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ORIGINAL PAPER

Voltammetric study of polyviologen and the application of polyviologen-modified glassy carbon electrode in amperometric detection of vitamin C

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Abstract The voltammetric behavior of viologen oligomers prepared from butylviologen dibromide and the factors influencing polyviologen film formation were investigated at a glassy carbon electrode (GCE). Based on the voltammetric observations, phosphoric acid is crucial to the formation of a stable polyviologen film on a GCE. The polyviologen-modified glassy carbon electrode (PVGCE) was employed to determine vitamin C (i.e., ascorbic acid) in order to demonstrate the electroanalytical application of the electropolymerized polyviologen film. The PVGCE was found capable of accumulating vitamin C at electrode surface in a slightly basic solution (pH = 7.8) and induce a negative shift of oxidation potential of vitamin C. Vitamin C was detected by hydrodynamic amperometry at +0.1 V (vs. Ag/AgCl) in a batch-injection cell; no accumulation time is required. The dependence of oxidation current on concentration was linear from 5.00×10^{-7} M to 1.22×10^{-4} M with a regression coefficient of 0.9993. Several real samples were analyzed and the results exhibit good agreement with those determined by iodimetric titration.

Keywords Polyviologen · Modified electrode · Vitamin C · Electropolymerization · Amperometric

1 Introduction

Vitamin C (i.e., ascorbic acid) is a common biological compound with good electroactivity in aqueous solutions. It presents in many foods, especially vegetables, fresh fruits and fruit juice beverages, and also distributes in animal liver, the anterior pituitary lobe and the human nervous system. Vitamin C is an efficient reducing agent and, therefore, an important antioxidant in organisms [1]. Apart from its vitamin activity, vitamin C is frequently used as an antioxidant in the food industry to prevent unwanted changes in color and in flavor. In addition, vitamin C has also clinical importance, e.g., prevention of scurvy [2, 3] and infectious diseases, and is vital to immune response and wound healing [4]. Recently, the antitumor and anticancer properties of organometallic ascorbic acid have also been investigated [5].

Due to the crucial role of vitamin C in biochemistry and in industrial applications, the determination of vitamin C is still a continuing research interest. For industrial applications, quick monitoring of vitamin C levels during production and quality control stages is important. Several methods have been developed for the detection of vitamin C including enzymatic method [6], oxidimetric titration [7], chromatography [8, 9] and chemiluminescence [10, 11]. The direct electrochemical oxidation of vitamin C is possible and thus electrochemical detection is feasible. Compared to other techniques, electrochemical analysis is relatively simple and sensitive; however, the inevitably high overpotential results in electrode fouling, poor reproducibility and sensitivity, and, most importantly, low selectivity. Electrochemical detection of vitamin C has been performed by using different electrode materials [12, 13] but suffered from the aforementioned defects. Although the glassy carbon electrode (GCE) has a minimal trend in such degenerative behavior, the requirement of a relatively higher overpotential limits the utilization of a bare GCE in the direct electrochemical detection of vitamin C. Therefore, several different strategies have been developed to modify the electrode surface in order to

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provide selective detection and prevent electrode fouling [14–24].

Based on the pK_{a1} (4.17) [25] of vitamin C, it should be in the anionic form when it is dissolved in an approximately neutral aqueous solution. An anion-exchange polymer might thus be a promising material for modifying bare GCE in order to enhance the detection sensitivity by accumulating vitamin C on the electrode surface through the anion-exchange characteristics. Among the ionic polymers, those that can be polymerized electrochemically (i.e. the electropolymerized polymers) have received attention because the usual dip-coating or spin-coating technique is not very convenient for electrode modification. Electropolymerized *N*,*N*-dimethylaniline, for example, is an anion-exchange polymer and has been used for amperometric detection of vitamin C [26]. Another electropolymerized anion-exchange polymer polyviologen (PV), however, is rarely reported. A polyviologen-modified glassy carbon electrode (PVGCE) has been used for electroanalysis [27]. However, the detailed study of PV film formation and the factors affecting film performance have not been discussed.

In this paper we demonstrate the electrochemical behavior of viologen oligomers at GCE and explain why PV film can only be formed in Britton-Robinson (BR) buffer solution if no cross-linking reagent is used. PVGCE was employed for amperometric detection of vitamin C in a batch-injection cell containing pH 7.8 H_3BO_3 solution and the applied potential was +0.1 V (vs. Ag/AgCl). Direct and fast detection is achieved without inordinate accumulation time.

2 Experimental

2.1 Instrumentation

All electrochemical experiments were performed with a CHI 621C electrochemical analyzer (CH Instruments) in conjunction with a CS-2 cell stand containing a three-electrode system, which consisted of a glassy carbon disk electrode (BAS MF-2012) with or without electropolymerized polyviologen (PV) film, an Ag/AgCl (saturated NaCl) reference electrode, and a platinum spiral counter electrode. Before the experiments were performed all glassware was soaked in 1:1 (v:v) nitric acid for at least one hour and then thoroughly rinsed with deionized water. A GCE was polished with alumina slurry on a polishing cloth. Afterwards, the GCE was cleaned ultrasonically in deionized water. These procedures were repeated between each experiment.

2.2 Reagents

Vitamin C (L(+)-ascorbic acid, 99%) was purchased from Acros Organics and used as received. pH 4.2 Britton-Robison (BR) buffer solution was prepared by mixing acetic acid (SOWA, 99.7%), phosphoric acid (SOWA, 85%), and boric acid (SOWA, 99.5%) with identical concentrations and then adjusted to the desired pH by adding sodium hydroxide (Riedel-de Haën, 99%) solution. Finally, each constituent except for the sodium hydroxide in the pH 4.2 BR buffer solution had a concentration of 0.032 M and this buffer solution was used for preparing the polyviologen (PV)-modified GCE. Phosphate buffer solution (0.2 M, pH 7.8) was prepared from Na_2HPO_4 (SOWA, 99%) and $NaH_2PO_4 \cdot 2H_2O$ (SOWA, 99%). Sodium chloride (99.9%) was obtained from J. T. Baker and used as received. All solutions were prepared with deionized water purified by Milli-Q Gradient system (Millipore).

Water-soluble viologen oligmers were prepared from butylviologen dibromide according to the procedures reported earlier [28] and the chemical structure is shown in Fig. 1a. According to earlier studies [27] n is 3–7.

2.3 Preparation of polyviologen-modified glassy carbon electrode (PVGCE)

The pH 4.2 BR buffer solution containing 0.1 wt% of viologen oligomers was employed for PV film preparation. The solution was deaerated by sparging argon for 4 min and then the PV film was formed on GCE by applying a polymerization potential of −1.00 V for 60 s. A deep blue film was formed on the electrode surface. Before using the PVGCE, it was rinsed with deionized water in order to remove any unreacted viologen oligomer. The BR buffer solution and GCE are essential for the formation of a stable PV film, and the reasons will be discussed latter.

2.4 Procedures of hydrodynamically amperometric analysis

Amperometric analysis was carried out in 4 ml of 0.1 M H_3BO_3 aqueous solution in which the solution pH was adjusted to 7.8 by adding NaOH solution. A potential of +0.1 V was applied to the PVGCE while the solution was vigorously stirred. After the background current decayed to a steady value, an appropriate volume of the standard vitamin C solutions or real samples were injected with a microsyringe into the electrochemical cell through a pinhole on the sidewall. Vitamin C anions were accumulated on the PVGCE by the anion exchange feature of polyviologen; however, no accumulation time was needed. A steady oxidation current was observed after each injection and the current was proportional to the vitamin C concentration in the cell.

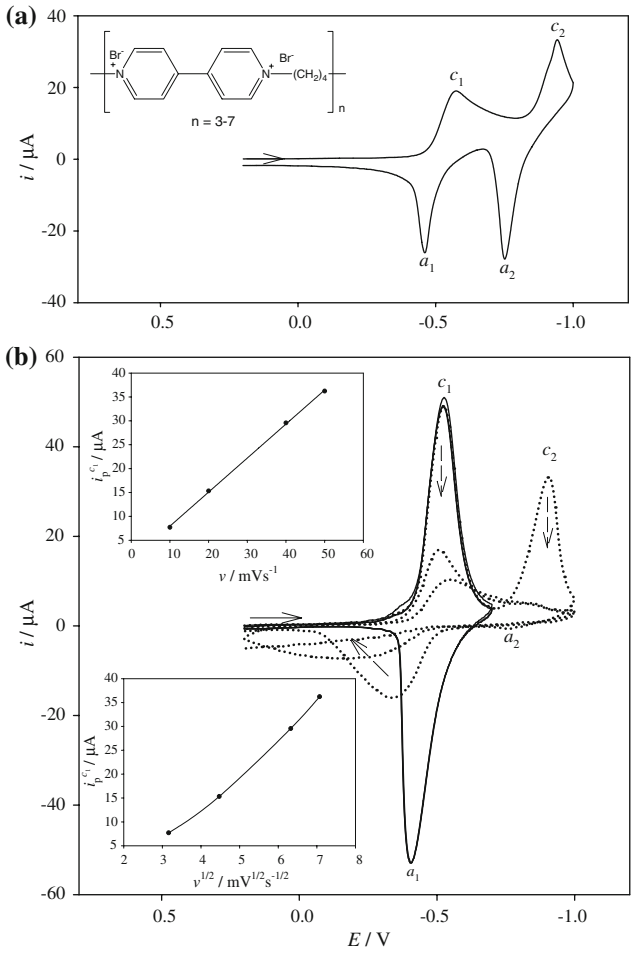

Fig. 1 Cyclic voltammograms of (**a**) deaerated pH 4.2 BR buffer solution containing 0.1 wt% of viologen oligomers at GCE, and (**b**) continuous scans (3 cycles) of PVGCE in the deaerated 0.1 M NaCl solution; potential was reversed at (——) −0.7 V and at (·····) −1.0 V, respectively. Scan rate: 100 mV s^{-1}. The insets in (**b**) show the plots of cathodic peak current $i_p^{c_1}$ vs. scan rate v, (and $v^{1/2}$, respectively. The PVGCE was fabricated by electropolymerizing at −1.0 V for 60 s in the solution used for (**a**)

3 Results and discussion

3.1 Voltammetric behavior of viologen oligomers and polyviologen at GCE

Figure 1a shows the cyclic voltammogram (CV) of viologen oligomers at GCE in deaerated pH 4.2 BR buffer solution. The arrow indicates the initial direction of the scan. Two redox couples (c_1/a_1 and c_2/a_2) were observed and they are related to the redox reactions of the respective nitrogen center in each bipyridine structure. Two nitrogen centers reduce at different potentials indicating that certain interactions take place between the two nitrogen centers through the delocalized π electrons. c_1 is a typical reduction wave produced from a soluble species; however, the second reduction wave c_2 seems to have an adsorptive behavior. In the reverse scan, the anodic waves, a_2 and a_1, exhibit the typical features of a stripping peak (sharp and symmetric) indicating that the reductive products of viologen oligomers adsorbed on the electrode surface. In other words, PV film was formed at a potential more negative than wave c_2. Several electrode materials were examined; however, PV film can only be formed on GCE. This limitation may result from the fact that PV film is only formed at potentials beyond the reductive wave c_2. For many metallic electrodes, this potential region is close to, or even exceeds, their cathodic potential limits. The indium-tin oxide (ITO) glass electrode, which has a similar cathodic limit to GCE, is also suitable for PV film formation. During electropolymerization, the resulting viologen moiety in the PV film is reduced to its cation radical so that the growing film on the electrode surface appears intensely blue [29, 30].

Figure 1b demonstrates that PV film is electroactive (the solid curves) but its electroactivity rapidly decreases if the switch potential exceeds the reduction wave c_2 (the dotted curves). Except for the first cycle, no noticeable change in the peak height (the solid curves) was observed indicating that PV film is highly stable. On the other hand, the dotted curves and dashed arrows indicate that the PVGCE rapidly lost its redox activity when the potential scans were reversed at the second reduction wave c_2. Based on the fact that PV film can only be formed by electrolysis in the potential region where the second reduction wave occurs, we thus suppose that the decrease in electroactivity was due to the change in chemical structure of the PV film when the potential was repeatedly scanned to the electropolymerization potentials. It should be emphasized that the PV film was still visible on the electrode surface after the successive potential scans; it merely lost its redox activity. The two insets in Fig. 1b demonstrate that the peak current of wave c_1, $i_p^{c_1}$, is proportional to the potential scan rate. This behavior implies that the PV film is really thin.

3.2 The crucial component in PV film formation

Earlier studies about the electropolymerization of viologen oligomers demonstrate the necessity of a cross-linking reagent in PV film formation [29, 31]. Recently, it was reported that a stable PV film can form on GCE in a BR buffer solution without cross-linking reagents but no investigation was provided [27]. In this study, it has been recognized that the crucial component in the PV film formation is phosphoric acid contained in the BR buffer solution. Without phosphoric acid, the two redox couples of viologen oligomers shown in Fig. 1a are still observed in any frequently-used deaerated electrolyte solution but no film can form.

To demonstrate the essentiality of H_3PO_4, a PVGCE was prepared in deaerated NaCl solution containing 32 mM H_3PO_4 and 0.1 wt% of viologen oligomers. 32 mM H_3PO_4 was used because the pH 4.2 BR buffer solution contains the same concentration of H_3PO_4. The identical electropolymerization conditions used in Fig. 1b were also employed. This PVGCE was immersed in a 0.1 M NaCl solution in which a CV was recorded as shown in Fig. 2a. An analogous redox couple c_1/a_1 shown in Fig. 1b was also observed indicating the formation of a PV film on the electrode. This redox couple is stable in continuous potential scans unless the switch potential exceeds the reduction wave c_2. A PV film cannot form on GCE in 0.1 M NaCl solution containing either 32 mM acetic acid or 32 mM boric acid, which is also the main component in BR buffer solution. A PVGCE as fabricated for Fig. 2a was immersed in the 32 mM H_3PO_4 solution and the successive CVs are shown in Fig. 2b. Figure 2a and b are very similar to each other except that the redox couple c_1/a_1 in Fig. 2b is less stable and the $i_p^{c_1}$ decayed more rapidly when the switch potential was −1.0 V. This behavior additionally suggests that phosphate is involved in PV film formation.

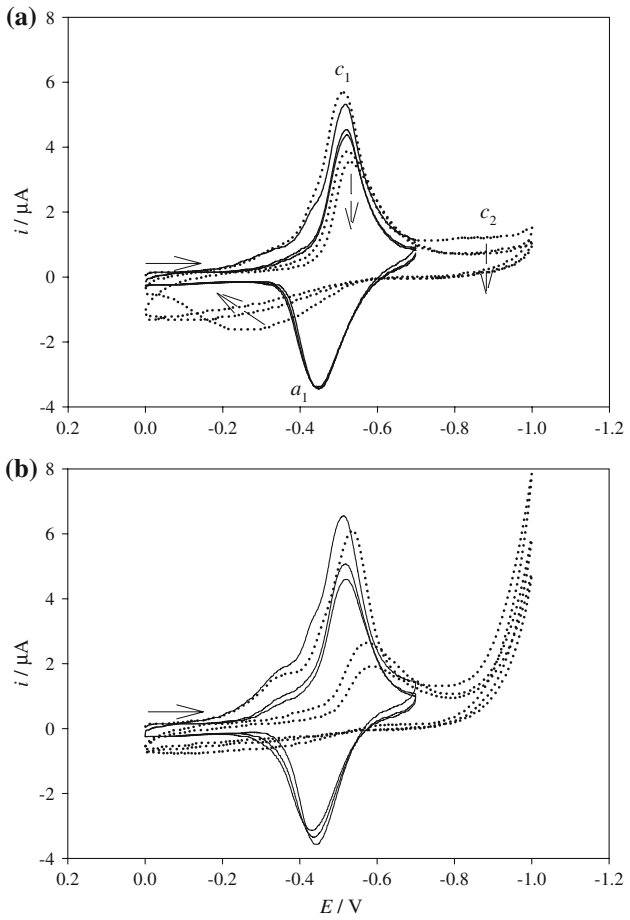

Fig. 2 Continuous cyclic voltammograms (3 cycles) of PVGCEs in (**a**) deaerated 0.1 M NaCl and in (**b**) deaerated 0.032 M H_3PO_4 solutions. The cathodic switch potential was (——) −0.7 V and (·····) −1.0 V, respectively. Scan rate: 100 mV s^{-1}. The PVGCEs were fabricated by electropolymerizing at −1.0 V for 60 s in the 0.1 M NaCl solution containing 0.032 M H_3PO_4 and 0.1 wt% viologen oligomers

Based on the crucial role of H_3PO_4 in PV film formation plus all aforementioned observations, we suppose that the phosphate ion may have an analogous function as the cross-linking reagents mentioned in earlier studies [29, 31]. In other words, the viologen oligomers perhaps polymerize through the phosphate cross-link. Unfortunately, no sufficient information was obtained to support this assumption. PV film is not conductive and thus only a limited thickness of polymer film can be formed so that no signal of phosphate and other functional groups can be detected in the FT-IR and FT-Raman spectra. Therefore, the chemical structure and polymerization mechanism of PV film cannot yet be determined. Some novel applications can be provided if the phosphate group is really connected between the viologen oligomers in the PV film because molecules with phosphate terminals may be bound to the electrode surface though this process. We intend to further study the role of the phosphate group in PV film formation.

The PV film is a cationic polymer [27] which is capable of accumulating anions by means of its anion-exchange properties. This feature was confirmed by performing cyclic voltammetry in solutions containing either $Fe(CN)_6^{4-}$ or Fe^{3+}. In the $Fe(CN)_6^{4-}$ solution, enhancement of the oxidation current was observed at the PVGCE; however, no reduction current of Fe^{3+} was detected at the same electrode in the Fe^{3+} solution.

3.3 Effects of electropolymerization time on the redox activity of PV film

It is supposed that the anion-exchange capacity of a PV film is directly proportional to the number of positively charged sites that are the quaternary nitrogen sites in the bipyridine structure, and these sites reflect the redox activity of a PV film. Thus, the best electropolymerization condition is that the maximum number of positively charged sites can be obtained. If the concentration of viologen oligomers is fixed, the potential and time of electropolymerization determine the number of positively charged sites. This number was reflected in the reduction peak current $i_p^{c_1}$ of PV films that were fabricated under different electropolymerization conditions. A higher $i_p^{c_1}$ indicates more positively charged sites in the PV film.

The solid circles shown in Fig. 3 demonstrate the connection between the $i_p^{c_1}$ of the PV film (electropolymerized at −1.0 V) and the electropolymerization time, t_e. Several electropolymerization potentials have been examined; however, the highest reduction peak current $i_p^{c_1}$ of PV film

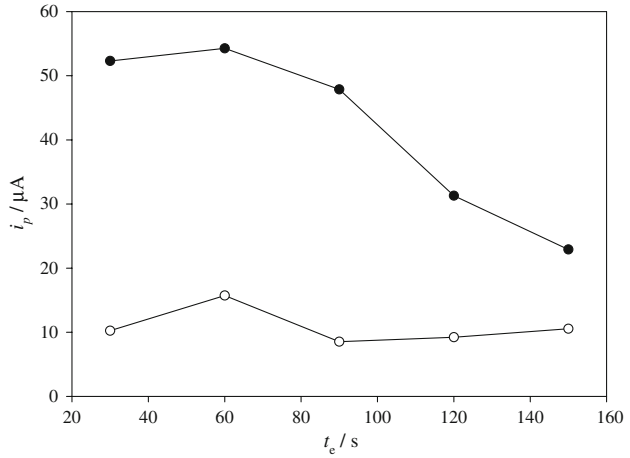

Fig. 3 Effects of electropolymerization time t_e on (•) first cathodic peak currents of the PVGCEs in 0.1 M NaCl solutions and on (○) anodic peak currents of $Fe(CN)_6^{4-}$ adsorbed on the relevant PVGCE. Scan rate: 100 mV s^{-1}. The electropolymerization solution is the same one indicated in Fig. 1

was always obtained when the electropolymerization potential was −1.0 V. The optimum electropolymerization time was 60 s.

$Fe(CN)_6^{4-}$ ions were used to demonstrate the correlation between the number of positively charged sites carried by the PV film and the anion-exchange capacity. Each PVGCE used to construct the solid circles in Fig. 3 were immersed in a 10 μM $Fe(CN)_6^{4-}$ solution for 60 s, rinsed with deionized water, and then transferred to a pure 0.1 M NaCl solution where the CVs of $Fe(CN)_6^{4-}$ adsorbed on the PVGCE were recorded. The numeric values of the oxidation peak current i_p of $Fe(CN)_6^{4-}$ were used for the comparison of anion-exchange capacities possessed by individual PVGCE. In Fig. 3, the blank circles illustrate the relation between i_p of the adsorbed $Fe(CN)_6^{4-}$ and the electropolymerization time employed for preparing the PVGCE. Obviously, the $i_p^{c_1}$ of the PV film has a positive correlation to the i_p of the adsorbed $Fe(CN)_6^{4-}$. Based on these observations electropolymerization was performed at −1.00 V for 60 s in the following experiments because the highest anion-exchange capacity can be obtained under this condition.

3.4 Voltammetric behavior of vitamin C at bare GCE and PVGCE

Based on the pK_{a1} value of vitamin C (4.71), it is believed that all vitamin C molecules are in their base form in slightly basic aqueous solutions. PV film thus is suitable for accumulating vitamin C molecules on an electrode surface under an alkaline environment. However, the PV film in basic solutions is not as stable as in acidic conditions. In basic solutions, the voltammetric response of the redox couple c_1/a_1 shown in Fig. 1b gradually diminished when multiple scans were performed. Based on this fact, a pH 7.8 aqueous solution composed of H_3BO_3 and NaOH was chosen as the medium for the following experiments because PV film is sufficiently stable and the detection sensitivity of vitamin C is notably enhanced under this condition.

Figure 4a shows the CVs of vitamin C at the GCE with and without PV film, respectively. The PVGCE significantly improves the current response. This improvement must be due to the enhancement of surface concentration. The power of PV film was more obviously revealed in the solution containing lower concentration of vitamin C. In Fig. 4b, the anodic current response of vitamin C at a bare GCE was almost indistinguishable from the background current; however, a peak-shape response was still observed at a PVGCE in the solution containing only 25 μM of vitamin C. Based on Fig. 4, the potential of +0.1 V was employed for the amperometric detection of vitamin C.

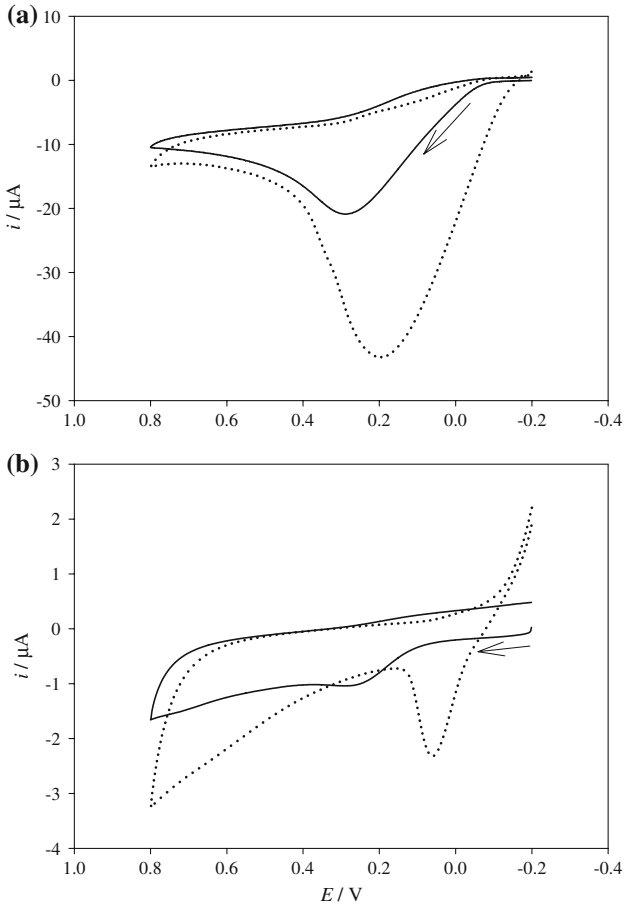

Fig. 4 Cyclic voltammograms of 0.1 M H_3BO_3 solutions (pH 7.8) containing (**a**) 1 mM and (**b**) 25 μM vitamin C at (——) bare GCE and (·····) PVGCE, respectively. Scan rate: 100 mV s^{-1}.

3.5 The hydrodynamic amperometric behavior of vitamin C

The detection of vitamin C was carried out in the solution composed of H_3BO_3 and NaOH because a higher and stabler current response can be obtained. This conclusion was demonstrated in Fig. 5 where the amperometric behavior of vitamin C was recorded at +0.1 V in four different solutions under vigorous stirring. In the electrochemical batch cell, 5, 10, 15, 20, 25, 25, and 25 µl of 1 mM vitamin C were successively injected into the cell; the injection volumes covered the concentration range from 1.25 to 30.3 µM. The current responses produced from higher concentration of vitamin C were still stable in the H_3BO_3 or H_3PO_4 solution. However, the current response recorded in the H_3BO_3 solution was higher. The pH 7.8 solution consisted of H_3BO_3 and NaOH was thus employed as the medium for amperometric detection of vitamin C.

3.6 Calibration

The amperomogram shown in Fig. 5 was employed to build the calibration curve where the current responses measured for a series injection of the vitamin C standard solutions were recorded as a function of vitamin C concentration. Such a calibration curve is shown in Fig. 6 where the curve exhibits a linear behavior in the front portion of the entire concentration range and then a saturation behavior is observed, indicating the limited anion-exchange capacity of PV film. The inset in Fig. 6 demonstrates a linear behavior between 5.00×10^{-7} M and 1.23×10^{-4} M with a slope (µA μM^{-1}) and a correlation coefficient of 0.4914 and 0.9993, respectively.

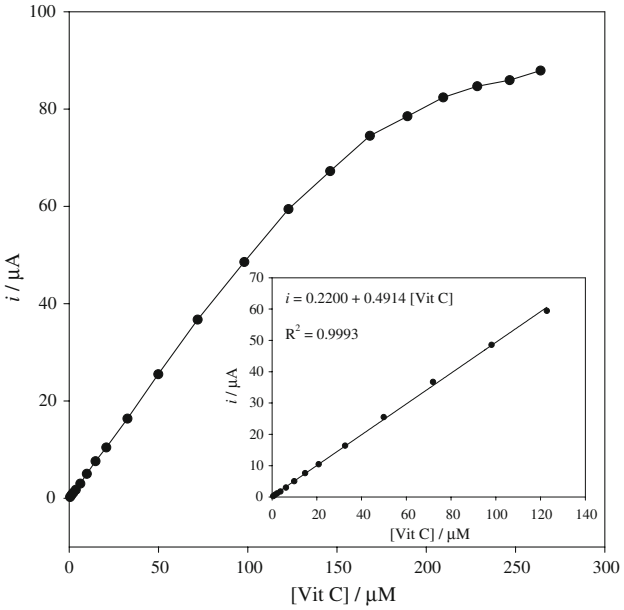

Fig. 6 The calibration curve of vitamin C. It was established based on the steady current response taken from the current transients produced by successive injections of vitamin C in a batch cell. The inset shows the linear range of the calibration curve

3.7 Determination of vitamin C in fruit juices and in pharmaceutical product

The procedures used for the vitamin C measurements were applied to determine vitamin C in fruit juices and in

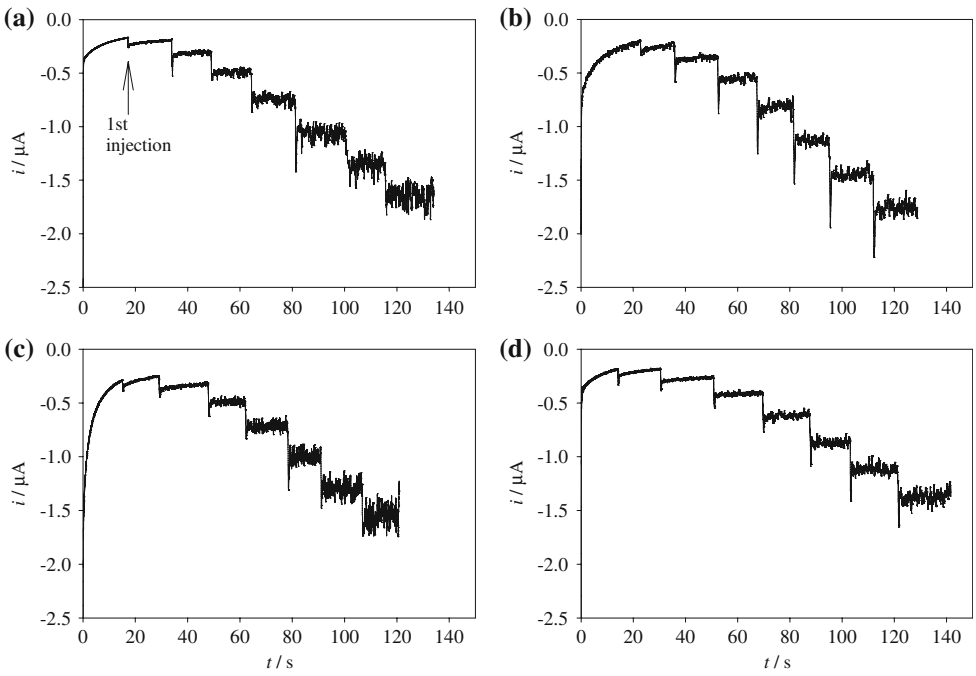

Fig. 5 Current transients recorded at +0.1 V with successive injections of vitamin C in a batch cell containing 4 mL 0.1 M (**a**) acetic acid, (**b**) H_3BO_3, (**c**) PBS, and (**d**) H_3PO_4 solutions. The pH of all solutions was 7.8

Table 1 Determination of vitamin C in several aqueous samples

	H_3BO_3 solution (pH = 7.8)	Canned apple juice	Fresh orange juice	Effervescent tablet (contain ascorbic acid and Ca)
Detected value, original (μM)	0	2017 ± 56 ($n = 3$)	1313 ± 49 ($n = 3$)	10,563 ± 532 (= 930 ± 47 mg) ($n = 3$)
Vitamin C added (μM)	6.1	–	–	–
Vitamin C found after added (μM)	6.2 ± 0.3 ($n = 4$)	–	–	–
Vitamin C found by titration (μM)	–	2071 ± 58 ($n = 3$)	1447 ± 96 ($n = 3$)	–
Vitamin C labeled on box	–	–	–	1000 mg
Recovery (%)	102 ± 4.9	–	–	–

pharmaceutical product. The experimental results were collected in Table 1. To demonstrate that this method is precise and accurate, a recovery test was carried out in pH 7.8 H_3BO_3 solution and the recovery ratio 102 ± 4.9% was obtained. The electrochemical detection of vitamin C was performed for two fruit juices. One was canned apple juice and the other was orange juice prepared from fresh oranges. Before the electrochemical detection was carried out, the pH values of the fruit juices were adjusted to 7.8 by adding NaOH. The apple juice was detected directly; however, the orange juice was filtered before the electrochemical detection. Amperometry was employed and steady current response was obtained under vigorous stirring. The fruit juices were also analyzed by iodimetric titration in order to provide a comparison. The results were collected in Table 1 and similar values were obtained between two different methods. A commercial effervescent tablet containing 1000 mg of ascorbic acid, 327 mg of calcium carbonate, and 1000 mg of calcium lactate-gluconate was dissolved in 500 mL deionized water and then diluted to appropriate concentration. The final solution pH was adjusted to 7.8. The solution was analysed by procedures identical to those mentioned above and the results are shown in Table 1. The value of 930 ± 47 mg of vitamin C was determined by the electrochemical analysis.

4 Conclusions

This study demonstrates that phosphoric acid contained in BR buffer solution is crucial to the formation of PV film on GCE. The PV film exhibits an anion-exchange property that is able to accumulate anionic species such as ferrocyanide and vitamin C. The PVGCE has been used for detection of vitamin C contained in fruit juices and in pharmaceutical product. Good results have been obtained in this study. It is assumed that phosphate is a cross-linking reagent involving in the chemical structure of PV film. However, further experiments are needed to investigate the real role of phosphate ion in the PV film formation.

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