFP in the serum samples. As can be seen in Table 1, the AFP concentration of dilution solution of human serum sample could be detected by the prepared immunosensor. After introduction of different concentrations of AFP solution into the samples, it is found that recoveries were in the range of 93.4~110.0%. These results indicated that this biosensor can be used in the practice sample analysis.

Table S1 Real sample detection and Recoveries

Sample (ELISA method)	**AFP concentration of the serum sample after dilution (ng/ml)**	**add AFP (ng/ml)**	**found (ng/ml)**	**Recovery (%)**
Human blood Serum (AFP32.23 ng/ml)	0.107	0 0.250 0.500 0.800	0.100 0.360 0.600 1.000	93.4 101.0 98.8 110.0

References

S1 Lin, Y., Dai, H., Yang, C., Lin, S., Chen, G., *2011. Electroanalysis,23, 1260–1266.*

S2 Zhang, B., Liu, C.Y., Liu, Y., *2010.J. Inorg. Chem. 22, 4411–4414.*

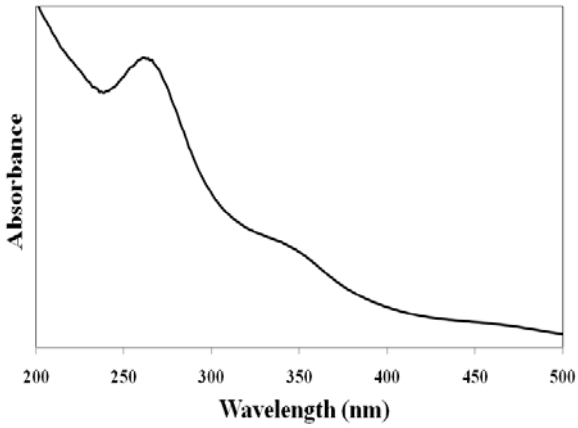
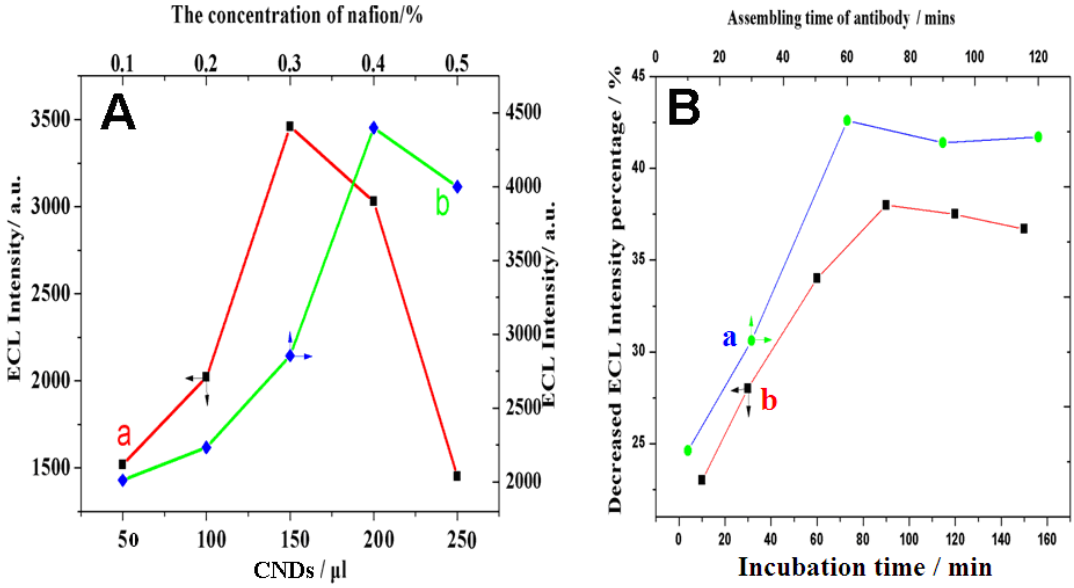


Figure S1 UV-vis absorption spectra of CNDs.



❖ **Figure S2 (A)**Effects of volum of CNDs(a) and the concentration of Nafion(b) on the ECL emission of luminol on CNDs/Nafion nanocomposite modified electrode. **(B)** Effects of the self-assembly time of anti-AFP(a) and the incubation time (b) on the decreased ECL intensity percentage of luminol.

Conditions: pH 8.0PBS contained 5×10^{-5} mol/L Luminol. AFP: 100μgml^{-1}. Potential windows: 0.2-0.8V, scan rate: 0.1V/s.