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Amperometric Sensor for the Determination of Ascorbic Acid Based on Cobalt Hexacyanoferrate Modified Electrode Fabricated through a New Route

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A new approach was attempted to prepare a chemically modified electrode using Cobalt hexacyanoferrate (CoHCF) as the redox mediator and to study its stability and electrocatalytic activity for ascorbic acid (AA) oxidation. The basic principle underlying the electrode modification is the coordination of cobalt ion with the amino nitrogen of aniline adsorbed on the surface of a graphite rod. This surface was subsequently derivatized with ferrocyanide to get CoHCF film on the electrode surface. The CoHCF modified electrode as prepared above was characterized using cyclic voltammetry. The effect of scan rate, supporting electrolyte and pH of the medium on the performance of the modified electrode was investigated. The CoHCF modified electrode exhibited good electrocatalytic activity towards the oxidation of ascorbic acid and gave a linear response from 5.52×10^{-5} M to 3.23×10^{-2} M with a correlation coefficient of 0.9929. The detection limit was found to be 3.33×10^{-5} M. Hydrodynamic voltammetry and chronoamperometry studies for the oxidation of ascorbic acid were also carried out. The electrode was highly stable and exhibited good reproducibility. This modified electrode was also applied for the determination of ascorbic acid in commercial samples.

Key words aniline; cobalt hexacyanoferrate; cyclic voltammetry; ascorbic acid

Ascorbic acid is a vital, ubiquitous substance in the life process. It is a water-soluble antioxidant which is chemically a simple carbohydrate material related to glucose of rather unique properties.[1] Ascorbic acid (AA) is found in high concentrations in citric fruits, black berries, tomatoes, black peppers, cabbages and green vegetables.[2] One of the main functions of AA in animal physiology is the maintenance of biochemical homeostasis under stress.[1] Also it is needed for wound healing, and strengthening of blood vessel walls, treatment of common cold, mental illness, infertility and cancer.[3] Considering its provital role into account, significant attention has been focussed on the determination of ascorbic acid and many analytical methodologies have been proposed for its determination including spectrophotometry,[4] titrimetry,[5] enzymatic analysis[6] and electrochemistry.[7,8]

Electrochemical techniques employing sensitive amperometric sensors are particularly suited for simple, inexpensive and rapid analytical tests for the determination of trace concentrations of biologically and clinically important compounds.[9] Chemically Modified Electrodes (CMEs) play an important role in reducing the high overvoltage required for the analyte determination.[10—12] Many methods have been reported for the determination of AA at CMEs.[13—16] All the above reported methods always aim either at the reduction of overpotential for ascorbic acid oxidation by varying the mediators or at increasing the shelf-life of the modified electrode by varying the method of fabrication. Conventional methods of electrode modification include electrodeposition,[17,18] mechanical immobilization,[19,20] paraffin composites,[21] sol-gel technique,[22,23] covalent immobilization,[24,25] carbon paste[26,27] and so on. There has been a continuing interest in the development of new fabrication techniques with an objective to promote a firm linkage between the mediator and the electrode surface. Among the variety of mediators used for the construction of modified electrodes, metal hexacyanoferrates are of particular interest in chemistry and material science.[28—31] Cobalt hexacyanoferrate (CoHCF) is an important member of the mixed valence metal hexacyanoferrates having similar characteristic as that of Prussian blue.[18] During this course of investigation, an entirely new strategy has been adopted in order to anchor CoHCF more firmly on the electrode surface.

It is generally known that carbon/graphite is a good adsorbent for aromatic compounds.[32,33] When aniline is adsorbed on the surface of a graphite rod, it is strongly held by the graphite and the surface exposes the amino functional group of aniline whose reactivity can be utilized for further modification. It has been reported that cobalt can co-ordinate with the terminal amino group of silane which is attached to glass surface.[34] In this work, an attempt was made to bind the cobalt ion with the amino group of aniline which is adsorbed on a graphite rod. The cobalt incorporated on the graphite surface was further derivatized with ferrocyanide to obtain a film of Cobalt hexacyanoferrate on the electrode surface.

The above CoHCF modified electrode was characterized using cyclic voltammetry. The application of the modified electrode for the electrocatalytic oxidation and also for the amperometric determination of ascorbic acid was examined. The analytical utility of the CoHCF modified electrode for the determination of AA in commercial samples has also been tested.

Experimental

Chemicals and Reagents Graphite rods (3 mm diameter) of spectroscopic grade were used for modification. Ascorbic acid, Aniline and $CoCl_2 \cdot 6H_2O$ were purchased from Merck. Aniline was distilled before use. All other chemicals were of analytical grade and used as received. All the solutions were made up with twice distilled water. Ascorbic acid solutions were prepared freshly before use. pH of the solutions was adjusted with HCl and NaOH. Solutions were deaerated by bubbling high purity nitrogen gas prior to the experiments.

Apparatus A three electrode system was employed with a platinum wire, a saturated calomel electrode (SCE) and the CoHCF modified electrode as the counter electrode, reference electrode and working electrode re-

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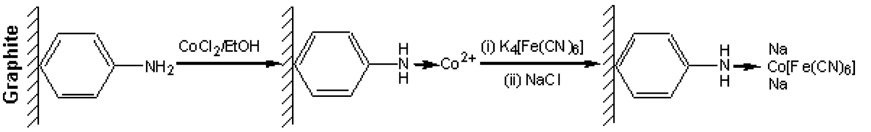

Chart 1. Scheme of Electrode Fabrication

spectively. All the potentials mentioned are with respect to the SCE. For cyclic voltammetry, hydrodynamic voltammetry and chronoamperometry studies, EG&G PAR Electrochemical system (Model 263A) equipped with GPIP (IEEE-488) interface port coupled with an IBM personal computer was used. pH of the solutions were checked using Elico pH meter (Model LI 120).

Fabrication of the Modified Electrode and Procedure Fabrication of the modified electrode was carried out as follows: The surface of the graphite rod was polished using a wet fine emery paper and washed with doubly distilled water. The polished surface of the graphite rod was then dipped in distilled aniline such that its lower surface alone is in contact with aniline for 45 min. The electrode surface was then washed with DMF and allowed to dry at room temperature for 2 h. The aniline adsorbed graphite rod was then dipped in ethanolic solution of cobalt chloride (0.01 M) for 4 h to bind the cobalt ion with aniline. The electrode was then rinsed with water in order to remove the adsorbed cobalt chloride. The graphite rod was then used as working electrode and the potential was cycled in the range of -0.2 to 1.0 V *vs.* SCE in 0.1 M KNO_3 containing 20 mM potassium ferrocyanide at a scan rate of 20 mVs^{-1}. The electrode surface was then washed with water and was subjected to potential cycling in 0.1 M NaCl solution (up to 25 cycles) in the potential range of 0 to 1.0 V *vs.* SCE at a scan rate of 20 mVs^{-1} in order to stabilize the derivatized film. During this cycling, potassium ions in the interstitial positions of CoHCF will be replaced by sodium ions.

Preparation and Analysis of Commercial Samples Commercially available Celin-500 mg (Glaxo Smithkline Limited, Mumbai, India) containing ascorbic acid was ground in a mortar, dissolved in twice distilled water and made up to 50 ml in a standard flask. This solution was then used for the determination of ascorbic acid using the CoHCF modified electrode. The same procedure was followed for Limcee (Sarabhai Pharmaceuticals, Ahmedabad, India) tablet. Each experiment was repeated 10 times in order to ascertain the precision of the method. AA content in the tablets was also determined by titration with Ceric ammonium sulphate using ferroin as indicator and the results are compared.[35)]

Results and Discussion

Electrochemical Studies on the CoHCF Modified Electrode Chart 1 shows the steps involved in the fabrication of the CoHCF modified electrode. The modified electrode thus fabricated was characterized as follows: Cyclic scans were conducted in an unstirred solution of 0.1 M NaCl at a scan rate of 20 mVs^{-1} in the potential range of 0 to 1.0 V *vs.* SCE, recording first the anodic scan and then the cathodic scan of the CoHCF modified electrode. Figure 1 (curves a and c) represents the typical cyclic voltammograms of the bare and the CoHCF modified electrode in 0.1 M NaCl medium. Two sets of redox peaks with formal potentials 0.34 V and 0.79 V *vs.* SCE were observed. The peaks at lower potentials can be attributed to the redox behaviour of the Co^{2+}/Co^{3+}pair and that at higher potentials are characteristic of the $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ pair. The results are analogous with that of the literature reported for CoHCF modified electrode prepared by electrodeposition.[17)]

To confirm the binding of CoHCF on the surface through aniline, another electrode was fabricated without the adsorption of aniline and further steps were carried out according to the procedure adopted for fabricating the CoHCF modified electrode. The cyclic voltammogram of this electrode also showed the presence of two sets of redox peaks, but on continuous cycling the peaks started to decrease and finally disappeared after 15 cycles. This study revealed that CoHCF film is anchored to the surface of the graphite rod only through the adsorbed aniline.

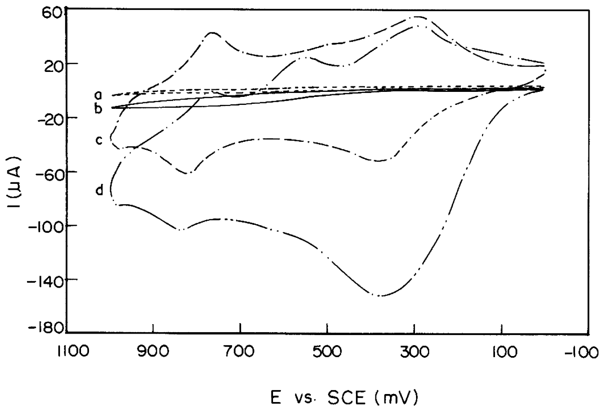

Fig. 1. Cyclic Voltammograms of (a) Bare Electrode in the Absence of AA (b) Bare Electrode in the Presence of 1.82×10^{-3} M AA (c) CoHCF Modified Electrode in the Absence of AA (d) CoHCF Modified Electrode in the Presence of 1.82×10^{-3} M AA in 0.1 M NaCl

Scan rate: 20 mVs^{-1}.

CoHCF has a zeolitic structure, which allows only certain ions to penetrate into and out of the channels. The shape of the voltammogram and the formal potential of the modified electrode varies in the presence of different cations. Among the alkali metal ions studied, well-defined peaks with lower $E^{0\prime}$ value for Co^{2+}/Co^{3+} redox couple were achieved with Na^+ ion which is also in accordance with the results reported earlier for CoHCF modified electrodes.[17,18)] Eventhough anions do not have considerable effects on the voltammetric response of the modified electrode, NaCl was chosen as the suitable supporting electrolyte for further experiments.

The electrochemical behaviour of the CoHCF modified electrode at different scan rates from 2 to 100 mVs^{-1} in 0.1 M NaCl medium in the potential range of 0 to 1.0 V *vs.* SCE was studied. Both the anodic as well as cathodic peak currents for both the redox pairs were proportional to the square root of scan rate indicating that both the redox processes occurring at the modified electrode are diffusion controlled. A study on the effect of pH in the range from 2 to 10 on the electrochemical behavior of the CoHCF modified electrode was undertaken. Both the peak currents and the peak potentials were found to be unaffected in this pH range studied. This reveals that in the range examined pH has no considerable effect on the voltammetric response of the CoHCF modified electrode.

Electrocatalytic Oxidation of Ascorbic Acid The electrocatalytic property exhibited by the CoHCF modified electrode for the oxidation of AA in 0.1 M NaCl is also shown in Fig. 1. It is seen that on bare graphite electrode, the oxidation of ascorbic acid occurs at a higher potential around 0.75 V (curve b) whereas on the CoHCF modified electrode, the oxi-

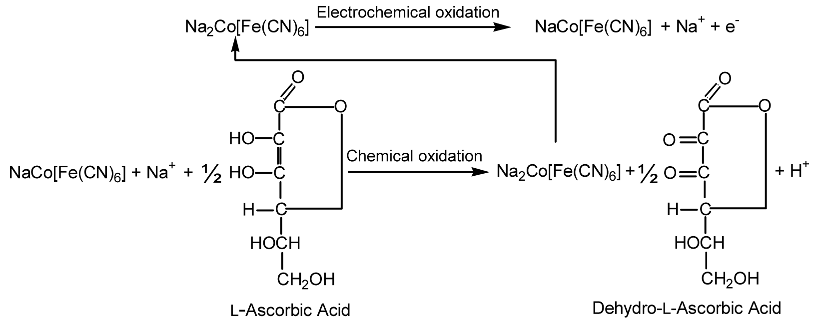

Chart 2. Electrocatalytic Oxidation of AA at the Modified Electrode

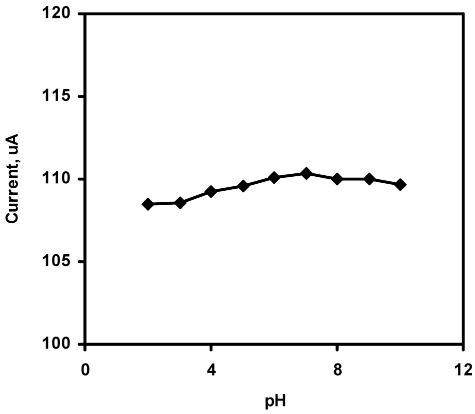

Fig. 2. Effect of pH on the Catalytic Oxidation of 1.96×10^{-3} M Ascorbic Acid at the Modified Electrode in 0.1 M NaCl

Scan rate: 20 mVs^{-1}.

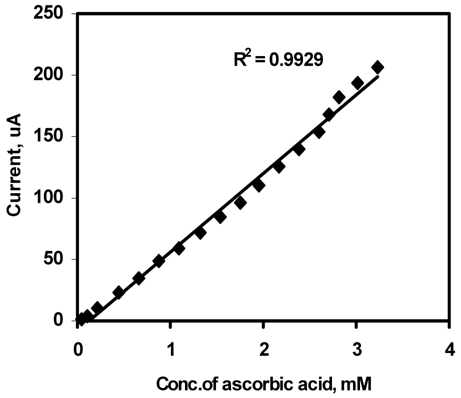

Fig. 3. Calibration Plot for the Determination of Ascorbic Acid

dation of AA occurs at about 0.38 V (curve d). Also the catalytic current at the modified electrode was nearly 10 times higher than that obtained at the bare electrode for AA oxidation. The increase in anodic current in the presence of AA at around 0.38 V which is the oxidation potential of Co^{2+}/Co^{3+} couple infers that the catalytic oxidation of AA is due to the Co^{2+}/Co^{3+} couple and not by the $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ pair. The electrocatalytic oxidation can be represented as in Chart 2.

The effect of pH on the electrocatalytic oxidation of AA at the CoHCF modified electrode is given in Fig. 2. The anodic current in the presence of 1.96×10^{-3} M AA was found to be almost the same in the pH range studied as the H^+ ions formed as a product of oxidation is very small in concentration and it does not alter the pH of the medium. This suggests that the CoHCF modified electrode has similar response towards AA both in acidic as well as in basic conditions. This negligible influence of H^+ on the catalytic oxidation at the modified electrode in this wide pH range may be attractive for practical aspects.

For each addition of AA, a corresponding increase in anodic current was observed followed by a decrease in cathodic current. The CoHCF modified electrode showed linear response for the catalytic oxidation of ascorbic acid. The linear range for the determination of ascorbic acid is from 5.52×10^{-5} M to 3.23×10^{-2} M with a correlation coefficient of 0.9929 (Fig. 3). The detection limit was found to be 3.33×10^{-5} M. The method is comparable to other methods (Table 1) and gives a better working range for pharmaceutical preparations.

Table 1. Comparison of the Working Range and Detection Limit of the Proposed CME with Reported CMEs

S. No.	CMEs based on	Working range	Detection limit	Reference
1	Nickel pentacyanonitrosyl ferrate	2.00×10^{-3} M to 5.00×10^{-2} M	—	36
2	Nickel (II) macrocycle	0 to 5.0×10^{-3} M	2.5×10^{-7} M	15
3	Ruthenium oxide	0 to 4.0×10^{-3} M	—	37
4	Cobalt pentacyanonitrosyl ferrate	1.00×10^{-5} M to 1.20×10^{-4} M	—	38
5	Polyaniline film	5.00×10^{-3} M to 5.00×10^{-2} M	—	39
6	Prussian blue	1.20×10^{-6} M to 1.12×10^{-3} M	2.49×10^{-6} M	8
7	Proposed method	5.52×10^{-5} M to 3.23×10^{-2} M	3.33×10^{-5} M	—

Hydrodynamic Voltammetry Studies Hydrodynamic voltammetric studies on the bare and the CoHCF modified electrode were carried out in the potential range between 0 and 1.0 V in 0.1 M NaCl in order to check the applicability of the modified electrode in flow systems. The bare electrode showed a poor response to AA oxidation whereas there was a considerable increase in response with the modified electrode around 0.38 V (Fig. 4). This is in accordance with the oxidation potential of Co^{2+}/Co^{3+} couple found in cyclic voltammogram of the modified electrode, revealing the mediating effect of Co^{2+}/Co^{3+} couple. Hence 0.38 V was chosen as the operating potential for amperometric studies.

Chronoamperometric Determination of Ascorbic Acid The chronoamperometric study of the CoHCF modified elec-

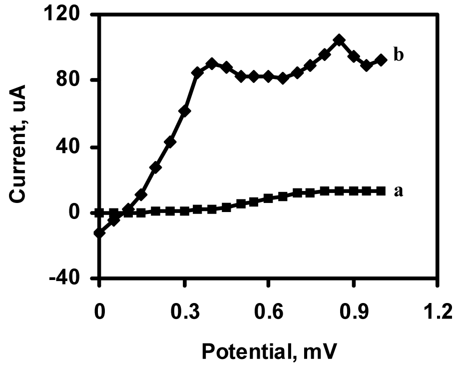

Fig. 4. Hydrodynamic Voltammograms of 6.2×10^{-4} M AA at (a) Bare and (b) CoHCF Modified Electrode in 0.1 M NaCl

Stirring rate: 300 rpm.

trode towards the quantification of ascorbic acid by electrocatalytic oxidation was also carried out and the results are depicted in Fig. 5. The figure shows the current-time recording obtained in a stirred solution of 0.1 M NaCl for successive addition of 0.5 ml of 0.05 M stock solution of AA at regular intervals. As can be seen, the CoHCF modified electrode responds very quickly to the changes in the AA concentration. Inset in Fig. 5 infers that under optimum conditions, the modified electrode showed a linear response to AA in the concentration range from 2.76×10^{-4} M to 2.38×10^{-3} M with a correlation coefficient of 0.9997. Linear amperometric response was obtained even for a wider concentration range from 5.0×10^{-5} M up to 3.23×10^{-2} M (Figure not shown).

Interference Studies To evaluate the interferences of foreign compounds in the determination of AA at 1.0×10^{-4} M level, a systematic study was carried out. Dopamine is found to interfere with AA in 1 : 1 ratio, whereas cysteine was found to interfere when its concentration exceeds twice that of AA. A 10-fold concentration of paracetamol, uric acid, glucose, fructose and H_2O_2 did not influence the current response of 1.0×10^{-4} M AA. The interference of surfactant was checked by the addition of Cetyl trimethyl ammonium bromide (CTAB) to the supporting electrolyte. For each 0.1 ml addition of CTAB (0.01 M), the peak currents decreased continuously and when the volume exceeds 0.5 ml, the peaks completely disappeared. Hence the electrode cannot be used if surfactant is present or used for sample preparation. Thus the CoHCF modified electrode is more suitable in pharmaceutical preparation only.

Analysis of Ascorbic Acid in Commercial Samples The analytical utility of the CoHCF modified electrode for the determination of ascorbic acid in commercial samples was tested by analyzing Celin and Limcee tablets. The amount of ascorbic acid present in the samples was determined and the results were compared with standard method (Table 2). The results clearly indicate that sufficient precision and accuracy has been achieved for the determination of ascorbic acid using the CoHCF modified electrode.

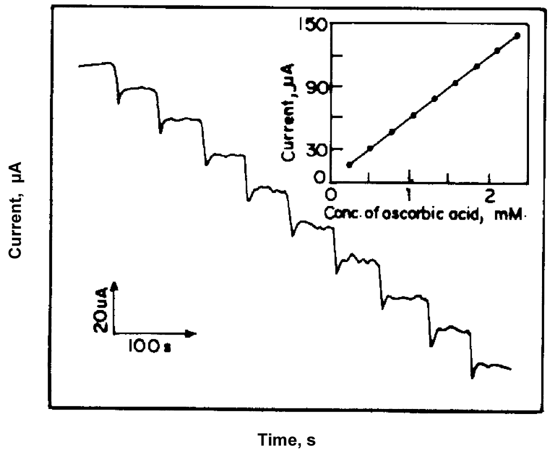

Fig. 5. Amperometric Response of the CoHCF Modified Electrode to Successive Injections of 0.5 ml of 0.05 M AA in Steps in the Supporting Electrolyte (0.1 M NaCl) at the Applied Potential of 0.38 V

Stirring rate: 300 rpm. Inset: Calibration plot for the determination of AA.

Table 2. Experimental and Statistical Results of Ascorbic Acid Determination in Commercial Samples

Sample	No. of measurements	Amount of ascorbic acid (mg)			Percentage recovery	Standard deviation
		Taken	Found (mean)			
			Present method	Titrimetry		
Celin	10	50	50.2	50.0	100.4	0.129
Limcee	10	50	49.7	49.8	99.4	0.094

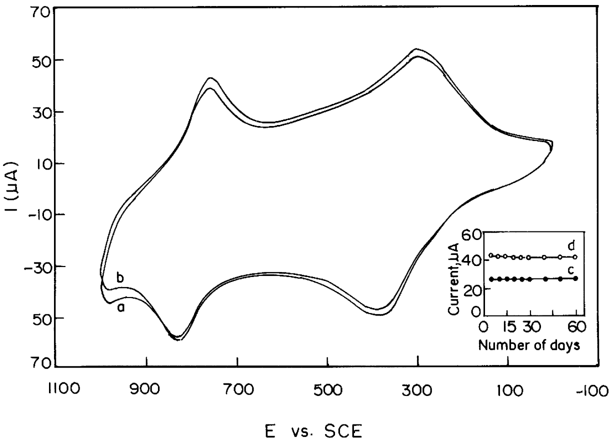

Fig. 6. Cyclic Voltammogram of the Modified Electrode in 0.1 M NaCl at (a) 1st and (b) 100th Cycle at a Scan Rate of 20 mVs^{-1}

Inset: Current response of the CoHCF modified electrode (c) in the absence of AA and (d) in the presence of 8.0×10^{-4} M AA.

Stability and Reproducibility The next important attention was given to the regeneration and stability of the CoHCF modified electrode. The voltammogram of 1st and 100th cycle of the modified electrode in 0.1 M NaCl in the potential range from 0 to 1.0 V *vs.* SCE is shown in Fig. 6. The decrease in peak current was negligible even on continuous cycling. The modified electrode when stored in air or supporting electrolyte did not show any change in its voltammetric response for more than two months (Inset in Fig. 6). The electrode was used daily for the oxidation of AA and its response was found to decrease only 2.6% even after 60 d. The above results indicate that the CoHCF modified electrode has a good storage and operational stability as an amperometric sensor.

Conclusion

A CoHCF modified amperometric sensor for ascorbic acid oxidation, based on the binding of cobalt ions to the adsorbed aniline on the surface of a graphite rod has been successfully achieved by means of a simple and easily reproducible procedure. The CoHCF modified electrode showed excellent electrocatalytic activity for the oxidation of ascorbic acid. The response of the modified electrode for the oxidation of ascorbic acid was found to be significantly unaffected for more than 60 d. The CoHCF modified electrode also possessed excellent stability and reproducibility for long-term use. Hydrodynamic and amperometric studies revealed the applicability of the modified electrode in flow injection analysis. Also the modified electrode was applied for the determination of ascorbic acid in commercial samples with good precision and accuracy. The proposed method furnishes a new route for the fabrication of chemically modified electrodes.

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