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Application of Poly (Fast Green) Based Sensor for the Detection of Dopamine in presence of Ascorbic Acid and Uric acid

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ABSTRACT: The electropolymerisation of fast green was prepared on the surface of graphite pencil electrode using cyclic voltammetric technique. This poly (fast green) modified graphite pencil electrode shows good electrocatalytic activity in the detection of dopamine when compared to bare graphite pencil electrode in 0.2 M phosphate buffer solution of pH 7.0. The effect of scan rate show that the electrode process was diffusion controlled. The concentration and pH effects on the voltammogram of dopamine were studied. The poly (fast green) modified graphite pencil electrode shows excellency in resolution of dopamine in presence of large excess of ascorbic acid and uric acid concentrations. The interfering study was made by increasing the concentration of one species and keeping concentration of other two species constant. The poly (fast green) film coated electrode shows outstanding potential difference for dopamine, ascorbic acid and uric acid in their mixture.

KEYWORDS: Fast green, graphite pencil electrode, electropolymerisation, dopamine, cyclic voltammetry.

I. INTRODUCTION

Electroanalytical methods have been used through the past three decades to explore the function of neurotransmitters in the brain due to their electroactive nature. Such methods offer great promise for the biomolecule detection with features that consist of high sensitivity, inherent miniaturization, inexpensive, independence of sample turbidity, minimal power demands and high compatibility with modern microfabrication technologies [1, 2]. In that chemically modified electrodes have been widely used as sensitive and favorable analytical methods [3, 4].

Electrode surface modification has been demonstrated to be an effective means to reduce the overpotential of many electrode processes [5]. Recently, carbon-based porous materials have been used in a variety of fields including catalysis, purification, separation and energy storage because of their high specific surface area, and high mechanical stability [6-8].

Electrodes coated with film of conducting polymer by electropolymerisation have been paid great attention in the past decades, due to their unique physical and chemical properties and some possible applications in organic batteries, microelectronic devices and electrocatalysis, as reviewed by several groups [9-13]. In the initial period, the principal thought was devoted to some basic representative materials, e.g., polypyrrole (PPy), polythiophene and polyaniline. Since these electroactive polymer films coated electrodes generally offer very dense redox sites, they can mediate electron transfer to a solution species. The selective response of this kind of polymers towards dissolved ions has made them useful as an important class of ion and molecular sensors. To attain this purpose, one can employ electrochemical reactions that cause a substantial change in the interaction between the ionic species and the polymer [14-20].

Thin polymer films can be formed at electrodes using electrochemical techniques via monomer electropolymerization. These film systems are an attractive area of study because the synthesis takes place at electrodes in aqueous media under electrochemical control. A significant focus of thin film electropolymerisation research has centered on forming and studying highly conjugated polymers. Examples consist of both conducting polymers and non-conducting polymer films formed from monomers such as phenol and its derivatives [21 - 25].

Dopamine (DA) is an important neurotransmitter molecule of catecholamines that is extensively distributed in the mammalian central nervous system for message transfer. Hence, it has been of attention to neuroscientists and chemists. Loss of DA-containing neurons may result in some serious diseases such as Parkinsonism [26 - 29]. Therefore, determination of the concentration of this neurochemical is a key task. The fact that DA and other catecholamines are easily oxidizable compounds makes their detection possible by electrochemical methods. A major problem of electrochemical detection of DA in genuine biological matrices is the coexistence of many interfering compounds. Among these, ascorbic acid (AA) is of particular importance. For example, in the extracellular fluid of the central nervous system, this species is present in excessive concentrations like 100–500 μM [30, 31]. Uric acid (UA) is the main end product of purine metabolism. Irregular levels of UA are symptoms of a number of diseases for instance hyperuricemia, gout, and Lesch–Nyan disease [32]. Other diseases, such as leukemia and pneumonia, are as well associated with improved urate levels [33]. Moreover in most of all bare electrodes, DA, AA and UA get oxidized at almost the same potential, resulting in overlapped voltammetric response. Simultaneous detection of DA, AA and UA is a complexity of critical importance not only in the field of biomedical chemistry and neurochemistry but also for diagnostic and pathological research. Recently Y. Zhou and co-workers developed the electropolymerised methyl red modified electrode for the electrochemical detection of DA [34]. But our interest is to study the sensor activity of poly (fast green) modified graphite pencil electrode for the investigation of DA.

II. EXPERIMENTAL

A. REAGENTS

Fast green, DA and AA were purchased from Himedia and Aldrich, UA and graphite powder from Fluka. 25mM fast green stock solution was prepared. $25 \times 10^{-4}\text{M}$ stock solution AA was prepared by dissolving in double distilled water and $25 \times 10^{-4}\text{M}$ stock solution of UA in 0.1M NaOH. The stock solution DA ($25 \times 10^{-5}\text{M}$) was prepared by dissolving in 0.1M perchloric acid solution. The phosphate buffer solutions of different pH were used as supporting electrolyte. Above mentioned all chemicals were of analytical grade and used without further purification. The double distilled water was used in the preparation of all aqueous solutions.

B. APPARATUS

The electrochemical experiments were carried out using a model-201 electroanalyser (EA-201 chemilink system). All the experiments were carried out in a conventional three electrode cell. The electrode system contained a working electrode was graphite pencil electrode (GPE) (0.5 mm in diameter), a platinum wire as counter electrode and saturated calomel electrode as reference electrode.

C. ELECTROPOLYMERISATION OF FAST GREEN

The electropolymerisation of fast green was performed by using cyclic voltammetric technique in the range of –1000 to 2000 mV at graphite pencil electrode. The saturated calomel electrode and platinum electrode were used as reference and counter electrode respectively. The 0.5 mM fast green monomer was placed in the three electrode electrochemical cell with phosphate buffer at pH 6.5. Followed by this, the electrode is made to suffer by applying 20 multiple cycles at 100 mVs^{-1} sweep rate. The prepared poly (fast green) modified graphite pencil electrode (poly (FG)/GPE) was set aside in pH 7 phosphate buffer solution for half an hour to eliminate unpolymerised monomers.

III EXPERIMENTAL RESULTS

A. ELECTROCATALYTIC RESPONSE OF DA AT POLY (FG)/GPE BY CYCLIC VOLTAMMETRY

Figure 1 shows the comparison of voltammograms for bare GPE and poly (FG)/GPE for 50 μM DA with 0.2M phosphate buffer of pH 7.0 supporting electrolyte in the potential range of –250 to 600mV at 100 mVs^{-1} sweep rate. At bare GPE, the electrochemical reaction of DA shows less sensitive and reproducible voltammogram. A pair of redox peak at bare GPE (solid line) for DA were obtained with anodic peak potential located at 220 mV and cathodic peak potential was at 120 mV (vs SCE). The separation of redox peaks (ΔE_p) was found to be 100 mV. However, at the poly (FG)/GPE a pair of redox peaks obtained with strong increased redox peak current signals (dashed line). The poly

(FG)/GPE showed reduction in the over potential compared to bare GPE. The anodic peak potential and cathodic peak potential were located at 190 mV and 134 mV respectively. At poly (FG)/GPE the anodic peak potential was shift negatively upto 30 mV and cathodic peak potential was positively upto 14 mV. The ΔE_p was found to be 56 mV, which shows the electrocatalytical activity of poly (FG)/GPE.

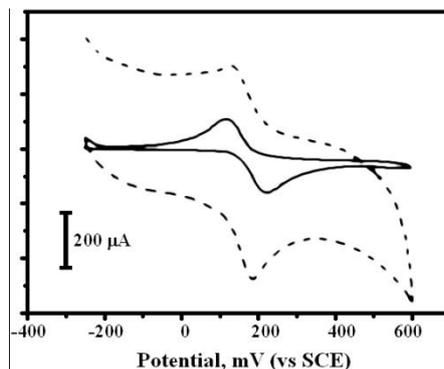


Figure 1. Cyclic voltammograms of 50 μM DA at poly (FG)/GPE (Dashed line) and bare GPE (solid line) in 0.2M phosphate buffer of pH 7.0 at 100 mVs^{-1} scan rate.

The effect of scan rate on the anodic peak current of 50 μM DA was studied by cyclic voltammetric technique with increasing the scan rate (50mVs^{-1} - 600mVs^{-1}). The graph of anodic peak current verses square root of scan rate ($v^{1/2}$) was plotted (data was not shown) and showed superior linearity between the square root of scan rate and anodic peak current. The correlation coefficient was found to be 0.9997 which indicates the electrode transfer reaction was diffusion controlled.

The electrochemical reaction of DA at poly (FG)/GPE is in general pH reliant. The voltammograms of 50 μM DA were recorded at 0.2 M phosphate buffer solutions of diverse pH by cyclic voltammetric method. The redox peak potentials were shift to less positive side with the increase in the pH value from 3.0-9.0. The potential diagram was constructed by plotting the graph of anodic peak potential (E_{pa}) vs pH of the solution. The graph has excellent linearity with a slope 53 mV/pH . This performance is nearly obeyed the Nernst Equation for equal number of electron and proton transfer reaction.

B. ELECTROCATALYTIC RESPONSE OF AA AND UA AT POLY (FG)/GPE BY CYCLIC VOLTAMMETRY

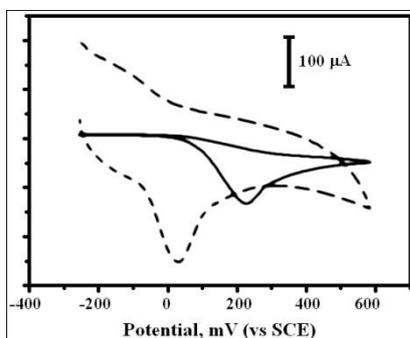


Figure 2. Cyclic voltammograms of 150 μM AA at poly (FG)/GPE (Dashed line) and bare GPE (solid line) in 0.2M phosphate buffer of pH 7.0 at 100 mVs^{-1} scan rate.

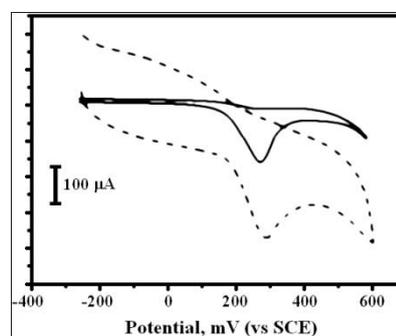


Figure 3. Cyclic voltammograms of 100 μM UA at poly (FG)/GPE (Dashed line) and bare GPE (solid line) in 0.2M phosphate buffer of pH 7.0 at 100 mVs^{-1} scan rate.

AA and UA are the interference compounds for the detection of DA. Hence it is essential to study their electrochemical behaviors. Figure 2 and 3 were the voltammograms recorded for 150 μM AA and 100 μM UA respectively at bare GPE (solid line) and poly (FG)/GPE (dashed line) in 0.2M phosphate buffer of pH 7.0 supporting electrolyte in the potential range of -250 to 600mV at 100 mVs^{-1} sweep rate. The individual electrochemical reaction of AA and UA were poor at bare GPE by means of overpotential and less sensitivity. The oxidation of AA and UA occurs at 225 mV and 295mV respectively. The poly (FG)/GPE has reduced the over potential compared to bare GPE. The anodic peak potentials of AA and UA were located at 30 mV and 295 mV respectively.

C. SIMULTANEOUS DETERMINATION OF DA, AA AND UA

AA and UA were present along with DA in mammalian brain. The concentration of AA and UA were much higher than that of DA. Since, the oxidation potential of AA and UA were nearly same as that of DA result is an overlapped voltammetric response at bare GPE. However, the poly (FG)/GPE has ability to separate the oxidation peak potentials of DA, AA and UA. Figure 4 shows the cyclic voltammograms for solution containing mixture of $50\text{ }\mu\text{M}$ DA, $100\text{ }\mu\text{M}$ UA and $200\text{ }\mu\text{M}$ AA in pH 7.0 phosphate buffer solution at sweep rate of 100 mVs^{-1} . The bare GPE (dashed line) showed only one broad and less sensible anodic peak for mixture of sample. The poly (FG)/GPE (solid line) has able to separate the oxidation peaks of DA, UA and AA by showing three well separated anodic peaks. The electrochemical response of DA was showed at 230 mV , AA was found to be at 20 mV and the UA at 310 mV by cyclic voltammetric techniques. The resolutions of this mixture of three samples were also performed by using differential pulse voltammetric technique (Figure 5). The oxidation potentials of DA, AA and UA were found at 160 mV , -45 mV and 310 mV respectively. The peak-to-peak separation between DA-AA was found to be 205 mV and between DA-UA was 150 mV .

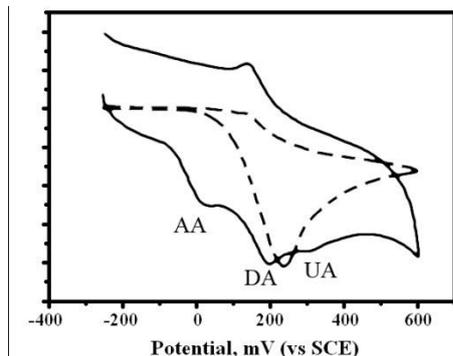


Figure 4. Cyclic voltammograms for simultaneous determination of $50\text{ }\mu\text{M}$ DA, $100\text{ }\mu\text{M}$ UA and $200\text{ }\mu\text{M}$ AA at 100 mVs^{-1} scan rate at bare GPE (dashed line) and at poly (FG)/GPE (solid line).

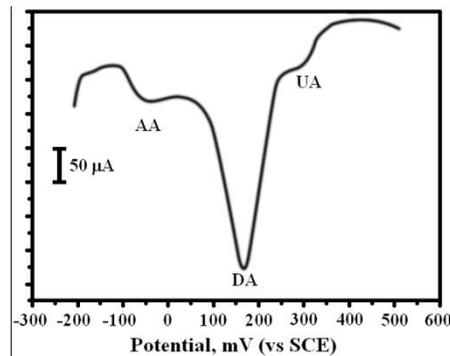


Figure 5. Differential pulse voltammograms for simultaneous determination of $50\text{ }\mu\text{M}$ DA, $100\text{ }\mu\text{M}$ UA and $200\text{ }\mu\text{M}$ AA at 20 mVs^{-1} scan rate at poly (FG)/GPE.

D. INTERFERENCE STUDY

The simultaneous determination of DA, AA and UA in the mixture was carried out at poly (FG)/GPE by keeping two species concentration constant and varying one species concentration [37]. From the Figure 6, it can be seen that the concentration of DA was increased from 5 to $70\text{ }\mu\text{M}$, while keeping the concentration of UA $100\text{ }\mu\text{M}$ and AA $200\text{ }\mu\text{M}$. The anodic peak current was proportional to concentration of DA and there were no changes in the peak current and peak potential was occur for AA and UA. The correlation coefficient was found to be 0.9989 . The limit of detection (LOD) was calculated by using the formula (1), where S is the standard deviation of blank solutions and M is the slope obtained by the peak current vs concentrations [37]. The LOD was found to be $4 \times 10^{-7}\text{ M}$. These experimentally obtained results were compared with other previously reported literatures and shown in table 1 [38-43].

$$\text{LOD} = 3S/M \tag{1}$$

Similarly, in the figure 7 shows the concentration effect of UA from 20 to $350\text{ }\mu\text{M}$ at constant $200\text{ }\mu\text{M}$ AA and $50\text{ }\mu\text{M}$ DA and figure 8 shows the concentration effect of AA from 50 to $450\text{ }\mu\text{M}$ at constant $50\text{ }\mu\text{M}$ DA and $100\text{ }\mu\text{M}$ UA. These results show that the DA, AA and UA exist independently in their mixtures of samples. The detection limit of AA and UA were found to be $5.2 \times 10^{-6}\text{ M}$ and $2 \times 10^{-6}\text{ M}$ respectively.

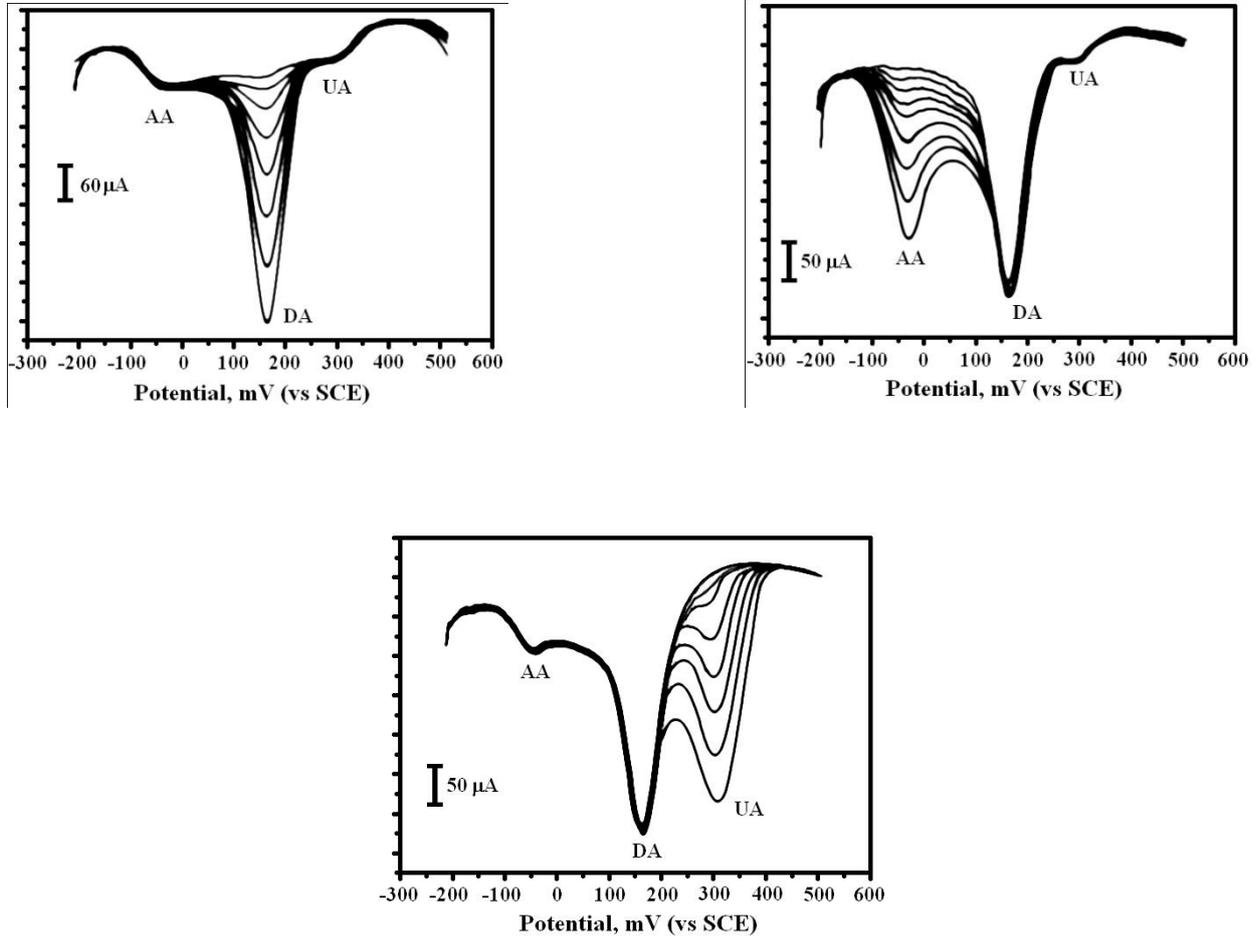


Figure 8. Differential pulse voltammogram of AA (a – g; 50 μM , 100 μM , 200 μM , 250 μM , 300 μM , 350 μM , 400 μM and 450 μM) in 0.2M phosphate buffer solution of pH 7.0 in the presence of 50 μM DA and 100 μM UA at poly (FG)/GPE.

Table 1. Comparison of different modified electrode for DA determination.

Electrode	Detection limit (mol/liter)	Method	References
Metallothioneins self-assembled gold electrode	6.0×10^{-6}	CV	[38]
Ionic liquid modified carbon paste electrode	7.0×10^{-7}	CV	[39]
poly(XO)MCPE	5.24×10^{-5}	CV	[40]
Sonogel-Carbon L-cysteine Modified Electrode	1×10^{-7}	SWV	[41]
Overoxidized Polypyrrole/Graphene Modified Electrodes,	0.1×10^{-6}	CV	[42]
Activated roughened glassy carbon electrode	8×10^{-8}	CV	[43]
Poly (FG)/GPE	5×10^{-7}	DPV	Present Work

IV CONCLUSION

The prepared poly (FG)/GPE exhibits higher electrocatalytic activity towards the detection of DA. The obtained results revealed that determination of DA can be easily performed using poly (FG)/GPE and this modified electrode has dramatically enhanced electrocatalytic activity towards DA with good stability. The LOD was found to be 4×10^{-7} M. The applicability of poly (FG)/GPE is able to identify the DA, AA and UA in mixture of sample. AA and UA are not interferences in the electrochemical investigation of DA. Poly (FG)/GPE also has good stability and this fabricated electrode can be applied for study of other bioactive molecules.

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development of electrochemical sensors for bio medical applications. He has published more than 151 papers in referred journals and at present working as Assistant Professor in Post-Graduate Studies and Research in Industrial Chemistry Department, Kuvempu University, Shimoga, Karnataka, INDIA from December 2006. He has guided 08 Ph.D's, 03 M.Phil's and presently 06 students are working for the Ph.D. programme. He served as referee and editorial board member in Journals. He completed four projects from different funding agencies and member of several university committees.

Dr. B.E. Kumara Swamy received his Master of Science in Industrial Chemistry in 1997 from Kuvempu University, Shimoga, Karnataka, INDIA, recipient of Prof. M.R. Gajendraghad Gold Medal and "Young Scientist Award" from Indian Council of Chemists in 2000 in Physical Chemistry Section. Later he received Ph.D. degree from the same department in August 2002. He joined as National Science Foundation (NSF) Post-Doctoral Research Associate in Department of Chemistry in Southern Methodist University, Texas, Dallas, USA (Feb 2003– Jan 2006) and Research Associate in University of Virginia, Virginia, USA (February 2006– ebruary 2006). The area of interest involved in the modification of carbon paste electrode by nanoparticles and some modifiers for the detection of neurotransmitters. Basically works on



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