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# Simultaneous determination of ascorbic acid, dopamine and uric acid using PEDOT polymer modified electrodes

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Dopamine, uric acid and ascorbic acid have been determined simultaneously on a poly(3,4-ethylenedioxythiophene) modified electrode using electrochemical techniques. On a bare glassy carbon electrode, all three undergo oxidation and appear as a single peak at around 200 mV, whereas on the modified electrode, distinct peaks with clear potential separation are observed, paving way for their simultaneous determination. Detection limits of 7.4 μ*M*, 1.2 μ*M* and 1.4 μ*M* for ascorbic acid, dopamine and uric acid, respectively are observed by pulse voltammetry. The possible interference from creatinine, norepinephrine, epinephrine, glucose, ascorbic acid, urea, oxalate and acetylsalicylic acid (aspirin) has been tested. The protocol developed using this modified glassy carbon electrode has been evaluated using the human blood serum samples for the simultaneous determination of dopamine and uric acid.

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Electrochemical sensors offer several advantages such as sensitivity, selectivity, fast response time and allow ease of preparation[1]. In recent times, there has been tremendous developments in the electrochemical sensing devices that are mainly used for environmental monitoring, clinical assays and quality monitoring. In clinical analysis, the major goal is the selective determination of dopamine and uric acid in serum samples. Dopamine (DA), the neurotransmitter plays a vital role in human nerves/brain as a messenger and a loss in DA containing neurons result in some serious diseases such as Parkinson[2]. The prime difficulty in the detection of DA in brain fluids using electrochemical methods is the co-existence of many interfering compounds. Among these, ascorbic acid (AA) and uric acid (UA) are of particular importance. These compounds are present in relatively high concentrations (100-500 μ*M* AA and 1-50 μ*M* UA), while the dopamine levels are of the order of 100 n*M*[3,4]. On the other hand, the detection of UA in serum is important from the clinical point of view since an increase in the uric acid content of the blood is usually regarded as a sign of gout. Apart from this, it is now recognized that the unusual uric acid content in the blood causes cardiovascular disorders[5]. Hence, the analysis of DA and UA in human blood serum samples assumes utmost importance, where again AA is the common interfering molecule.

Several approaches have been examined in order to overcome this problem and detect DA and UA simultaneously. In our earlier work, poly(3,4-ethylenedioxythiophene) (PEDOT) film was employed as the sensing material for dopamine and uric acid detection independently with sensitivity at n*M* levels being achieved in presence of AA at 1:1000 ratio[6-8]. Further, it is observed that the PEDOT matrix alone can be used as a material for the simultaneous determination of these biologically important molecules. A careful analysis of the results using other sensing materials, shows that PEDOT offers good scope for the simultaneous determination of these biologically important molecules.

Voltammetric differentiation between AA, DA and UA has not been reported so extensively as that between AA-DA and AA-UA; probably due to increased complexity arising from the interference. However, a few studies have been reported for the detection of these biologically important molecules, using glassy carbon electrode (GCE) modified with 2,2-bis (3-amino-4-hydroxyphenyl) hexafluoropropane (BAHHFP)[9], carbon nanotube ionic liquid gel modified electrode[10], tetrabromo-*p*-benzoquinone modified carbon paste electrode[11], poly (vinyl alcohol) covalently modified GCE[12], carbon-polyvinylchloride composite electrode[13], temperature assisted peak separation using screen printed carbon electrode[14], oracet blue modified glassy carbon electrode[15] and titanate nanotube films[16]. However, a clear voltammetric separation of oxidation of these molecules on the modified electrodes has not been demonstrated.

Herein, we report the simultaneous determination of AA, DA and UA using PEDOT modified GCE. This analytical protocol has been evaluated using human blood serum with possible interfering molecules.

## Experimental

Sample of 3, 4-ethylenedioxythiophene (EDOT, Baytron M) was a gift from Bayer-AG (Germany). Dopamine (Aldrich), ascorbic acid (Aldrich), uric acid (Aldrich), tetrabutylammonium perchlorate (TBAPC) (Aldrich), potassium dihydrogen phosphate (E-Merck) and sodium hydroxide (E-Merck) were used as received. The aqueous solutions were prepared using Milli-Q water (18.3 MΩ) (Millipore).

For voltammetric studies, a glassy carbon working electrode (ϕ 3 mm, BAS, Inc.), a platinum wire coil auxiliary electrode and an Ag | AgCl (3M NaCl) reference electrode were used. The potential values mentioned in this text are referred to this reference electrode unless otherwise mentioned. Phosphate buffer solution (PBS, 0.1 *M*) of *p*H: 7.4 was employed as the electrolytic medium.

Electrochemical experiments were carried out using a Potentiostat/Galvanostat Autolab PGSTAT-30 instrument (Eco-Chemie B.V., The Netherlands) at ambient temperature (25±1°C). To record the differential pulse voltammograms (DPV), the following input parameters were used: scan rate: 30 mV $s^{-1}$, sample width: 17 ms, pulse amplitude: 50 mV, pulse width (modulation time): 50 ms, pulse period (interval): 200 ms and quiet time: 2 s. Peak currents were determined by subtraction of a manually added baseline.

### Preparation of PEDOT modified GCE

The GC electrode surface was polished first on fine emery paper and then with 1.0 and 0.06 μm alumina powder, and finally sonicated with Milli-Q water for 5 min. Before electropolymerization, the polished electrode was pretreated by cycling it for 10 min between –0.9 and +1.5 V at 10 V/s in acetonitrile containing TBAPC. Then, PEDOT was electrodeposited on the electrode from a solution of 10 m*M* EDOT + 0.1 *M* tetrabutyl ammonium perchlorate in acetonitrile by potential cycling between –0.9 to 1.5 V versus Ag wire pseudo-reference electrode. The PEDOT film was allowed to grow on the GC surface for five successive scans, as inferred from the increasing anodic and cathodic peak current densities.

## Results and discussion

### Electrooxidation of AA, DA and AA using PEDOT modified electrodes

Figure 1 shows the oxidation of AA, DA and UA (1 m*M* each) on GCE with and without PEDOT modification. On the bare GCE, all three molecules are oxidized at around 200 mV and the oxidation peaks coalesce to appear as a single peak making the peak potentials for these molecules indistinguishable at bare GCE. Therefore, it is impossible to deduce any information from the broad and overlapped voltammetric peak. On the other hand, on the PEDOT modified GCE, the oxidation peaks of the individual molecules are resolved into three well-defined peaks at three distinct potentials, viz., AA oxidized at –2 mV, DA oxidized at 202 mV and UA oxidized at 370 mV vs Ag | AgCl. The clear peak potential separation favours the individual analyte determination in the presence of others. It can be seen that the electrochemical signals are independent from each other and the peak separations were 204 mV, 168 mV and 372 mV between DA and AA, DA and UA, and AA and UA, respectively. These peak separations are large enough to determine AA, DA and UA simultaneously using the PEDOT modified electrode.

### Effect of variation of concentration

When the concentrations of AA, DA and UA are increased by successive additions, the peak currents at the PEDOT modified GCE increase proportionately at a constant sweep rate of 50 $mVs^{-1}$ (Fig. 2), satisfying Randle-Sevcick relation[17]. It can be seen that the peak currents for AA, DA and UA increase linearly with increase in their respective concentrations. This

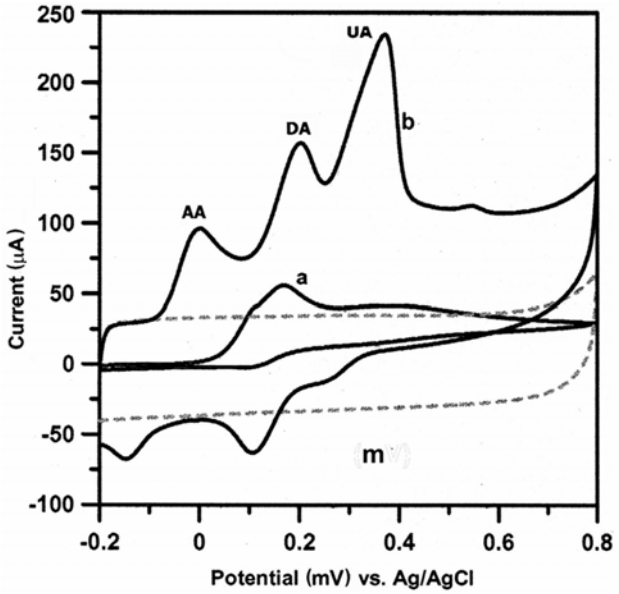


Fig. 1—Cyclic voltammetric behaviour of AA (1 m*M*) + DA (1 m*M*) + UA (1 m*M*) at (a) bare GC, (b) PEDOT modified GCE in PBS of *p*H 7.4. [Scan rate 0.05 V $s^{1}$(dotted line shows the behaviour of PEDOT modified GCE in PBS of *p*H 7.4)].

behaviour shows the analytical importance of the PEDOT modified electrodes for use in the electroanalysis of these molecules. The intercept on the current axis is suggestive of a possible interference of background capacitive current arising from the polymer matrix[18].

**Simultaneous determination of AA, DA and UA**

The electro-oxidation processes of DA, AA and UA in the mixture have been investigated simultaneously by varying the concentration of the individual analyte species. DPV has a much higher current sensitivity and better resolution than cyclic voltammetry and hence simultaneous determination of AA, DA and UA could be carried out at lower concentration levels. Moreover, contribution of the charging current to the background current, which is a limiting factor in the analytical determination, is negligible in DPV mode.

The differential pulse voltammogram (Fig. 3) shows the oxidation behaviour of AA, DA and UA at various concentrations at PEDOT modified GCE. It can be seen that the peak current individual analyte increases with increase in concentration. From these experimental results, it can be discerned that the peak responses for AA, DA and UA oxidation at PEDOT modified GCE are clearly separated from each other when they co-exist at *p*H PBS 7.4. Thus, simultaneous determination of AA, DA and UA molecules at a PEDOT modified electrodes is feasible. The analytical parameters for the simultaneous determination of AA, DA and UA are listed in Table 1.

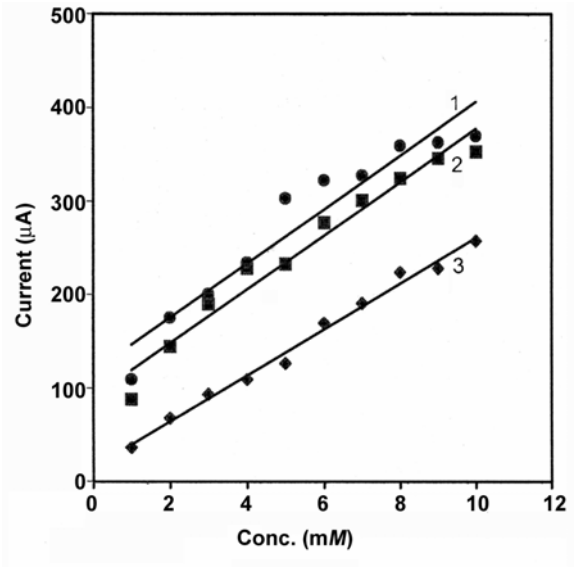


Fig. 2—Effect of concentration of AA, DA and UA on their oxidative voltammetry at PEDOT modified GCE. [1, ③ UA; 2, ⑤ DA; 3, ❶ AA].

**Effect of interferants**

For investigating the effect of interferents, several endogenous and exogenous compounds such as creatinine, epinephrine, nor-epinephrine, L-dopa, glucose, urea, oxalate, acetaminophen, acetyl salicylic acid (aspirin) were chosen. The concentrations of these interfering compounds were chosen to be ten times higher than the therapeutic concentration levels. Interference studies were conducted by exposing the PEDOT modified GCE in a solution containing

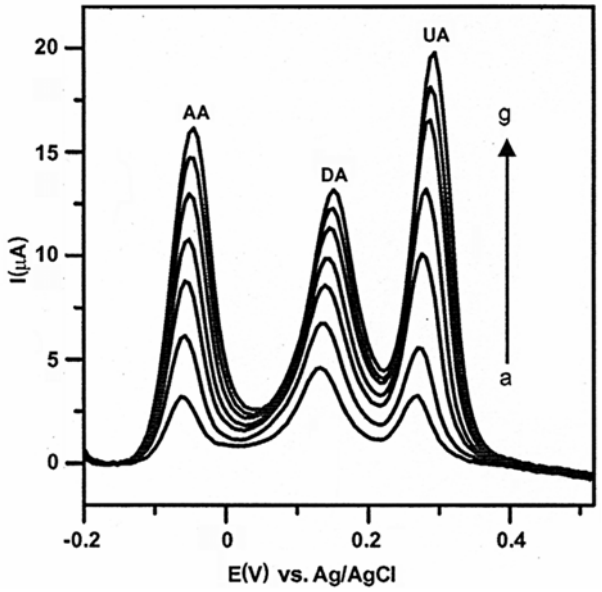


Fig. 3—Differential pulse voltammograms of AA, DA, UA at PEDOT modified GCE electrode in PBS (*p*H 7.4). [AA concentrations (from a to g): 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 m*M* respectively; DA concentrations (from a to g): 20, 30, 40, 50, 60, 70, 80 μ*M* respectively; UA concentrations (from a to g): 20, 30, 50, 70, 90, 110, 130 μ*M* respectively].

Table 1—Analytical parameters derived for simultaneous determination of DA, AA and UA

| Analyte | Linear range ($\mu mol^{-1}$) | Linear regression eq. $i$ (μA), $C$ ($\mu mol\ L^{-1}$) | Correlation coefficient | Detection limit ($\mu mol\ L^{-1}$) |
|---|---|---|---|---|
| AA | 500-3500 | $i_{AA} = 1.4677 + 0.004C$ | 0.99 | 7.4 |
| DA | 20-80 | $i_{DA} = 2.1313 + 0.0965C$ | 0.98 | 1.2 |
| UA | 20-130 | $i_{UA} = 0.7955 + 0.136C$ | 0.97 | 1.4 |

Table 2—Effect of interferants on the DPV responses of DA and UA

| Interferant | Conc. (mg/L) | Peak current (μA) DA | UA |
|---|---|---|---|
| No interferant (DA + UA) | 10 + 10[a] | 47.11 | 16.13 |
| Ascorbic acid | 1761 | 49.57 | 15.52 |
| Creatinine | 30 | 39.85 | 12.59 |
| Epinephrine | 20 | 41.64 | 12.87 |
| Nor-epinephrine | 20 | 45.44 | 16.35 |
| L-dopa | 100 | 42.21 | 14.25 |
| Glucose | 100 | 46.53 | 14.67 |
| Urea | 50 | 48.75 | 15.64 |
| Acetylsalicylic acid (aspirin) | 50 | 48.06 | 15.01 |

[a]in μ*M*

Table 3—Determination of uric acid in blood serum samples

| Sample | Uric acid (mg/L) By electrochemical method | By enzymatic method |
|---|---|---|
| Blood serum 1 | 3.51 | 3.6 |
| Blood serum 2 | 5.43 | 5.0 |
| Blood serum 3 | 4.39 | 4.7 |
| Blood serum 4 | 4.51 | 4.7 |
| Blood serum 5 | 4.15 | 4.3 |

50 μ*M* AA, 10 μ*M* DA and 10 μ*M* UA which includes the specified interfering molecule. The DPV responses resulting from the presence of interfering molecule were then compared with those obtained for the AA + DA + UA (Table 2). The studies on the influence of interferents on the voltammetric responses were carried out by making three replicate measurements.

Even though we have analyzed ascorbic acid earlier, we have included ascorbic acid in the interference studies since it is usually coexists with the DA and UA in many samples and it strongly affected the determination of UA and AA at a bare GCE[6-8]. In this study, since the peak potential of AA is shifted more negatively, it did not show any significant interference when present in 1:1000 ratio and neither did it affect the analytical measurements. Incidentally, this is the highest potential shift we have observed using the present PEDOT modified GCE. Similarly, other compounds such as glucose, urea, acetaminophen, aspirin, creatinine did not show any interference in the detection of DA and UA. However, the dopamine like compounds such as epinephrine, nor-epinephrine, L-dopa, creatinine showed apparent interference on the determination of DA and UA when the concentrations of the interferants present was 10-fold in excess of the physiological/therapeutic concentration levels.

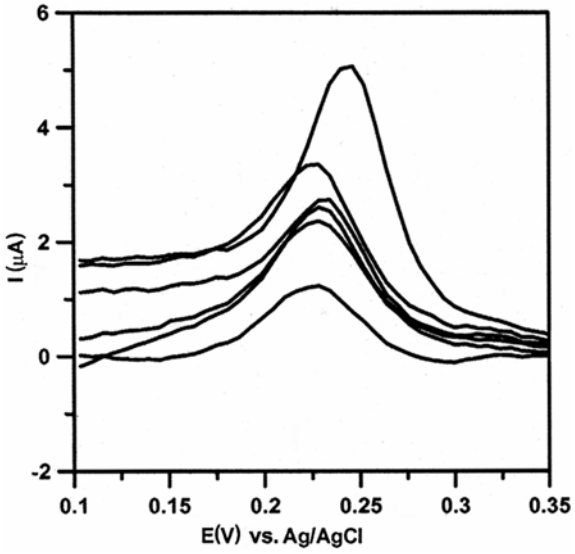


Fig. 4—DPV signal of UA in blood serum samples diluted 20 times with PBS at *p*H 7.4.

**Analysis of real samples**

The utility of the PEDOT modified electrode for the determination of UA in real samples was tested by measuring the UA concentration in the blood serum. Dopamine was spiked into the serum sample and analyzed using DPV protocol.

The serum samples were diluted to 20 times with PBS solution (*p*H 7.4) before measurement to prevent matrix effects of the serum samples. The DPV responses obtained for the serum sample (Fig. 4) shows a peak at ~220 mV which corresponds to UA. A small broad wave is also observed at around - 15 mV corresponding to the oxidation of AA present in the serum sample, which does not affect the measurement of the other two molecules present in the serum sample. The concentration of UA observed by the present method is in close agreement with the value obtained by an enzymatic method (using uricase peroxidase)[19] (Table 3). Further, the recovery of the DA spiked in the samples was found to be more than 83% (Table 4). Apart from the main interfering molecule AA, the presence of other substances, such as proteins and glucose did not interfere with the determination of DA.

The above results show that the proposed method can be efficiently used for the determination of DA and UA in analysis of serum samples.

**Stability and reusability of PEDOT modified GCE**

The stability of the PEDOT modified GCE in the presence of AA, DA and UA was tested by a series of 20 repetitive voltammetric measurements of sample

Table 4—Determination of dopamine in human blood serum samples

| Sample (%) | DA spike ($\times 10^{-5}$ *M*) | DA found ($\times 10^{-5}$ *M*) | Recovery (%) | R.S.D. |
|---|---|---|---|---|
| Blood serum 1 | 20 | 17 | 83.16 | 1.6 |
| Blood serum 2 | 20 | 18 | 88.69 | 1.3 |
| Blood serum 3 | 20 | 24 | 119.79 | 2.1 |
| Blood serum 4 | 20 | 18 | 89.60 | 0.9 |
| Blood serum 5 | 20 | 18 | 88.24 | 1.5 |

solutions containing AA (50 μ*M*), DA (10 μ*M*) and UA (10 μ*M*). The coefficient of variation was found to be 1.6%, indicating that the PEDOT modified GCE is stable and does not suffer from surface fouling by the oxidation products in the time frame of voltammetric measurements. It is interesting to note that preservation of these electrodes by immersing in phosphate buffer at neutral *p*H helped to increase its stability. To ascertain the reproducibility of the results further, six sets of PEDOT-modified GCEs and their response towards the oxidation of AA, DA and UA were tested by 10 repeated measurements of the sample solution containing AA (50 μ*M*), DA (10 μ*M*) and UA (10 μ*M*). The separation between the voltammetric peaks of AA-DA, DA-UA and AA-UA was found to be 205 ± 5 mV, 170 ± 5 mV and 375 ± 5 mV, respectively. The observed peak current showed a relative standard deviation of 1.6%, proving reproducibility of the results. Since the electrode preparation involves simple electrodeposition of the conducting polymer and also the electrode is very stable in aqueous solutions without showing any redox activity (*cf.* Fig. 1, PEDOT modified GCE response in PBS 7.4), its use in clinical analysis for the determination of DA, AA and UA is promising. Further, development of disposable screen-printed PEDOT electrodes for clinical analysis is underway.

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