# Simultaneous Electroanalysis of Ascorbic Acid, Dihydroxyphenylacetic Acid, Homovanillic Acid and Uric Acid Using Gold Electrode Modified with Cationic Self-Assembled Monolayers

Chellappan Retna Raj and Takeo Ohsaka*
*Department of Electronic Chemistry, Interdisciplinary Graduate School of Science and Engineering, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama 226-8502*

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Cationic self-assembled monolayers (SAMs) of cystamine (CYST) and dithiobishexaneamine (DTH) are utilized for the simultaneous electroanalysis of four different anions, ascorbic acid (AA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and uric acid (UA).

There has been considerable interest in the development of voltammetric sensor for the detection of neurotransmitters and their metabolites and uric acid.[1–3] The neurotransmitter metabolites released into the cerebrospinal fluid (CSF) can be a sensitive indicator of neuronal functioning in nearby diencephalon structures.[4] Measurements are, however, complicated due to the coexistence of high concentration of ascorbic acid (AA), which oxidizes almost at the same potential as the other analytes. Uric acid (UA) is one of the principal final products of the purine metabolism in the human body. Abnormal levels of UA are symptoms of several diseases such as gout, hyperuricemia.[2] It is generally believed that the direct oxidation of these analytes at bare electrodes is not reversible and it requires large overpotentials. Moreover the direct oxidation at the bare electrodes very often suffers from a pronounced fouling effect by the oxidation products, which results in rather poor selectivity and sensitivity. Various approaches have been made to overcome these problems.[1–3] An attractive approach for the modification of electrode surface, which has been explosively used for the last decade, involves the formation of self-assembled monolayers (SAMs) on gold (Au) electrode.[5] Substantial efforts have been devoted to the development of electrochemical sensor based on electrodes modified with SAMs.[5–7] Here we report the simultaneous electroanalysis of AA, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and UA using cationic self-assembled monolayers of cystamine (CYST) and dithiobishexaneamine (DTH). As all these analytes are anionic at the physiological pH and are almost the same in size, it is a challenging task to simultaneously detect all these anions.

The SAMs of CYST, mercaptoethanol (ME) and diethyl disulfide (DEDS) were fabricated by immersing the clean Au electrode (0.02 $cm^2$) into an aqueous or ethanol solution of 1 mM respective thiol/disulfide for 1 h. The SAM of DTH was formed by immersing the Au electrode into an ethanol solution of DTH for 12 h. The SAMs of CYST and DTH are expected to be positively charged (p$K_a$ of CYST is 8.35) and the SAMs of DEDS and ME will exist as neutral at physiological pH.

The electrochemical measurements were carried out using a two-compartment three-electrode cell with an Au working electrode (diameter 1.6 mm), a Pt wire auxiliary electrode and a NaCl saturated Ag/AgCl reference electrode. The Au working electrodes were polished with alumina powder (1.0 and 0.06 μm) and sonicated in water for 5–10 min. The polished electrodes were then electrochemically cleaned by cycling the potential scan between –0.2 and 1.5 V in 0.05 M $H_2SO_4$ at the scan rate of 10 V/s for 10 min or until the CV characteristics for a clean Au electrode were obtained. The following instrumental parameters were used to record Osteryoung square-wave voltammograms: Square wave amplitude: 25 mV; Frequency: 15 Hz; Step potential: 4 mV; Quiet time: 2 s.

Figure 1 shows the Osteryoung square-wave voltammograms obtained for a mixture of AA, DOPAC, HVA and UA at the bare and monolayer modified Au electrodes. The bare electrode showed an indistinguishable voltammetric peak at ca. 0.43 V for all the four analytes (Figure 1a) and it underwent a progressive deterioration upon the repeated potential scan. Fouling of electrode surface by the adsorption of oxidation products could be the reason for the observed behavior at the bare electrode. On the other hand, the cationic SAM modified electrodes (CYST and DTH) successfully separated the voltammetric peaks of all the four analytes. Although all the four analytes are negatively charged at the physiological pH, the cationic SAMs can clearly distinguish the voltammetric peaks of the four analytes. Therefore the simultaneous detection of these analytes is feasible at the present cationic SAM electrodes. The neutral monolayers (ME and DEDS) failed to separate the voltammetric signals of these analytes.

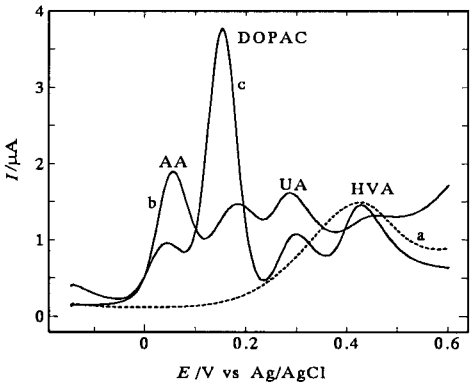


**Figure 1.** Osteryoung square-wave voltammograms for the oxidation of AA, DOPAC, HVA and UA (0.1 mM each) at (a) bare, (b) CYST-Au and (c) DTH-Au electrodes in 0.1 M phosphate buffer (pH 7.2).

At the bare Au electrode the oxidation of AA occurs at around 0.5 V and the electron transfer kinetics is rather sluggish possibly due to the fouling of electrode surface. However, at the CYST monolayer-modified electrode the electron transfer is very fast and the oxidation potential is shifted to less positive potential (~0.05 V). The electrostatic interaction of anionic AA with the cationic monolayer facilitates the electron transfer.[8]

Moreover, the SAM of CYST on Au electrode prevents the fouling of electrode surface, which favors the oxidation of AA at less positive potential. In the cases of neutral monolayers, the oxidation of AA occurs at more positive potential (~0.21 and ~0.32 V at ME and DEDS monolayers, respectively) than those at the cationic SAM electrodes. The oxidation of AA at these monolayers is relatively fast, compared to the bare electrode, presumably due to the prevention of the fouling of electrode surface. Therefore it can be concluded that the enhanced oxidation of AA at the cationic SAM electrodes is due to both the electrostatic interaction of anionic AA with the cationic monolayers and the prevention of the fouling of electrode surface.

Considerable acceleration in the oxidation of DOPAC is observed at the cationic monolayer-modified electrodes. That is, the peak to peak separation ($\Delta E_p$) at the bare Au electrode is more than 150 mV, but it becomes 86 ± 2 and 44 ± 4 mV at the CYST and DTH monolayers, respectively. The electrostatic interaction between DOPAC and the cationic self-assemblies favors its oxidation. The DTH monolayer shows relatively large voltammetric signal for DOPAC (Figure 1(c)) compared to the CYST monolayer. The adsorption of DOPAC into the monolayer could be the reason for the observed large current, because the DTH monolayer used for the oxidation of DOPAC showed voltammetric signal for DOPAC when it was transferred to the pure supporting electrolyte. The oxidation of DOPAC at the neutral monolayers is rather slow, compared with the cationic monolayer.

In the case of HVA, although there is no significant change in the oxidation potential at the monolayer-modified electrodes compared to the bare electrode, an enormous enhancement in the peak current was observed at the cationic monolayer-modified electrodes. At the neutral monolayers the peak current was little increased compared to the bare electrode. These results obtained for HVA suggest that the electrostatic interaction of anionic HVA with cationic monolayer facilitate the oxidation of HVA. On the other hand, a ca.130-mV negative shift in the peak potential for the oxidation of UA was observed at the cationic monolayers, while it was almost unchanged at the neutral monolayers. Although all the four analytes are negatively charged at physiological pH, the oxidation of AA is significantly influenced at the cationic monolayer-modified electrodes. As the thermodynamic potential of AA is more negative (–0.2 V)[9] than other analytes, it is reasonable to expect a large negative shift in the oxidation potential of AA at the cationic monolayer-modified electrodes.

As the concentration of AA is higher than those of neurotransmitter metabolites in the brain and is a major interference in the detection of neurotransmitters and their metabolites, we measured the square-wave voltammetric signals of HVA and DOPAC in the presence of high concentration of AA. As shown in Figure 2, the voltammetric signal of AA is almost unchanged in the presence of different concentrations of HVA and DOPAC, indicating that the present SAM electrode can be used for the detection of these neurotransmitter metabolites in the presence of high concentration of AA. The sensitivities of

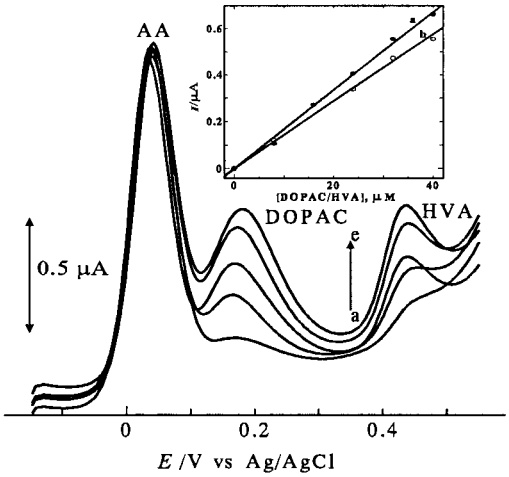


**Figure 2.** Osteryoung square-wave voltammograms of DOPAC and HVA in the presence of AA (0.1 mM) at CYST-Au electrode in 0.1 M phosphate buffer (pH 7.2). [HVA] = [DOPAC]: (a) 8, (b) 16, (c) 24, (d) 32 and (e) 40 μM. Inset shows the calibration plots for (a) DOPAC and (b) HVA.

the CYST electrode towards the sensing of DOPAC and HVA in the presence of 0.1 M AA were found to be 0.0167 ± 0.0005 and 0.0144 ± 0.0003 μA/μM, respectively. Linear increases in the peak currents were observed while simultaneously changing the concentrations of all the analytes.

The present work demonstrates the possible application of cationic SAMs for the simultaneous electroanalysis of AA, DOPAC, UA and HVA. Further studies are underway.

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