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Direct amperometric determination of L-ascorbic acid (Vitamin C) at octacyanomolybdate-doped-poly(4-vinylpyridine) modified electrode in fruit juice and pharmaceuticals

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Abstract

The glassy carbon electrode (GCE) modified with $Mo(CN)_8{}^{4-}$-incorporated-poly(4-vinylpyridine) ($PVP/Mo(CN)_8{}^{4-}$), which has been recently shown to possess several attractive attributes as an efficient electrocatalytic electrode for L-ascorbic acid oxidation and its estimation, is used for L-ascorbic acid estimation directly in orange fruit juice and Celin tablet in a 0.1 M H_2SO_4 acid solution without any special treatment. Constant potential amperometry at 570 mV (saturated calomel electrode, SCE) in stirred solutions is used for this purpose. A good correlation is attained with the official titrametric method. To understand the possible electrocatalytic reaction mechanism for the electro-oxidation of L-ascorbic acid, calibration graphs over the range 1×10^{-5} to 1×10^{-2} mol dm^{-3} L-ascorbic acid are compared for the three electrodes, ca. $PVP/Mo(CN)_8{}^{4-}$, undoped PVP, and GCE; the curvature at high ascorbic acid concentration for the $PVP/Mo(CN)_8{}^{4-}$ electrode is explained in terms of Michaelis–Menten (MM) saturation kinetics. The apparent MM constant (K_M), the maximum catalytic current (i_M), the complex decomposition rate constant (k_c), and the heterogeneous modified electrode rate constant (k'_{ME}) are calculated from three different approaches. A reasonably high value of $\approx 1 \times 10^{-2}$ cm s^{-1} is obtained for k'_{ME}, indicating efficient L-ascorbic acid mediation at the $PVP/Mo(CN)_8{}^{4-}$ electrode, thus accounting for quite a high sensitivity of this modified film electrode compared to several other modified electrodes.

Keywords: $PVP/Mo(CN)_8{}^{4-}$ film modified electrode; L-Ascorbic acid; Real sample analysis; Hydrodynamic amperometric determination; Michaelis–Menten saturation kinetics

1. Introduction

L-Ascorbic acid (AH_2), which is present in body fluids and also in cerebral fluids along with various neurotransmitters, their precursors, and their metabolites, is one of the most important biological compounds, taking part in several significant biological processes, such as immune response, wound healing, and therapeutic purposes [1]. It is also one of the most frequently taken vitamins to supplement dietary intake. Besides, ascorbic acid is a powerful anti-oxidant present in food and beverages, and it is also used as a marker chemical in evaluating food deterioration and product quality. In view of these several functionalities, the determination of AH_2 has attracted great attention in biomedical engineering, food and pharmaceutical industries [2]. Various methods and techniques, such as titrimetry [3], fluorimetry [3], spectrometry [4], and high performance liquid chromotography [5], have been employed in its measurements. In recent times, the development of several convenient and rapid procedures for measuring AH_2 levels using electrochemical methods has been the subject of considerable interest mainly as a result of high sensitivity and selectivity associated with the electrochemical techniques.

The estimation of ascorbic acid electrochemically by its direct oxidation on conventional electrodes, such as Hg [6], Au [7], Pt [8], and glassy carbon electrode (GCE) [9], is almost too difficult because of surface fouling by oxidation products and sluggish electron transfer [10,11]. This problem is overcome especially for carbon electrodes by adopting several electrode surface pretreatment methods, including

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electrochemical [12], laser radiation [13], vacuum heat treatment [14], dispersion of metal oxide particles on the surface [15], etc. As another approach, electron transfer mediators, which shuttle electrons between AH_2 and the electrode, have been studied, both in solution soluble form [16–18] and as monolayers confined to electrode surfaces [19–24], to estimate AH_2 at reduced overpotentials. Notable among them extended for real sample analysis are solution soluble benzoquinone [17], and carbon electrode surface modified ferrocene [20], TCNQ, tetrathiafulvalene, or 1,1-dimethylferrocene [24].

Recently, with the emergence of polymer modified electrodes, in which the active mediators are strongly entrapped three-dimensionally, several electrocatalytic systems with excellent mediation activity for L-ascorbic acid oxidation have been reported [25–36]. In most of the studies [25–32] ascorbic acid oxidation has been used as a bench mark reaction primarily to understand the mechanism of electrocatalysis at electroactive polymer modified electrodes in the context of Andrieux–Saveant [37] and Albery–Hillman [38] models. Nevertheless, a few polymer modified electrodes have been described also as electrochemical sensors for AH_2 [32–36]. However, even among them [32–36], an $[Os(bpy)_2(PVP)_{10}Cl]Cl$ (where bpy: 2,2′-bipyridyl and PVP: poly(4-vinylpyridine)) redox polymer coated electrode [32], electropolymerized conducting polypyrrole film electrodes doped with chloride or dodecylbenzene sulphonate ion [33], a $Fe(CN)_6{}^{4-}$-incorporated-perfluoro-anionic exchange membrane, Tosflex [34], and $SO_4{}^{2-}$-doped-electropolymerized polyaniline on Ni [35] have all been limited to the usual feasibility studies, establishing them as candidates for ascorbic acid estimation, but they have not been extended to application for ascorbic acid estimation in real samples. Only the Cl^- ion-doped-polyaniline sensor electrode has been demonstrated by O'Connell et al. [36] for useful estimation of Vitamin C content in several real samples.

In a recent preliminary work [39], we have developed an octacyanomolybdate-doped-poly(4-vinylpyridine) (PVP/$Mo(CN)_8{}^{4-}$) modified electrode for electrocatalytic oxidation of L-ascorbic acid and its several attractive potentialities have been described, such as strong binding capacity for $Mo(CN)_8{}^{4-}$, less-restricted AH_2 diffusion in the film, efficient mediation with the complete utilization of immobile mediator sites in the film, highly sensitive and selective electrocatalytic ability, and better stability. The analytical parameters of the PVP/$Mo(CN)_8{}^{4-}$ system are comparable with several other modified electrodes [39]. In this article we report the use of this composite electrode for direct estimation of L-ascorbic acid in fruit juice and pharmaceutical samples in acidic aqueous solutions without any special treatment. Also, hydrodynamic chronoamperometric and calibration curves are compared for PVP/$Mo(CN)_8{}^{4-}$, undoped PVP, and bare GC electrodes to understand the mechanism of oxidation of L-ascorbic acid at the modified electrode in terms of Michaelis–Menten (MM) saturation kinetics. The associated MM kinetic constants K_M, substrate–catalyst complex dissociation rate constant (k_c), maximum catalytic current (i_M), and the heterogeneous modified electrode rate constant (k'_{ME}) are estimated.

2. Experimental

2.1. Chemicals

PVP was obtained by the polymerization of 4-vinylpyridine monomer (Fluka) using 2,2′-azobis(isobutyronitrile) (Fluka) as the initiator. Its weight average molecular weight (M_w) determined viscometrically was 5.2×10^5 [40]. $K_4Mo(CN)_8 \cdot 2H_2O$ was prepared following the method suggested by Furman and Miller [41]. L-Ascorbic acid (SRL, India) was recrystallized twice from distilled water. All other chemicals were of analytical grade and were used without further purification. Double distilled water passed through activated charcoal column was used to prepare all experimental solutions. All the experimental solutions were de-aerated with oxygen-free nitrogen.

2.2. Apparatus

The cyclic voltammetry unit was a Wenking potentiostat (ST 72) coupled with a Wenking signal generator (VSG 83) and a Graphtec X-Y-t recorder (WX 2300). The electrode rotator was obtained from Pine Instruments Co., USA. A GCE of 0.196 cm^2 geometric area was used as the working electrode. A Pt electrode of large surface area and a saturated calomel electrode (SCE) were used as the counter and reference electrodes, respectively.

2.3. Preparation of $Mo(CN)_8{}^{4-}$-doped-PVP modified electrode

Before the modification procedure, the GCE was abraded with increasingly fine grades of emery papers (1/0, 2/0, 3/0, 4/0, and 6/0 Kohinoor products, India) down to mirror finish, degreased with trichloroethylene, smoothed on a polishing cloth, washed with double distilled water, and dried. The PVP films were prepared on the above pretreated GCE surface by droplet evaporation of 7 μl of a 0.2% (w/v) methanolic PVP solution, followed by drying for 12 min at room temperature. $Mo(CN)_8{}^{4-}$ ions were incorporated into the PVP coating by immersing the electrode in 0.1 M H_2SO_4 containing 2 mM $Mo(CN)_8{}^{4-}$ for 30 min. The amount of $Mo(CN)_8{}^{4-}$ incorporated into the film (Γ_{MoCN}) was calculated with the following equation [42]:

$$\Gamma_{MoCN} = \frac{Q_t}{nFA_e} \qquad (1)$$

where Q_t is the charge consumed, obtained from integrating the peak area in cyclic voltammograms under the background correction recorded at slow scan rate (5 mV s^{-1}) in pure 0.1 M H_2SO_4, n is the number of electrons consumed ($n = 1$ in the present case), and A_e is the electrode geometric area. Note that the true surface area of the electrode instead of its geometric area needs to be applied for calculations. However, as the same surface polishing method was adopted throughout the present work, it is reasonable to consider that the GC electrode surface of nearly same true surface area is produced for each experiment. Thus, in order to maintain uniform treatment of results, geometric area of GCE was used for calculations throughout the work.