High-Sensitive Glutamate Biosensor Based on NADH at Lauth's Violet/Multiwalled Carbon Nanotubes Composite Film on Gold Substrates

M. M. Rahman,*,† Ahmad Umar,*,‡,§ and Kazuaki Sawada†

Venture Business Laboratory, Department of Electrical and Electronic Engineering, Toyohashi University of Technology, Toyohashi, Aichi 441-8580, Japan, Department of Chemistry, Faculty of Science, Najran University, Najran, Saudi Arabia, and School of Semiconductor and Chemical Engineering, BK21 Centre for Future Energy Materials and Devices, Chonbuk National University, Jeonju 561-756, South Korea

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A highly sensitive amperometric L-glutamate biosensor based on the electrocatalytic oxidation of reduced nicotinamide adenine dinucleotide has been developed on Lauth's Violet (known as thionine)/multiwalled carbon nanotubes (Th-MWCNTs) composite film, which is used as a mediator and an enzyme immobilization matrix. The glutamate biosensor, which is fabricated by immobilizing glutamate dehydrogenase (GLDH) on the surface of Th-MWCNTs, displayed a precipitous response (ca. 3 s), a low detection limit (15.9 nM), a wide linear dynamic range (0.1 to 500 μM), and high sensitivity of 281.6 μAmM^{-1} cm^{-2}, higher biological affinity, as well as good stability and repeatability. Interferences from other biological compounds were also studied for the fabricated sensor. The Th-MWCNTs system exemplifies a simple and efficient approach to the assimilation of GLDH and electrodes, which can provide analytical access to a large group of enzymes for wide range of bioelectrochemical applications in health care fields.

1. Introduction

Glutamate is an amino acid recognized as an important energy and nitrogen source in eukaryotic and mammalian cells. It functions as an excitatory neurotransmitter in the central nervous system and is believed to be responsible for certain fundamental processes such as learning, memory, neurodevelopment, biomaterial sensitivity, and synaptic plasticity.[1,2] An increase in glutamate levels in the cerebrospinal fluids has been observed in neurological disorders, such as stroke, Alzheimer's, and Parkinson's diseases.[3,4] L-Glutamate is one of the 20 standard amino acids used by all organisms, which plays an important role in food processing and clinical applications. It is a well-known flavor enhancer present in many seasoning, soy sauce, chicken broth cubes, and soup base of instant noodles. The excessive intake of this flavor enhancer, commonly known as monosodium glutamate, can cause allergic effects such as headache and stomach pain.[5−7]The main excitatory neurotransmitters (L-Glutamate) in the central nervous system and neuronal pathways in the brain, plays also a major role in various neural functions, and relates to various neurological disorders, as well as to memory and learning processes.[8,9] Therefore, its determination is of interest to both the biological science and food industries. Much effort has been focused on developing suitable methods for precisely monitoring the glutamate level in its biological environment. Those approaches involve chromatographic techniques,[10] capillary biosensors,[11] chemiluminescence,[12] and capillary electrophoresis,[13] etc. All those methods are considered to be time-consuming and laborious and require sophisticated and expensive instruments. More recently, several amperometric biosensors for glutamate monitoring have been reported. Those biosensors are usually based on the glutamate oxidase,[14,15] or GLDH.[16,17] Basu et al. developed a glutamate biosensor based on immobilized GLOx and GLDH on the polycarbonate membrane by the cross-linking procedures.[18]

Several techniques such as mass spectrometry, capillary electrophoresis, microdialysis, and biosensors have been developed to detect glutamate in a range of complex matrices.[19,20] Of various techniques developed, biosensors are one of the most rapidly growing techniques developed for the detection of glutamate. Various types of amperometric biosensors have been also fabricated for the detection of glutamate in various applications such as food processing,[21] cell cultures,[22] and extracellular brain fluid.[23] Although these biosensors are sensitive and selective, elaborate and complex steps for the preparation of enzymatic electrodes are usually needed. Unlike optical biosensors, amperometric biosensors are simple to fabricate, easy to use, and suitable for on-site detection. Quantification of glutamate is achieved via electrochemical oxidation of the liberated H_2O_2. However, the oxidation of H_2O_2 usually requires a relatively high positive potential. Many other electro-active species usually coexisting in the biological fluids can also be oxidized at the high potential and their electrochemical signals thus severely affect the selectivity of the biosensors. Therefore, interference elimination represents a main task to be solved for this type of biosensors. The interference can be partially overcome by coating the biosensor with a membrane impermeable to interferents[24] or oxidizing the interferents before they reach the biosensor.[25] Coating with polymeric films leads to lower signals and longer response time due to the additional diffusion barrier, whereas using electrochemical preoxidation displays a risk of oxidizing the substrate of interest. To eliminate this risk, the oxidation of the interferents can be accomplished by enzymatic preoxidation, which is achieved either by incorporating specific enzymes in the sensor configuration or immobilizing them in reactors, thus complicating the biosensor fabrication procedures. Thionin (Th, known as thionin acetate

* To whom correspondence should be addressed. E-mail: (A.U.) ahmadumar@chonbuk.ac.kr; (M.M.R.) mmrahman@vbl.tut.ac.jp. Tel: +82-63-270-2439. Fax: +82-63-270-2306.
† Toyohashi University of Technology.
‡ Najran University.
§ Chonbuk National University.

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or Lauth's violet) is a strongly staining metachromatic dye that is widely used for biological staining, which is used to fabricate composite film with MWCNTs for catalyzed the sensitive glutamate detection in biological system. The GLDH-based biosensors involve the electrochemistry of NAD^+ (NAD^+: the oxidation form of β-nicotinamide adenine dinucleotide). The biosensing of glutamate using GLDH requires highly sensitive NADH transducer because the signal of the biosensor is based on detection of the anodic current of enzymatically generated NADH. The selective detection of glutamate in the presence of other interfering compounds is successful only if NADH can be detected at a lower potential. However, the direct oxidation of NADH at unmodified electrodes is not straightforward and requires high overpotential (usually +0.7 to +1.0 V) owing to the sluggish electron-transfer kinetics.[26] Bare electrodes very often suffer from fouling by the adsorption of oxidation products. Various methodologies have been developed to enhance the electron-transfer kinetics of the oxidation of NADH.[27,28] A conventional way is to fabricate material-based electrodes that can promote the electron-transfer kinetics. In this way, much effort has been devoted to identifying/developing new materials that can effectively overcome the kinetic barriers for the oxidation of NADH and regenerate the enzymatically active NAD^+.[29]

Carbon nanotubes (CNTs) are promising materials for biosensing applications due to several intriguing properties.[30] Various design methodologies for CNTs-based electrochemical biosensors and their performance characteristics, advantages, and employment for the detection of a number of analytes have been developed.[31,32] CNTs have received considerable attention for this purpose. Previously, many studies have demonstrated that CNTs can be used to fabricate the structured macroscopic electrodes, biosensors, and nanobioelectronic devices due to their nanometer size, lack of toxicity, good electrocatalytic properties, and efficient accumulation of biomolecules as well as minimization of surface fouling.[33] Although CNTs-based electrodes are known to decrease the overpotential for the oxidation of NADH, the extent of decrease is not sufficient enough for the selective detection of NADH.[34] The oxidation of NADH at potential of more than 0.34 V often suffers from much interference. Therefore, it is essential to develop an electrode, which can catalyze the oxidation of NADH at less positive potential. It is demonstrated that Th and MWCNTs could synergistically catalyze the electrochemical oxidation of NADH with an anodic potential of less than +0.24 V. The Th-MWCNTs-coated gold electrode allowed highly sensitive amperometric detection of NADH. These results suggest that Th-MWCNTs can be used as a biocompatible platform for development of dehydrogenase-based amperometric biosensors. Here, a glutamate biosensor (GLDH-Th-MWCNTs/GE) based on Th-MWCNTs and GLDH is developed. The characteristics of the biosensor to the detection of glutamate are presented.

2. Experimental Section

FE-SEM images were obtained with a Cambridge Stereoscan 240 at KBSI. Cyclic voltammetric and chronoamperometric measurements were carried out in a conventional three-electrode cell with the electrochemical analyzer (BAS100B/W, BASi, USA). The working electrode was a gold disk (GE, diameter 3.0 mm) electrode. An Ag/AgCl and a Pt wire were used as reference and counter electrodes, respectively. Amperometric detection was performed under at constant applied potential of +0.145 V. The solution was continuously stirred using a magnetic bar at a speed of 100 rpm. A higher speed leads to increase of the noise while a lower speed results to a longer responsive time. Therefore, it is better to choose an appropriate stirring speed of 100 rpm to obtain a good single-to-noise ratio and a short responsive time. The response current was taken as the change between the steady state and background currents. Buffer was purged with high-purity nitrogen for at least 30 min prior to each experiment and the nitrogen environment was then kept over solution to prevent it from oxygen.

GLDH (from bovine liver, EC 1.4.1.3, lyophilized powder, approximately 25 units mg-1 protein), NAD^+ (trihydrate), bovine serum albumin (BSA), glutaraldehyde (25% in water), and L-glutamate (monosodium salt, 99%) were purchased from Sigma. Ascorbic acid (AA), glucose (Gl), uric acid (UA), 4-acetamidophenol (AP), and thionine (Th) were obtained from Sigma-Aldrich Company. These chemicals were used as received. MWCNTs (10∼25 nm in diameter, purchased from Carbon Nanotechnologies Inc., U.S.A.) were purified following the reported method.[35] All other chemicals were of analytical grade. The buffer solutions of 0.1 M phosphate buffer solution (PBS, 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4) were used as supporting electrolyte. All aqueous solutions were prepared with doubly distilled water, which was obtained from a Milli-Q water purifying system (18.2 MΩcm). Solutions of GLDH and NAD^+ were freshly prepared using PBS (pH 7.4) immediately before each experiment.

Th-MWCNTs composite and the Th-MWCNTs/GE electrode were fabricated as reported previously.[36] Briefly, Th-MWCNTs were prepared by dispersing 2.0 mg of purified MWCNTs into 1.0 mL of Thionine aqueous solution (0.5 mM), and stirring for ca. 20 min. Afterward, the composites were collected by centrifugation, thoroughly washed at least thrice with water, and finally dried under vacuum at ambient temperature overnight. The Th-MWCNTs/GE electrode was fabricated by casting 2.0 μL of Th-MWCNTs suspension (2.0 mg/ml in water) onto the surface of pretreated gold electrode (GE, 3.0 mm in diameter). Before use, the solvent was allowed to be evaporated. The MWCNTs/GE electrode was prepared using a similar procedure.

Several parameters were optimized to obtain the best voltammetric response and the high performance of biosensor. Typically, GLDH solution (5.0 mg/ml in phosphate buffer solution, pH 7.4) was first mixed with 1% of BSA with a volume ratio of 1 to 1. Then, 5.0 μL of the mixture was coated the surface of the Th- MWCNTs/GE electrode. To prevent the leakage of the enzyme from the electrode surface, the electrode was cross-linked with 4.0 μL of 20.0 mM of glutaraldehyde, which could form covalent bonds with BSA and GLDH, respectively, and hold the GLDH on the electrode surface stably. The excess glutaraldehyde can lead to denaturing of the enzyme. The aim of addition of BSA is to bind with the excess glutaraldehyde and prevent the GLDH from denaturation. The resulting electrode was denoted as GLDH-Th-MWCNTs/GE electrode, and stored at 4.0 °C when it was not in use. The GLDH-MWCNTs/GE and GLDH/GE electrodes were fabricated by the similar procedures. The schematic representation of GLDH-Th-MWCNTS/GE fabrication is shown in the Scheme 1.

3. Results and Discussion

SEM images can provide information of morphologies of Th-MWCNTs and the GLDH-Th-MWCNTs on the surface of the GE electrode. The image of Th-MWCNTs indicates that the composite distributes almost uniformly on the entire surface of the GE electrode exhibiting a special three-dimensional structure with small bundles entangling each other (Figure 1A). The

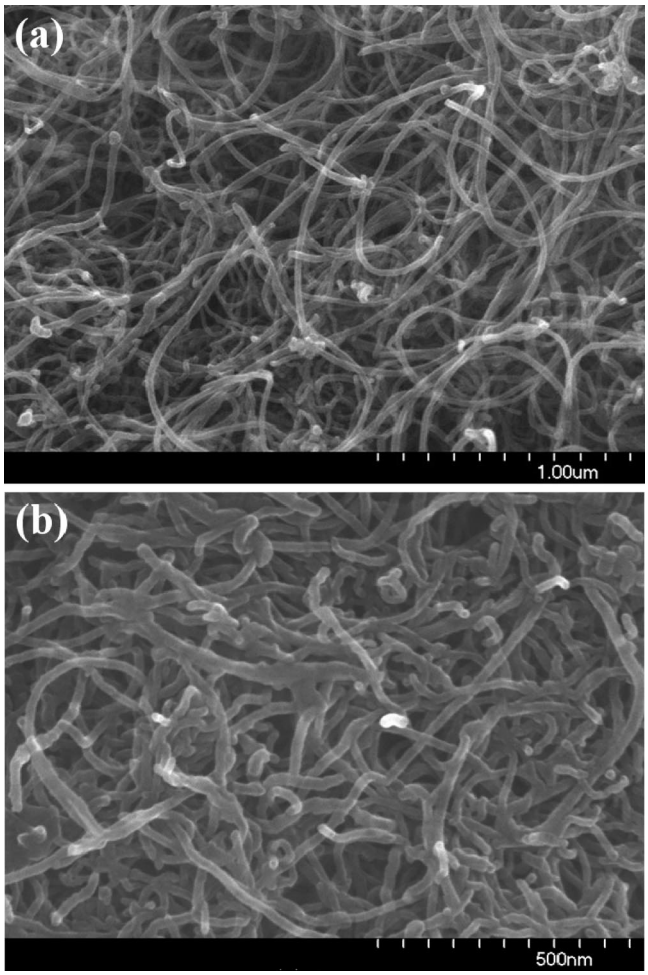
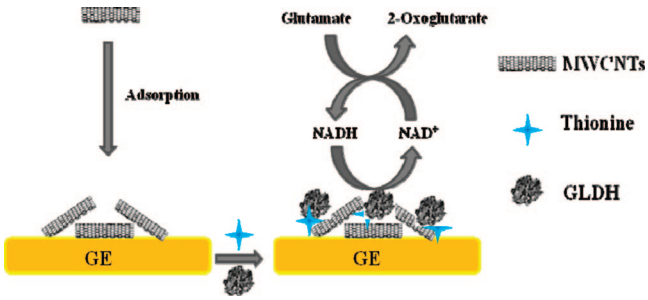


Figure 1. Typical SEM images of Th-MWCNTs (a) and GLDH/Th-MWCNTs (b) on gold electrode.

SCHEME 1: Schematic Representation of the Enzymes, Thionine, and MWCNT Immobilization Steps for the Fabrication of GLDH-Th-MWCNTs/GE Composite Electrodes



morphology is different from that reported by Li et al.[37] They showed that thionine-CNTs composite could not uniformly distribute on the electrode surface representing some agglomerations. The result of Figure 1A indicates that the Th-MWCNTs composite prepared in this work has a good dispersive ability in aqueous solution and can form a well-distributed film on the surface of the electrode. The uniform structure can significantly increase the effective surface of the electrode for loading of biomolecules and accelerating electron-transfer. After GLDH was deposited on the surface of Th-MWCNTs, some small and uniformly distributed aggregated structures appear (Figure 1B). These nanostructures are the aggregates of the trapped GLDH molecules at the defect areas or terminals, indicating that GLDH

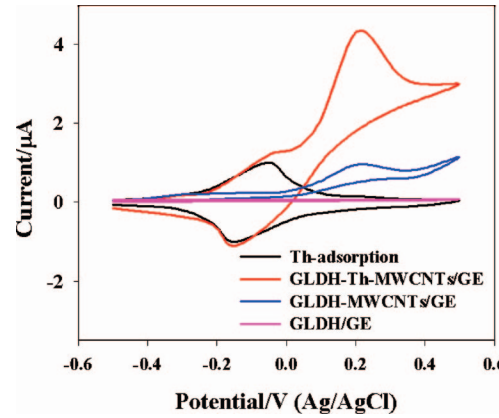


Figure 2. Voltammetric responses of the GLDH-Th-MWCNTs/GE electrode in phosphate buffer solution (0.1 M, pH 7.4) containing 2.0 mM NAD^+ in the absence (black line) and presence (red line) of 0.1 mM glutamate. Blue and pink lines are cyclic voltammograms of the GLDH-MWCNTs/GE and GLDH/GE electrodes, respectively, in phosphate buffer solution (0.1 M, pH 7.4) containing 2.0 mM of NAD^+ and 0.1 mM of glutamate. The scan rate is 10 mV/s.

has been effectively immobilized on the surface of Th-MWCNTs. Moreover, the morphology of GLDH-Th-MWCNTs on the electrode surface processes the similar three-dimensional structure to that of Th-MWCNTs. Such a structure is expected to be very attractive for the detection of substrates because each of the Th-MWCNTs is fully and easily accessible to substrates, and thus can be used as an electrochemical sensing unit, yielding a high ratio of signal-to-noise for electrochemical determination.

As known, the biosensing of glutamate using GLDH requires highly sensitive NADH transducer because the transduced signal of the biosensor is based on the detection of enzymatically generated NADH, according to the following reactions

$$\text{Glutamate} + NAD^+ + H_2O \xrightarrow{\text{GLDH}} \text{2-oxoglutarate} + NADH + NH_3 + H_2O_2$$

Electrocatalytic response is not observed at the GLDH-Th-MWCNTs/GE electrode in phosphate buffer solution (0.1 M, pH 7.4) containing 2.0 mM NAD^+ at a scan rate of 10 mV/s in the potential range of interest (−0.5 to +0.5 V). The cyclic voltammogram of the GLDH-Th-MWCNTs/GE electrode is characterized by a pair of well-defined redox peaks with the cathodic (E_{pc}) and anodic (E_{pa}) peak potential of −148 and −56 mV (at 10 mV/s), respectively (Figure 2, black line). This pair of redox peaks is due to the electrochemical reaction of adsorbed thionine.

Upon addition of 0.1 μM glutamate, the shape of cyclic voltammogram of the GLDH-Th-MWCNTs/GE electrode changes significantly and is characterized by a large anodic peak at ca. 200 mV (Figure 2, red line). In the presence of NAD^+, the GLDH catalyzes the oxidation of glutamate to 2-oxoglutarate, and produces NADH simultaneously. Thus, the anodic peak of Figure 2 (red line) is resulted from the electrocatalytic oxidation of NADH. The anodic peak potential for the NADH oxidation is comparable to that obtained at the chitosan-azure c-CNTs-based electrode (160 mV),[38] and peptide nanotube-based electrode,[39] respectively. The onset potential is slightly positive than that obtained at meldola's blue-CNTs-based electrode (ca.−60 mV). The increase of glutamate concentration leads to the enhancement of the anodic current. These characteristics are the typical features of electrocatalytic reactions. Control experiments show that electrocatalytic oxidation of glutamate does

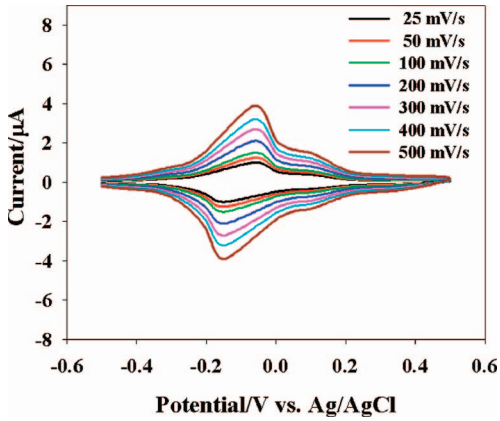


Figure 3. Cyclic voltammograms of the GLDH-Th-MWCNTs/GE electrode in phosphate buffer solution (0.1 M, pH 7.4) at scan rate of 25, 50, 100, 200, 300, 400, and 500 mV/s.

not occur at the Th-MWCNTs/GE (without GLDH) electrode. The electrocatalytic activities of the GLDH-MWCNTs/GE and GLDH/GE electrodes toward oxidation of glutamate were also recorded and compared with that of the GLDH-Th-MWCNTs/GE electrode. The activity of GLDH/GE electrode is too low to be analyzed quantitatively (Figure 2, pink line). The cyclic voltammogram of the GLDH-MWCNTs/GE electrode in phosphate buffer solution (0.1 M, pH 7.4) containing 2.5 mM NAD^+ and 0.1 mM glutamate shows an anodic peak at ca. 205 mV (Figure 2, blue line). The peak potential is more positive than those obtained at the GLDH-Th-MWCNTs/GE electrode (Figure 2, red line). Moreover, the electrocatalytic current obtained at the GLDH-MWCTs/GE electrode is about 1/4 of that at GLDH-Th-MWCNTs/GE electrode (All of the currents were measured by subtracting the background current from the peak current). These results suggest that the electrocatalytic activity of the GLDH-Th-MWCNTs/GE electrode is much higher than that of the GLDH-MWCNTs/GE electrode. The high activity of the GLDH-Th-MWCNTs/GE electrode may come from the synergistic characteristics of Th and MWCNTs toward the oxidation of NADH.

The cathodic and anodic peak potentials are almost equal at all scan rates (υ) and increase linearly from 50 to 500 mV/s, indicating that the overall electrochemical process is controlled by a surface reaction. The values of E_{pc} and E_{pa} remain almost invariable when υ is lower than 100 mV/s. The E_0' (-36 ± 2 mV) is almost independent of υ. The small redox pair at ca. 110 mV (Figure 3) is ascribed to the reaction of Th polymer on the surface of MWCNTs,[40] indicating that there are some Th polymers on the surface of MWCNTs. These features are in good agreement with those obtained at the Th-MWCNTs/GE electrode, indicating that the electrochemical characteristics of Th on the surface of MWCNTs remain unchangeable even after GLDH was immobilized. The electrochemical response of the electrode is fairly stable since the shape and size of the redox peaks remain invariant even after the electrode was continuously scanned for a long time (ca. 20 cycles at 10 mV/s). This characteristic is important when the electrode is used as a biosensor to sense the substrate.

The activity of dehydrogenase usually depends on the solution pH, performance temperature, and the concentration of coenzyme. In this work, these conditions were optimized. The response was independently tested for each pH, temperature, and the concentration of NAD^+, and an average value was calculated. The electrocatalytic current increases with the concentration of NAD^+ up to 3.5 mM. It reaches a relatively

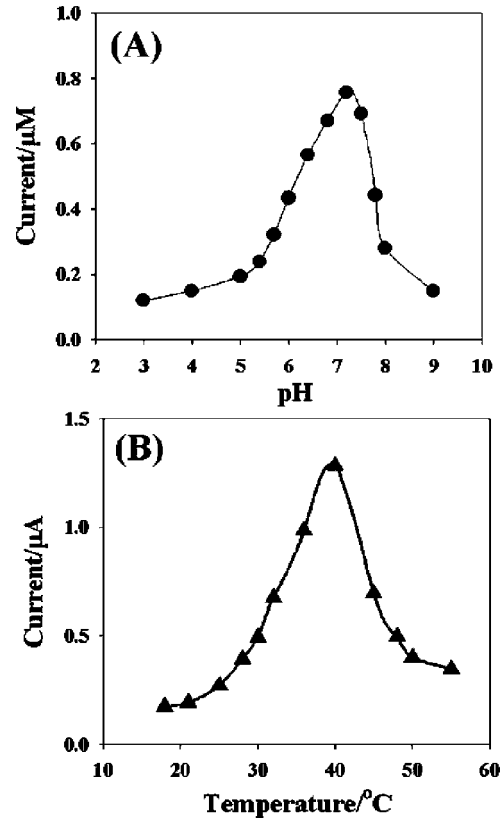


Figure 4. Optimization: the dependence of the electrocatalytic current of the GLDH-Th-MWCNTs/GE electrode on the solution of pH (A) and the performing temperature (B), respectively.

stable value when the concentration of NAD^+ increases to 2.0 mM, and stays practically constant afterward. The highest electrocatalytic response corresponds to a pH 7.4 (Figure 4A) and temperature of 41.0 °C (Figure 4B). The optimal pH is almost simillar to that for GLDH in solution (pH 8.1).[41] The optimal temperature obtained in this work is also similar to that obtained by immobilizing GLDH in a carbon paste working electrode (42.0 °C).[42]

Thus, phosphate buffer solution (0.1 M) with pH 7.4, temperature of 41.0 °C), and an NAD^+ concentration of 2.0 mM are chosen as the optimal condition for the GLDH-Th-MWCNTs/GE electrode sensing glutamate. Bovine liver GLDH is a hexameric enzyme containing chemically identical subunits.[43] Its activity is affected by a variety of anions and cations. For the cation effects, the Ca^{2+} and Mg^{2+} were reported to affect stability of the enzyme. Zn^{2+} can induce a conformation change and thus causes inhibition of the enzyme.[44] Lanthanide ions La^{3+} and Eu^{3+} can enhance the activity of the enzyme. However, those results were obtained with GLDH in solution. This work chooses Zn^{2+} and Eu^{3+} as the models to study the effects of cations on the activity of GLDH immobilized on the surface of Th-MWCNTs. The catalytic currents have a clear decrease if Zn^{2+} is added into solution (the results are not shown here), suggesting that Zn^{2+} has a significant inhibitory effect on the activity of the immobilized GLDH. Moreover, the catalytic current further decreases with the increase of the concentration of Zn^{2+}. These features are similar to those reported previously for GLDH in solution. The effects of Eu^{3+} on the activity of immobilized GLDH are also studied. Eu^{3+} can slightly enhance the activity of the enzyme since the catalytic currents increase slowly with the concentration of Eu^{3+}. This characteristic is in agreement with that obtained with GLDH in solution.

TABLE 1: Comparison of the Performance of Some Amperometric Glutamate Biosensors Constructed Based on Different Modification on Electrodes

electrode modification	sensitivity (μA mM^{-1} cm^{-2})	detection limit (nM)	response time (s)	K_M^{app} (mM)	references
GLDH-Th-MWCNTs/GE	281.6	15.9	3.0	1.67	current work
GLDH-Pt-DENs-CNTs-Ppy	51.48	40.0	3.0		54
GLDH-CHIT-CNT	0.0059	2000			55
GLOx-CHIT	85.0	100	2.0		56
GLDH/GCE	21.0	1000			57

Figure 5 shows the amperometric response of the GLDH-Th-MWCNTs/GE electrode at 0.145 V to the successive addition of 0.1 μM glutamate in solution (pH 7.4). The detection potential, which is chosen from the plateau potential in the hydrodynamic voltammogram (not shown here), is similar to those at GLDH-MWNTs-chitosan-based (0.14 V),[45] and GLDH-Os-gel-HRP-based electrode (0.24 V).[46] However, it is 0.25 V lower than that used for a glutamate biosensor based on self-assembling GLDH on Pt nanoparticles (0.44 V).[47] The low detection potential will significantly diminish the influence of those easily oxidizable species. Immediately after the addition of glutamate, the response increases and reaches a steady state within ca. 3 s. The response time is much lower than that obtained with glutamate oxidase (20−30 s),[48] suggesting that the electrode responds rapidly to the change of the substrate concentration. The electrode linearly responds to glutamate at lower concentration and attains saturation level at higher concentration as expected for Michaelis−Menten type enzyme kinetics (Figure 5 (inset)). The apparent Michaelis−Mentan constant (K_M^{app}) is estimated to be 1.67 mM from the Lineweaver−Burk plot. The response displays a linear range from 0.1 to 500 μM with a correlation coefficient of 0.9989. Therefore, the sensitivity is calculated to be (281.6 ± 5.7) μAmM^{-1} cm^{-2}.

The apparent surface area of the electrode is estimated using the method reported previously.[49] The linear range is useful since the normal glutamate concentration in extracellular space is in the range between 1 and 80 μM,[50] suggesting that the GLDH-Th-SWNTs/GC electrode can be used as a biosensor to sense glutamate in the physiological level. The limit of detection is estimated to be ca. 15.9 nM (at S/N = 3), which is much lower than that obtained at a glutamate microbiosensor based on GLOx (2.5 μM).[51] The linear dynamic range is 0.1−500 μM, which is wider than that of 0.2−250 μM reported previously.[52] The sensitivity is also much higher than that acquired previously (0.71 ± 0.08 μA mM-1).[53] The observed sensitivity is significantly higher and detection limit is comparatively lower than other previously reported glutamate biosensors based on different modified electrodes. For comparing the performances of the fabricated glutamate biosensor based on Th/MWCNTs nanocomposite film, the properties of the previously reported glutamate biosensors based on the utilization of different modification as the working electrode are summarized in the Table 1.[54−57] Different aspects regarding the characteristics of the GLDH-Th-MWCNTs/GE electrode are evaluated. The RSD (relative standard deviation) is ca. 3.9% estimated from the response of five different and freshly prepared electrodes, revealing an acceptable repeatability in the construction of the electrode.

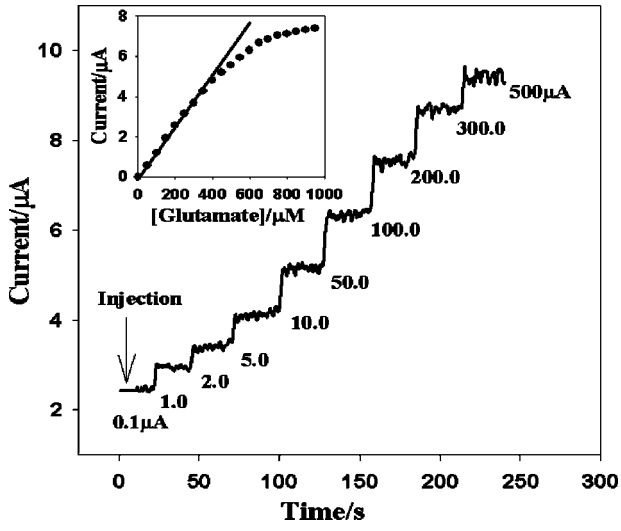


Figure 5. Successive amperometric responses of the GLDH-Th-MWCNTs/GE electrode to the glutamate in phosphate buffer solution (0.1 M, pH 7.4) at an applied potential of 0.145 V. The each addition of glutamate is 0.1 μM. The inset shows the dependence of the response of the GLDH-Th-MWCNTs/GE electrode on glutamate concentration (calibration plot) under the optimal condition.

The assay precision of the biosensor was examined by five determinations at a glutamate concentration of 100.0 μM. A RSD of ca. 4.3% is obtained. The batch-to-batch repeatability is estimated with the slopes of calibration plots obtained from five independent modified electrodes. The RSD of these slopes is ca. 4.7%. Also, the stability of the electrode is considered. When the electrode was not in use, it was stored in buffer at 4.0 °C. Its response to 100.0 μM glutamate was recorded at 3∼5 days interval. The response decreases rapidly at the initial few days (∼5 days), and afterward the response tends to be practically constant and can still retain ca. 96.2% of its original response even after two weeks' storage. Even after the biosensor has been used continually for one week (four measurements a day), its response still remains at ca. 89.7% of the initial value. In general, these results on the stability can be advantageously compared with those reported for the CNTs-based biosensors. The high stability can be attributed to the capability of the Th-MWCNTs composite adsorbing GLDH molecules and retaining their biological activities. This improved stability may also be attributed to the special three-dimensional structure of the GLDH-Th-MWCNTs (Figure 1B), which is beneficial for the substrates getting access to the enzyme molecules. In addition, the fast diffusion of species from nanotubes and the protection of enzyme by BSA and glutaraldehyde may also contribute to the improvement in stability of the electrode. In real samples, some coexisting electroactive species, for example, ascorbic acid, uric acid, glucose, and acetaminophene, etc., might affect the biosensors response. The selectivity and anti-interference advantages of the biosensor are demonstrated (figure not shown), which compares amperometric responses of the relevant electroactive species (at a level of 100.0 μM) and glutamate (also at a level of 100.0 μM). The addition of glutamate results a well-defined response; however, the successive addition of each interfering species does not bring out discernible current response. This feature is largely attributed to the low operating

potential used in the determination. Thus, highly selective response to glutamate is obtained without the use of permselective membrane or enzymatic preoxidation. This is another advantage of the proposed biosensor over those reported previously. These results indicate the suitability of the proposed glutamate biosensor to practical applications in the health care fields.

4. Conclusions

Successful fabrication of highly sensitive amperometric glutamate biosensor (GLDH-Th-MWCNTs/GE) based on the immobilization of GLDH on the surface of Th-MWCNTs composite has been demonstrated. The biosensor exhibited fast response time, large linear dynamic range, low detection limit with satisfactory stability, repeatability, and selectivity. It would have potential applications in glutamate determination in health care biological fields. The fabrication method of the biosensor has many advantages such as ease of fabrication, enhanced electrocatalysis, efficiently preserving the activity of biomolecules.

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