Electrocatalytic oxidation of ascorbic acid by immobilized silver nanoparticles on self-assembled L-cysteine monolayer modified gold electrode

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The electrocatalytic oxidation of ascorbic acid in phosphate buffer solution (pH 7.0) by immobilized silver nanoparticles (Ag@CTAB) on L-cysteine modified gold electrode is reported. The modified electrode has been characterized electrochemically using redox couple [Fe(CN)6]3-/4- and [Mn(NH3)6]3+/4+. The electrocatalytic activity of the prepared electrodes is studied using cyclic voltammetry and electrochemical impedance spectroscopy. Electrochemical measurements show that the modified electrode (Au/L-cysteine/AgNPs) is highly active towards ascorbic acid oxidation. The oxidation peak of ascorbic acid at the Au/L-cysteine/AgNPs electrode is highly stable upon repeated potential cycling. The oxidation current of ascorbic acid increases upon each increment (0.05–0.35 µM) in differential pulse voltammetry experiments. The oxidation current shows a linear relationship with the concentration of ascorbic acid with a correlation coefficient of 0.996. The detection limit of ascorbic acid was found to be 2×10⁻¹² M. Common physiological interferents such as glucose, tartaric acid, citric acid and cysteine do not show any interference within the detection limit of ascorbic acid. The silver nanoparticles modified gold electrode has been used to determine the amount of ascorbic acid present in fruit and vegetable juices.

Keywords: Electrocatalytic oxidation, Ascorbic acid, Silver nanoparticles, L-Cysteine, Self-assembled monolayer, Gold electrode

Ascorbic acid (AA), the most ubiquitous vitamin present naturally in fruits and vegetables, is important in forming the protein collagen, and plays a paramount role as an antioxidant and preservative. Ascorbic acid has been widely used in food industry, pharmaceutical formulation and cosmetic applications. Therefore, an accurate, reliable and rapid method is needed for accurate determination of ascorbic acid. Different methods can be used for the quantitative estimation of AA such as volumetric titration, spectrophotometry, fluorimetry, high performance liquid chromatography, flow analysis, terbiometry, etc. The detection of AA by voltammetric methods has received much attention mainly due to their interesting electrocatalytic and biosensing applications. Detection of ascorbic acid using gold nanoparticles attached to glassy carbon (GC) electrode modified with 4-aminobenzoic acid followed by coupling with 4-aminophenol has been reported with the detection limit of 2.8 µM AA. The detection limit of 0.1 mM AA was reported at multilayers of AuNPs/redox polymers immobilized on GC electrode. Electrocatalytic oxidation of AA by the immobilised citrate capped AuNPs on 1,6-hexanedithiol (HDT) modified Au electrode has also been reported with a detection limit of 1 µM for AA.

Amongst all metal nanoparticles, silver nanoparticles (AgNPs) are of high interest because of their catalytic properties. Traditionally, AgNPs have been employed as catalyst in different reactions. Also, Ag exhibits the highest electrical and thermal conductivity among all metals. Recently, modification of electrode surface with noble metal nanoparticles has received much attention because of their interesting electrocatalytic and biosensing applications. Detection of ascorbic acid using gold nanoparticles attached to glassy carbon (GC) electrode modified with 4-aminobenzoic acid followed by coupling with 4-aminophenol has been reported with the detection limit of 2.8 µM AA. The detection limit of 0.1 mM AA was reported at multilayers of AuNPs/redox polymers immobilized on GC electrode. Electrocatalytic oxidation of AA by the immobilised citrate capped AuNPs on 1,6-hexanedithiol (HDT) modified Au electrode has also been reported with a detection limit of 1 µM for AA.
Experimental

L-cysteine (Sisco Research Laboratories) and AgNO₃ (Qualigen Fine Chemicals) were used as received. Sodium borohydride and ascorbic acid were purchased from SD Fine Chemicals Ltd., while cetyltrimethyl ammonium bromide (CTAB) was obtained from Central Drug House (P) Ltd. All other chemicals were of analytical grade and used without further purification. Phosphate buffer saline (PBS) solution was prepared by mixing 0.1 M NaClO₄ and 0.01 M H₃PO₄ using Smalley method. The pH of the solution was adjusted with 0.11 M NaOH. The Au electrode was polished using aqueous slurries of gamma alumina oxide (0.05 micron) and sonicated in doubly distilled water. The mechanically polished Au electrode was then cleaned electrochemically by cycling the potential between −0.2 V and 1.6 V versus Ag/AgCl reference electrode in 1 M H₂SO₄ at a scan rate 100 mV/s until the characteristic reproducible voltammograms were obtained.

Electrochemical measurements were performed with a conventional three-electrode cell with a gold electrode as working electrode, a Pt wire as counter electrode and Ag/AgCl (0.03 M KCl) as reference electrode. All electrochemical experiments were carried out with CH Instrument-660C electrochemical workstation. UV-vis spectra were recorded on a Shimadzu UV-3101PC spectrophotometer.

CTAB capped Ag nanoparticles (Ag@CTAB) were prepared by adding 2 mL of ice cold solution of 0.1 M NaBH₄ to 1.25 mL of 10⁻² M AgNO₃ prepared in 48.75 mL of 0.01 M CTAB solution under vigorous stirring for eight hours. The colourless solution changed slowly to yellow, indicating the formation of AgNPs. The solution was stored at 4 °C in a dark bottle until further use. Characteristic UV-vis absorption of the CTAB capped Ag nanoparticles, with the sharp peak appearing at 410 nm was observed (Supplementary data, Fig. S1).

The electrochemically cleaned electrode was immersed in 10 mM L-cysteine solution containing 0.1 M HClO₄ for 24 h to allow the chemisorptions of the reagent on to the gold. The modified gold was then rinsed thoroughly with ethanol and water. Immobilisation of Ag nanoparticles on Au/L-cysteine modified SAM electrode was done by dipping the Au/L-cysteine electrode into an Ag colloidal solution for 4 h. The resultant electrode was washed with doubly distilled water and used for electrochemical measurements of AA.

Results and discussion

Figure 1 shows the cyclic voltammograms of 0.5 mM [Fe(CN)]₃⁴⁻⁻ obtained for bare Au, Au/L-cysteine and Au/L-cysteine/AgNPs electrodes in PBS at pH 7. The bare Au electrode exhibits a quasi-reversible voltammetric response for [Fe(CN)]₃⁴⁻⁻ redox couple with a peak separation of 116 mV (curve 1) at a scan rate of 100 mV/s. The cathodic peak current was significantly decreased (2.4×10⁻⁶ A to 8.6×10⁻⁷ A) in the case of Au/L-cysteine SAM modified electrode as compared to bare Au electrode. This suggests that the monolayer of L-cysteine was densely packed on Au electrode surface and thus effectively blocked the electronic communication between the [Fe(CN)]₃⁴⁻⁻ in solution and the underlying gold electrode surface. After the immobilisation of AgNPs on Au/L-cysteine electrode the cathodic current peak increased from 8.6×10⁻⁷ A to 3.5×10⁻⁶ A with a peak separation of 67 mV (curve 3), indicating that the Ag nanoparticles were successfully immobilized on the L-cysteine modified gold electrode and a good electronic communication was achieved between the redox species [Fe(CN)]₃⁴⁻⁻ in solution and the underlying Au electrode through AgNPs.

The influence of pH of electrolyte solution on the electrochemistry of immobilized AgNPs over Au-L-cysteine SAM was studied. The cathodic peak current reached the maximum value at pH 7.0. Beyond this pH, the cathodic peak current decreased. It is known that the isoelectric point of native cysteine is 5.06 and with a negative charge on cysteine in the
pH domain of 6.0−7.5. In other words, the L-cysteine coated gold electrode carries negative charge in this pH range. At pH 7.0, the interaction between positively charged Ag nanoparticles and the negatively charged L-cysteine reached a maximum as is observed from the cathodic peak current. At low pH, a poor response was obtained, as shown by low currents. This is due to poor interaction between the carboxyl group and the positively charged Ag nanoparticles. A representative cyclic voltamogram at varying pH (2.0 to 7.0) is given in Fig. S2 (Supplementary data). At the higher pH range (7.5−10.0), the free amino group may interact with the gold surface and weaken the interaction between amine group and AgNPs, showing low current height. At high pH (> 10.0) the signal was unstable and disappeared.

The cyclic voltammograms obtained in the presence of 0.5 mM ascorbic acid, 0.1 M PBS and at pH 7, on bare Au, Au/L-cysteine SAM electrode and Au/L-cysteine/AgNPs modified electrode are represented in Fig. 2. An irreversible oxidation of AA occurred at 0.34 V at Au/L-cysteine/AgNPs modified electrode, which was 100 mV less positive than the oxidation of AA (0.44 V) at Au/L-cysteine electrode; the oxidation current of AA increased from 4.6 µA to 5.4 µA. This behaviour demonstrates electrocatalytic activity for Au/L-cysteine/AgNPs electrodes towards AA oxidation. No reduction peak appeared for ascorbic acid on bare or modified electrodes. This confirms the data reported in literature that electrochemical oxidation of ascorbic acid is an irreversible process.

Electrochemical impedance spectroscopy (EIS) was carried out in 0.5 M ascorbic acid at pH 7.0 (PBS buffer) using modified working electrodes (bare Au electrode, Au/L-cysteine SAM electrode and Au/L-cysteine/AgNPs modified electrode) where the frequency range was 0.01 Hz to 100000 Hz and $E_{ac} = 10$ mV. The diameter of the semicircle observed in the Nyquist plot corresponds to the charge transfer resistance, $R_{ct}$; the smaller the semi-circle, faster is the charge or electron transfer. Figure 3 shows that the semi-circle decreases upon immobilisation of AgNPs on Au/L-cysteine SAM electrode surface. The decrease in semi-circle ($R_{ct}$) shows the following trend: bare Au (2.518×10^6 Ω) > Au/L-cysteine (13.776×10^6 Ω) > Au/L-cysteine/AgNPs (7.574×10^6 Ω). The observed trend is due to the fact that the modified electrodes facilitate electron transfer rate for the oxidation of ascorbic acid to dehydroascorbic acid. Impedance measurements clearly show that Au/L-cysteine/AgNPs exhibits lower resistance as compared to the bare Au and Au-L-cysteine SA modified electrodes. This study shows that the Au/L-cysteine/AgNPs modified electrode is an efficient electrocatalyst for AA oxidation.

The dependence of voltammetric response on the ascorbic acid concentrations at Au/L-cysteine/AgNPs modified electrode is shown in Fig. 2. A representative cyclic voltammogram at varying pH (2.0 to 7.0) is given in Fig. S2 (Supplementary data). At the higher pH range (7.5−10.0), the free amino group may interact with the gold surface and weaken the interaction between amine group and AgNPs, showing low current height. At high pH (> 10.0) the signal was unstable and disappeared.

![Fig. 2 — Cyclic voltammogram of 0.5 mM ascorbic acid in 0.1 M PBS solution at pH 7 using different working electrodes.](image1)

![Fig. 3 — Nyquist plot ($-Z'$ versus $Z'$) of 0.5 mM ascorbic acid in 0.1 M PBS solution at pH 7 using various working electrodes obtained from impedance measurements.](image2)
modified electrode was studied using DPV. Figure 4 shows the DPV responses of the modified electrode towards AA at varying concentrations. The oxidation current of AA increases linearly in the range of 0.05–0.35 μM, (linear regression equations: \(I_p (\mu A) = 18.821 C (\mu M) + 1.4286\) with a correlation coefficient of 0.996, as shown in Fig. 4 (inset)). Detection limit of \(2 \times 10^{-12} M\) for AA was obtained using \(3\sigma/m\), where \(\sigma\) is the standard deviation (1.259×10\(^{-11}\) A) of the peak current in blank solution, \(n = 25\) and \(m\) (18.826 μA/μM) is the slope of the calibration curve). The detection limit was further confirmed by chronamperometry (Supplementary data, Fig. S3).

The application of Au/L-cysteine/AgNPs modified electrode for the determination of AA at 0.5 μM concentration was also checked in the presence of 0.5 mM concentration of glucose, tartaric acid, citric acid, and cysteine as interferents in PBS solution (pH 7).

In the presence of interferents, the oxidation peak potential of AA was stable and the current response of AA was also not affected (Supplementary data, Fig. S4). Thus, Au/L-cysteine/AgNPs modified electrode may be successfully used to determine the concentration of AA in the presence of physiologically common interferents.

Fruit (orange, lemon, apple, and grape) and vegetable (tomato, cabbage, and cauliflower) juices were obtained by pressing. The stock solution (1 mL juice was diluted with 9 mL PBS buffer solution; pH = 7) was prepared and DPV was recorded in each case with the Au/L-cysteine/AgNPs modified electrode. The ascorbic acid content was calculated by measuring the peak currents obtained for sample solutions and after addition of standard AA solution, using the equation

\[I = KC\]

where \(I\) is the current obtained for sample solution and \(K\) is the increased amount of current after addition of standard AA solution and \(C\) is the unknown concentration. The obtained results are presented in Table 1 and agree with the data reported in literature\(^{34-36}\). The accuracy of the method was also verified by recovery studies adding standard ascorbic acid solutions to samples. Recoveries of 99.91–105.85% were achieved. A representative DPV for AA in orange juice solution and after addition of standard AA solution is shown in Fig. S5 (Supplementary data). Also, recovery and reproducibility of these measurements were satisfactory.

The present study demonstrates an excellent approach for the development of a novel silver nanoparticles/L-cysteine modified gold electrode for the electrocatalytic oxidation of ascorbic acid. Fast electron transfer and high stability for the oxidation of ascorbic acid were achieved at the Au/L-cysteine/AgNPs modified electrode. The electrocatalytic oxidation of AA showed the following order of the studied electrode: Au/L-cysteine/AgNPs > Au/L-cysteine > bare Au. EIS results are consistent.

### Table 1— Determination of ascorbic acid content in various fruit and vegetable juices using DPV

<table>
<thead>
<tr>
<th>Sample (juice)</th>
<th>Initial current (μA)</th>
<th>Current after addition of standard AA solution (μA)</th>
<th>AA acid conc. (mg/100 mL juice)</th>
<th>Ascorbic acid (×10(^{-3}) M)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange</td>
<td>-0.6891</td>
<td>-0.8395</td>
<td>39.91</td>
<td>1</td>
<td>105.85</td>
</tr>
<tr>
<td>Lemon</td>
<td>-0.8171</td>
<td>-0.9855</td>
<td>47.52</td>
<td>1</td>
<td>99.96</td>
</tr>
<tr>
<td>Apple</td>
<td>-0.6566</td>
<td>-0.8335</td>
<td>34.41</td>
<td>1</td>
<td>99.95</td>
</tr>
<tr>
<td>Grape</td>
<td>-0.6523</td>
<td>-0.8480</td>
<td>51.05</td>
<td>1</td>
<td>99.91</td>
</tr>
<tr>
<td>Tomato</td>
<td>-0.6603</td>
<td>-0.7552</td>
<td>29.18</td>
<td>1</td>
<td>100.03</td>
</tr>
<tr>
<td>Cabbage</td>
<td>-0.4534</td>
<td>-0.5227</td>
<td>18.59</td>
<td>1</td>
<td>99.96</td>
</tr>
<tr>
<td>Cabbage</td>
<td>-0.6538</td>
<td>-0.7922</td>
<td>26.00</td>
<td>1</td>
<td>99.92</td>
</tr>
</tbody>
</table>

Fig. 4 — Overlaid DPV with increasing ascorbic acid concentration (0.05—0.35 μM) in 0.1 M PBS (pH 7) at Au/L-cysteine/AgNPs modified electrode. [Inset: Plot of current as a function of concentration of ascorbic acid with linear trend line (\(R^2 > 0.99\)).]
with the CV responses. The DPV results indicate that the Au/L-cysteine/AgNPs modified electrode has a superior detection limit \(2.0 \times 10^{-12} \text{ M}\) than earlier reported gold nanoparticles modified electrode systems. This electrode selectively determines ascorbic acid in presence of physiologically common interferents and has been successfully used for analysis in fruit and vegetable juices with good recovery. The sensor displays good storage stability if kept in aqueous medium at room temperature. The Au/L-cysteine/AgNPs modified electrode retained its initial activity after one–two weeks of storage.

**Supplementary data**

Supplementary data associated with this article, i.e., Figs S1-S5, are available in the electronic form at http://www.niscair.res.in/jinfo/ijca/IJCA_53A(01)57-61_SupplData.pdf.

**References**