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Amperometric glutamate biosensor based on self-assembling glutamate dehydrogenase and dendrimer-encapsulated platinum nanoparticles onto carbon nanotubes

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Abstract

A novel amperometric biosensor based on self-assembling glutamate dehydrogenase (GLDH) and poly(amidoamine) dendrimer-encapsulated platinum nanoparticles (Pt-PAMAM) onto multiwall carbon nanotubes (CNTs) has been developed for the determination of glutamate. The formation of the self-assembled $(GLDH/Pt\text{-}PAMAM)_n$/CNTs construction was investigated by ζ-potential and high resolution transmission electron microscopy (HRTEM). The results indicated the uniform growth of the layer-by-layer nanostructures onto carboxyl-functionalized CNTs. The electrocatalytic property of the $(GLDH/Pt\text{-}PAMAM)_n$/CNTs modified electrode to glutamate in presence of NAD^+ (β-nicotinamide adenine dinucleotide, 0.1 mM) was investigated at a low overpotential 0.2 V by electrochemical measurements. The results showed it had series of attractive characteristics, such as a large determination range (0.2–250 μM), a short response time (within 3 s), a high sensitivity (433 $\mu A/mM^{-1}\,cm^2$) and good stability (85% remains after 4 weeks).

Keywords: Multiwall carbon nanotubes; Poly(amidoamine)-encapsulated platinum nanoparticles; Glutamate dehydrogenase; Self-assembly; Biosensor

1. Introduction

Glutamate is an important neurotransmitter in the mammalian central nervous system and neuronal pathways in the brain which is related to several neurological disorders, such as schizophrenia, Parkinson's disease, epilepsy and stroke [1–3]. It also has widespread use in food as a flavour enhancing food additive. Thus, glutamate is an important analyte in medicine and food analysis which involves neurotransmission and protein metabolism.

Many spectrophotometric, fluorimetric and chromatographic methods have developed for the assay glutamate concentration, but they are considered to be time-consuming and laborious [4,5]. Several types of amperometric biosensors for glutamate have been reported. More recently, glutamate oxidase incorporated into modified electrode has been used for rapid estimation. Mesophilic and thermophilic glutamate dehydrogenase has also been used for the analysis of glutamate [6–9].

For achieving a rapid and high sensitive measurement of substrate, development of immobilization strategy and implementation of novel materials for the construction of desirable biosensor have been the subjects of intensive research.

Enzymes are immobilized on transducer or support matrices by physical and chemical methods [9,10], such as covalent binding, direct adsorption, cross-linking or entrapment, which often denature the enzymes and alter their conformations considerably. Recently, layer-by-layer (LbL) technique for constructing multilayer films has attracted much attention since it can provide a favorable microenvironment to keep the bioactivity of enzyme and prevent enzyme molecule leakage. This technique involves an alternate adsorption of enzyme and another electrolyte onto an electrode surface through electrostatic force and covalent bonding [11–15]. In addition, the efficient immobilization materials can prolong the use of the sensor and extend storage and working stability. They have recently been extended to a variety

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of materials, including redox mediators, conducting polymers, nanomaterials, dendrimers and so on [16,17].

A wide variety of nanomaterials with unique chemical and physical properties have found broad application in the construction of novel and improved biosensor. Metal nanoparticles have been intensively studied for the electrode modification owing to their extraordinary catalytic activity, especially Pt, Au nanoparticles [18–20]. Carbon nanotubes [21], another class of promising nanomaterials, have been drawn to considerable attention due to their unique electronic, chemical and mechanical properties [22]. Recent studies have demonstrated that carbon nanotubes can enhance the electrochemical reactivity of biomolecules and promote the electron transfer reactions of proteins [23–28].

Highly branched dendritic macromolecules poly(amidoamine) (PAMAM) could also be used to modify electrode surface due to their good biocompatibility and adequate functional groups for chemical fixation. It was reported that the material was capable of increasing the concentration of hydrophobic molecules at the electrode–solution interface, improving the sensitivity as well as the selectivity of certain specific electrochemical reactions [29,30,34].

Considering their truly enormous potential and unique properties, in this context, poly(amidoamine) dendrimer-encapsulated platinum nanoparticles (Pt-PAMAM) self-assembled onto multiwall carbon nanotubes (CNTs) were applied to the immobilization of glutamate dehydrogenase in biosensors for the first time (Fig. 1). The modification of CNTs and Pt-PAMAM not only increased the effective area of the electrode, but also may act as an efficient promoter used to enhance the electrochemical reaction, thereby increasing the rate of heterogeneous electron transfer.

2. Experimental

2.1. Chemicals and materials

Glutamate dehydrogenase (GLDH, EC 1.4.1.3, MW 300,000, from bovine liver, suspension in 2.0 M $(NH_4)_2SO_4$ solution, 48 units/mg prot.), L-glutamate (monosodium salt, 99%) and β-nicotinamide adenine dinucleotide (NAD^+) were purchased from Sigma–Aldrich. Multiwall CNTs were from UNILAB, State Key Laboratory of Chemical Engineering, East China University of Science and Technology. Pt-PAMAM was synthesized according to the previous literature [29–32]. Phosphate buffer was used for immobilization and electrochemical measurements 0.1 M potassium phosphate (PBS), pH 7.4. The other chemicals were of analytical grade. Doubly-distilled and deionized water was used through this work.

2.2. Instruments

Electrochemical measurements were performed by using a CHI 660C (CH Instruments, Chenhua Inc., Shanghai, China) connected to a personal computer. A three-electrode configuration was employed, consisting of a multilayer modified glassy carbon (GC) electrode (3 mm diameter) serving as a working electrode, whereas Ag/AgCl (3 M KCl) and platinum wire served as the reference and counter electrodes, respectively. Batch electrochemical experiments were carried out in a 10 mL voltammetric cell at room temperature (25 °C).

The HRTEM images were recorded on a JEOL-2100 transmission electron microscope. The electrophoretic mobilities of the adsorbed CNT suspension were measured using a Malvern Zetasizer 3000HS.

2.3. Synthesis of CNTs/Pt-PAMAM heterostructures

The CNTs were first shortened and functionalized by sonicating in a mixture of concentrated HNO_3 and H_2SO_4 (v/v, 1/3) for 6 h followed by extensive washing in deionized water several times and finally dried under vacuum, dispersed in double-distilled water in a concentration 5 mg/mL. After the acid treatment, the CNTs were shorter and left with the carboxylic groups that impart a hydrophilic nature and facilitate further functionalization by Pt-PAMAM. The Pt-PAMAM/CNTs heterostructures were attached covalently by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), which yield stable conjugates, since they form amide linkages between them (Fig. 1a). The EDC reaction was carried out for 8 h at 50 °C under continuous mixing. The heterostructures were characterized by HRTEM.

2.4. Preparation of $(GLDH/Pt\text{-}PAMAM)_n$/CNTs nanocomposites

The isoelectric point (Ip) of GLDH lies around pH 4.83, and therefore at pH > Ip, it is net negatively charged, while at pH < Ip, it is positively charged. So GLDH can be used as polyanionic material for preparing LbL films around pH 7.4. Here, GLDH was immobilized on the positive charged Pt-PAMAM/CNTs surface by alternatively assembling a GLDH layer and a Pt-PAMAM layer (Fig. 1b). First, GLDH/Pt-PAMAM/CNTs film was attained by mixing the Pt-PAMAM/CNTs heterostructures with the negatively charged GLDH (0.2 unit/mL) in a PBS (pH 7.4) for 20 min, centrifuged at 7000 rpm for 10 min, and then washed with distilled water twice to remove the supernatant. Using the same procedure, positively Pt-PAMAM and negatively GLDH were alternately absorbed onto the CNTs until suitable layer was obtained. The resulting structures were characterized by ζ-potential measurement, HRTEM. Finally, the resulting nanocomposite was thoroughly washed with PBS (pH 7.4) and stored in PBS below 4 °C for future use.

2.5. Electrode preparation

Glass carbon (GC, 3 mm diameter, Model CHI 104, CH Instruments) electrodes were polished with 1.0, 0.3 and 0.05 μm alumina powder cleaned in a piranha solution (a 1:3 mixture of 30% H_2O_2 and concentrated H_2SO_4) and finally sonicated thoroughly in double-distilled water. After that, the electrode was cycled 10 times between −0.800 and 0.800 V in a phosphate buffer solution (pH 7.4) at 50 mV/s. The biosensors were prepared by casting the GCE with 20 μL of the resulted nanocomposite solution dispersion by sonicating for 5 min. The

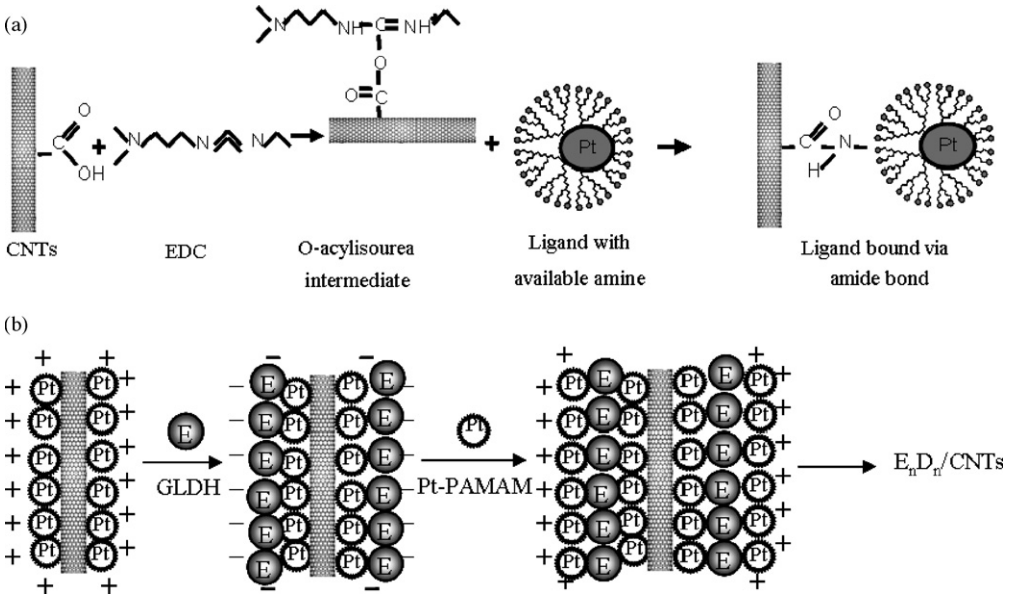

Fig. 1. Schematic showing the procedure of immobilizing Pt-PAMAM onto CNTs (a) and the layer-by-layer self-assembly of GLDH and Pt-PAMAM onto CNTs (b). Pt-PAMAM/CNTs heterostructures were attached covalently via EDC, Pt-PAMAM and GLDH were alternately deposited until suitable layers were obtained.

coated electrode was dried at room temperature and then kept under dry conditions at 4 °C before use.

3. Results and discussion

3.1. Electron micrographs of the Pt-PAMAM/CNTs heterostructures

A key element for the exploration of the nanoparticles–CNTs composites as sensing or catalyst materials is the ability in effective and controllable assembly of nanoparticles on the surface of CNTs, which is the focus of the present work. As demonstrated previously, the oxidation procedure used for treating carbon nanotubes, mainly to remove metal catalyst and amorphous carbon, also results in partial oxidation of the carbon atoms to produce oxygen-containing groups (such as carboxylic acid groups) especially in the open ends of the nanotubes. These groups electrostatically and covalently interact with the Pt-PAMAM. TEM (Fig. 2a) showed well-organized nanosized platinum nanoparticles, with a particle diameter of approximately 3 nm and a narrow distribution on the surface of CNTs. This demonstrates the assembly route linked via EDC is effective and controllable for the assembly of the monolayer-caped platinum nanoparticles on CNTs. Similar results have been reported in the literature [33,34].

3.2. Multilayers assembling and characterization

Microelectrophoresis measurements were conducted to qualitatively follow adsorption of the layers on the CNTs templates. The ζ-potentials of the hybrid layer were calculated from the mobilities that were measured after deposition of each layer. Fig. 3 shows the ζ-potentials change regularly and alternately as the outmost layer is adsorbed via a layer-by-layer approach. Weak positively charged Pt-PAMAM/CNTs showed a ζ-potential of 3.09 mV. Subsequent adsorption of GLDH enzyme resulted in ζ-potential of about −14.55 mV. Further depositions cause the ζ-potentials to alternate in sign from positive to negative, depending on whether the outmost layer is GLDH or Pt-PAMAM. Alternate adsorption of Pt-PAMAM and GLDH finally got a reversal cyclic change. This provides qualitative evidence for the successful adsorption of Pt-PAMAM (positive values) and GLDH (negative values).

HRTEM confirmed the success of the attachment of $(GLDH/Pt\text{-}PAMAM)_n$ multilayers onto the CNTs. As shown in Fig. 2(b), it can be seen that almost all of the tubes were evenly coated with $(GLDH/Pt\text{-}PAMAM)_n$ layers, and the thickness of the $(GLDH/Pt\text{-}PAMAM)_n$ layers is about 10 nm. HRTEM images provide strong evidence for the formation of LbL nanocomposite.

3.3. Electrochemical study of the $(GLDH/Pt\text{-}PAMAM)_3$/CNTs/GCE biosensor

To demonstrate the function of CNTs and Pt-PAMAM in the process of electrocatalytic process, different electrode has been prepared. Fig. 4 depicts the oxidation of glutamate at the GLDH/Pt-PAMAM/CNTs/GC (curve 4), Pt-PAMAM/CNTs/GC (curve 3), CNTs modified (curve 2) and bare GC (curve 1) electrodes in the PBS (pH 7.4) containing 0.1 M NAD^+. Glutamate dehydrogenase occurs irreversibly with a large overpotential at the bare electrode (curve 1), indicating the slow electron transfer rate at bare GC electrode. The CNTs or/and Pt nanoparticles modified electrodes show low overpotential and high current density (curves 2–4), suggesting that CNTs and Pt nanoparticles have promotion effect on the electrocatalytic activity of GLDH to the oxidation of glutamate [17]. The superior promotional effect of CNTs and Pt-PAMAM to the electrocatalytic activity of GLDH, when compared with other forms of carbon, such as GC and ACP, may be due to their small dimen-

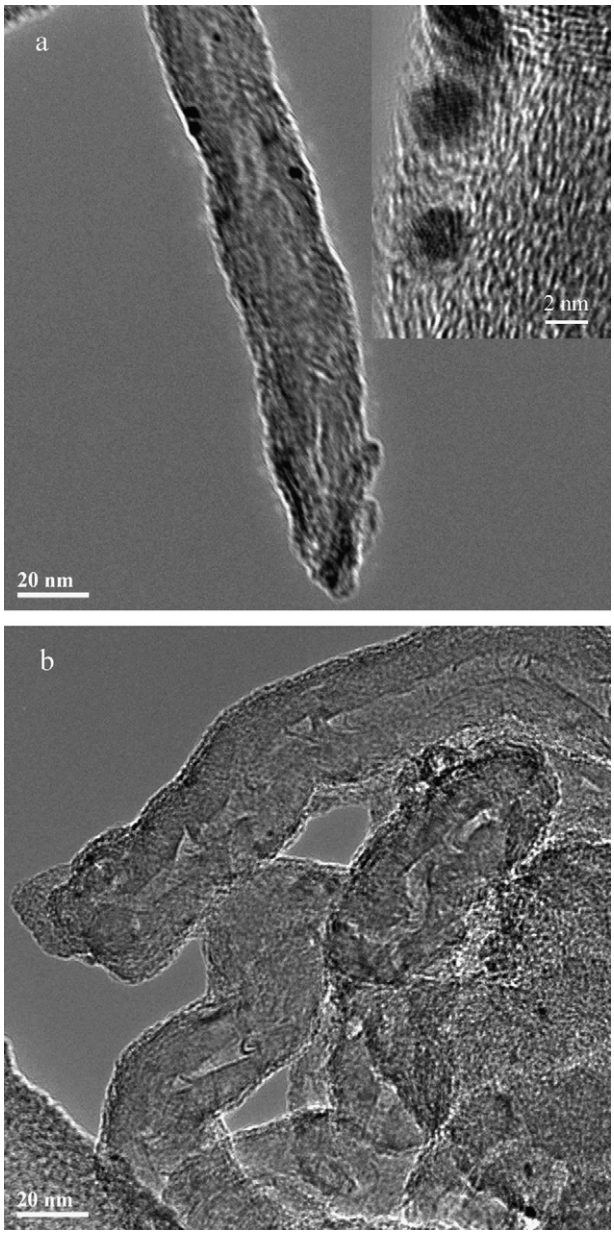

Fig. 2. HRTEM image of CNTs incorporated with Pt-PAMAM (a), and $(GLDH/Pt\text{-}DENs)_3$/CNTs hybrid nanocomposite (b). *Inset*: A magnified image of the Pt-PAMAM/CNTs heterostructure.

sion, their unique chemical structure, their distinctive electronic characteristics, and their high electrical conductivity, and importantly, also to some oxygen-contained groups present on their surface [35], even though the exact reason is not fully understood at the present time [36].

The layer number of the self-assembled nanocomposite had an important effect on sensitivity of the sensor. In this experiment, we found that with the increasing of layer numbers of enzyme, the oxidation peak potential stayed at 0.2 V and the current response increased gradually, but the response time would also be increased. So the $(Pt\text{-}PAMAM/GLDH)_3$/CNTs/GC electrodes were selected for the electrochemical measurements.

Fig. 5 shows the electrocatalytic behavior of the $(Pt\text{-}PAMAM/GLDH)_3$/CNTs nanocomposite modified GC elec-

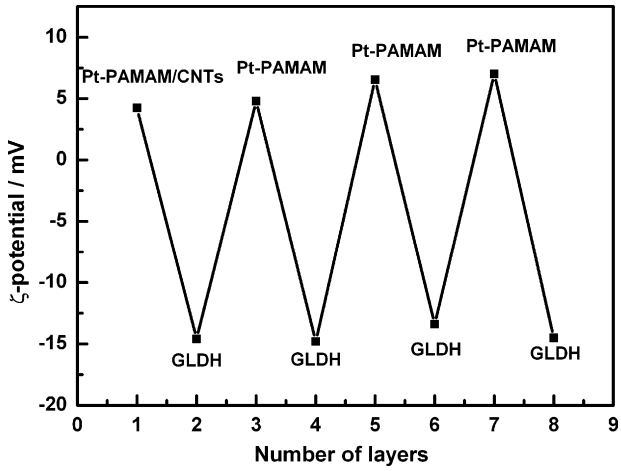

Fig. 3. ζ-Potentials changed as the multilayers were alternatively adsorbed via a layer-by-layer approach.

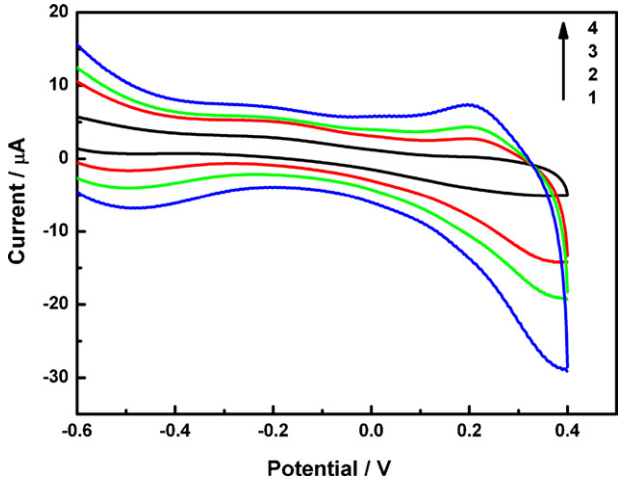

Fig. 4. The cycle voltammograms of $(GLDH/Pt\text{-}PAMAM)_n$/CNTs nanostructure assembled onto GC electrode in pH 7.4 PBS 0.1 mM NAD^+ and 0.1 mM glutamate. The numbers (1, 2, 3, 4) refer to the bare GCE, CNTs/GCE, Pt-PAMAM/CNTs/GCE, GLDH/Pt-PAMAM/CNTs/GCE, respectively.

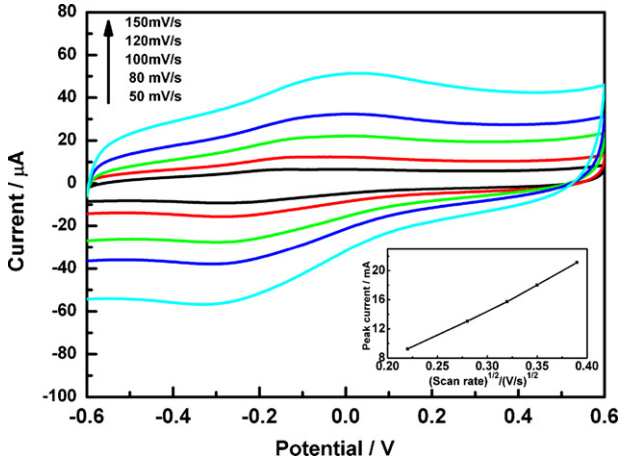

Fig. 5. The cycle voltammograms for oxidation of glutamate at $(GLDH/Pt\text{-}PAMAM)_3$/CNTs nanocomposite modified GC electrode in pH 7.4 PBS containing 0.1 mM NADH at different scan rates: 50, 80, 100, 120 and 150 mV/s. *Inset*: The relationship between the peak currents and square root of potential scan rate.

trode that changes with the scan rate. With the scan rate increasing from 50 to 150 mV/s, the peak current increased, while the peak potential shifted positively slightly. To confirm the anodic current response is coming from the enzymatic reaction between GLDH and the substrate glutamate, a comparison experiment was performed with the Pt-PAMAM/CNTs/GC electrode and GLDH/Pt-PAMAM/CNTs/GC electrode to glutamate at the working potential 0.2 V in the presence of NAD^+. We found that no response was obtained with the Pt-PAMAM/CNTs GC electrode on adding the substrate glutamate, while a very good response was obtained with GLDH/Pt-PAMAM/CNTs GC electrode. In Fig. 5, one notices that the peak current was linear with the square root of potential scan rate in a rage from 50 to 150 mV/s (shown in the inset), demonstrating that the electrochemical oxidation of glutamate is a diffusion-controlled process at the assembled CNTs.

The effect of pH value on the performance of the $(GLDH/Pt\text{-}PAMAM)_3$/CNTs/GCE biosensor has been investigated using 0.1 M PBS. It is found that the response of the resulting electrode is pH dependent. The maximum current response of the enzyme electrode was obtained at pH 7.4. To avoid the interference from other electroactive species, obtain the maximum current response and keep the good activity, a 0.2 V working potential and a pH 7.4 were used in the flowing.

The amperometric responses of the $(GLDH/Pt\text{-}PAMAM)_3$/CNTs modified biosensor were investigated by successively adding glutamate at an applied potential of 0.2 V versus Ag/AgCl, in a PBS (pH 7.4) supported electrolyte containing 0.1 mM NAD^+. It indicates that the $(GLDH/Pt\text{-}PAMAM)_3$/CNTs modified biosensor showed an increase in measured currents with the addition of glutamate. The resulting calibration curve plot displays linearly from 0.2 up to 250 μM with a correlation coefficient of 0.9992. The lowest detectable concentration of this biosensor was 10 nM, when the signal-to-noise characteristics of these data (S/N ratio) were 3. Also the time required to reach the 95% steady-state response of this biosensor was only 3 s.

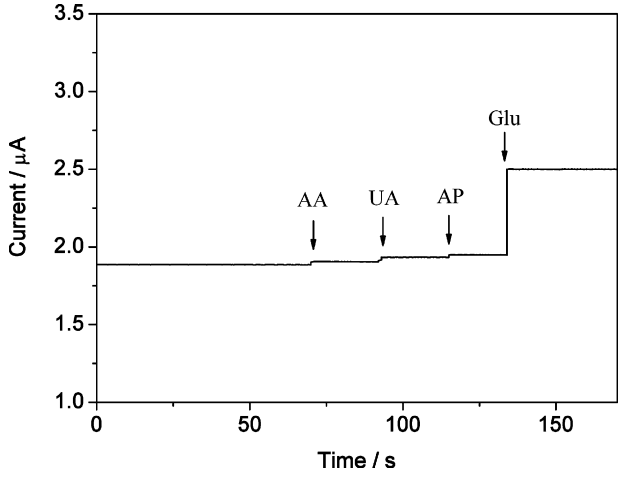

Fig. 6. Amperometric responses of the biosensors upon subsequent additions of 0.1 mM L-ascorbic acid (AA), 0.1 mM uric acid (UA), 0.1 mM acetaminophen (AP) and 0.1 mM glutamate (Glu) in PBS (pH 7.4) at 0.2 V vs. Ag/AgCl.

In real samples, some co-existing electroactive species might affect the biosensor response, for example, ascorbic acid (AA), uric acid (UA), acetaminophen (AP), L-cysteine (L-Cys), etc. The selectivity and anti-interference advantages are demonstrated in Fig. 6, which compares amperometric responses for three relevant electroactive species (UA, AA, AP, 0.1 mM, respectively) and L-glutamate (0.1 mM) at a modified GC electrode at a working potential of 0.2 V. The successive addition of each interfering species brought out fairly discernible current response and a well-defined L-glutamate response was obtained, which is largely attributed to the low working potential of 0.2 V used in the determination of glutamate. Reproducibility of the biosensor system was evaluated from the response for 10 μM glutamate at 10 different biosensors and an acceptable R.S.D. value of 4.8% was observed. We stored the enzyme electrode under 4 °C for 4 weeks, and the sensitivity of electrode was reduced to 85% of our former assay. These results indicate the suitability of the nanocomposite modified glutamate biosensor to practical applications.

4. Conclusion

In this work, we presented a novel amperometric glutamate biosensor based on functionalized carbon nanotubes by alternatively electrostatic or/and covalently self-assembling Pt-PAMAM and GLDH layers on CNTs surface. The LbL film-modified enzyme sensors have been shown to exhibit a good reproducibility, stability and high sensitivity, which can efficiently preserve the activity of enzyme molecules and prevent enzyme leaking. The fabrication method of biosensor opens a new opportunity for the development of simple and reliable other enzymes. Moreover, the film is expected to have potential applications in the construction of screen-printed biosensor, in virtue of many advantages such as ease of fabrication, enhanced electrocatalysis, etc.

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