

## Congo Red as a Modifier for Amperometric Determination of Ascorbic Acid

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In this paper, the fabrication and characterization of congo red chemically modified electrode are described. A carbon-paste electrode (CPE) chemically modified with the congo red was used as an electrochemical sensor for the determination of ascorbic acid. The electrochemical characterization of this modified electrode exhibits stable redox behaviour for congo red-CPE. The electrochemical behaviour of congo red-CPE was studied, using cyclic voltammetric, chronoamperometric, differential pulse voltammograms (DPV) and hydrodynamic amperometric techniques. The experimental results showed that ascorbic acid was electro-oxidized at the congo red modified carbon paste electrode. The electrode has shown a stable response in the long-term. At a fixed potential (0.6 V vs. SCE) under stirred conditions, the calibration plot was linear over the ascorbic acid concentration range 50-6000  $\mu\text{M}$ . The detection limit of the method is 29  $\mu\text{M}$ . The relative standard deviation for 60  $\mu\text{M}$  ascorbic acid (3 repetitions using the same electrode) is 4.3 %.

**Key Words:** Congo red, Ascorbic acid, Amperometric determination.

### INTRODUCTION

The anionic dye, congo red (1-naphthalenesulfonic acid, 3,3'-(4,4'-biphenylenebis-(azo))bis(4-aminodisodium salt), as azo dye with one central biphenyl group and two symmetric naphthalenic groups, known as a laboratory reagent, has been used as a test for the estimation of the functional state of the reticulo-endothelial system and as a chemotherapeutic reagent for septicaemia and as a hemostatic agent in cases of bleeding<sup>1</sup>. Congo red is water-soluble dye, yielding a red colloidal solution with high absorption band in visible spectrum located around 500 nm. Pharmacological studies on congo red were first conducted by Petroff<sup>2</sup>, who examined the detoxification effect of various organic dyes against curare and discovered that congo red had the highest detoxification ability. Congo red protects animals against poisoning from curare. It was reported that congo red could be used to prepare a modified electrode using silica/aniline xerogel<sup>3</sup> and water-soluble carbon nanotube<sup>4</sup>. In order to further study, an attempt is made to use congo red-CPE in acidic solution and studied its electrochemical and electrocatalytic behaviour.

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The carbon paste electrode was introduced by Adams<sup>5</sup>. The carbon paste electrode (CPE) always contains a polyethylene tube with a well into which is inserted a platinum, copper, steel or graphite contact<sup>6</sup>. The well is filled with a paste made by mixing powdered graphite with a suitable mulling liquid. By adding modifier materials in paste, we can improve the electrode selectivity and sensitivity. The modifier amount in the paste usually varies between 10 and 30 % (w/w), depending on the character of modifying agent and its capability of forming enough active sites in modified paste<sup>7</sup>.

Ascorbic acid as a water-soluble vitamin is necessary in the redox process taking place in cell. Ascorbic acid is sensitive to light. Therefore, oxygen and temperature must be protected from them. Ascorbic acid is known for its reductive properties and for its use on a wide scale as an antioxidant agent in foods and drinks; it is also important for therapeutic purposes<sup>8</sup>. Due to the wide use of ascorbic acid in soft drinks and drugs<sup>9</sup>, its determination has received great attention in analytical chemistry and several methods have been used for this purpose<sup>10-17</sup>.

This paper deals with the preparation of a CPE as congo red-CPE and presents a detailed study of the electrochemical behaviour of the electrode. Congo red as a dye is strongly retained in CPE and not easily leached from the CPE matrix in acidic solution.

## EXPERIMENTAL

Congo red (99.5 % from Aldrich), HCl, KNO<sub>3</sub> and other chemicals were of analytical grade from Merck and used without further purification. A solution of hydrochloric acid pH 1.0 solution was used as supporting electrolyte. The electrochemical experiments were carried out using a potentiostat/galvanostat (BHP-2063 electrochemical analysis system, Behpajoo, Iran). A conventional three-electrode cell was used at room temperature. A saturated calomel electrode and platinum wire were used as reference and auxiliary electrodes, respectively.

**Preparation of the modified electrode:** For preparation of working electrode, first congo red was mixed with hydrochloric acid (pH = 1) and then stirred solution, the congo red precipitate was then filtered, washed and dried. The resulting precipitate was stable in aqueous solution at acidic pH settings well about 1. The carbon paste electrode was prepared by intimately homogenising a mixture of graphite powder and congo red precipitate with 5:1 ratio and 3 drop of paraffin oil. The obtained paste then packed into a polyethylene tube. The electrode surface was renewed by extrusion of approximately 1 mm carbon paste from the holder and smoothing with a filter paper. Typically, such mechanical renewal of the carbon paste surface was made when starting a new series of measurements.

## RESULTS AND DISCUSSION

**Electrochemical characteristics of congo red-CPE:** It was reported that congo red could be used to prepare a modified electrode using silica/aniline Xerogel<sup>3</sup> and

Water-Soluble Carbon Nanotube<sup>4</sup>. To further study this, we attempted to use congo red-CPE in acidic solution and studied its electrochemical and electrocatalytic behaviour. Congo red is strongly retained in CPE and not easily leached from the CPE matrix in acidic solution. A typical cyclic voltammogram of congo red-CPE is shown in aqueous 0.5 M HCl from 0.1 to 0.6 V vs. SCE with scan rate of 0.05 V s<sup>-1</sup> (Fig. 1). The voltammogram exhibits a pair of peak with formal potential,  $E^{\circ} = (E_{pa} + E_{pc})/2$ , of 320 mV and peak potential separation,  $\Delta E_p = E_{pa} - E_{pc}$ , is 70 mV vs. SCE. The peak current of the voltammogram is linearly proportional to the scan rate up to 0.2 V s<sup>-1</sup> (data not shown) and the ratio  $i_{pa}/i_{pc}$  remains almost equal to unity, as expected for a surface-type behaviour. Congo red also examined as a sol-gel carbon composite electrode, in this case the results not suitable and cannot obtain a reversible cyclic voltammogram. Therefore, congo red in CPE in acidic medium is used.

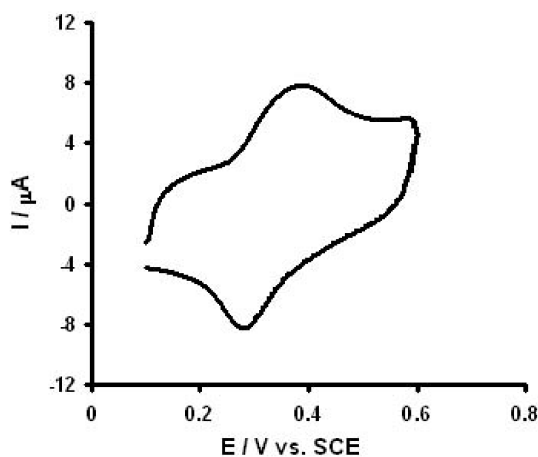


Fig. 1. Cyclic voltammograms of a CR-CP electrode with scan rate of 0.05 V s<sup>-1</sup> in 0.5 M HCl

The electrochemical behaviour of the CR-CPE modified electrode was studied in a different concentration of supporting electrolyte. The experimental results showed that the response of electrode depended on the concentration of the supporting electrolyte. When the concentration of HCl changed from 0.1 to 0.6 M, both redox peaks showed a negative shift of the peak potential of differential pulse voltammograms (DPV) from 0.40 V to 0.20 V vs. SCE and the shape of the DPV voltammetric curves did not change appreciably. Continuous scanning of the modified electrode suggested its stability during continuous use.

The surface coverage ( $\Gamma$ ) of CR-CPE was evaluated from the following equation:

$$\Gamma = Q/nFA \quad (1)$$

where Q is charge obtained by integrating the anodic peak at 0.02 V s<sup>-1</sup> scan rate and the other symbols have their usual meaning. In the present case, the calculated value of  $\Gamma$  is  $9.82 \times 10^{-8}$  mol cm<sup>-2</sup>.

The stability of CR-CPE checked by repetitive scanning. The height of redox peak decreased with the continuously cycles, after about 100 cycles the cyclic voltammograms reached to a steady state. In this case only 1.0 % of peak height decreased, this may be due to the gradually leaking of some congo red molecules from surface, which does not suitably entrap into the CPE electrode.

For preparation of CR-CPE we examined congo red with different weight per cent, the optimum amount of the modifier was 20 % (w/w). The repeatability of the congo red-CPE response was evaluated by measuring the current values in prepared electrode the relative standard deviation (RSD) of 3.8 % was obtained.

**Electrode reproducibility and stability:** To characterize the reproducibility of the congo red-CPE preparation, repetitive preparation of congo red-CPE were carried out. The results of 4 successive preparations in different days showed a relative standard deviation of 4.9 %, indicating that the preparation of congo red-CPE is good producible. The modified electrode also shows high stability. For example, the modified electrode signal showed a 5 % decrease of its initial response.

**Electrocatalytic properties of congo red-CPE:** Chronoamperometry as an analytical technique has been used for the evaluation of the kinetics. Double step potential chronoamperometry and other electrochemical methods were employed for investigation of electrochemical processes at chemically modified electrodes<sup>8,18,19</sup>. Chronoamperometric measurements of ascorbic acid at congo red-CPE electrode were completed by setting the working electrode potential at 0.5 V vs. SCE and used for the measurement of the diffusion coefficient of ascorbic acid. The catalytic oxidation of ascorbic acid by a congo red-CPE was studied by double potential-step chronoamperometry. A double potential-step technique at an initial potential of 0.5 and final potential of 0.1 V (vs. SCE) was used for this purpose.

For an electroactive material with diffusion coefficient, D, the current corresponding to the electrochemical reaction (under diffusion control) have described by Cottrell's law<sup>20</sup>

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

where C is the bulk concentration (mol cm<sup>-3</sup>). The plot of I vs.  $t^{-1/2}$  will be linear and from the slope the value of D can be obtained. The mean value of D was found to be  $4.4 \times 10^{-6}$  cm<sup>2</sup> s<sup>-1</sup> which is in good agreement with the values reported<sup>21,22</sup>. At intermediate times ( $t = 2-4$  s in the present work) from chronoamperograms in presence of ascorbic acid the catalytic current ( $I_{cat}$ ) and in absence of ascorbic acid the limit current ( $I_L$ ) is determined and according to the method described in the literature<sup>23</sup> from the slope of the  $I_{cat}/I_L$  vs.  $t^{1/2}$  plot (Fig. 2), we can calculate the value of catalytic rate constant, k, for a given concentration of ascorbic acid. The value for k was found to be  $2.5 \times 10^3$  M<sup>-1</sup> s<sup>-1</sup>.

**Application as a hydrodynamic amperometric sensor:** In order to assess the effect of the working potential on the current, the constant potential amperometry was used to investigate the determination of ascorbic acid. When the potential was lower than 0.4 V, the current of the oxidation was very small. The current increased

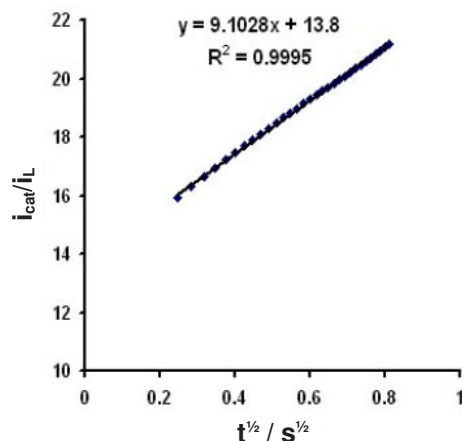


Fig. 2. A typical plot if  $i_{cat}/i_L$  vs.  $t^{1/2}$  derived from chronoamperograms of congo red in absence (a) and presence (b) 10 mM of ascorbic acid in 0.5 M HCl

quickly when the potential was over 0.5 V. When the potential was higher than 0.7 V, the detection signal was disturbed by the background noise. As a result, the detection potential of 0.6 V was selected to give the best signal.

The proportion of the congo red in modified electrode is also investigated. The 20 % congo red was used as the optimal proportion. In this case, the modified electrode has good activity. The hydrodynamic amperometric method was applied to the determination of ascorbic acid in vitamin C tablets. Under the best conditions, calibration plots for ascorbic acid at pH 1, in 0.5 M HCl were linear up 50-6000  $\mu\text{M}$  ascorbic acid with a detection limit (S/N) (3) of 29  $\mu\text{M}$ .

The solid samples, with a nominal ascorbic acid in mass from 50 to 250 mg, in 100 mL volume were dissolved in distilled water. The spiked samples were prepared by adding the standard solution on the sample solution with equal volume.

The proposed method applied to determination of ascorbic acid in vitamin C tablets. For this purpose, the tablets dissolved in distilled water and after running hydrodynamic amperometric technique applied to determination of ascorbic acid in tablets (Fig. 3), the standard solution of ascorbic acid added to the sample solution in electrochemical cell (Fig. 4). The recoveries of ascorbic acid in solid samples were good enough (98 %) for practical use in all determinations.

### Conclusion

In this paper, congo red was used as a modifier in preparation of CPE. Congo red in different conditions was inserted in carbon paste, but occasionally congo red leaked to solution. However, when we used congo red in hydrochloric acid solution, congo red precipitate was stable in CPE. The electrochemical behaviour of congo red-CPE studied using cyclic voltammetric method. It is shown that ascorbic acid can be detected using a congo red modified carbon paste electrode by hydrodynamic amperometric technique.

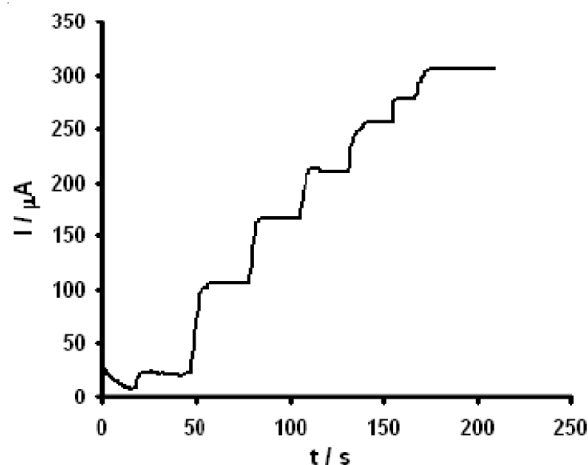


Fig. 3. A typical hydrodynamic amperograms showing the current response for addition of different concentration of ascorbic acid standard solution to 10 mL of sample in 0.5 M HCl

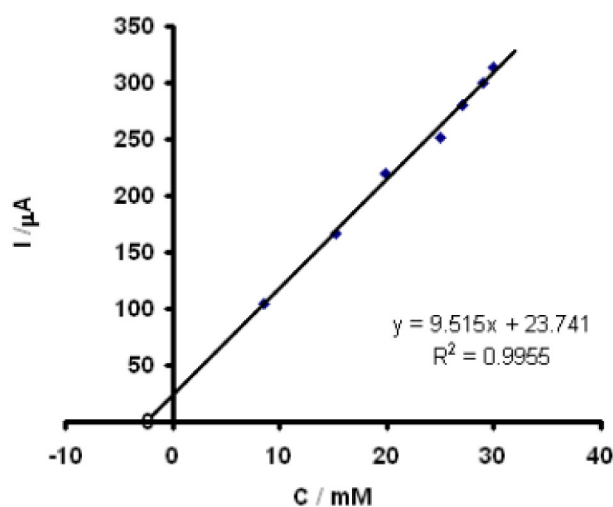


Fig. 4. A typical, standard addition calibration plot for hydrodynamic amperogram

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