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Amperometric Sensor Based on Carbon Nanotubes and Polycations for the Determination of Vitamin C

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Detection of vitamin C (VC) is necessary because of its wide use in chemical, biological, and pharmaceutical engineering. Here, we describe a sensing system for the determination of VC. Instead of a bare glassy carbon electrode (GCE), a prepared multi-walled carbon nanotube-polycation-GCE was used to detect VC in samples with higher sensitivity, better repeatability, and with a lower detection limit. Under the optimal conditions, the obtained sensor presented a linear response to VC in the range of 1–100 μM with a detection limit of 500 nM. The proposed electrode also successfully detected VC concentrations in real samples.

Keywords: Electrochemical detection; vitamin C; polycation; nanocomposite; protamine

1. INTRODUCTION

Improving the selectivity and sensitivity of the monitoring techniques of electrochemical sensors for target samples is currently the focus of considerable research [1-4]. For example, the determination of vitamin C (VC) is of great importance. VC, also known as ascorbic acid (AA), is readily found in our daily lives, whether in fresh fruits and vegetables [5,6] or in the form of pharmaceutical products such as VC supplements or multivitamin tablets. VC is a strong antioxidant that can reduce oxidative stress in the body and is, therefore, believed to lower cancer and cardiovascular risks [7,8]. VC deficiency has been reported in certain chronic disorders such as sickle cell anaemia [9].

Because of the biological and technological importance of VC, its accurate detection is essential to food quality and health care. Various chemical sensors for VC detection have been developed using appropriate signal transductions such as spectroscopy [10], chromatography [11],

titrimetry [12], photometry [13], and polarimetry[14]. Nevertheless, these methods require either expensive and sophisticated instrumentation or complicated sample preparation processes. Since amperometric techniques allow for the sensitive, simple, and inexpensive detection of analytes, they are a promising alternative for the analysis of VC in real samples [15,16]. Unfortunately, solid electrode fouling often occurs with the adsorption of oxidized products, ultimately leading to poor stability and repeatability of the solid electrodes. Furthermore, AA, dopamine (DA), and uric acid (UA), which have very similar electrochemical properties, always coexist in biological samples. In order to improve the selectivity, a variety of electrodes modified with nanocomposites have been recently designed and constructed to either reduce the overpotential of AA oxidation or to prevent the approach by DA and UA to the electrode surface [17-19].

Our group has shown that multi-walled carbon nanotubes (MWCNTs)/protamine bionanocomposites (abbreviated MWCNTs-Pro) can be employed for the modification of a GCE for the determination of dihydronicotinamide adenine dinucleotide (NADH) with submicromolar detection limits [20]. In this work, we employed the MWCNTs-protamine-GCE for the detection of AA as well. AA is adsorbed onto the MWCNTs-Pro and then oxidized on the protamine surface due to electron transfer between AA and protamine, converting the OH of AA into a carbonyl group [21]. Due to the charged character of DA (pK_a = 8.87), UA (pK_a = 5.75), and AA (pK_a = 4.10), the MWCNTs-Pro modified electrode was more sensitive toward the negatively charged AA and UA than to the positively charged DA.

The aim of this study was to validate a simple method for the determination of VC in real samples by direct electrochemical oxidation. This method should not only be reliable, but also as rapid and simple as possible in order to be useful for in situ testing of VC.

2. EXPERIMENTAL

2.1. Materials

L-Ascorbic acid, dopamine, and uric acid were procured from Aladdin. Carbon nanotubes (diameter 40–60 nm; length < 2 μm; purity > 97%) were procured from Shenzhen Nanotech Port (China), and protamine was obtained from Sigma. Phosphate buffered saline (PBS, 0.1 M, pH 7.0) was used as the supporting electrolyte solution. All other chemicals and reagents were analytical grade and were prepared using ultrapure water.

2.2. Apparatus and measurements

Electrochemical experiments were performed with a 760D electrochemical workstation (CH Instruments, Chenhua, Shanghai, China). All electrochemical measurements were carried out with a three-electrode system comprised of a modified or unmodified GCE (3 mm diameter) as the working electrode, a platinum wire as an auxiliary electrode, and Ag/AgCl (3 M KCl) as the reference electrode.

2.3. Preparation of modified GCEs

Prior to modification, the GCE was polished with alumina slurries of 0.3 and 0.05 μm and sonicated in distilled water for 30 s. After successive sonication in anhydrous ethanol and ultrapure water, the electrode was rinsed with ultrapure water and allowed to dry at room temperature. For the detection of VC, the modified GCE was prepared as previously described [20].

2.4.Electrochemical measurements

All Electrochemical experiments were performed at room temperature in a cell containing 5.0 mL phosphate buffer solution (PBS, pH 7.0) using an electrochemical instrument (CHI 760D, chenhua, shanghai). A three-electrode system comprising of a platinum wire as the auxiliary, an Ag/AgCl as the reference and the modified GCE as the working electrode was used for the electrochemical experiments. Cyclic voltammetric and differential pulse voltammetric measurements were carried out with three electrodes in PBS. The cyclic voltammograms were recorded by cycling the potential between 0.0 and 1.0 V at a scan rate of 0.1 V/s. The differential pulse voltammetric measurements were performed by applying a sweep potential from 0.0 to 0.6 at pulse amplitude of 50 mV and pulse width of 0.1s. The modified electrode could be used repeatedly after rinsed with doubly distilled water and blotted with filter paper.

3. RESULTS AND DISCUSSION

3.1. Electrocatalytic oxidation of AA, DA, and UA at the MWCNTs-Pro nanocomposite modified electrode

The electrochemical responses of the bare GCE and modified GCE to AA, DA, and UA measured by cyclic voltammetry（CV） in 0.1 M PBS buffer solution (pH = 7) are shown in Fig. 1A. For the ternary mixture of AA, DA, and UA, an overlapping oxidation peak can be seen with the bare GCE. In contrast, three separate well-defined anodic peaks corresponding to the ternary mixture oxidations are clearly observed at the MWCNTs modified GCE with potential separations of 200 mV and 150 mV for AA/DA and DA/UA, respectively, which are large enough separations to be useful for the compounds' simultaneous determination in a mixture. However, no advantage in the determination of AA was found.

At the MWCNTs-Pro modified GCE, the peak currents of AA were approximately five times higher than those obtained on the MWCNTs modified GCE, and the anodic oxidation peaks of AA shifted to more negative potentials. It is believed that the existence of protamine, a polypeptide rich in arginine residues with an overall charge of about +20, is essential for enhancing the sensitivity and specificity of the GCE to AA. Due to the charged character of AA, DA, and UA, the protamine-modified electrode was more sensitive toward negatively charged AA, whereas positively charged DA was more inhibited. Thus, the modified electrode could more efficiently catalyse the electro-oxidation

of AA than of DA. In addition, AA oxidation is an inner-sphere reaction, and the electron transfer kinetics is sensitive to the electrode surface properties [21]. Therefore, a remarkable enhancement of the oxidation peak current of AA was obtained, which is beneficial for the selective determination of AA over DA and UA.

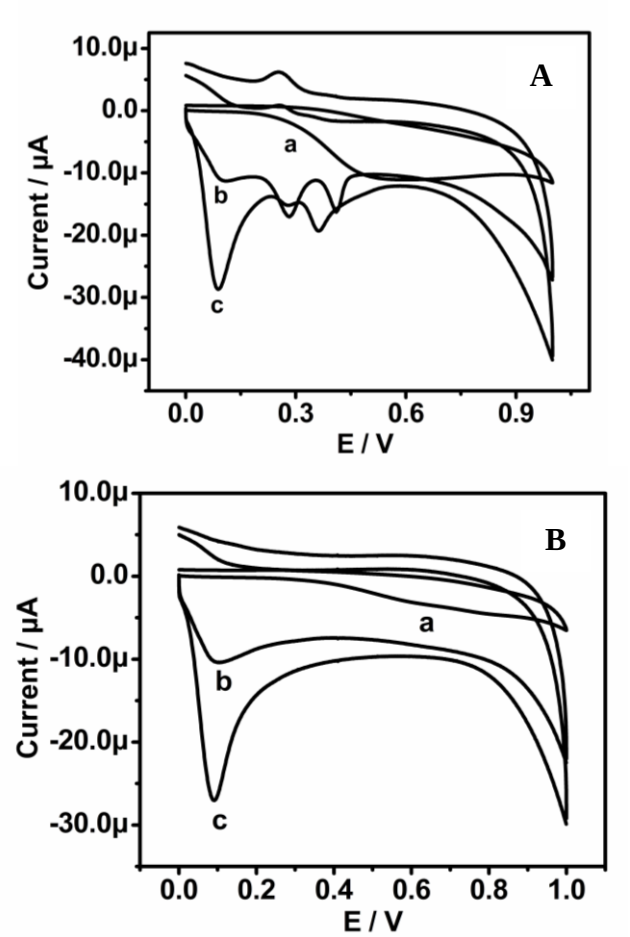

Figure 1. A Cyclic voltammetric responses at bare GCE (a), MWCNTs/GCE (b) and MWCNTs-Protamine/GCE (c) in 0.1 M PBS containing 1mM AA, 0.1 mM DA and UA. Scan rate, 100 mVs^{-1}. Fig.1B Cyclic voltammetric responses at bare GCE (a), MWCNTs/GCE (b) and MWCNTs-Protamine/GCE (c) in 0.1 M PBS containing 1mM AA. Scan rate, 100 mVs^{-1}.

The electrocatalytic oxidation of AA alone on the MWCNTs-Pro modified GCE was also investigated by CV. Figure 1B shows the CV responses of 1 mM AA in 0.1 M PBS buffer solution (pH = 7) at the bare GCE, MWCNTs modified GCE, and MWCNTs-Pro modified GCE. As seen in the figure, the MWCNTs-Pro modified GCE exhibits a sharp and well-defined oxidation peak with low background for AA alone.

3.2. Effect of scan rate

To further characterize the oxidation of AA at the MWCNTs-Pro modified GCE, the kinetic and transport characteristics of MWCNTs-Pro-GCE were further investigated by performing cyclic voltammetry experiments with different scan rates.

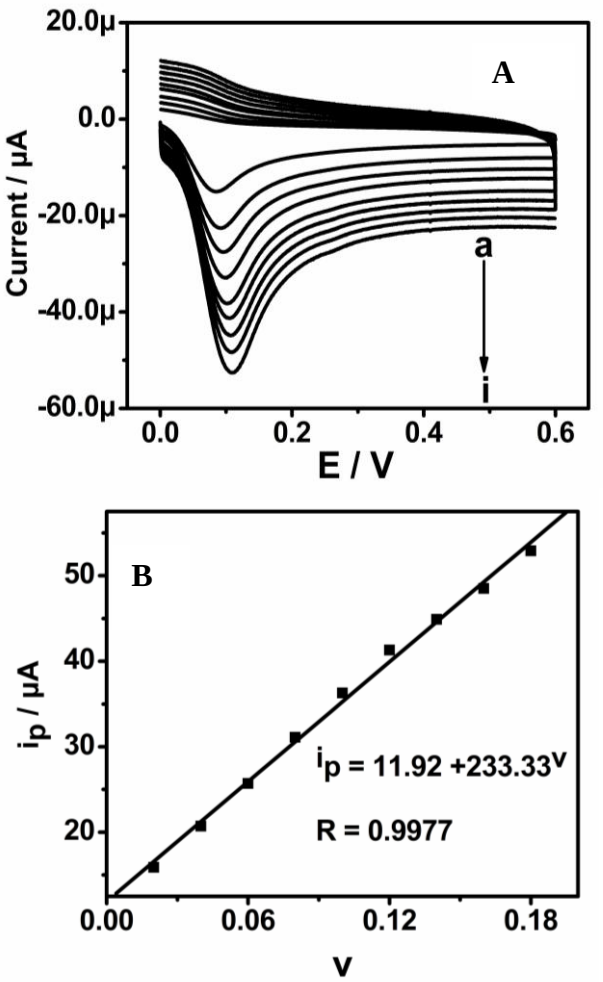

Figure 2. (A) Cyclic voltammograms at(a) 20, (b)40, (c)60, (d)80, (e)100, (f)120, (g)140, (h)160, (i)180mVs^{-1} on the Protamine-MWCNTs/GCE in the presence of 1 mM AA in 0.1M phosphate buffer solution. (B) A anodic peak currents of AA vs. the square root of scan rate.

Figure 2A shows the CVs of the MWCNTs-Pro-GCE at different scan rates (20–180 mVs^{-1}) for AA. The oxidation peak current was linearly proportional to the scan rate (Fig. 2B). The regression equation was i_p = 11.92 + 233.33v. These results show that the electrochemical oxidation of AA at the MWCNTs-Pro-GCE is a surface-controlled process and not a diffusion-controlled process [22].

3.3. Calibration of ascorbic acid concentration

A wide linear range of AA concentrations could be measured using CV, as shown in Fig. 3. This technique also allows for the fast and selective detection of AA. The calibration graph was plotted as the peak current of AA vs. AA concentration, and the sensitivity was found to be 23.58 ± 0.60 μA/mM. A linear concentration range of two orders of magnitude (0.1–10 mM) is quite useful for the development of further applications.

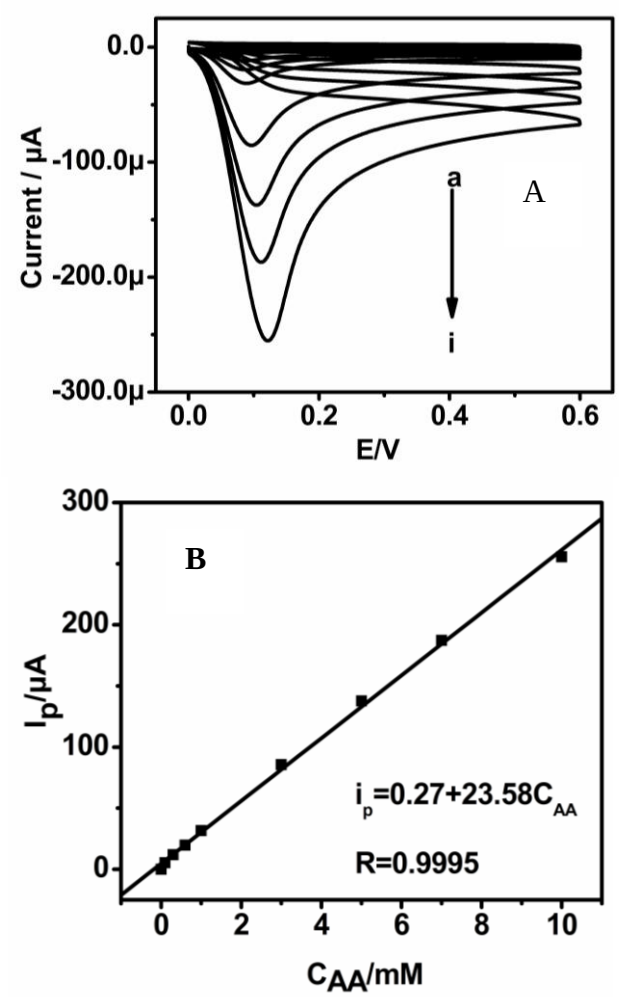

Figure 3. (A) Cyclic voltammograms of the MWCNTs-Pro-GCE in PBS as a function of AA concentration (a)0, (b)0.1(c) 0.3(d) 0.6(e)1(f)3(g)5(h)7(i)10 mM. (B) Calibration curves of the AA concentration vs. the anodic peak currents.

In examining the reproducibility of the proposed electrode, the relative standard deviations (RSD) of 10 determinations of 1 mM AA was found to be 5.8%. To assess the consistency of the MWCNTs-Pro modified GCE, cyclic voltammograms were recorded on one day, and again a week later under the same conditions, and compared. The peak currents of AA changed less than 6.2%, which indicated that the prepared electrode had good stability. These results indicating that the modified electrode is not subject to surface fouling by the oxidation products, which are notorious for their surface fouling effects at the bare electrode [23].

The amperometric response of the MWCNTs-Pro-GCE to successive additions of AA was further evaluated under the optimized experimental conditions. Figure 4 shows the amperometric current-time response of AA at 0.1 V. As illustrated, upon addition of AA into PBS (pH = 7.0), the oxidation current increases steeply and reaches a steady-state current within an average response time of 10 s. The amperometric signal displays a good linear correlation to AA concentration in the range of 1 μM to 100 μM. The linear regression equation is expressed as I = 0.0078 + 0.34 C_{AA}, with a correlation coefficient of R = 0.9968. The detection limit is 500 nM at a signal-to-noise ratio of three. For electrochemical sensors, most previous reports have focused on the improvement of limits of

detection [2,26,28]. However, very few efforts have concentrated on the range of detection. This reported system not only had lower detection limit, but also had longer linear range than most other electrode for Vitamin C. Thus, AA could be quantifiably measured by CV and amperometric according to our requirement. Analysis of unpretreated, real world samples that span a broad range of concentrations requires the sensors with wide range detection capabilities [24]. In table 1, some of the analytical characteristics obtained in this work are compared with those previously reported in the literature. The proposed sensor exhibited lower detection limit and broader linear range for AA, which are due to the excellent electrocatalytic activity of MWCNTs-Pro nanocomposites. Furthermore, the current response becomes stable in less than 5s, which indicates a significantly rapid response of MWCNTs-Pro-GCE towards AA.

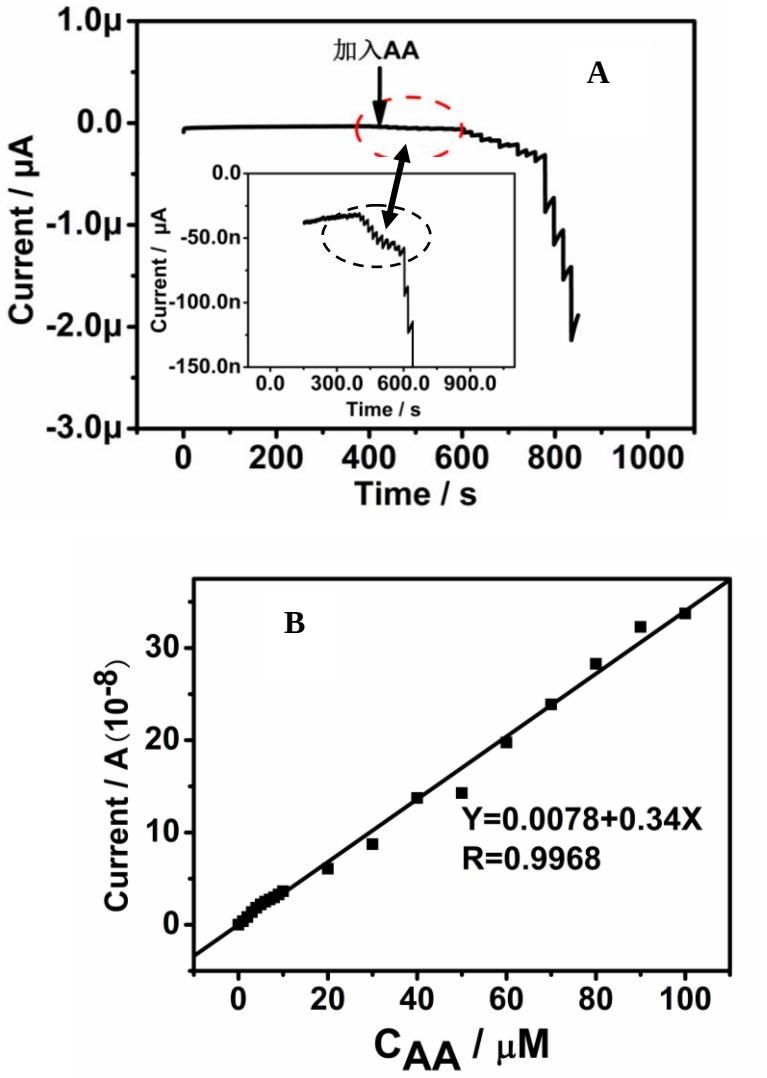

Figure 4. (A) Amperometric response of MWCNTs-Pro-GCE for the oxidation of AA at +0.1 V in 0.1 M PBS (pH 7.0). (B)The calibration plot of the concentration of AA with current at MWCNTs-Pro-GCE.

Table 1. Analytical figures of mertt for nanocomposite-modified electrodes for VC.

Sensor	Limit of detection(μM)	linear range （μM）	Reference
Carbon fibers/ZnO coaxial nanocable microelectrode	156.7	600-1800	25
Recessed gold nanoelectrode array	7.5	30-190	2
NiCoO2/C modified GCE	0.5	20-2410	21
Graphene flowers modified carbon fibers	24.7	45.4-1489.23	30
Overoxidized polyimidazole/grapheme oxide copolymer modified electrode	18	75-2275	29
beta-cyclodextrin/Au nanoparticles/graphene-modified electrodes	2	50-900	28
Polyaniline/nickel composite film modified electrode	0.4	2-1210	27
CTAB functionalized grapheme oxide multiwalled carbon nanotube composite modified electrode	1.0	5.0-300	26
Multiwalled carbon nanotube-protamine/GCE	0.5	100–10000 1-100	This work

3.4. Determination of AA in the presence of DA

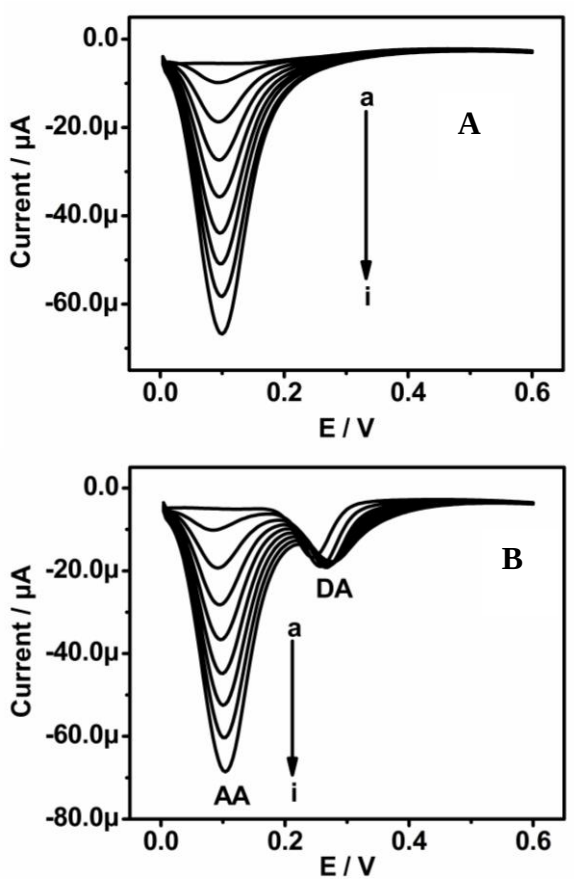

Figure 5. (A) DPVs of MWCNTs-Pro-GCE in PBS solution (0.1 M, pH 7.0) containing (a)0, (b)0.1, (c)0.3, (d)0.5, (e)0.7, (f)0.9, (g)1.1, (h)1.3 and (i)1.5 mM AA. (B) DPVs of MWCNTs-Pro-GCE in PBS solution (0.1 M, pH 7.0) containing mixed concentrations of 0.1 mM DA and (a)0, (b)0.1, (c)0.3, (d)0.5, (e)0.7, (f)0.9, (g)1.1, (h)1.3 and (i)1.5 mM AA.

For the selective determination of AA at the modified electrode, differential pulse voltammetry (DPV) was carried out to study the interference of DA. Figure 5A shows the DPV response for the different concentrations of AA in the presence of a fixed concentration of 0.1 mM DA. A linear relationship between peak currents and AA concentrations was observed in the range of 0.1–1.5 mM.

MWCNTs-Pro nanocomposite, as an anionic exchanger at the GCE surface, selectively attracts anionic AA and allows it to pass through to the electrode surface [31]. As a result, the presence of DA and UA did not interfere in the AA determination in the phosphate buffer solution of pH=70, as shown in Figure 5B. It is interesting to note that the sensitivities and linear equation of the modified toward AA in the absence and presence of DA are approximately the same, which indicates the facts that the selective and sensitive determination of AA in the presence of DA is feasible at the modified electrode.

3.5. Real sample analysis

In order to examine the practical applicability of the proposed method, electrochemical determinations of AA in samples of VC tablets and vegetables were performed.

3.5.1. Analysis of pharmaceutical samples

Several commercial VC tablets were weighed and finely pulverized. A pill of VC was dissolved in 200 mL of the carrier solution. The solution of VC effervescent tablets was directly detected, and the solution of Guowei C was diluted by a factor of 10 for the analysis of VC. Cyclic voltammetic measurements were taken for VC effervescent tablets and Guowei C, and the analyte concentrations were quantified according to the calibration curve of the proposed electrode. The results obtained by the proposed method agree with the potency specification of the VC tablets (Table 2). These results of VC determination in commercial vitamin C tablets indicate that the fabricated biosensor could be employed for the determination and analysis of VC content in pharmaceutical samples.

A

Table 2. Results of AA in VC tablets

Sample	Detected/mM	In real sample (mg/g)	Potency of the tablets (mg/g)
VC effervescent tablets	2.72	23.81	25.00
Guowei C	0.29	133.22	127.70

3.5.2. Analysis of vegetables

Vegetables were cut into small pieces, and 10 g samples were homogenized with 100 mL of the carrier solution, filtered, and then diluted with the same reagent by a factor of 100 for the analysis of VC. The proposed method was applied to the detection of VC in vegetables (using the method of standard addition with satisfactory results). In order to avoid the interferences of the real samples

matrix, and to fit into the linear range of VC, only diluted samples were added into the electrochemical cell. To establish the correctness of the results, certain amounts of VC were added into the above-mentioned diluted samples, and were then detected. The results are shown in Table 3. It reveals that the inorganic MWCNTs-Pro modified electrode would be more suitable for practical application.

Table 3. Results of AA in vegetables

Sample	Detected/μM	Added/μM	Found/μM	Recovery
Tomato	2.42±0.15	2.00	4.4±0.22	103%

4. CONCLUSIONS

This paper describes the development of a simple and efficient nanostructured platform based on polycations that was used as an analytical sensor for the determination of VC. The proposed sensor is easy to prepare, inexpensive, and has low detection limit, long linear range and a short analysis time. Compared with most reported methods, the present modified electrode possesses lower detection limit and longer linear range and could be employed for practical applications.

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