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Electrochemical Sensor for Determination of Ascorbic Acid Using a 2-Chlorobenzoyl Ferrocene/Carbon Nanotube Paste Electrode

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A chemically modified carbon paste electrode with 2-chlorobenzoyl ferrocene (2CBF) and carbon nanotube (2CBFCNPE) was employed to study the electrocatalytic oxidation of ascorbic acid in aqueous solution using cyclic voltammetry, square wave voltammetry and chronoamperometry. The diffusion coefficient ($D = 1.42 \times 10^{-6}$ cm^2 s^{-1}), and the kinetic parameter such as the catalytic rate constant ($k = 3.7 \times 10^{3}$ M^{-1} s^{-1}) of ascorbic acid oxidation at the surface of 2CBFCNPE were determined using electrochemical approaches. It has been found that under an optimum condition (pH 4.0), the oxidation of ascorbic acid at the surface of such an electrode occurs at a potential about 85 mV less positive than that of an unmodified carbon paste electrode. Applying square wave voltammetry, in phosphate buffer solution (PBS) of pH 4.0, the oxidation current increases linearly with two concentration intervals of ascorbic acid, one is 1.0×10^{-7}-2.5×10^{-6} M and the other is 2.5×10^{-6}-7.0×10^{-5} M. Detection limit (3δ) was obtained 64.0 nM. This method was also examined for determination of ascorbic acid in some real samples.

Keywords: Ascorbic acid, Voltammetry, Carbon nanotubes paste electrode, Drug analysis

INTRODUCTION

Ascorbic acid as a water-soluble vitamin is an extremely important substance which plays a unique redox and electrochemical role. It is known as an antioxidant [1-4]. It is one form of vitamin C. Eating foods rich in vitamin C is important for overall health, especially if one is at risk for high blood pressure, common cold, Alzheimer's disease, mental illness, cancer, infertility and cancer [5-8]. Results of scientific studies on whether ascorbic acid is helpful for preventing heart attack or stroke indicated that it does not lower cholesterol levels or reduce the overall risk of heart attack, but evidence suggests it may help protect arteries against damage. Results of many population-based studies suggest that ascorbic acid may be associated with lower rates of cancer, including skin cancer, cervical dysplasia (changes to the cervix which may be cancerous or precancerous, picked up by pap smear), and, possibly, breast cancer [9,10].

Due to its importance, many analytical techniques have been developed and reported for the determination of ascorbic acid in pharmaceutical preparations, biological fluids, food and beverages. The methods are: chromatography, spectrophotometry, mass spectrometry, flow injection, chemiluminescence and electrochemical methods [11-13]. Among these methods, electrochemical approaches are used extensively for the especial and sensitive properties because other methods usually need sample pretreatment (*e.g.*, extraction, complex formation) that is time-consuming and grinding [14]. It is generally

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believed that direct redox reaction of this specie at bare electrodes such as carbon or those metallic ones, Hg, Au, Pt require a high overpotential. Thus, there have been numerous attempts to enhance the electrode kinetics using various chemically modified electrodes (CMEs) [15-20]. In recent years, chemically modified carbon paste electrodes have received increasing attention due to their potential applications in various analyses, as well as their low background current (compared to solid graphite or noble metal electrodes), high sensitivity, facility to prepare, low cost, stable response, large potential window and simple surface renewal process [21,22].

Since the discovery of carbon nanotubes (CNTs), great investigations have been centralized on the studies of their properties and applications [23-27], because CNTs possess several unique properties such as small size, high electrical and thermal conductivity, high chemical stability, high mechanical strength and high specific surface. Moreover, the subtle electronic behavior of CNTs reveals that they have the ability to promote electron-transfer reaction and have a high electrocatalytic effect when used as electrode materials [28-31]. All these fascinating properties make CNTs suitable candidates for the modification of electrodes [32-35].

In the present work, following the idea of searching new methods for ascorbic acid detection, we describe the preparation of a new electrode composed of 2-chlorobenzoyl ferrocene modified carbon nanotube paste electrode (2CBFCNPE). We describe initially the preparation and suitability of a 2CBFCNPE as a new electrode in the electrocatalysis and determination of ascorbic acid in an aqueous buffer solution. Finally this new constructed electrochemical sensor is used for determination of ascorbic acid in the real samples.

EXPERIMENTAL

Apparatus and Chemicals

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System (GPES) software. A conventional three electrode cell was used at 25 ± 1 °C. An Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and 2CBFCNPE were used as the reference, auxiliary and working electrodes, respectively. A Metrohm 710 pH meter was used for pH measurements.

Ascorbic acid and all other reagents were of analytical grade and were obtained from Merck (Darmstadt, Germany). The buffer solutions were prepared from *ortho*-phosphoric acid and its salts in the pH range of 2.0-9.0. Multi-walled carbon nanotubes (purity more than 95%) with o.d. between 10 and 20 nm, i.d. between 5 and 10 nm, and tube length from 10 to 30 μm were prepared from Nanostructured & Amorphous Materials, Inc. 2CBF was synthesized in our laboratory as reported previously [36].

Preparation of the Electrode

The 2CBFCNPEs were prepared through mixing 0.01 g of 2CBF with 0.89 g graphite powder and 0.1 g carbon nanotubes with a mortar and pestle. Then, ~0.7 ml of paraffin was added to the above mixture and mixed for 20 min until a uniformly-wetted paste was obtained. The paste was then packed into the end of a glass tube (*ca*. 3.4 mm i.d. and 10 cm long). A copper wire inserted into the carbon paste provided the electrical contact. When necessary, a new surface is obtained by pushing an excess of the paste out of the tube and polishing with a weighing paper.

For comparison, 2CBF modified CPE electrode (2CBFCPE) without carbon nanotubes, carbon nanotubes paste electrode (CNPE) without 2CBF, and unmodified CPE in the absence of both 2CBF and carbon nanotubes were also prepared in the same way.

RESULTS AND DISCUSSION

Electrochemical Behavior of 2CBFCNPE

2CBFCNPE was constructed and its electrochemical properties were studied in a 0.1 M PBS (pH 4.0) using CV (Fig. 1). The experimental results show well-defined and reproducible anodic and cathodic peaks with E_{pa}, E_{pc} and E°´ of 655, 540 and 597 *vs.* Ag/AgCl/KCl (3.0 M), respectively. The observed peak separation potential, $\Delta E_p = (E_{pa} - E_{pc})$ of 110 mV, was greater than the value of 59/n mV expected for a reversible system [37] suggesting that the redox couple of 2CBF in 2CBFCNPE has a quasi-reversible behavior in aqueous medium.

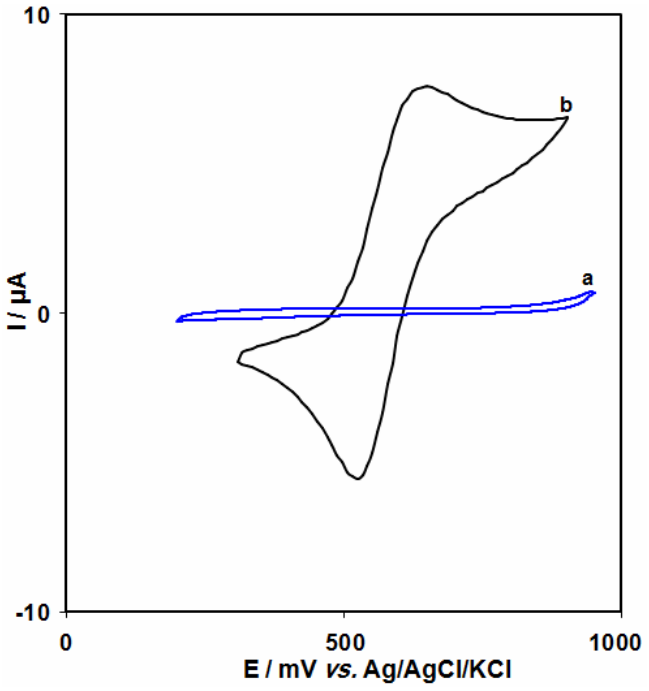

Fig. 1. CVs of CPE (a) and 2CBFCNPE (b) in 0.1 M PBS (pH 4.0) In all cases, the scan rate is 100 mV s^{-1}.

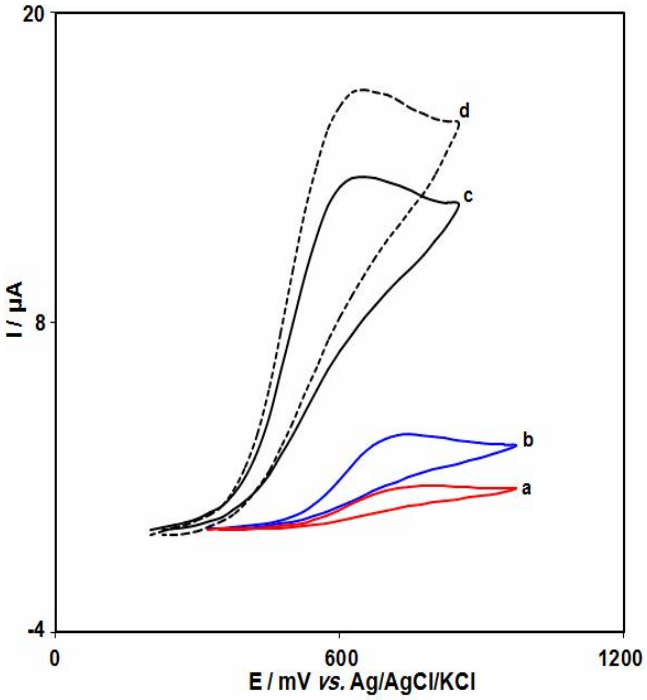

Fig. 2. CVs of CPE (a), CNPE (b), 2CBFCPE (c) and 2CBFCNPE (d) in 0.1 M PBS (pH 4.0) containing 50.0 µM ascorbic acid. In all cases, the scan rate is 10 mV s^{-1}.

Electrocatalytic Oxidation of Ascorbic Acid at a 2CBFCNPE

Figure 2 depicts the CV responses for the electrochemical oxidation of 50.0 µM ascorbic acid at unmodified CPE (curve a), CNPE (curve b), 2CBFCPE (curve c) and 2CBFCNPE (curve d). As can be seen, while the peak potential for ascorbic acid oxidation at the CNPE, and unmodified CPE are 690 and 740 mV, respectively, the corresponding potential at 2CBFCNPE and 2CBFCPE is ~655 mV. These results indicate that the peak potential for ascorbic acid oxidation at 2CBFCNPE and 2CBFCPE shifts by ~35 and 85 mV toward negative values compared to CNPE and unmodified CPE, respectively. However, 2CBFCNPE shows a much higher anodic peak current for the oxidation of ascorbic acid compared to 2CBFCPE, indicating that the combination of carbon nanotube and mediator (2CBF) has significantly improved performance of the electrode toward ascorbic acid oxidation.

2CBFCNPE, in 0.1 M PBS (pH 4.0) and withoutascorbic acid in solution (Fig. 1 curve b), exhibited a well-behaved redox reaction and with addition of 50.0 µM ascorbic acid, increased the anodic peak current (Fig. 2 curve d), indicating a strong electrocatalytic effect [37].

The effect of scan rate on the electrocatalytic oxidation of ascorbic acid at the 2CBFCNPE was investigated by LSV (Fig. 3). As can be observed in Fig. 3, the oxidation peak potential shifted to the more positive potentials with increasing scan rate, confirming the kinetic limitation in the electrochemical reaction. Also, a plot of peak height (I_p) *vs.* the square root of scan rate ($v^{1/2}$) was found to be linear in the range of 2-35 mV s^{-1}, suggesting that at sufficient overpotential, the process is diffusion rather than surface controlled [37].

Chronoamperometric Measurements

Chronoamperometric measurements of ascorbic acid at 2CBFCNPE were carried out by setting the working electrode potential at 0.75 V *vs.* Ag/AgCl/KCl (3.0 M) for the various concentrations of ascorbic acid in 0.1 M PBS (pH 4.0) (Fig. 4). For an electroactive material (ascorbic

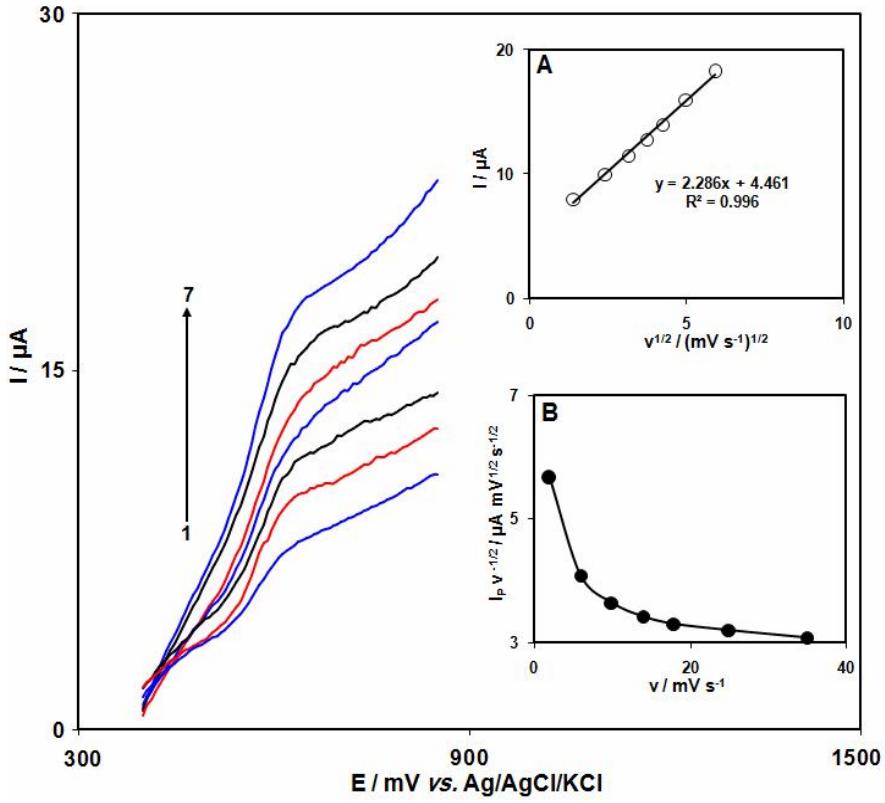

Fig. 3. LSVs of 2CBFCNPE in 0.1 M PBS (pH 4.0) containing 2.0 µM ascorbic acid at various scan rates; numbers 1-7 correspond to 2, 6, 10, 14, 18, 25 and 35 mV s^{-1}, respectively. Insets: Variation of (A) anodic peak current *vs.* $\nu^{1/2}$ and (B) normalized current ($I_p/\nu^{1/2}$) *vs.* ν.

acid in this case) with a diffusion coefficient of D, the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell equation [37],

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2} \quad (1)$$

where D and C_b are the diffusion coefficient (cm^2 s^{-1}) and the bulk concentration (mol cm^{-3}), respectively. Experimental plots of I *vs.* $t^{-1/2}$ were employed, with the best fits for different concentrations of ascorbic acid (Fig. 4A). The slopes of the resulting straight lines were then plotted vs. ascorbic acid concentration (Fig. 4B). From the resulting slope and Cottrell equation the mean value of the D was found to be 1.42×10^{-6} cm^2 s^{-1}.

Chronoamperometry can also be employed to evaluate the catalytic rate constant, k, for the reaction between ascorbic acid and 2CBFCNPE according to the method described by Galus [38]:

$$I_C/I_L = \gamma^{1/2}[\pi^{1/2}\,\mathrm{erf}(\gamma^{1/2}) + \exp(-\gamma)/\gamma^{1/2}] \quad (2)$$

where I_C is the catalytic current of ascorbic acid at 2CBFCNPE, I_L is the limited current in the absence of ascorbic acid and $\gamma = kC_bt$ is the argument of the error function (C_b is the bulk concentration of ascorbic acid). In cases where γ exceeds the value of 2, the error function is almost equal to 1 and therefore, the above equation can be shortened to:

$$I_C/I_L = \pi^{1/2}\gamma^{1/2} = \pi^{1/2}(kC_bt)^{1/2} \quad (3)$$

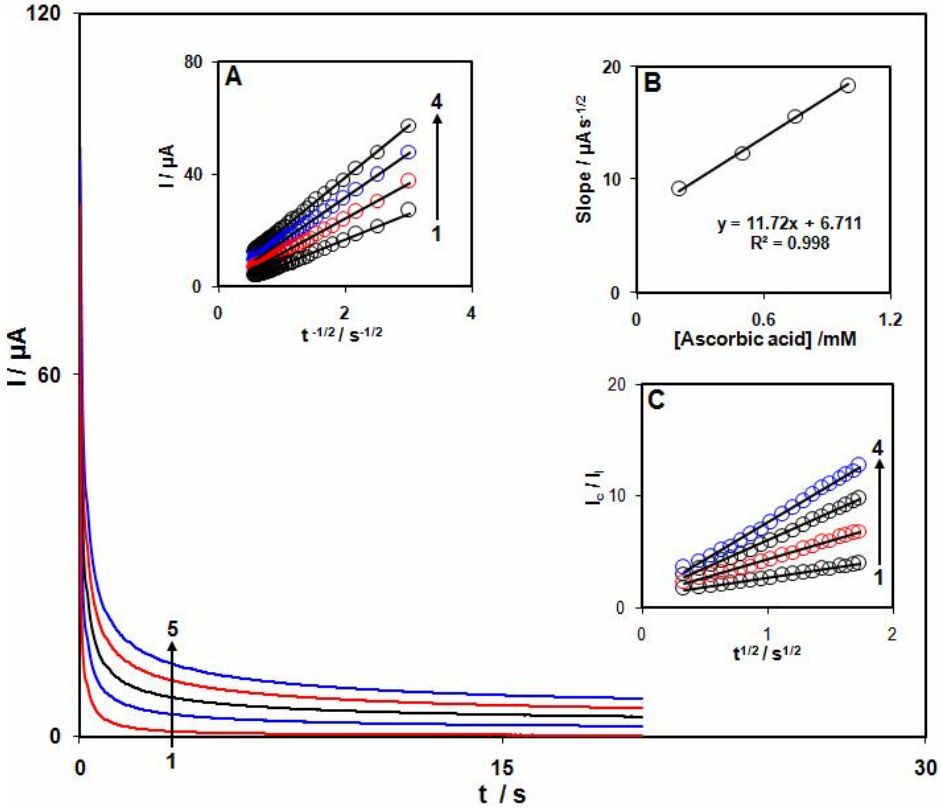

Fig. 4. Chronoamperograms obtained at 2CBFCNPE in 0.1 M PBS (pH 4.0) for different concentrations of ascorbic acid. The numbers 1-5 correspond to 0.0, 0.2, 0.5, 0.75 and 1.0 mM of ascorbic acid. Insets: (A) Plots of I *vs.* $t^{-1/2}$ obtained from chronoamperograms 2-5. (B) Plot of the slope of straight lines against ascorbic acid concentration. (C) Dependence of I_c/I_l on $t^{1/2}$ derived from the data of chronoamperograms 1-5.

where t is the time elapsed. The above equation can be used to calculate the rate constant, k, of the catalytic process from the slope of I_C/I_L *vs.* $t^{1/2}$ at a given ascorbic acid concentration. From the values of the slopes (Fig. 4C), the average value of k was found to be 3.7×10^3 $M^{-1} s^{-1}$.

Calibration Plot and Limit of Detection

The electrocatalytic peak current of ascorbic acid oxidation at the surface of 2CBFCNPE can be used for determination of ascorbic acid in solution. Therefore, square wave voltammetry (SWV) experiments were performed using modified electrode in 0.1 M PBS (pH 4.0) containing various concentration of ascorbic acid (Fig. 5).

The plot of peak current *vs.* ascorbic acid concentration includes two linear segments with slopes of 1.7021 and 0.0664 µA μM^{-1} in the concentration ranges of 1.0×10^{-7}-2.5×10^{-6} M and 2.5×10^{-6}-7.0×10^{-5} M, respectively. The detection limit (3σ) of ascorbic acid was found to be 6.4×10^{-8} M.

Real Sample Analysis

In order to evaluate the analytical applicability of the proposed method, it was also applied to the determination of ascorbic acid in ascorbic acid injection and urine samples. The results are listed in Table 1. Satisfactory recovery of the experimental results was found for ascorbic acid. The reproducibility of the method was demonstrated by the mean relative standard deviation (R.S.D.).

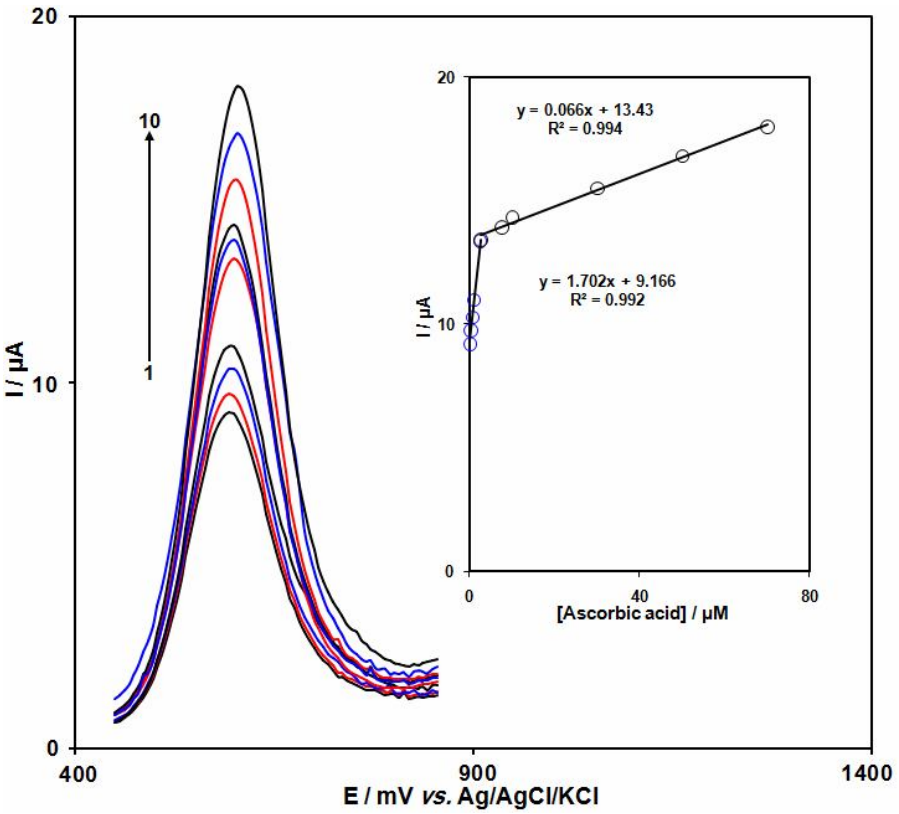

Fig. 5. SWVs of 2CBFCNPE in 0.1 M PBS (pH 4.0) containing different concentrations of ascorbic acid. Numbers 1-10 correspond to: 0.1, 0.25, 0.75, 1.0, 2.5, 7.5, 10.0, 30.0, 50.0 and 70.0 µM. Insets: plots of Ip *vs.* ascorbic acid concentrations in the range of 0.1-70.0 µM.

Table 1. The Application of 2CBFCNPE for Determination of Ascorbic acid in Ascorbic Acid Injection and Urine Samples (n = 5)

Sample	Spiked (µM)	Found (µM)	Recovery (%)	R.S.D. (%)
Ascorbic acid injection	0.0	15.0	-	3.1
	5.0	19.9	99.5	2.2
	10.0	25.7	102.8	2.6
	20.0	34.1	97.4	1.8
	30.0	45.3	100.7	2.9
Urine	0.0	ND[a]	-	-
	5.0	5.1	102.0	2.3
	10.0	9.9	99.0	3.5
	15.0	14.6	97.3	1.9
	20.0	20.2	101.0	2.6

[a]ND: Not detected

CONCLUSIONS

2CBFCNPE was prepared and used for the investigation of the electrochemical behavior of ascorbic acid. Two pairs of well-defined redox peak were obtained at 2CBFCNPE. 2CBFCNPE showed excellent electrocatalytic activity for the ascorbic acid. The SWV currents of ascorbic acid at 2CBFCNPE increased linearly with the ascorbic acid concentration in the range from 1.0×10^{-7}-7.0×10^{-5} M with a detection limit of 6.4×10^{-8} M. Finally, this method was used for the determination of ascorbic acid in some real samples.

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