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Short communication

Hydroquinone modified chitosan/carbon film electrode for the selective detection of ascorbic acid

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ABSTRACT

A redox active polymer, hydroquinone modified chitosan (Q-chitosan) was synthesized and characterized by IR and ^{1}H NMR spectroscopy. The nanocomposite of Q-chitosan with carbon was prepared and used to construct a stable conductive film on the electrode surface. The SEM studies confirms that the Q-chitosan/C composite covers the electrode surface with polymer embedded 50 nm size carbon particles. The formal redox potential, E^0 of the Q-chitosan/C composite modified electrode is evaluated to be 0.09 V vs. Ag/AgCl at pH 7 and the composite electrode shows an excellent electrocatalytic activity toward the ascorbic acid (AA) at 0.0 V vs. Ag/AgCl. It is more negative potential than the similar AA biosensors and the lower potential oxidation of AA enabled the selective detection of AA over major interferences such as dopamine and uric acid. Using amperometric method, the linear range for ascorbic acid is estimated to be 10 μM to 5 mM with the detection limit of 3 μM and the sensitivity is 0.076 μA μM^{-1} cm^{-2}.

1. Introduction

Biomolecule ascorbic acid (AA) and dehydroascorbic acid are known as a vitamin C, which plays a vital role in human body as a radical scavenger and an antioxidant. Its deficiency leads to the diseases like scurvy and protect much kind of infections. The recommended daily intake ascorbic acid is about 70–90 mg for adults (Levine, Wang, Padayatty, & Morrow, 2001). Traditional procedures for the AA determination are generally based on enzymatic (Marchesini, Montuori, Muffato, & Maestri, 1974) and chromatographic methods with fluorimetric detection (Tsao & Young, 1985). The electroanalytical methods have been applied for the AA detection (Finley & Duang, 1981), because of its simplicity and high detection limit. In electroanalytical methods, the electrode modification plays an important role in the detection process. Several reports are available on successful modification of electrode surface by co-immobilization of catalyst along with mediators for the sensing of AA (Fernandez & Carrero, 2005; Zhang & Dong, 2004; Zhang, Yang, Huang, Jiao, & Li, 2008). AA oxidation on the conventional electrodes yields the dehydroascorbic acid (Turyan & Kohen, 1995) and this oxidized form of AA adsorbed on the electrode surfaces results in the fouling of electrode and decrease in sensitivity. The detection of AA is also limited by interfering species like dopamine and uric acid, so proper modification of the electrode surface is essential for the selective determination of AA with wide detection range and superior sensitivity (Kumar, Lo, & Chen, 2008; Lin et al., 2008; Manjunatha, Shivappa, Melo, D'Souza, & Venkatesha, 2010).

Quinone compounds have been known as perspective mediators because of their relatively negative redox potentials compared to those of ferrocene and some of osmium derivative. Hydroquinone immobilized through spacer to the polyacrylic acid polymer grafted on carbon was prepared and used for the bio-fuel cell applications (Tamaki, Ito, & Yamaguch, 2007). Further quinone based compounds are immobilized on electrodes and employed as a mediator for the electrochemical sensing of the AA, and NADH (Murthy & Sharma, 1997).

Chitosan, isolated from chitin, is the linear and partly acetylated (1–4)-2-amino-2-deoxy-β-D-glucan (Muzzarelli, 1977, 2012). Degree of deacetylation can be used to tune the properties of chitosan (Bodnar, Hartmann, & Borbely, 2005). Nanocomposite of chitosan with carbon nanotube was reported for the synergetic action on the biosensor response (Zhang et al., 2008). Ferrocene modified chitosan derivatives as a redox mediator are synthesized and used for the enzyme based biosensor construction and mediators for the electrocatalysis (Yang, Zhou, & Sun, 2008). Cetyltrimethylammonium bromide with chitosan was used for the selective determination of the AA (Zou, Luo, Ding, & Wu, 2007).

Abbreviations: Q-chitosan, hydroquinone modified chitosan; C, carbon; AA, ascorbic Acid; DA, dopamine; UA, uric acid.

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In the present study, we synthesized a hydroquinone modified chitosan (Q-chitosan) polymer by a simple method and its composite with carbon (Q-chitosan/C) film modified electrode was prepared for an electrochemical sensor to detect AA.

2. Materials and methods

2.1. Materials and instruments

Chitosan (448869), 2,5-dihydroxybenzaldehyde, sodium cyanoborohydride ($NaCNBH_3$), acetic acid, ascorbic acid, dopamine hydrochloride, uric acid and sodium hydroxide were obtained from Sigma–Aldrich Chemicals. Carbon black was obtained from the Jin chemicals company. All the other chemicals are of analytical grades and used without further purification. Water purified from Millipore system was used in all experiments.

^{1}H NMR was recorded with Bruker-500 spectrometer. FT-IR spectra were measured on Nicole Avatar 330 FTIR (Thermo Electron Corporation) using dry KBr pellet. SEM images were obtained with Hitachi S-4300, CHI 900 potentiostat was used for electrochemical measurements. The elemental analysis was performed with Thermo Flash 1112 CHNS analyzer. For preparing composite electrode, 2 μL of Q-chitosan/C composite solution was drop coated on glassy carbon (GC) (3 mm bioanalytical system) or plasma treated printed carbon electrode (PCE) (5 mm, SE 100, Zensor R&D, Taiwan) and dried at room temperature. Cyclic voltammetry and amperometric measurements were performed in 0.1 M phosphate buffer (PB) pH 7 with Ag/AgCl (3 M KCl) reference and platinum wire counter electrodes.

2.2. Synthesis of hydroquinone modified chitosan (Q-chitosan)

Chitosan is known to react with aldehydes and ketones (Schiff reaction) in particular with 3,4-dihydroxybenzaldehyde (Muzzarellli, 1988; Ravi Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). 38 mg of 2,5-dihydroxybenzaldehyde (0.27 mmol) was dissolved in 15 mL methanol and the solution was added dropwise over 30 min into 15 mL of 5 mg/mL chitosan in 1% acetic acid solution followed by stirring overnight to ensure the complete reaction. The molar amount of the amine groups in the chitosan is about twice as the amount of the aldehyde. Excess of sodium cyanoborohydride was added to reduce the imine linkage to amine and stirred over 24 h at room temperature. The resulting reaction mixture was filtered and collected by 10,000 molecular weight cut-off polycarbonate membrane and repeatedly washed with distilled water till the pH of the filtrate reached to 6. The polymer solution was collected and dried under the vacuum.

2.3. Treatment of carbon

Commercially available carbon black powder (50–100 nm and sulfur free) was acid treated according to the procedure reported (Wang, Yin, Zhang, Wang, & Gao, 2007) with a slight modification. Carbon black powder was boiled in 5 M HNO_3 overnight, filtered and repeatedly washed with deionized water. It was dried in air followed by drying in vacuum.

2.4. Preparation of electrode

The polymer composite (Q-chitosan/C) was prepared by mixing 200 μL of 1% Q-chitosan (10 mg/mL) in 1% acetic acid and 2 mg of carbon powder (particle size 50–100 nm) and sonicating for 1 min. The mass ratio of Q-chitosan to carbon is 1 to 1 in the resulting composite solution. 2 μL of the composite solution was drop coated on a PCE and dried at room temperature.

3. Results and discussions

3.1. Synthesis and characterization of Q-chitosan polymer

The Q-chitosan polymer was synthesized as 2,5-dihydroxybenzaldehyde easily reacts with the amino group of chitosan through Schiff base condensation. Subsequent reduction of Schiff base by sodium cyanoborohydride ($NaCNBH_3$) gives the hydroquinone functionalized chitosan (Q-chitosan). The modification of the chitosan was characterized by FT-IR (Fig. S1). The IR spectrum of 2,5dihydroxy benzaldehyde at 1665 cm^{-1} for the characteristic C=O of free aromatic aldehyde group and the absorption bands at 1582 cm^{-1} for aromatic ring vibrations and the broad peak at 3400 cm^{-1} for the OH stretching vibrations. The principal IR absorptions bands of chitosan observed for an amide at 1654 cm^{-1} (amide I) and 1596 cm^{-1} (amide II). CH_3 bands at 2926 cm^{-1} and 1381 cm^{-1}, C–N axial deformation at 1422 cm^{-1} and C–N amino groups axial deformation 1323 cm^{-1}, besides the three characteristic polysaccharide bands, 1156 cm^{-1}, 1074 cm^{-1} and 1030 cm^{-1} sharp intense peak at 2370 cm^{-1} which correspond to the –CN group, Q-chitosan shows the IR bands of chitosan with the additional bands of aromatic ring of hydroquinone at 1450–1550 cm^{-1} and 778 cm^{-1} and 822 cm^{-1} conforms the modification of the chitosan.

^{1}H NMR spectrum of Q-chitosan was recorded in $CD_3COOD + D_2O$ (1:20) Fig. S2 exhibits the characteristic polysaccharide protons of chitosan at δ 1.88 (s, 3H NHCOCH3), δ 3.035 (s, H, H2 and H2′), δ 4.43 to δ 3.49 (7H of NH-CH2, H3, H4, H5, H6 and H6') along with the aromatic proton at δ 7.50 to δ 6.71 (3H, ph) confirmed the modification of the chitosan polymer by 2,5-dihydroxybenzaldehyde. From the elemental analysis, the percentage of C, H, and N found to be 42.01%, 6.20%, and 5.29%. For Q-chitosan polymer. These values are well correlated with the extent of substitution of 50%.

Fig. 1 Q-chitosan film preserves the film-forming characteristics of chitosan and shows smooth morphology. Q-chitosan/C composite matrix shows homogeneous three dimensional porous structures due to the carbon particles embedded in Q-chitosan and the size of the particles is around 50–100 nm (Fig. 1B). The preparation of homogeneous and stable Q-chitosan/C film is attributed to the electrostatic interaction between the carbon particles and Q-chitosan.

3.2. Electrochemical characterization of Q-chitosan/C

The E^0 of the Q-chitosan/C composite modified PCE is close to the E^0 of previously reported hydroquinone immobilized on carbon grafted polyacrylic acid (Tamaki et al., 2007). Fig. 2A shows the cyclic voltammograms of Q-chitosan/C composite at different scan rate in the range of 1–50 mV/s. The redox peak current peak to peak separation was linearly increased with the scan rate. Fig. 2B shows the linear dependence of the redox peak current to the scan rate suggests that the charge transport through the film occurred under the surface confined phenomenon of the electron transfer at the Q-chitosan/C modified electrode. Similar behavior was reported by Zhou, Yang, and Sun (2008) and Zou et al. (2007), for the chitosan modified ferrocene polymer embedded with carbon nano tubes.

3.3. Electrocatalytic oxidation of AA

Q-chitosan/C modified electrode was used for the electrocatalytic oxidation of AA, Fig. 3A shows the electrocatalytic response of Q-chitosan/C modified PCE (a) and unmodified PCE (b) in the presence of 5 mM AA. The unmodified PCE showed the electrochemical oxidation of the AA at 0.14 V vs. Ag/AgCl which is near to the reported value by Prasad, Muthuraman, and Zen (2008)

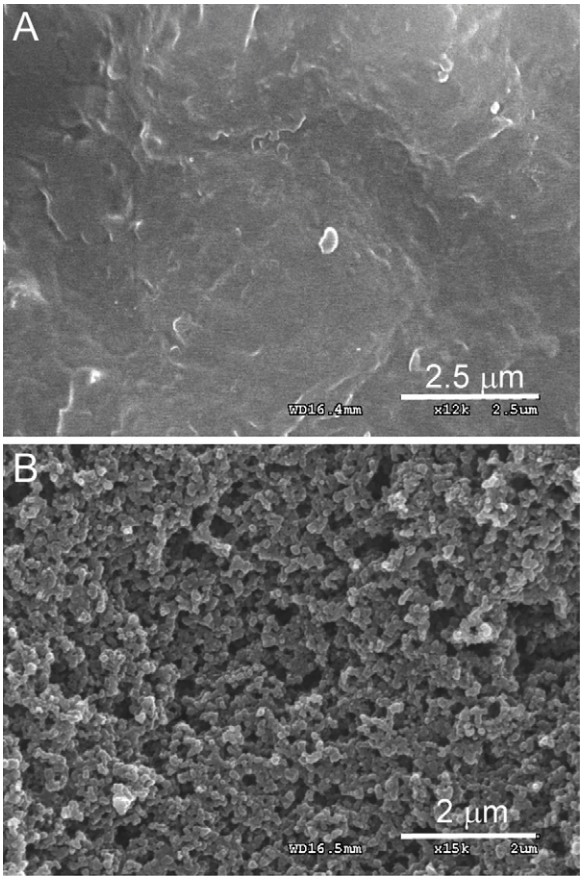

Fig. 1. SEM images of Q-chitosan (A) and Q-chitosan/C (B) film modified electrode, surface view.

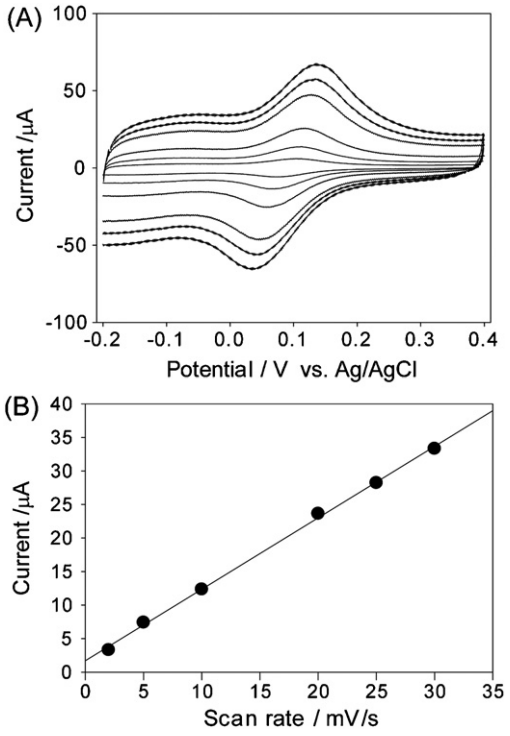

Fig. 2. CVs of Q-chitosan/C electrode at different scan rates; 2, 5, 10, 20, 25 and 30 mV/s in 0.1 M PB at pH 7 (A) and plot of I_{pa} vs. scan rate (B).

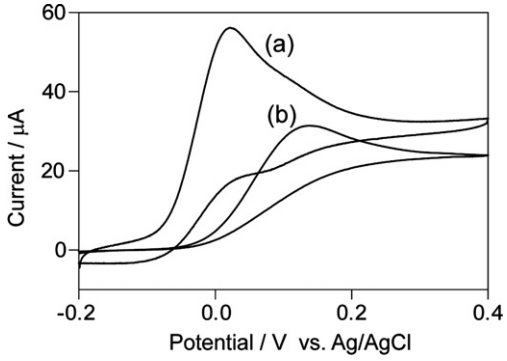

Fig. 3. Electrochemical oxidation of 5 mM AA at Q-chitosan/C modified electrode (a) and bare PCE (b).

while the Q-chitosan/C modified electrode oxidized AA at 0.02 V with anodic peak current of 56 μA. The Q-chitosan/C composite shifted the electro-oxidation of AA to more negative potential with enhanced anodic peak current.

3.4. Amperometric determination of ascorbic acid

The amperometric response of the Q-chitosan/C electrode for the AA oxidation was measured at an applied potential of 0.1 V vs. Ag/AgCl with incremental addition of concentrated AA in 0.1 M PB during constant stirring. It shows fast catalytic response and 95% of the steady state current was reached within less than 3 s (Fig. 4A). The response is linear in the range of 10 μM and 5 mM (Fig. 4B) and

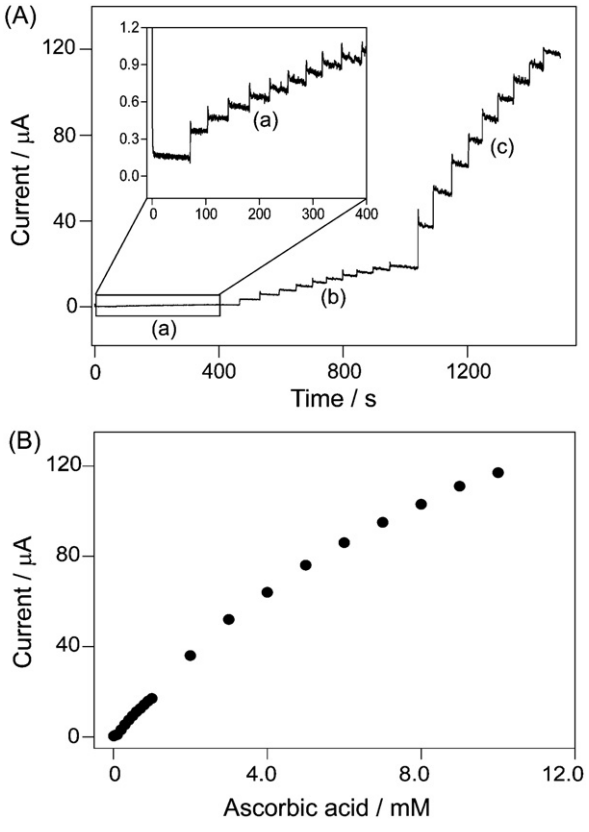

Fig. 4. (A) Amperometric responses of Q-chitosan/C electrode at 0.1 V to successive addition of 10 μL of concentrated AA solutions; 5 mM in region (a), 50 mM in (b), and 500 mM in (c) into 5 mL of pH 7, 0.1 M PB. (B) Calibration curve for ascorbic acid concentration measurement.

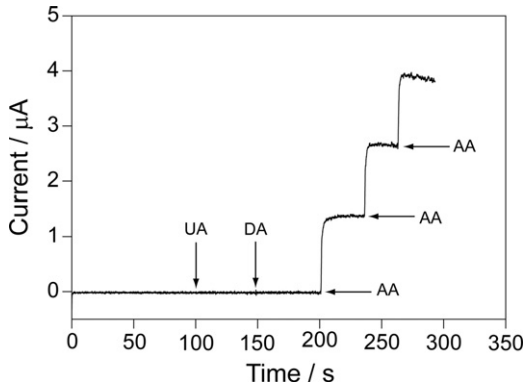

Fig. 5. Amperometric response of the Q-chitosan/C electrode to AA at an applied potential of 0.0 V. The UA, DA, and AA were successively added to make 100 μM solutions at each arrow into pH 7, 0.1 M PB.

the sensitivity was 0.076 μA μM^{-1} cm^{-2}. The detection limit was found to be 3 μM on signal-to-noise ratio of 3.

3.5. Interferences and analytical response

The effect of interferences on Q-chitosan/C electrode was demonstrated by the typical amperometric *i–t* curve recorded in continuously stirred PB at 0.0 V. 100 μM DA (dopamine) and UA (uric acid) showed no significant change in amperometric response current at such a low potential but the same amount of AA in the same solution showed the significant increased in current as shown in Fig. 5. These results convincingly indicated that the Q-chitosan/C electrode can be used as a sensor for the selective determination of the AA in the presence of interferences like dopamine and the uric acid.

4. Conclusions

Hydroquinone modified chitosan/carbon film modified electrode was fabricated and its electrochemical properties were investigated. It was found that the peak current responses of the ascorbic acid were shifted toward the more negative potential on Q-chitosan/C modified electrode. Steady state current shows that UA and DA are not interfering in AA detection at Q-chitosan/C film electrode. The Q-chitosan/C film shows wide range of AA detection from 10 μM to 10 mM with the detection limit of 3 μM and the sensitivity is 0.076 μA μM^{-1} cm^{-2}. This Q-chitosan/C film modified electrode has good stability, reproducibility and easily can be prepared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.09.024.

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