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Synergetic effects in the flow injection analysis determination of catechol in the presence of excess ascorbic acid by series dual-band amperometric detection[1]

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Abstract

The deviations at high concentrations ($>10^{-5}$ M) in the calibration curves for the determination of catechol in catechol/ascorbic acid mixtures by flow injection analysis using series dual-band poly(3-methylthiophene)-coated electrodes has been re-examined. The cyclic voltammetry (at pH≅7.4) of catechol/ascorbate and catechol/urate mixtures and NMR measurements show these deviations are the result of the simultaneous homogeneous catalytic reaction of dehydrocatechol with ascorbate.

Keywords: Flow injection analysis; Dual band electrodes; Polymer electrodes; Catechol; Ascorbic acid; Uric acid; Synergetic effects; Micro electrodes

1. Introduction

Recently we reported a flow injection analysis (FIA) method for the determination of catechol in the presence of ascorbic acid using series dual-band poly(3-methylthiophene) electrodes (P3MT) [1]. Interference from the more easily oxidized ascorbic acid was eliminated for amperometric detection based on the fact that the catechol redox couple is virtually reversible at a P3MT electrode while the ascorbic acid couple is totally irreversible [2]. The upstream band electrode was held at a sufficiently positive potential to oxidize both the catechol and the ascorbic acid. By poising the downstream electrode at a current-limiting negative potential, a signal corresponding only to the reduction of the dehydrocatechol was observed. This method was effective for dilute solutions of catechol ($<10^{-5}$ M) with up to a tenfold excess of ascorbic acid (actually in the form of ascorbate ion, A^- in pH=7.4 buffer) [1]. However, for more concentrated mixtures ($>10^{-5}$ M), significant deviations from ideal behavior were found for both the anodic and cathodic calibration curves as shown in Fig. 1. This behavior was also

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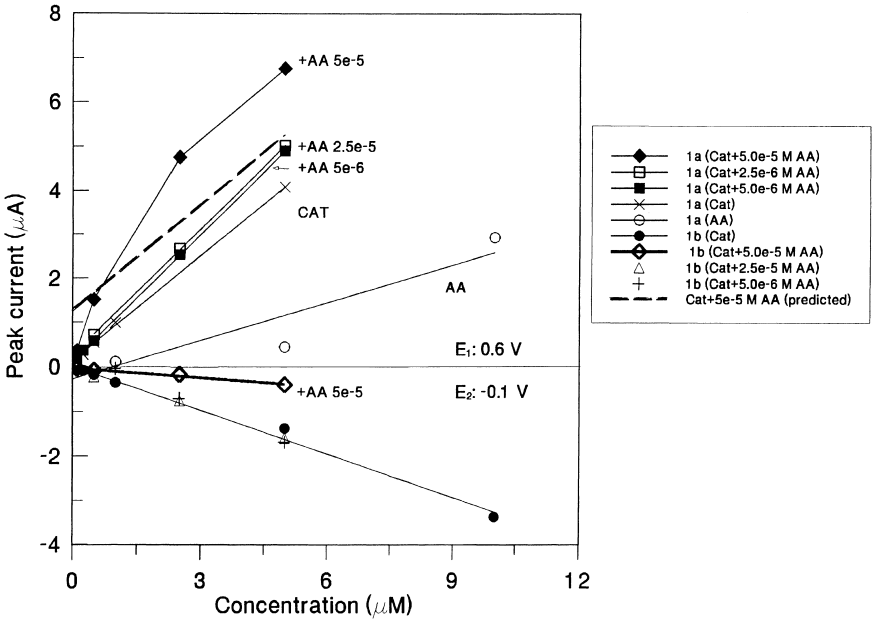

Fig. 1. Calibration curves for the determination of catechol in the presence of ascorbic acid ranging from 5.0×10^{-6} to 5.0×10^{-4} M using series dual-band electrode flow-amperometry at pH=7.4 (interelectrode gap 150 μm, band size approximately 100 μm×2.5 mm. Potential of the upstream, E_1, and downstream, E_2, electrodes were 0.40 and −0.10 V vs Ag/AgCl. Mobile phase: 0.1 M phosphate buffer and 0.1 M NaCl; flowrate 1.0 ml min^{-1}; sensitivity 11 μA.

observed on gold, carbon and platinum dual-band microelectrodes as well as on Pt macro dual disk electrode units. It had been suggested previously that the negative deviation of the cathodic calibration curve (based on the response of the downstream electrode) might be explained by hydrogen bonding and/or charge–dipole interactions [1]. The fact that these deviations or synergetic effects disappeared in acidic media (pH=1.6) was used to support this hypothesis. This paper examines the nuclear magnetic resonance (NMR) spectra of catechol/ascorbate and catechol/dehydroascorbate mixtures in various ratios of concentration. Also, the cyclic voltammetric (CV) behavior at a P3MT ultramicroelectrode of such mixtures and those of catechol/uric acid (actually urate anion at pH=7.4) mixtures are examined. A unified mechanism is presented which explains both the anodic and cathodic deviations.

2. Experimental

With the exception of the preparation of the P3MT microelectrodes for the CV experiments and the NMR experiments, all experimental conditions and instrumentation were identical to those previously reported [1–3].

2.1. Electropolymerization of 3-methylthiophene

Electrochemical polymerization was carried out in a one-compartment cell containing deaerated acetonitrile, 0.075 M tetrabutylammonium tetrafluoro-borate, and 0.05 M 3-methylthiophene. Film growth on glassy carbon fiber ultramicroelectrodes (GC UMEs), which were 8 μm in diameter and approximately 1 mm in length, was achieved by applying a constant potential of +1.7 V vs Ag/AgCl for 10 s at room temperature using an EG&G Model 173 potentiostat/galvanostat.

2.2. NMR experiments

The 400 MHz ^{1}H NMR spectra were obtained on a Bruker AMX-400 MHz multinuclear spectrometer using a 5 mm ^{1}H probe. The sample temperature was maintained at 303.1 K and a presaturation sequence was used for solvent suppression. Chemical

shifts were recorded with respect to an internal standard $(CH_3)_4NI$ at 3.0 ppm.

3. Results and discussion

The NMR spectra of millimolar solutions of catechol, dehydroascorbate, and ascorbic acid at pH=7.4 were obtained as well as those for mixtures of catechol/ascorbate and for catechol/dehydroascorbate. The mixtures examined were in the ratios 1:1, 1:2, 1:5 and 5:1. The chemical shifts for all of the mixtures were the same as those of the pure compounds within ±0.5 Hz (about 0.01 ppm), Thus, there is *no interaction* of any kind between catechol, ascorbate and dehydroascrobate.

All attempts to synthesize and purify dehydrocatechol in order to extend the NMR investigation were unsuccessful. Although several published synthetic routes were tried [4–6], in all cases, brown to dark-red oils were obtained indicating decomposition when recrystallization was attempted. Further effort was considered unnecessary as it is highly unlikely, in view of the total lack of interaction of the above three compounds, that the dehydrocatechol NMR spectra would have indicated any significant hydrogen bonding with these other compounds.

As previously discussed [1], the positive deviation of the anodic calibration curve as shown in Fig. 1 can be explained by the observation of Wightman and coworkers [7] that the electrochemical oxidation of catechols in the presence of ascorbate also produced a homogeneous catalytic oxidation of solution phase ascorbate (A^-) by the electrogenerated dehydrocatechol (DHC). The dehydrocatechol is regenerated in a cyclic manner at the electrode, E_1, of Fig. 2, which results in the current enhancement. This homogeneous reaction of DHC with A^- also explains the negative deviation of the current response at the downstream electrode at high reactant concentrations. In the gap between the two-band electrodes, DHC continues to diffuse into the solution phase and react with A^- but DHC is no longer being electrogenerated. Thus, the concentration of DHC in the diffusion layer is significantly decreased as the solution reaches the downstream electrode, E_2. The reaction sequence in the dual-band detector is shown schematically in Fig. 2. The fact that this negative deviation is not observed at pH=1.6 is a result of the much slower kinetics of the reaction of DHC with ascorbic acid, as expected.

Cyclic voltammetric studies of catechol/ascorbate and catechol/urate ion mixtures at pH=7.4 using P3MT-coated carbon fiber microelectrodes were used

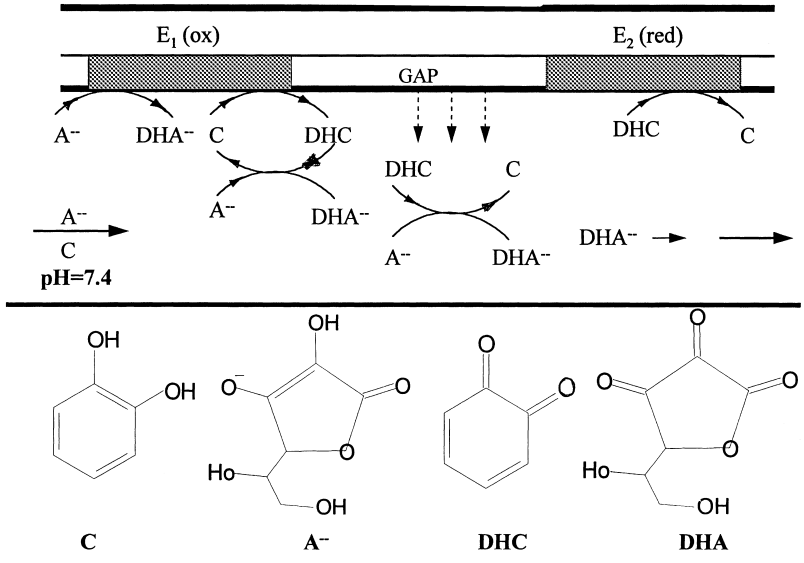

Fig. 2. Schematic diagram of cross-section of the series dual-band flow cell and the reaction sequences in the stream.

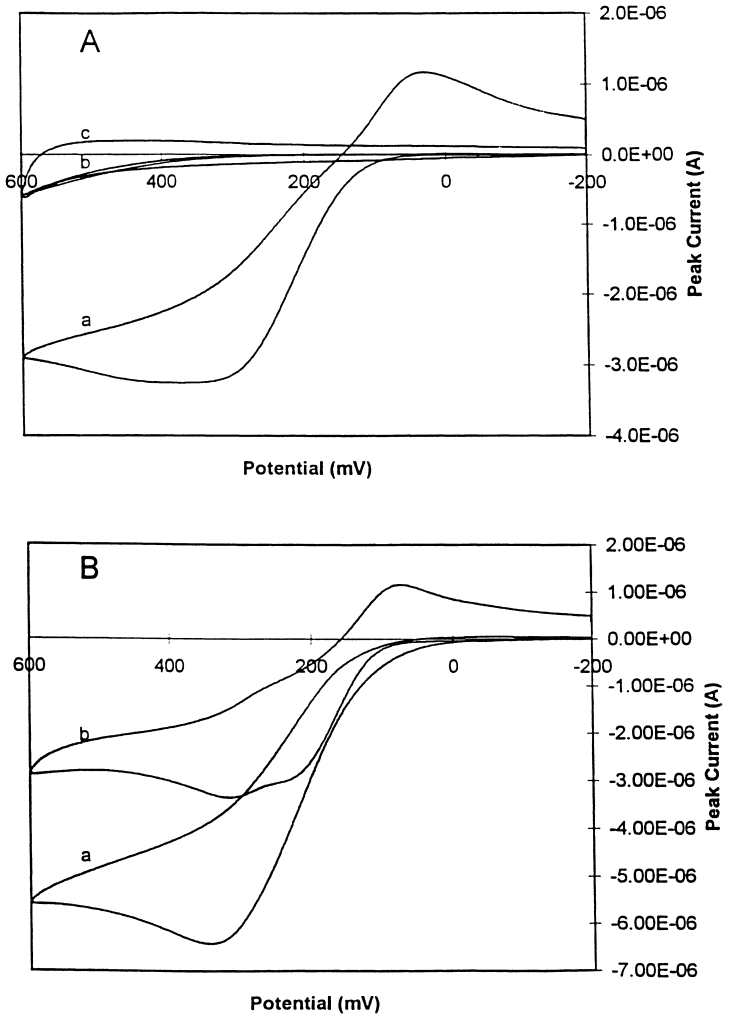

Fig. 3. (A) Cyclic voltammograms of: (a) 5 mM catechol at a P3MT-coated glassy carbon ultramicroelectrode; (b) 5 mM catechol at a bare glassy carbon electrode; (c) 0.1 M phosphate buffer (pH=7.4)+0.1 M NaCl at P3MT-coated glassy carbon electrode. (B) Cyclic voltammograms of: (a) 5 mM-catechol+10 mM ascorbate; (b) 5 mM catechol+5 mM urate at a P3MT-coated glassy carbon ultramicroelectrode. The background-electrolyte was the same as in (A). All scans carried out at 500 mV^{-1}.

to confirm this explanation of the series dual-band synergetic effects. Fig. 3(A) shows that the typical chemically reversible behavior of catechol. Fig. 3(B), curve a, shows the CV for a catechol/ascorbate mixture. In this case, the reverse wave for the reduction of DHC is completely missing. The electrogenerated DHC is totally consumed in the diffusion layer by a homogeneous follow-up reaction with A^-. However, for the catechol-urate mixtures, the CV (Fig. 3(B), curve b) shows that catechol is more easily oxidized than urate (two peaks in the anodic wave) and the DHC reverse wave is present. (The urate oxidation product is electroinactive.) DHC cannot oxidize urate, and thus, is not chemically lost in the detector gap region.

Therefore, based on the CV and NMR data for the compounds investigated, the explanation for the deviations from linear behavior lies in the homogeneous cyclic regeneration of catechol by reaction between dehydrocatechol and ascorbate.

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