Int. J. Electrochem. Sci., 7 **(2012)** 12752 - 12763

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Electrochemical Determination of Ascorbic Acid Using Poly(Xanthurenic Acid) and Multi-Walled Carbon Nanotubes

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Received: 6 October 2012 / *Accepted:* 22 October 2012 / *Published:* 1 December 2012

Poly(xanthurenic acid) (polyXa) and multi-walled carbon nanotubes (MWCNT) hybrid composite have been successfully prepared to form polyXa-MWCNT for the determination of ascorbic acid (AA). One well defined redox couple has been recognized as the polyXa redox processes. This composite is stable in various scan rates and different pH conditions. It is surface-confined with the surface coverage of 2.3×10^{-9} mol cm^{-2} at polyXa-MWCNT/GCE. This electrode shows lower over-potential and higher current response to AA when compared to the bare electrode. Linearity is also found in other electrochemical techniques using linear sweep voltammetry (LSV) and amperometry. Applied potential at +0.3 V, it shows the sensitivity of 160.2 μA mM^{-1} cm^{-2} for the linear concentration range of 1×10^{-6}–1.52×10^{-3} M, and detection limit of 0.1 μM (S/N = 3).

Keywords: Xanthurenic acid (Xa), Multi-walled carbon nanotubes (MWCNT), Ascorbic acid (AA), Electroanalysis, Electrochemical sensor

1. INTRODUCTION

Ascorbic acid (AA), also known as vitamin C, is one of the most important vitamins widely exist in various plants and takes part in some important biological reactions such as free radical scavenging, cancer preventing and improving immunity. This compound exists widely in food, plant and animal tissues, but cannot be synthesized by the human body. A recommended daily intake of AA is about 70–90 mg. Inadequate intake will result in the symptoms of scurvy, gingival bleeding, and so on; excess AA intake will also lead to urinary stone, diarrhea and stomach convulsion [1]. Due to the importance of AA in life cycle, its determination in aqueous solution is very important. Traditional procedures for AA determination are generally based on enzymatic methods [2], on titration with

oxidizing agents, like iodine or 2, 6-dichlorophenolindopheno and HPLC analysis with fluorimetric [3] or UV–vis detection [4]. Recently there is a considerable interest to develop chemical sensors for electrochemical detection of AA. AA can be easily oxidized electrochemically at conventional electrodes which have been used to detect AA [5,6]. However, direct oxidation of ascorbic acid at bare electrode requires high over-potential which results in electrode fouling by its oxidation products with poor reproducibility, low selectivity and sensitivity. In addition, some of biological molecules, e.g. dopamine and uric acid, undergo oxidation within same potential window as AA. To dissolve these problems, chemically modified electrodes have been developed and reported with various functional materials such as conducting polymers [7–11], ionic liquid [12,13], metal nanoparticles [8], carbon nanotubes [9,13,14] and macrocyclic compounds [15,16].

Xanthurenic acid (Xa) is a product of the tryptophan–NAD pathway. It is related to various pathological conditions; although the biological function of this compound remains obscure [17–20]. Xa acts as a potent iron chelator and has been shown to have prooxidant actions [20]. This compound can form a variety of possible dimers after oxidation, leading to radicals or cations, which can couple with phenoxy radicals, with other dimer radicals or with unconverted compounds to produce polymers adhering strongly to the electrode surface, as evidenced for other phenols [21,22]. Therefore, an investigation into the use of the Xa-modified electrode to oxidize important antioxidants, especially for AA, is a worthwhile endeavor.

Carbon nanotubes (CNTs), discovered by Iijima [23] in 1991, are good electrode materials. They exhibit unique characteristics, including electrical properties, obvious quantum effects, large specific surface areas, high stabilities and strong adsorption properties [24]. Carbon nanotubes (CNT) have been extensively used in electrode modification for electrochemical studies[25-37].These remarkable properties of CNTs endow them with a wide range of potential applications in different fields that include electrochemical biosensor. For the fabrication of electrochemical biosensor, CNTs have been used as an electrode modifying material on the surface of platinum [38,39], indium tin oxide (ITO) electrode [9], glassy carbon [14,26,40], graphite [41,42] and gold [43] electrodes. CNT modified electrodes have been shown to possess improved sensitivity and selectivity toward the determination of many biomolecules, including L-glutathione [13], glucose [41,44], H_2O_2 [45], uric acid [40], dopamine [39,43] and ascorbic acid [16].

Electrochemical sensing has been proven as an inexpensive and simple analytical method with remarkable detection sensitivity, reproducibility, and ease of miniaturization. It would be greatly interesting to further study the electrocatalytic properties and enhancement using the hybrid composites of polyXa and CNTs.

In this work, the polyXa-MWCNT film was prepared on electrode surface to determine ascorbic acid (AA). Poly(xanthurenic acid) (polyXa) was firstly immobilized on electrode surface by the electropolymerization of xanthurenic acid (Xa) monomers. Then the well dispersed MWCNT was coated on this electrode to form polyXa-MWCNT composite for investigation. It was characterized and discussed by cyclic voltammetry (CV), linear sweep voltammetry (LSV), and amperometry in the electrode interface, electrochemical process, and stability. The electrocatalytic oxidation of AA has been completed and discussed in this work.

2. MATERIALS AND METHODS

2.2. Reagents

Ascorbic acid (AA), xanthurenic acid (Xa), and multi-walled carbon nanotubes (MWCNT) were purchased from Sigma-Aldrich (USA). All other chemicals (Merck) used were of analytical grade (99%). Double distilled deionized water was used to prepare all the solutions. A phosphate buffer solution (PBS) of pH 7 was prepared using Na_2HPO_4 (0.05 M) and NaH_2PO_4 (0.05 M).

2.2. Apparatus

All electrochemical experiments were performed using CHI 1205a potentiostats (CH Instruments, USA). The working electrode was glassy carbon electrode (GCE) using BAS GCE (with diameter of 0.3 cm, geometric surface area of 0.07 cm^2, Bioanalytical Systems, Inc., USA). Electrochemical experiments carried out with a conventional three-electrode system which consisted of an Ag/AgCl (3 M KCl) as a reference electrode, a GCE as a working electrode, and a platinum wire as a counter electrode. The buffer solution was entirely altered by deaerating with nitrogen gas atmosphere. The electrochemical cells were kept properly sealed to avoid the oxygen interference from the atmosphere. Prior to modification, the GCE was mechanically polished with BAS polishing kit (Bioanalytical Systems, Inc., USA) and alumina powder (0.05 μm) to mirror finish and ultrasonicated in double distilled water for 3 min. Prior to the electrochemical experiments, the buffer solution was deoxygenated with nitrogen for 10 min.

2.3. Preparation of polyXa-MWCNT film modified electrode

The polyXa modified electrode was prepared by the electropolymerization of Xa monomers in acidic solution using bare GCE electrode. The electro-active system was electro-generated in situ from Xa oxidation, the electrode applied in the potential range of -0.15–1.1 V (vs. Ag/AgCl) with scan rate of 100 mV s^{-1} and 10 scan cycles in 0.1 M sulfuric acid (pH 1.5) containing 1×10^{-3} M Xa monomers. Hence the polyXa/GCE was prepared.

The polyXa/GCE was further coated with the well dispersed MWCNT to form polyXa-MWCNT modified electrode. Prior to the immobilization the MWCNT was functionalized with carboxylic group by acidic treatment. It was well dispersed in the neutral PBS (1 mg ml^{-1}). 10 μl of the solution was loaded on the polyXa modified electrode and then dried it out in the oven at 40 ℃. The prepared electrode, polyXa-MWCNT/GCE, was stored in the refrigerator at 4 ℃ for further study in this work.

3. RESULTS AND DISCUSSION

3.1. Formation of polyXa and polyXa-MWCNT composites

The polyXa film formation involving the electropolymerization of Xa monomers was investigated by cyclic voltammetry using GCE.

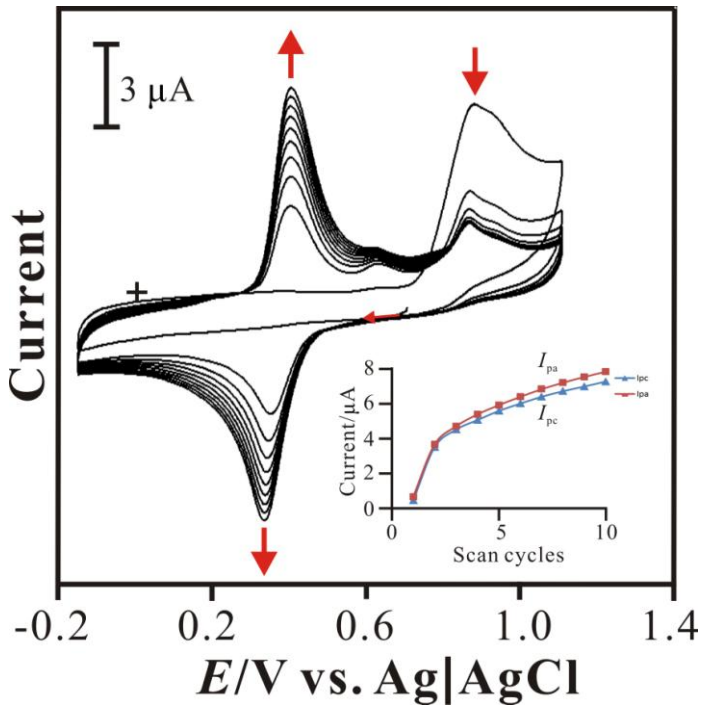

Figure 1. Cyclic voltammograms of polyXa form on glassy carbon electrode in sulfuric acid solution (pH 1.5) containing 1×10^{-3} M Xa. Scan rate = 0.1 Vs^{-1}. Inset: the plot of the peak currents (I_{pa} & I_{pc}) vs. scan cycles.

Fig. 1 displays the consecutive cyclic voltammograms of Xa electropolymerzation in pH 7 PBS using GCE. One redox couple ($E^{0'}$ = +365 mV) was found with an anodic peak at E_{pa} = +400 mV and a cathodic peak at E_{pc} = 330 mV after the first scan segment when the initial potential was started from +0.7 V with negative scan. It is recognized as the polyXa redox processes. After scanning to more positive potential at +0.85 V, both anodic and cathodic currents developed as increased the scan cycles. This is due to the sufficient positive potential to initiate Xa monomers to Xa radical results in the electropolymerization. Therefore, the voltammogram was characterized by one well-defined redox couple, with the formal potential occurring at $E^{0'}$ = +365 mV (vs. Ag/AgCl) at polyXa/GCE. It is attributed to the redox processes between reduced and oxidized forms of polyXa. No too much peak shifting in the polyXa electro-deposition at GCE. This result is similar to the previous related work [46]. It is expected to contain a quinonoid redox system (QRS) for a bifunctional electroactive polymer, poly(5-hydroxy-1,4-naphthoquinone(juglone)-co-5-hydroxy-3-thioacetic acid-1,4-naphthoquinone) [47]. It indicates that the polyXa formed on electrode surface very well. Inset shows the correlation between peak currents (I_{pa} & I_{pc}) and scan cycles, the current is increasing as the increase of scan cycle on GCE. Hereafter, this electrode was further coated with well dispersed MWCNT to form polyXa-MWCNT for further study. In order to have the hydrophilic and well-dispersed MWCNT, the acidic treatment of MWCNT was necessary to have functional carboxylic group for MWCNT and it had following procedure [36]. Here the MWCNT mentioned are all representative for the MWCNT with functional carboxylic group.

3.2. Electrochemical characteristics of polyXa-MWCNT composite

The polyXa-MWCNT composite was examined with various scan rates and different pH conditions by cyclic voltammetry.

Fig. 2 shows the cyclic voltammogram of the polyXa-MWCNT/GCE examined with various scan rate of 10–100 mV s^{-1} in pH 7 PBS. The formal potential of the redox couples is found similar to those in the polyXa redox couple. Both anodic and cathodic peak currents are also directly proportional to scan rate up to 100 mV s^{-1} (inset of Fig. 2) as expected for surface confined. This also means that this process is diffusion-less controlled and stable in the electrochemical system. The observation of well-defined and the persistent cyclic voltammetric peaks indicate that polyXa-MWCNT/GCE exhibits electrochemical response characteristics of redox species confined on the electrode surface.

This film modified electrode shows the linear regressing equation of peak currents (I_{pa} & I_{pc}) and scan rate (v) can be expressed as follows:

At polyXa-MWCNT/GCE:

$$I_{pc}(\mu A) = 0.0006v(\text{mV s}^{-1}) + 0.001 \ (R^2 = 0.9989) \quad (1)$$

$$I_{pa}(\mu A) = -0.0006v(\text{mV s}^{-1}) - 0.001 \ (R^2 = 0.9989) \quad (2)$$

Moreover, the ratio of oxidation-to-reduction peak currents is nearly unity in this redox couple and formal potentials do not change with increasing scan rate in this pH condition. By the result, it means that the active redox species is surface-confined and very stable in the scan rate tests.

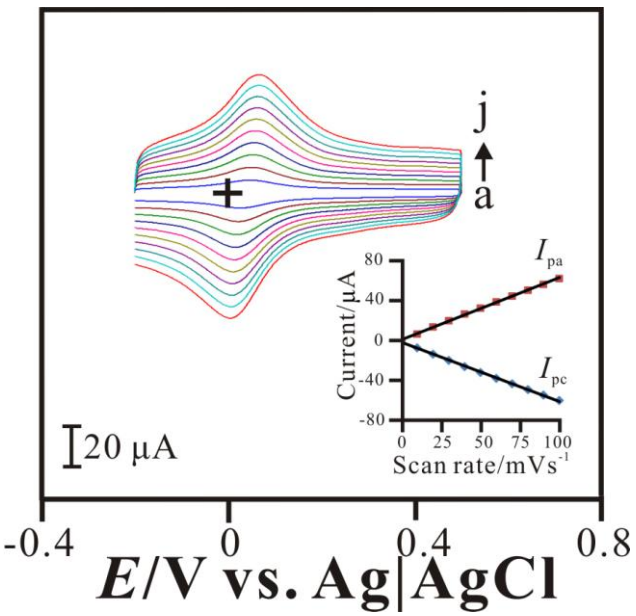

Figure 2. Cyclic voltammograms of polyXa-MWCNT/GCE examined in pH 7 PBS with various scan rates from 10 to 100 mVs^{-1} (a ➔ j). Inset: the plot of the peak currents (I_{pa} & I_{pc}) vs. scan rate.

This result reveals that the electron transfer kinetics is very fast on the electrode modified surface.

We have estimated, the apparent surface coverage (Γ), by using Eq. (3):

$$I_p = n^2F^2vA\Gamma/4RT \quad (3)$$

where, I_p is the peak current of the polyXa-MWCNT composite electrode; n is the number of electron transfer; F is Faraday constant (96485 C/mol); v is the scan rate (V s^{-1}); A is the area of the electrode surface (0.07 cm^2); R is gas constant (8.314 J mol^{-1} K^{-1}); and T is the room temperature (298.15 K). The calculated surface coverage (Γ) was $\Gamma = 2.3\times10^{-9}$ mol cm^{-2} for assuming a two-electron process at polyXa-MWCNT/GCE. This information also means that the compactable and stable structure between polyXa and MWCNT in the composite.

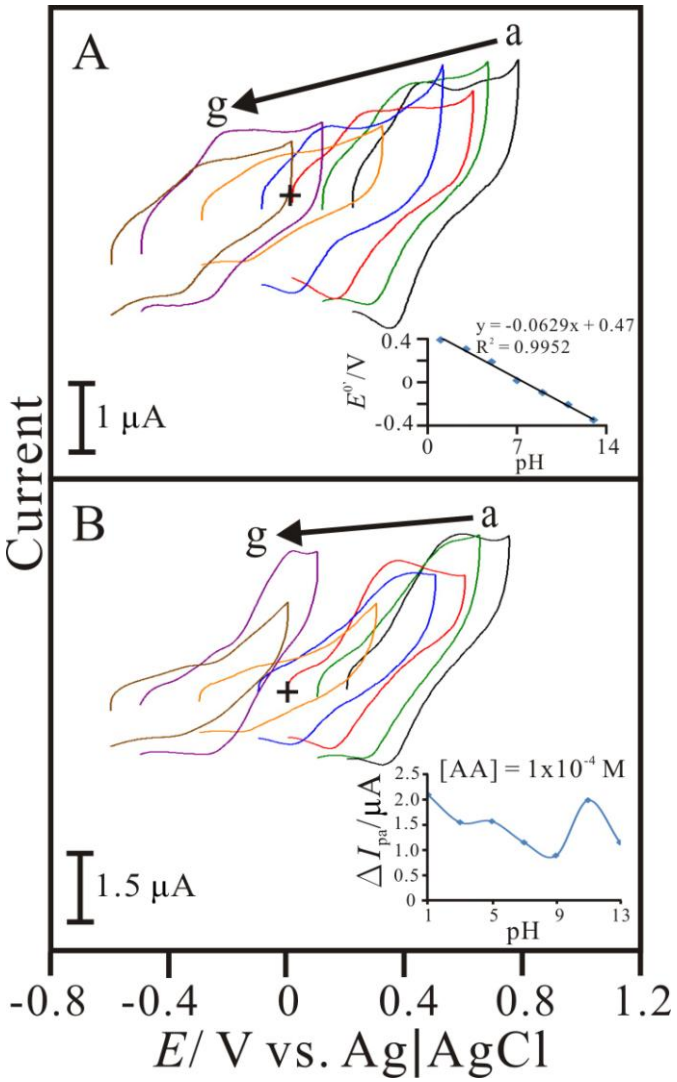

Figure 3. Cyclic voltammograms of polyXa-MWCNT/GCE examined with various pH conditions from pH 1 to 13 (a ➔ g) in the presence of (A) [AA] = 0 M and (B) [AA] = 1×10^{-4} M, respectively. Scan rate = 0.1 Vs^{-1}. Inset: the plots of the formal potential ($E^{0'}$) vs. pH and the net current (ΔI_{pa}) vs. pH.

Fig. 3A displays the pH-dependent voltammetric response of polyXa-MWCNT modified electrode. In order to ascertain this, the voltammetric responses of polyXa-MWCNT electrode were obtained in the solutions of different pH values varying from 1 to 13. The formal potential of the redox couples is pH-dependent with negative shifting as increasing pH value of the buffer solution. The inset in Fig. 3A shows the formal potential ($E^{0'}$) of the polyXa-MWCNT/GCE plotted over a pH range of 1 －13. It shows a slope of -62.9 mV pH^{-1}, which is close to that given by the Nernstian equation for equal number of electrons and protons transfer processes. The phenomenon indicates that two electrons and two protons transfer involving the redox processes. The above result shows that the polyXa-MWCNT film is stable and electrochemically active in the aqueous buffer solutions.

3.3. Electrocatalytic oxidation of ascorbic acid by polyXa-MWCNT film

The electroactive species, polyXa-MWCNT, was investigated for the electrocatalytic oxidation of AA.

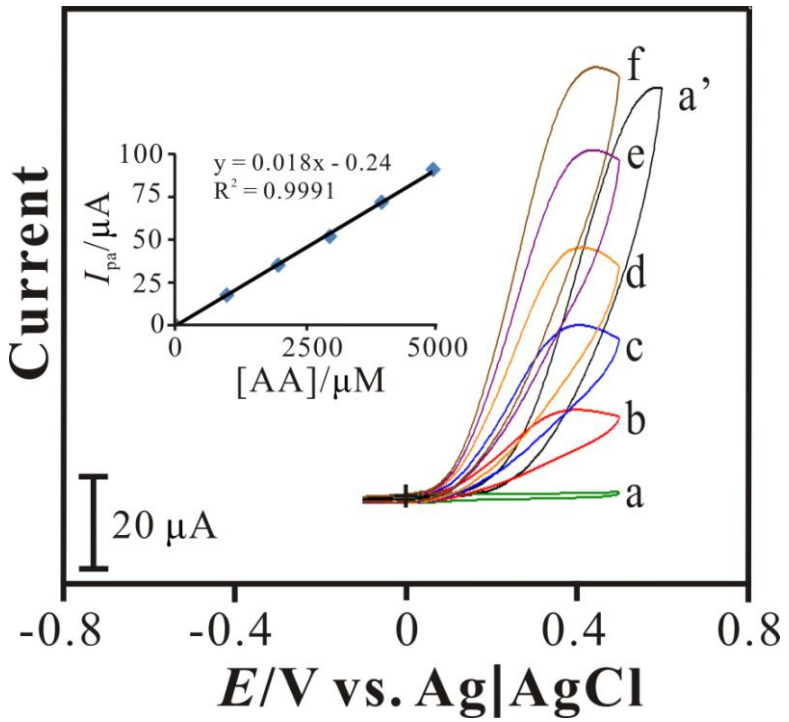

Figure 4. Cyclic voltammograms of polyXa-MWCNT/GCE examined in pH 7 PBS containing [AA] = (a) 1000, (b) 2000, (c) 3000, (d) 4000, and (e) 5000 μM, respectively. (a') is the bare GCE examined in the presence of of 5×10^{-3} M. Scan rate = 0.1 Vs^{-1}. Inset: the plot of the anodic peak current (I_{pc}) vs. [AA].

Fig. 4 shows the cyclic voltammograms of AA examined in pH 7 PBS using polyXa-MWCNT/GCE. This electrode exhibits obviously electrocatalytic oxidation peak at about +0.3 V. It can be found that the anodic peak current is increasing as the increase of AA concentration (as shown

in the inset of Fig. 4). Particularly, the polyXa-MWCNT/GCE shows lower over-potential and higher current response to AA as compared with the result done by bare electrode. The optimized pH condition for AA determination using this electrode was also investigated. Fig. 3B shows the cyclic voltammograms of polyXa-MWCNT/GCE examined with different pH buffer solutions in the presence of 1×10^{-4} M AA. The net currents (ΔI) were estimated by the subtraction of absent/present AA anodic peak currents correlated to each pH condition. From the inset of Fig. 3B, one can know that higher net current contribution is found in the strong acidic condition except of pH 11. This kind of AA sensor prefers to more strong acidic condition. Considering the approach of neutral condition in the practical application, it is also noticed about that the relative high net current occurred at pH 5 which might be the option to maintain good electrocatalytic current response for AA oxidation. Although it does not exhibit higher current response in pH 7, the neutral condition is chosen to study in this work due to the practical application should be tested in it.

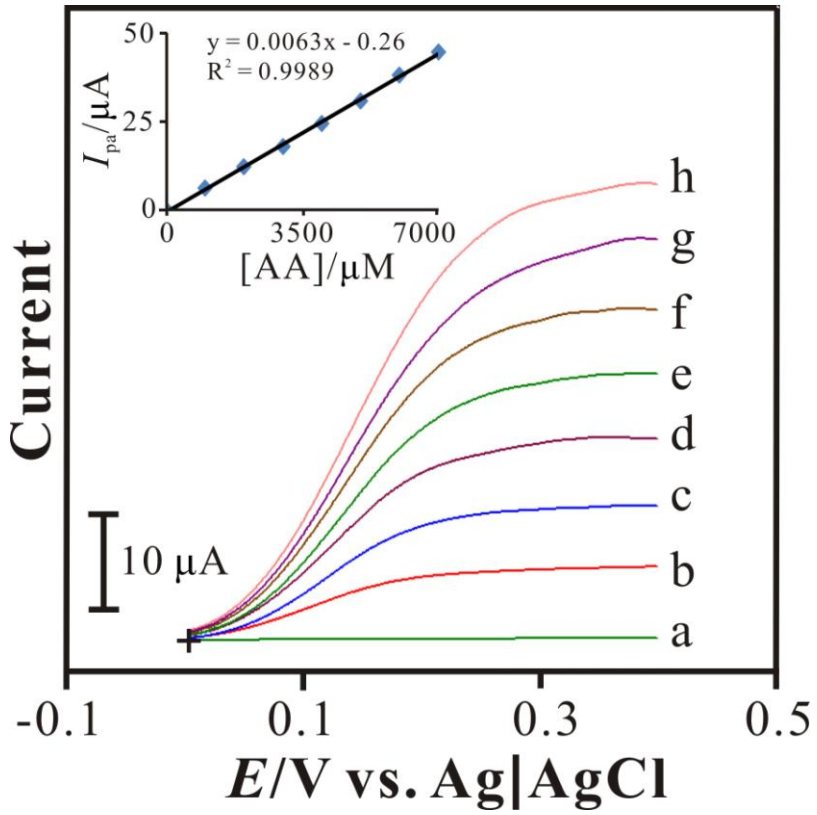

Figure 5. Linear sweep voltammograms of polyXa-MWCNT/GCE examined in pH 7 PBS containing [AA] = (a) 0, (b) 1000, (c) 2000, (d) 3000, (e) 4000, (f) 5000, (g) 6000, and (h) 7000 μM, respectively. Inset: the plot of the anodic peak current (I_{pa}) vs. [AA].

Fig. 5 shows the linear sweep voltammograms (LSV) of AA using polyXa-MWCNT/GCE in pH 7 PBS. The polyXa-MWCNT/GCE exhibits electrocatalytic oxidation peak at about +0.1 V for AA. The oxidation current increased more obviously by this method. It can be also found that the oxidation peak current is increasing as the increase of AA concentration. As compared with previous

work [48], it is competitive to determine AA. By above results, one can conclude that the polyXa-MWCNT has lower over-potential and higher current response to AA.

The polyXa-MWCNT electroactive species was further immobilized on rotating glassy-disk electrode to study the amperometric current response by amperometry.

Fig. 6 shows the amperograms of polyXa-MWCNT/GCE examined in pH 7 PBS with several additions of AA (10 μM per 50 seconds) when applied potential at +0.3 V and set electrode rotation speed at 1000 rpm.

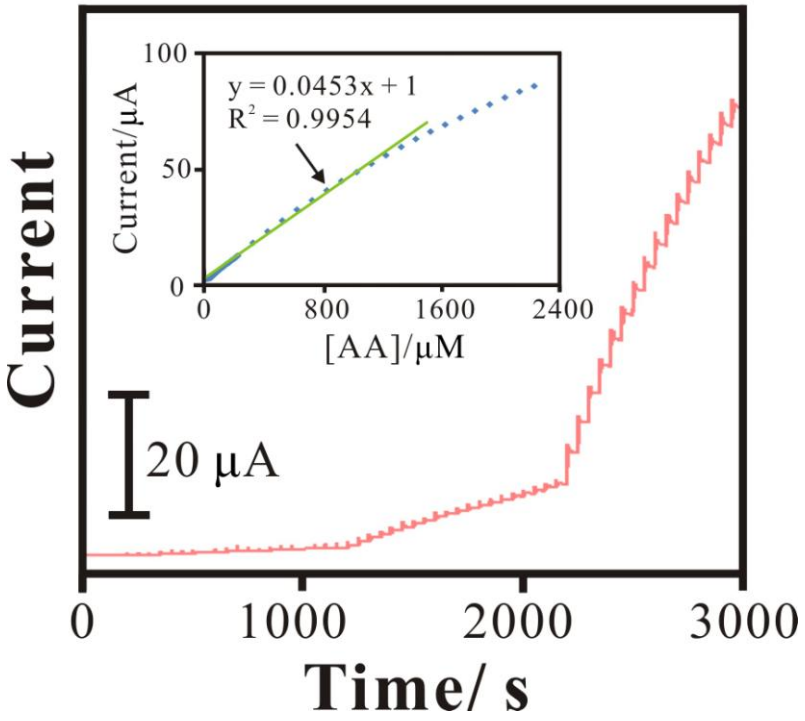

Figure 6. Amperograms of polyXa-MWCNT/GCE examined in pH 7 PBS with several additions of [AA] = 1×10^{-6}—2.22×10^{-3} M, respectively. Electrode rotation speed = 1000 rpm. $E_{app.}$ = +0.3 V. Inset: the plot of the peak currents vs. [AA].

Table 1. The detection limits and linear concentration ranges of different modified electrodes for the determination of AA

Modified electrodes	LOD[a]/μM	LCR[b]/μM	Ref.
MWCNT/CCE	7.71	15–800	[49]
CNF-CPE	2	2–64	[50]
PGE	13	25–500	[51]
OMC/Nafion	20	40–800	[52]
Pd/CNF-CPE	15	50–4000	[53]
ZnO/RM	1.4	15–240	[54]
Pt-Au hybrid	103	103–165	[55]
p-ATD	2.01	30–300	[56]
Chitosan-graphene	50	50–1200	[57]

NG	2.2	5－1300	[58]
polyXa-MWCNT/GCE	0.1	1－1520	This work

[a]LOD: the limit of detection.
[b]LCR: the linear concentration range.

This film modified electrode shows linear correlation between the electrocatalytic oxidation current (I_p) and species concentration (C_i), and the linear regressing equation can be expressed as follow:

$$I_p\,(\mu A) = 0.0453 C_i(\mu M) + 1 \ (R^2 = 0.9954) \qquad (4)$$

It is calculated with the sensitivity of 160.2 μA mM^{-1} cm^{-2} for the linear concentration range of 1×10^{-6}－1.52×10^{-3} M, and detection limit of 0.1 μM (S/N = 3). This data are competitive to other electrode materials compared as Table 1.

By above results, one can conclude that the polyXa-MWCNT is a good electroactive species as catalyst to determine AA with lower over-potential and higher current response.

4. CONCLUSIONS

The polyXa-MWCNT composite has been successfully prepared on electrode surface by the electropolymerization of Xa and adsorption of functionalized MWCNT. This composite is surface-confined and stable in various scan rates and different pH conditions. Its modified electrode shows lower over-potential and higher current response to AA when compared to the bare electrode. Linearity is also found in other electrochemical techniques using LSV and amperometry except of CV. This electroactive species modified electrode can be used as an AA electrochemical sensor due to active properties with lower over-potential and higher current response.

ACKNOWLEDGEMENTS
This work was supported by the National Science Council of Taiwan (ROC).

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