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Selective determination of 3,4-dihydroxyphenylacetic acid in the presence of ascorbic and uric acids using polymer film modified electrode

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Abstract. We report here the highly sensitive and selective electrochemical determination of 3,4-dihydroxyphenylacetic acid (DOPAC), one of the dopamine metabolites in the presence of important interferents ascorbic acid (AA) and uric acid (UA) using an ultrathin electropolymerized film of 5-amino-1,3,4-thiadiazole-2-thiol (p-ATT) modified glassy carbon (GC) electrode in 0.20 M phosphate buffer solution (pH 5.0). The bare GC electrode fails to resolve the oxidation peaks of AA, DOPAC and UA in a mixture. Further, the oxidation peak potentials of them were shifted to more positive potential with decreased peak currents in the subsequent cycles. On the other hand, the p-ATT modified electrode not only separated the voltammetric signals of AA, DOPAC and UA but also enhanced their peak currents. The amperometric current response was increased linearly with increasing DOPAC concentration in the range of 4.0×10^{-8} to 1.0×10^{-5} M and the detection limit was found to be 150 pM ($S/N = 3$).

Keywords. 5-Amino-1,3,4-thiadiazole-2-thiol; 3,4-dihydroxyphenylacetic acid; ascorbic acid; uric acid; amperometry.

1. Introduction

3,4-dihydroxyphenylacetic acid (DOPAC) is an important catabolite of dopamine (DA).[1] The major pathway responsible for DA catabolism in the brain involves deamination by monoamine oxidase type A and B to form DOPAC, which is then converted to homovanillic acid (HVA) metabolite by *O*-methylation via the membrane bound and soluble forms of catecholamine-*O*-methyltransferase.[2] The DA metabolism can be assessed in the prefrontal cortex by measuring the extracellular levels of DOPAC.[1,3–6] Therefore, the concentration of DOPAC is a sensitive indicator for neuronal functioning in nearby diencephalons structures.[7] Usually, DOPAC exists as very low concentration along with AA and UA in blood serum and urine.[8,9] Thus, the selective and sensitive determination of DOPAC is very important not only in the fields of biomedical chemistry and neurochemistry but also for diagnostic and pathological research.[10] Several techniques have been used for the determination of DOPAC including electrochemical,[11–19] high-performance liquid chromatography,[20] chemiluminescence[21] and fluorometry.[22] Among these methods, electrochemical methods of detection have attracted incessantly in recent years because they are more selective, sensitive and less time-consuming.

At unmodified electrodes, AA, DOPAC and UA were oxidized almost at the same potential and also they often suffered from the fouling effect due to the accumulation of oxidized products on the electrode surface, which resulted in rather poor selectivity and sensitivity.[14] In order to avoid these problems, chemically modified electrodes have been employed for the selective and stable determination of DOPAC including poly(N,N'-dimethylaniline) modified boron-doped diamond electrode,[11] short chain thiol/disulfides,[12] 6-mercaptonicotinic acid,[13] cationic self-assembled monolayer[14] and gold nanoparticles immobilized on sol–gel network[15] modified Au electrodes, nitrogen-doped carbon nanotubes,[16] single-wall carbon nanotubes[17] and protein-polysaccharide hybrid modified GC electrodes[18] and TiO_2 nanostructured film modified silicon substrate.[19]

Although several reports have been published for the determination of DOPAC, fabrication of an electrochemical sensor with high sensitivity is still one of the challenging tasks for the researchers because the concentration of DOPAC present in our body fluids is very low (3 nmol/L in the lumber cerebrospinal fluid and 60 nmol/L in the ventricular cerebrospinal fluid).[23]

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Recently, we have prepared a nanostructured film of 5-amino-1,3,4-thiadiazole-2-thiol (ATT) by potentiodynamic method on GC electrode[24] and demonstrated its excellent electrocatalytic properties toward L-cysteine,[24] dopamine,[25] folic acid,[26] epinephrine[27] and nitrite.[28] Further to exploit the application of this polymer film, we have examined its electrocatalytic activity towards DA catabolite, DOPAC. It was found that the p-ATT modified electrode dramatically enhanced the DOPAC oxidation current nearly 1.5 fold higher with 80 mV less positive potential when compared to bare GC electrode. Further, p-ATT film modified electrode showed an excellent selectivity toward the determination of DOPAC in the presence of 50-fold excess of AA and UA. Using amperometric method, we achieved the detection of 40 nM and detection limit of 150 pM ($S/N = 3$) for DOPAC at p-ATT modified electrode.

2. Experimental

2.1 *Chemicals*

5-amino-1,3,4-thiadiazole-2-thiol (ATT), 3,4-dihydroxyphenylacetic acid (DOPAC), ascorbic acid (AA) and uric acid (UA) were purchased from Aldrich and were used as received. All other chemicals used in this investigation were of analytical grade. pH 5.0 phosphate buffer (PB) solution was prepared using Na_2HPO_4 and NaH_2PO_4. Double distilled water was used to prepare the solutions used in this investigation.

2.2 *Instrumentation*

Electrochemical measurements were performed in a conventional two compartment, three electrode cell with a mirror polished 3 mm GC as the working electrode, Pt wire as counter electrode and a NaCl saturated Ag/AgCl as reference electrode. The electrochemical measurements were carried out with CHI Model 643B (Austin, TX, USA) Electrochemical Workstation.

2.3 *Fabrication of p-ATT modified GC electrode*

Prior to modification, the GC electrode was polished with 0.50 and 0.05 μm alumina slurries and rinsed thoroughly with water. Then, the electrode was sonicated in water for 5 minutes to remove the adsorbed alumina particles on the electrode surface. Electropolymerization of ATT on the GC electrode was carried out by 15 successive potential sweeps between −0.20 and +1.70 V at a scan rate of 50 mV s^{-1} in 1 mM ATT containing 0.10 M H_2SO_4.[24] By this method, an ultrathin film of p-ATT with a thickness of 25 nm can be prepared on GC electrode.[24]

3. Results and discussion

3.1 *Electrochemical behaviour of DOPAC at bare and p-ATT modified GC electrodes*

The present electrochemical sensor was optimized in terms of thickness of the p-ATT film and pH. The p-ATT film deposited by 15 and 50 cycles, resulted in the thickness of 25 nm and 40 nm, respectively.[24] The p-ATT film deposited by 15 potential cycles showed higher electrocatalytic activity towards AA, DOPAC and UA than the films deposited by more than 15 potential cycles. Further, we have performed the redox reaction of DOPAC and oxidation of AA and UA at different pH using p-ATT film. At pH 5.0, we observed higher oxidation currents for these analytes. Thus, in the present study, we have chosen the p-ATT film with a thickness of 25 nm and pH 5.0 for the determination of AA, DOPAC and UA. Figure 1 shows the cyclic voltammograms (CVs) obtained for 0.50 mM DOPAC at bare and p-ATT modified GC electrodes in 0.20 M

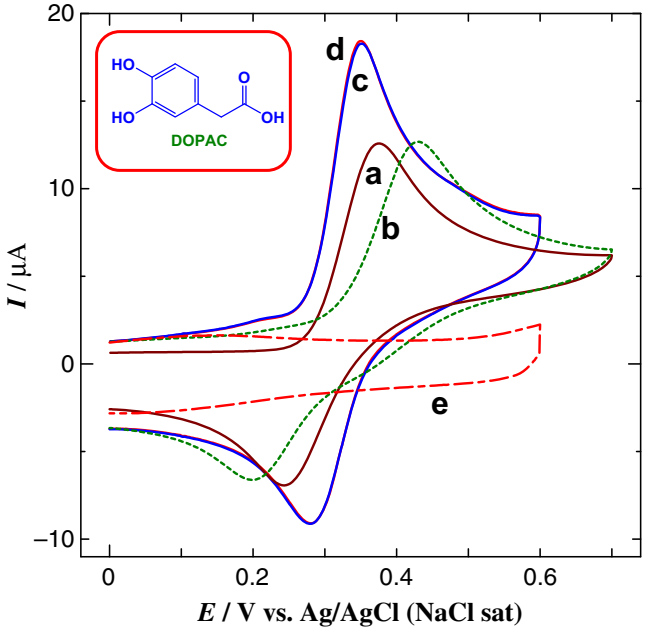

Figure 1. (a) CVs obtained for 0.50 mM DOPAC at bare and p-ATT modified GC electrodes after 1st (a and c) and 5th (b and d) cycles in 0.20 M PB solution (pH 5.0) at a scan rate of 50 mV s^{-1}. (e) CV obtained for p-ATT modified electrode in 0.20 M PB solution. Inset: Structure of 3,4-dihydroxyphenylacetic acid (DOPAC).

PB solution (pH 5.0). At bare GC electrode, oxidation and reduction peaks were observed for DOPAC at 0.38 V and 0.24 V, respectively with a peak to peak separation of 140 mV in the first cycle (curve a). The oxidation peak was shifted to more positive potential whereas the reduction peak was shifted to less positive potential in the subsequent cycles. After 5 cycles, the oxidation and reduction peaks were observed at 0.43 V and 0.20 V, respectively with a peak to peak separation of 230 mV (curve b). In addition, the reduction peak current was slightly decreased while the oxidation peak current remained same. The observed 230 mV peak to peak separation suggests that the redox reaction of DOPAC is sluggish at bare GC electrode. However, a well-defined redox peak as well as enhanced electrochemical response was observed for DOPAC at p-ATT modified electrode with a peak to peak separation of 70 mV (curve c). This revealed that the redox reaction of DOPAC was very much faster at p-ATT modified electrode than at bare GC electrode. It showed an oxidation and reduction peaks for DOPAC at 0.35 V and 0.28 V, respectively. When compared to bare GC electrode, nearly 1.5-fold higher oxidation peak current with 80 mV less positive potential shift was observed for DOPAC at p-ATT modified electrode. Further, the redox peak of DOPAC is very much stable at p-ATT modified electrode in the subsequent cycles as evidenced from curve d. The observed redox peak corresponds to two-electron oxidation of DOPAC to DOPAC *o*-quinone and the subsequent reduction of DOPAC *o*-quinone to DOPAC.[17] The p-ATT film does not show any pronounced electrochemical response between 0 V and 0.60 V in 0.20 M PB solution (curve e).

The effect of scan rate on the redox reaction of DOPAC was studied at p-ATT modified electrode. The redox peak current of DOPAC was increased while increasing the scan rate (figure S1 in the Supporting information). A good linearity between the anodic peak current and square root of scan rate was obtained within the range from 50 to 1000 mV s^{-1} for DOPAC with a correlation coefficient of 0.9965 as shown in inset of figure S1 in the Supporting information. This indicated that the electrode reaction process is controlled by the diffusion of DOPAC.

3.2 *Electrochemical behaviour of AA, DOPAC and UA in a mixture at bare and p-ATT modified GC electrodes*

Figure 2 displays the linear sweep voltammograms (LSVs) obtained for 0.50 mM AA, DOPAC and UA

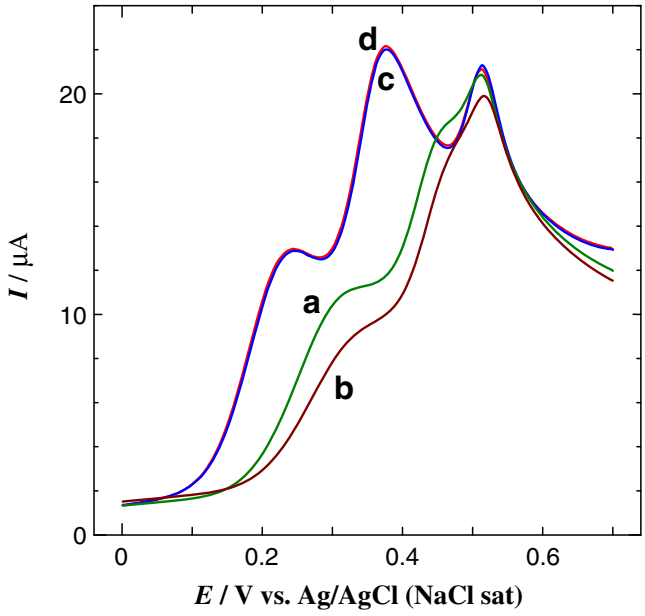

Figure 2. LSVs obtained for 0.50 mM each AA, DOPAC and UA at bare and p-ATT modified GC electrodes after 1st (a and c) and 5th (b and d) cycles in 0.20 M PB solution at a scan rate of 50 mV s^{-1}.

at bare and p-ATT modified GC electrodes in 0.20 M PB solution (pH 5.0). In the first cycle, bare GC electrode showed two shoulder waves around 0.32 V and 0.45 V along with an oxidation peak at 0.51 V for AA, DOPAC and UA, respectively in a mixture (curve a). In the subsequent cycles, the oxidation peak currents of them were shifted to more positive potential. After 5 cycles, the oxidation peaks were appeared at 0.33, 0.46 and 0.52 V with decreased peak currents (curve b). This divulged that bare GC electrode is not suitable for the determination of AA, DOPAC and UA in a mixture. On the other hand, the p-ATT modified electrode successfully separated the oxidation peaks of AA, DOPAC and UA and their oxidation peaks were appeared at 0.24, 0.38 and 0.51 V, respectively (curve c). Further, the oxidation peaks of them were more stable at p-ATT modified electrode in the subsequent cycles (curve d). The oxidation peak potential difference between AA and DOPAC was 140 mV and DOPAC and UA was 130, which were more than enough for the simultaneous determination of AA, DOPAC and UA in a mixture. This indicated that the oxidation of AA, DOPAC and UA at p-ATT modified electrode is independent and therefore simultaneous or independent measurement of the three analytes is possible without any interference. It is well known that AA, DOPAC and UA were present as anonic forms in pH 5.0.[13,29,30] It is expected that the negatively charged AA, DOPAC and

UA were electrostatically attracted by the positively charged back bone of the p-ATT film.[24] Thus, enhanced oxidation peak currents were observed for them at modified electrode when compared to bare GC electrode. Similar suggestion was recently reported at poly(N, N'-dimethylaniline) modified boron-doped diamond electrode[11] and dithiobishexaneamine monolayer modified Au electrode.[12]

3.3 *Simultaneous determination of AA, DOPAC and UA using p-ATT modified electrode*

One of the objectives of the present study is to determine the concentrations of AA, DOPAC and UA simultaneously using p-ATT modified electrode. An accurate determination of these analytes is very important in the clinical point of view, because these analysts are coexisted in blood serum and urine.[10] Figure 3 shows the differential pulse voltammograms (DPVs) obtained for the simultaneous determination of AA, DOPAC and UA at p-ATT modified electrode. It showed the oxidation peaks at 0.14, 0.30 and 0.44 V for 20 μM AA, 2.5 μM DOPAC and 10 μM UA, respectively (curve a). Based on LSV results in figure 2, the order of sensitivity of these molecules at p-ATT modified electrode was found to be DOPAC > UA > AA. Thus, we have chosen 2.5 μM DOPAC, 10 μM UA and 20 μM AA as increments. The oxidation peak of UA remained same whereas a slight shift in the oxidation potentials of AA and DOPAC were observed for further addition of the respective analytes (curves b–o). When the concentration of AA was increased from 20 to 300 μM, DOPAC was increased from 2.5 to 37.5 μM and UA was increased from 10 to 150 μM, the peak currents of AA, DOPAC and UA were increased linearly with a correlation coefficient of 0.9994, 0.9997 and 0.9992, respectively.

3.4 *Selective determination of DOPAC in the presence of AA and UA*

The main intention of the present study is to determine the concentration of DOPAC in the presence of very high concentrations of AA and UA. It is well-known that AA and UA are present along with DOPAC in our body fluids and further their concentrations are very much higher than that of DOPAC.[31] Therefore, for the clinical point of view, the determination of DOPAC in the presence of high concentrations of AA and UA is very important. Figure 4 shows the DPVs obtained for the increment of 10 μM DOPAC in the presence of 500 μM each AA and UA. A very clear signal was observed for 10 μM DOPAC in the presence of 500 μM

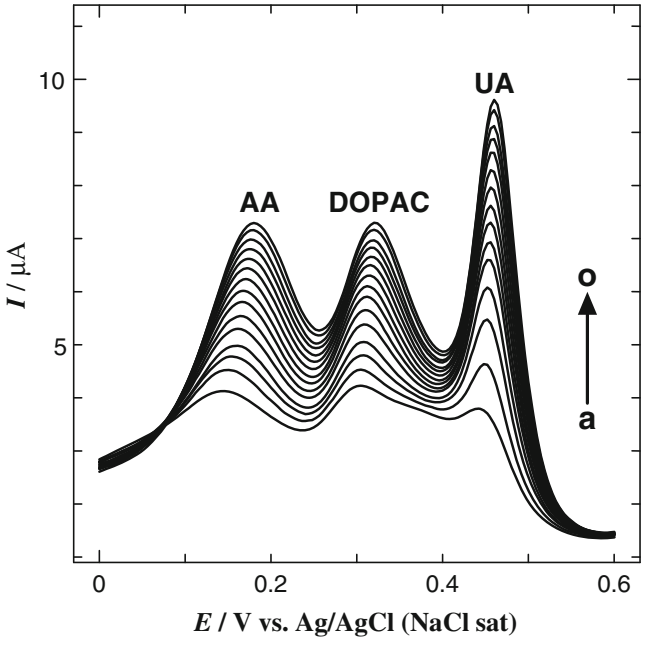

Figure 3. DPVs obtained for the increment of 20 μM AA, 2.5 μM DOPAC and 10 μM UA in 0.20 M PB solution at p-ATT modified GC electrode. Pulse width = 0.06 s, amplitude = 0.05 V, sample period = 0.02 s and pulse period = 0.2 s.

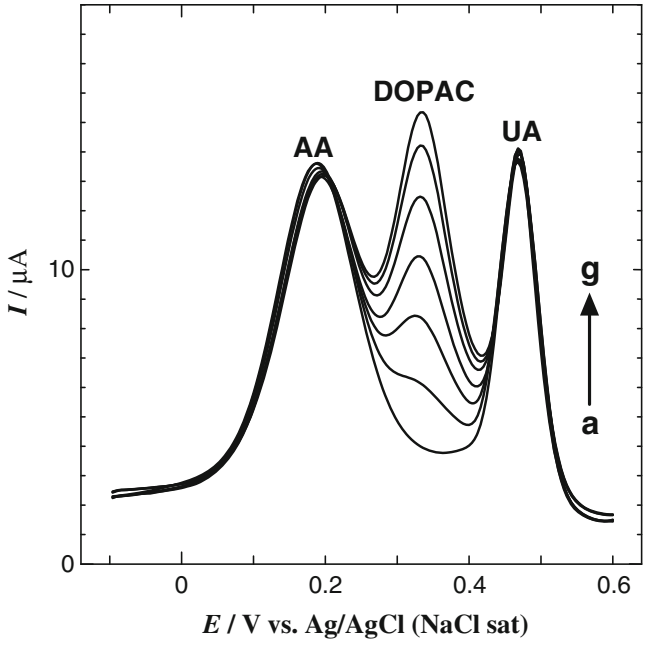

Figure 4. DPVs obtained for the increment of 10 μM DOPAC to 500 μM each AA and UA in 0.20 M PB solution at p-ATT modified electrode (a) 0, (b) 10, (c) 20, (d) 30, (e) 40, (f) 50 and (g) 60 μM. Pulse width = 0.06 s, amplitude = 0.05 V, sample period = 0.02 s and pulse period = 0.2 s.

each AA and UA in figure 4 (curve b), which revealed that the detection of very low concentration of DOPAC is possible even in the presence of 50-fold excess of each AA and UA. The increment of 10 μM DOPAC to a solution of 500 μM each AA and UA increases the current of DOPAC with a correlation coefficient of 0.9998, respectively while the peak current of AA and UA remain unchanged. Usually, AA and UA are present 20-fold excess than DOPAC in extracellular fluid.[31] In the present study, we have determined DOPAC even in the presence of 50-fold excess of AA and UA. These results showed that p-ATT modified electrode is more suitable for practical applications.

3.5 *Amperometric determination of DOPAC along with AA and UA*

Amperometric method was performed to examine the sensitivity of p-ATT modified electrode towards the detection of DOPAC individually and also along with AA and UA. Figure 5 depicts the amperometric *i–t* curve for DOPAC at p-ATT modified electrode in a homogeneously stirred 0.20 M PB solution at an applied potential of + 0.50 V. The modified electrode showed the initial current response due to 40 nM DOPAC and further addition of 100 nM DOPAC into the same solution with a sample interval of 50 s, the current response was increased and a steady state current response was attained within 3 s. Again the current response was increased for further addition of 200, 400, 800, 3000, 6000 and 10000 nM DOPAC to the same solution with a sample interval of 50 s. The amperometric current was linearly increased while increasing the concentration of DOPAC from 4.0×10^{-8} to 1.0×10^{-5} at p-ATT modified electrode with a correlation coefficient of 0.9956 and the detection limit was found to be 0.15 nM (inset of figure 5).

The amperometric method was also performed to determine the concentration of DOPAC along with AA and UA. Figure 6 displays the amperometric current response for the alternative addition of AA, DOPAC and UA in 0.20 M PB solution at an applied potential of + 0.60 V. The p-ATT modified electrode showed the initial current response due to 75 nM AA and addition of 40 nM DOPAC to this solution with a sample interval of 50 s, the current response was increased. The current response was again increased after the addition of 60 nM UA to the same solution. The amperometric current of DOPAC does not change in the presence of AA and UA. Further, the observed systematic increase in the current responses due to the addition of AA, DOPAC and UA indicated that the determination of any

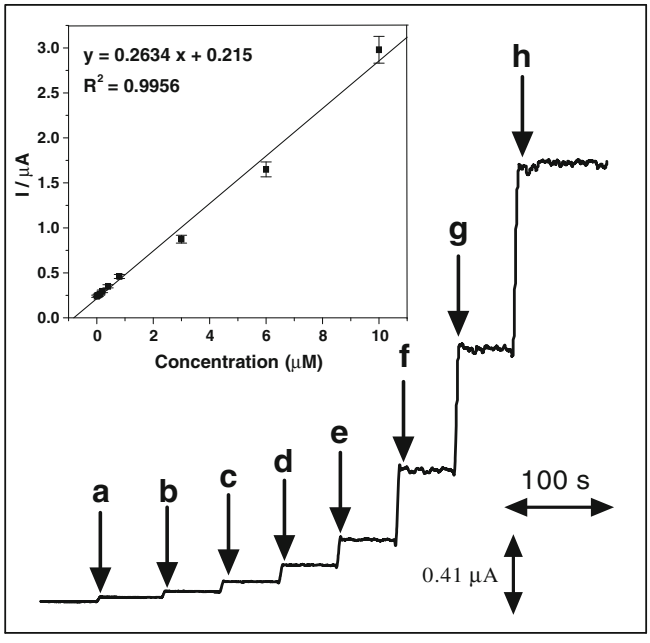

Figure 5. Amperometric *i–t* curve for the determination of DOPAC at p-ATT modified electrode in 0.20 M PB solution. Each addition increases the concentrations of DOPAC (a) 40, (b) 100, (c) 200, (d) 400, (e) 800, (f) 3000, (g) 6000 and (h) 10000 nM at a regular interval of 50 s. E_{app} = +0.50 V.

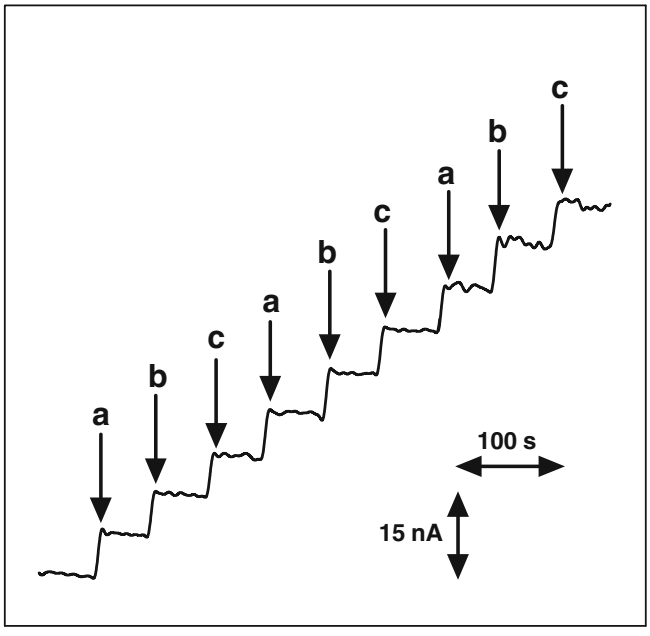

Figure 6. Amperometric *i–t* curve for the alternative addition of AA, DOPAC and UA at p-ATT modified GC electrode in 0.20 M PB solution. Each addition increases the concentration of (a) 75 nM AA, (b) 40 nM DOPAC and (c) 60 nM UA at a regular interval of 50 s. E_{app} = +0.60 V.

one of the analytes is possible in the presence of other analytes using p-ATT modified electrode. We have estimated the current response for 75 nM AA as 7.3 nA, 40 nM DOPAC as 7.1 nA and 60 nM UA as 7.2 nA from figure 6.

The sensitivity of 0.084 μA at poly(N, N'-dimethylaniline) modified boron-doped diamond electrode,[11] 0.016 μA at short chain thiol/disulfides modified Au electrode[12] and 0.040 μA at tyrosinase-conjugated polysaccharide hybrid film modified GC electrode[32] per μM of DOPAC were reported. Further, the lowest detection limit of 400 and 3 nM ($S/N = 3$) were reported at single-wall carbon nanotubes[17] and tyrosinase-conjugated polysaccharide hybrid film[32] modified GC electrodes, respectively. When compared to the reported modified electrodes, the present modified electrode showed the sensitivity of 0.18 μA per μM with a lowest detection limit of 0.15 nM ($S/N = 3$) for DOPAC. To the best of our knowledge, this is the first report for the very high sensitivity and lowest detection limit for DOPAC.

3.6 *Anti-interference ability of the p-ATT film*

The anti-interference ability of the p-ATT film was tested towards the detection of DOPAC from various physiological interferents such as glucose, urea, oxalate, valine, alanine, phenylalanine, proline, glycine, Ca^{2+}, Mg^{2+} and Na^{+} by amperometric method. No change in the amperometric current response was observed for 40 nM DOPAC in the presence of 100 μM of aforesaid analytes. This revealed that the present modified electrode is highly selective towards the determination of DOPAC even in the presence of 2500-fold excess of common physiological interferents (figure not shown).

3.7 *Reproducibility and stability of the p-ATT film*

The DPVs for 0.25 mM DOPAC in 0.20 M PB solution were recorded for every 3 min interval to evaluate the stability of the p-ATT modified electrode. It was found that the oxidation peak current of DOPAC remained same with a relative standard deviation of 1.23% for 25 times repetitive measurements indicating that this electrode has a good stability. Further, to find out the reproducibility of the results, three different GC electrodes were modified with the p-ATT film and their response towards the oxidation of 0.50 mM AA, UA and DOPAC was tested by 25 repeated measurements. The separation between the voltammetric peaks of AA-DOPAC and DOPAC-UA were the same at all the three electrodes. The peak current obtained in the 25 repeated measurements of three independent electrodes showed a relative standard deviation of 1.21%, confirming that the results are reproducible. The stability of the p-ATT film was also examined. While we keeping p-ATT modified electrode in 0.20 M PB solution at room temperature, no apparent decrease in the current response of DOPAC was observed in first two days and 6.6% of current was decreased after 2 weeks. These results showed that the present modified electrode was very much stable and reproducible towards the determination of AA, DOPAC and UA.

It is worth to compare the determination of DOPAC at p-ATT modified electrode with the reported chemically modified electrodes. The fabrication of p-ATT film on GC electrode surface is very simple and less time consuming ($\sim$20 min) when compared to reported modified electrodes for the determination of DOPAC. For example, for the preparation of tyrosinase-conjugated polysaccharide hybrid film modified GC electrode, 2 mg/ml chitosan solution and a 0.60 mg/ml tyrosinase solution mixed well under sonication. Then the 20 μL aliquot of the composite solution was evenly cast onto a GC surface and dried overnight under room temperature.[32] Similarly, for the preparation of functionalized self-assembled monolayer modified electrode, the Au was immersed into ethanol solution of 10 mM dithiobishexaneamine and 0.5 mM macrocylic nickel(II) complex for 12 h.[12]

4. Conclusions

Highly sensitive determination of DOPAC in the presence of AA and UA using p-ATT modified GC electrode in 0.20 M PB solution was reported for the first time. The bare GC electrode failed to resolve the voltammetric signals of AA, DOPAC and UA whereas p-ATT modified electrode successfully resolved the voltammetric signals of them. The modified electrode separated the voltammetric signals of AA, DOPAC and UA with potential differences of 140 mV between AA and DOPAC and 130 mV between DOPAC and UA. The oxidation currents of AA, DOPAC and UA were remarkably increased at p-ATT modified electrode when compared to bare GC electrode due to the presence of strong electrostatic attraction between positively charged p-ATT film and negatively charged analytes. The modified electrode exhibited an excellent sensitivity and selectivity towards DOPAC even in the

presence of 50-fold excess of each AA and UA. The detection of 40 nM DOPAC was achieved at modified electrode using amperometric method. The amperometric current response was increased linearly with increasing DOPAC concentration from 4.0×10^{-8} to 1.0×10^{-5} M and the detection limit was found to be 150 pM ($S/N = 3$).

Supplementary information

For figure S1 see www.ias.ac.in/chemsci website.

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