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Chemically modified electrodes based on macrocyclic compounds for determination of Vitamin C by electrocatalytic oxidation

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Abstract

The voltammetric behavior of Vitamin C (L-ascorbic acid) at carbon paste electrodes and electrodes modified with aza macrocycles have been studied. The use of zinc complexes, formed in situ at the electrode surface reduced the overpotential for the oxidation of ascorbic acid by about 200 mV and enhanced the peak currents. Linearity was observed over the range of 0.6–500 $\mu g/cm^3$ with a detection limit of 0.1 $\mu g/cm^3$ by differential pulse voltammetry. The modified electrode was used for the determination of Vitamin C in multivitamin–multimineral pharmaceutical preparations, fruit juices and wine samples.

Keywords: Vitamin C; Chemically modified electrodes; Macrocyclic compounds; Voltammetry; Electrocatalysis

1. Introduction

Vitamin C or L-ascorbic acid is distributed widely in both the plant and animal kingdoms. In vegetable cells, it occurs in free form and also often bound to protein as "ascorbigen". Among animal organs, the liver, leukocytes and anterior pituitary lobe show highest concentration of ascorbic acid. Vitamin C, a water-soluble vitamin that is widely required in metabolism and consumed on a large scale is electroactive and a variety of methods have been developed for its analysis. Spectrophotometric methods have been developed for the analyses of Vitamin C in pharmaceutical preparations and fruit juices [1,2]. Enzyme based electrodes [3], polymer based electrodes [4], dye based electrodes [5], etc. have been proposed for the electrochemical detection and determination of ascorbic acid. The electrocatalytic oxidation of ascorbic acid at polypyrrole coated platinum disc electrodes [6] doped with chloride and dodecylbenzene sulphonate have been studied by Lyons and co-workers, Lorenzo and co-workers have carried out mechanistic studies [7] of the electrocatalytic oxidation of β-nicotinamide adenine dinucleotide and ascorbate at glassy carbon electrodes modified with electrodeposited films derived from 3,4-dihydroxybenzaldehyde and applications of its other isomers in real analyses [8]. Glassy carbon electrodes modified with a cellulose acetate film [9] and screen-printed [10] carbon electrodes bearing 2,6-dichlorophenolindophenol have been developed and used for analyses of various samples. Carbon paste electrodes modified with an aqueous insoluble redox mediator, ruthenium(III)diphenyldithiocarbamate showed a linear response over the range 2.5–125 mg/dm^3 [11]. Voltammetric methods utilizing electrogenerated ferricinium carboxylic acid [12] and gold microelectrodes [13] have been proposed for the assay of Vitamin C.

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Electrodes modified with transition metal — macrocyclic complexes have been used to catalyze the oxidation of ascorbic acid and enhance the voltammetric signals, thereby enabling lower detection limits. The metal here acts as an electron mediator. Graphite–epoxy composite electrode modified with cobalt phthalocyanine [14], 1,5,8,12-tetraaza-2,4,9,11-tetramethylcyclotetradecinatonickel(II) — polymer modified glassy carbon electrode [15] and metalloporphyrin [16] based electrodes have been developed, which show quite low levels of detection.

In the present work, an attempt has been made to simplify the method of electrode fabrication by making use of carbon paste instead of electropolymerization methods and we have made use of in situ complexation of the transition metal ions, rather than synthesizing the macrocyclic complexes and incorporating them into the electrode. The macrocyclic compounds studied were 1,4,8,11-tetraazacyclotetradecane (**I**); 5,6,14,15-dibenzo-1,4-dioxa-8,12-diazacyclopentadeca-5,14-diene (**II**); 7,16-dibenzyl-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane (**III**); 1,4,7-tritosyl-1,4,7-triazacyclononane (**IV**); and 1,4,7,10-tetratosyl-1,4,7,10-tetraazacyclododecane (**V**). Of the chemically modified electrodes (CMEs) studied, the CME based on (**IV**) showed the best response and was used to assay the Vitamin C content in multivitamin pharmaceutical preparations.

2. Experimental

2.1. Chemicals and reagents

All chemicals used were of analytical-reagent grade. Double distilled, deionized water was used for preparation of all solutions. 1,4,8,11-tetraazacyclotetradecane; 5,6,14,15-dibenzo-1,4-dioxa-8,12-diazacyclopentadeca-5,14-diene; 7,16-dibenzyl-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane were bought from Fluka and used as such. 1,4,7-Tritosyl-1,4,7-triazacyclononane and 1,4,7,10-tetratosyl-1,4,7,10-tetraazacyclododecane were synthesized by the methods described by Searle and Geue [17]. The amino acids, cysteine, methionine, glutathione and glutamic acid were obtained from Centron Research Laboratories.

All the voltammetric studies were carried out in Britton Robinson buffer of pH 1.5 at $25 \pm 0.2^{\circ}$C.

2.2. Apparatus

The voltammetric system used for the studies was EG&G Princeton Applied Research 264A potentiostat with model 303A electrode assembly and X–Y chart recorder RE0089. The carbon paste electrodes were prepared as before [18] with 5% of the modifier in graphite–nujol matrix and used in conjunction with a saturated calomel reference electrode and a platinum counter electrode.

2.3. Procedure

Initially, cyclic voltammograms were recorded for the unmodified electrode and the electrode modified with (**I**) in the buffer solutions containing 4.95×10^{-3} M and 1.99×10^{-3} M ascorbic acid at different scan rates. Then, another set of experiments was carried out to study the effect of catalysis by the incorporated metal ion in the macrocyclic ring. For this purpose the CME was dipped in a 0.05 M $Zn(NO_3)_2$ solution for 2 min, rinsed with distilled water and the voltammograms were then recorded for ascorbic acid solutions of identical concentrations at varying scan rates. In another set, cyclic voltammograms were recorded for the same ascorbic acid solutions, but the supporting electrolyte used was, the buffer solution containing 0.05 M $Zn(NO_3)_2$. Similarly, CMEs containing other macrocycles were studied using the buffer containing 0.05 M $Zn(NO_3)_2$ as it showed maximum current enhancement.

Differential pulse voltammetry (DPV) was used for the quantification of ascorbic acid. Between successive runs the surface of the CME was renewed by pressing out a small amount of the paste, scraping off the excess and polishing the tip on a zero grade polishing paper until the surface had a shiny appearance. Before each scan the solution was purged with dry nitrogen for 2 min.

2.4. Determination of Vitamin C in the multivitamin preparations

The pharmaceutical preparations analysed were ‘A to Z’ multivitamin–multimineral tablets (Indo Prop-

kem Ltd., India) and 'ZiComplex' capsules (Mexin Medicaments Ltd., India). In addition to ascorbic acid, 'ZiComplex' contained Vitamins B_2, B_3, B_6, B_{12}, folic acid, zinc sulphate and calcium pantothenate whereas 'A to Z' contained Vitamins A and E, B_1, zinc oxide, cupric oxide, sodium selenate, manganese chloride and chromium(III)chloride also along with binding agents.

Each tablet/contents of capsule were crushed with a mortar and pestle and dissolved in the supporting electrolyte, which was then filtered through a Qualigens (615) filter paper. The standard addition method was employed for the quantification of ascorbic acid, wherein 0.3 cm^3 of the filtrate was taken into a cell containing 10.0 cm^3 of the supporting electrolyte and additions of 0.2 cm^3 ascorbic acid (1000 μg/cm^3) were done. DPV with a pulse amplitude of 50 mV and scan rate of 20 mV/s was used to record the peaks.

Similarly, a sport/health drink 'Glucose-D' (Tushar Health Care, India) containing dextrose, Vitamin C and minerals was analyzed.

Juices from oranges, lemons, grapes and tomatoes were squeezed out using a squeezer and passed through the built-in filter to clarify the solutions. Then suitable aliquots (1–2 cm^3) of the clear juices were immediately taken into the supporting electrolyte solution and analyzed as mentioned above. A weighed quantity of jam (2–3 g) was taken into a small volume of supporting electrolyte, stirred for 1 h to ensure homogeneity (without exposing to air), filtered and analyzed. Aliquots of red and white wines, and lemon squash were directly analyzed by the above method. Aliquots from the same batch of juices/jam/wines/squash were simultaneously analyzed by the 2,6-dichloroindophenol titrimetric method [19].

3. Results and discussion

Fig. 1 shows the cyclic voltammograms for ascorbic acid obtained by the plain carbon paste electrode (PCPE) and the CME-I based on (**I**) at a scan rate of 50 mV/s. It is seen that the oxidation potential E_p is shifted to less positive values with an increase in the peak currents I_p, indicating the effect of catalysis by the incorporated metal ion. We have used Zn complex as the electron mediator for Zn(II) happens to be one of the major components in the multivitamin–multimineral pharmaceutical formulations. We also found that this gave similar performance to that of Ni complexes (when $Ni(NO_3)_2$ was used instead of $Zn(NO_3)_2$). Table 1 shows the comparison of peak potentials E_p and peak currents I_p of CME-Ia (in absence of Zn) and CME-Ib (with Zn incorporated by dipping in Zn(II) solution for 2 min). It was observed that even without Zn, CME-I shows an increase in I_p over that of PCPE, which could be

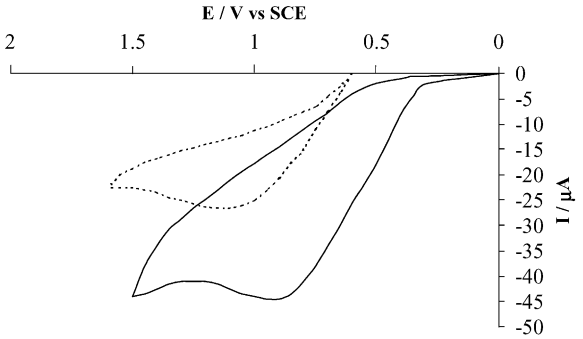

Fig. 1. Cyclic voltammograms for 4.95×10^{-3} M ascorbic acid obtained by PCPE (···) and CME-I (—) in the supporting electrolyte containing buffer pH 1.5 and 0.05 M $Zn(NO_3)_2$ at 50 mV/s.

Table 1
Effect of scan rate at CME-I in the absence (CME-Ia) and presence (CME-Ib) of Zn(II) in 4.95×10^{-3} M ascorbic acid

Scan rate (mV/s)	CME-Ia			CME-Ib		
	I_p (μA)	E_p (V)	$I_p/C\nu^{1/2}$	I_p (μA)	E_p (V)	$I_p/C\nu^{1/2}$
10	6.0	0.60	383.3	11.0	0.60	702.7
20	12.0	0.63	542.1	15.0	0.65	677.6
50	22.0	0.70	628.5	23.5	0.93	671.4
100	32.0	0.75	646.5	29.0	1.15	585.9
200	49.5	0.80	707.1	33.0	1.23	471.4

Table 2
Effect of scan rate on peak potentials for 4.95×10^{-3} M ascorbic acid in supporting electrolyte containing buffer and 0.05 M $Zn(NO_3)_2$

Scan rate (mV/s)	PCPE E_p (V)	CME-I E_p (V)	CME-III E_p (V)	CME-IV E_p (V)	CME-V E_p (V)
10	0.90	0.55	0.60	0.55	0.70
20	1.00	0.65	0.70	0.60	0.80
50	1.10	0.90	0.85	0.70	0.95
100	1.25	1.10	0.95	0.75	1.05
200	1.35	1.20	1.10	0.83	1.15

Table 3
Effect of scan rate on peak currents for 4.95×10^{-3} M ascorbic acid in supporting electrolyte containing buffer and 0.05 M $Zn(NO_3)_2$

Scan rate (mV/s)	PCPE I_p (μA)	CME-I I_p (μA)	CME-III I_p (μA)	CME-IV I_p (μA)	CME-V I_p (μA)
10	4.0	14.0	9.5	7.5	9.5
20	7.0	19.0	13.0	10.5	13.0
50	12.0	28.5	20.0	15.5	17.5
100	16.5	37.0	26.0	19.0	21.0
200	22.0	41.0	31.0	22.5	26.5

due to adsorption [20] and the amine–acid interaction, as confirmed by the increase in $I_p/C\nu^{1/2}$ with scan rate. Formation of an in situ complex at the surface of CME-I by dipping in $Zn(NO_3)_2$ solution, gave a further increase in I_p. But when $Zn(NO_3)_2$ was added to the buffer itself, maximum enhancement in peak currents was observed which over shadowed the adsorption effects Tables 2 and 3.

In order to determine whether the current was controlled by diffusion of ascorbic acid to the electrode surface, plots of I_p and $I_p/C\nu^{1/2}$ versus $\nu^{1/2}$ (where C represents the concentration of ascorbic acid and ν is the scan rate) were constructed. Figs. 2 and 3 show the I_p versus $\nu^{1/2}$ and $I_p/C\nu^{1/2}$ versus $\nu^{1/2}$ plots, respectively for CME-I in the supporting electrolyte (0.05 M $Zn(NO_3)_2$ in buffer of pH 1.5). Fig. 2 shows an initial linearity which curves off at higher scan rates, suggesting that, the reaction is initially diffusion controlled, but at faster scan rates the electron transfer becomes rate determining. In addition, the $I_p/C\nu^{1/2}$ plot shows a negative slope which is typical of a catalytic process involving a chemical reaction followed by an electron transfer process [14] and also the E_p values are shifted to more positive values.

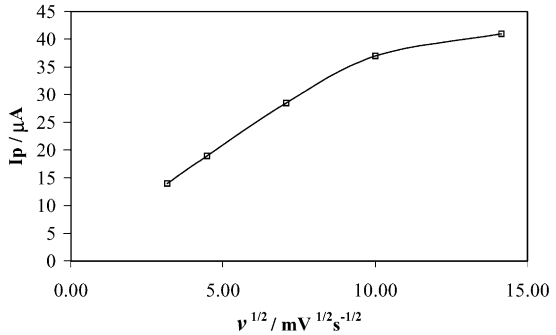

Fig. 2. Plot of I_p vs. $\nu^{1/2}$ for 4.95×10^{-3} M ascorbic acid obtained by CME-I in the supporting electrolyte containing buffer pH 1.5 and 0.05 M $Zn(NO_3)_2$.

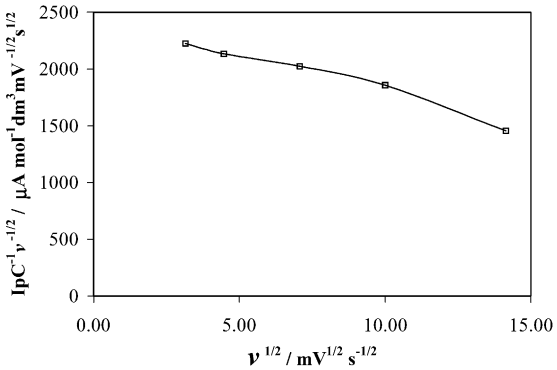

Fig. 3. Plot of $I_p/C\nu^{1/2}$ vs. $\nu^{1/2}$ for 4.95×10^{-3} M ascorbic acid obtained by CME-I in the supporting electrolyte containing buffer pH 1.5 and 0.05 M $Zn(NO_3)_2$.

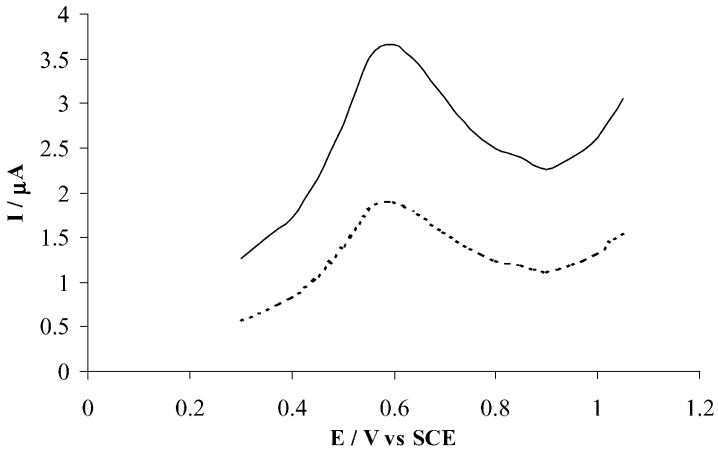

Fig. 4. Peaks obtained by CME-IV for 4 μg/cm^3 ascorbic acid at pulse amplitude of 50 mV and scan rates of 20 mV/s (···) and 10 mV/s (—).

However, when we attempted to exploit this enhancement in current for analyses at low concentrations by DPV, a very large background current was observed due to which, peaks corresponding to 60 μg/cm^3 and below, merged with the base line. We suspected this to be due to the amine–acid interaction, so to minimize this we fabricated CME-II and CME-III based on (**II**) and (**III**), respectively. CME-II showed a very erratic behavior and hence was discarded. CME-III showed lower peak currents (Table 3) than CME-I, but a better response in the DPV mode. However, the aim of lower working ranges and low detection limits could not be achieved. Other buffers like tetrabutylammonium perchlorate + perchloric acid, KCl + HCl, KNO_3 + HNO_3, sodium acetate + acetic acid, sodium dihydrogen phosphate + phosphoric acid, were tried, but were fruitless.

Following a clue from the work of Wickstrøm and others [21], who found that aza-crown ethers with a *p*-toluene sulphonyl group showed a better response due the prevention of protonation of nitrogen, we synthesized (**IV**) and (**V**). The cyclic voltammetric responses of CME-IV and CME-V showed lower catalytic peak currents, than CME-III. Nevertheless, the background current was greatly reduced. CME-IV gave the lowest background currents and the overpotential for oxidation was also greatly reduced (Table 3). Using this CME, we could get a detection limit of 0.1 μg/cm^3 (R.S.D. = 4.6%) and a linear working range from 0.6 to 500 μg/cm^3 (coefficient of correlation = 0.9897), at a scan rate of 20 mV/s and pulse amplitude of 50 mV. By lowering the scan rate (Fig. 4) to 10 and 5 mV/s and increasing the pulse amplitude, sharper peaks were obtained and a detection of 10 ng/cm^3 ascorbic acid was possible.

Interference effects were studied by adding large excesses of sugars, amino acids and metal ions in lower oxidation states to a solution containing 10 μg/cm^3 of ascorbic acid. It was observed that the sugars (dextrose, lactose and galactose), metal ions (Mn(II), Co(II) and Cr(III)) and glutamic acid do not interfere even when present in 100 times excess concentrations.

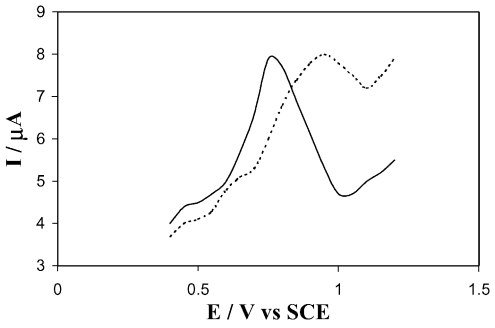

Fig. 5. Peaks obtained by CME-IV for a solution containing 10 μg/cm^3 ascorbic acid and 500 μg/cm^3 glutathione at pulse amplitude of 50 mV and scan rates of 20 mV/s (···) and 5 mV/s (—).

Table 4
Assay of Vitamin C in pharmaceutical preparations

Vitamin preparation	Quoted content	Observed content
A to Z tablets	100 mg/tablet	97.7 ± 3.2 ($n = 5$)
ZiComplex capsules	150 mg/capsule	146.2 ± 2.2 ($n = 5$)
Glucose-D powder	17.5 mg/35 g	16.9 ± 3.1($n = 5$)

Table 5
Vitamin C content (mg/100 cm^3) obtained by the present voltammetric method and the standard 2,6-dichloroindophenol titrimetric method [19]

Sample	Present method	Standard method
Lemon squash	33.2	33.5
Lemon juice	19.3	19.0
Orange juice	25.5	26.4
Tomato juice	12.2	11.8
Green grape juice	3.5	3.3
Black grape juice	2.2	2.1
Red wine	4.1	End point not distinct
White wine	9.6	9.4
Mixed fruit jam	119.8 (mg/100 g)	118.1 (mg/100 g)

Sulphur containing amino acids like cysteine and methionine cause lowering of the peak heights by about 8–10%, when present in 50 times excess concentration; but if the standard addition method is used, it is not very serious. Fifty times excess glutathione happens to cause severe effect (though it is not oxidized within the potential range studied) as the shape of the peak changes (Fig. 5), but can be overcome up to some extent by using a scan rate of 5 mV/s to get well defined peaks. Nevertheless, in real samples these are not present at such high concentrations, except the sugars.

CME-IV, at a scan rate of 20 mV/s and pulse amplitude 50 mV, was used for the purpose of determination of Vitamin C in pharmaceutical preparations having a complex matrix. Though, a lower scan rate and higher pulse amplitude showed greater sensitivity, it was not considered necessary here, as it also increased the analysis time. Table 4 shows the accuracy and precision of the method used for the analysis of pharmaceutical preparations. Table 5 shows the comparison of analyses of fruit juices, jam, lemon squash and wines by the present method and the standard method [19].

4. Conclusion

Redox processes of organic compounds often have slow charge transfer rates, leading to poorly defined voltammetric responses. Modification of the electrode surface by a redox mediator reduces the overpotential for the redox processes, and significantly lowers the detection limits. The modified electrode CME-IV has been successfully applied to the determination of Vitamin C in complex matrices such as multivitamin–multimineral pharmaceutical formulations, fruit juices, jam and wines. The linear working range and the detection limits are comparable to that of previous workers and in some cases better. In the present investigation, it has been possible to obtain a detection limit of 0.1 μg/cm^3 at room temperature in contrast to Bae and co-workers [15] who had optimized the method at 45°C. Also, the electrode preparation is simpler than the process of modification by electropolymerization [4,15,22], as it is a simple mixing of the required ingredients. The carbon paste approach permits convenient mixing of different ligands and as the ligand is homogeneously mixed in the bulk of the paste, renewal of the surface is done simply by pressing out the paste from the syringe, which is easier and faster. Moreover, the electrode so fabricated can be stored for about six months in an airtight container. Though, screen printed electrodes [10] are advantageous from the point of view of mass production, the carbon paste electrodes are easily within the reach of any ordinary laboratory as the ligands are now commercially available.

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