Selective Determination of Dopamine in Presence of Ascorbic Acid Using Nafion Coated Methylene Blue Functionalized Graphite Electrode

G. Sivasankari[*], K. Sivasankari, T. Nalini, P. Padmapriya and M. Nadhiya

Department of Chemistry, Cauvery College for Women, Thiruchirappalli-620018, India

*Corresponding author: E-mail: sivakspm@gmail.com

An electrochemical sensor has been developed using methylene blue (MB) functionalized graphite for the selective determination of dopamine in the presence of ascorbic acid. The immobilization of methylene blue on graphite was done by carbodiimide coupling method. The methylene blue functionalized graphite was characterized by SEM, FTIR spectroscopy and UV-visible spectroscopy. The immobilization of methylene blue functionalized graphite (MB-G) on the electrode surface was made by drop casting a small volume of MB-G dispersed methanol solution on the electrode surface. About 5 L of 0.5 % Nafion solution was also dropcasted for the stability and selectivity. The MB-G modified electrode was characterized by cyclic voltammetry. The modified electrode promotes the electrocatalytic oxidation of dopamine at a lower potential of 160 mV. The selective determination of dopamine in the presence of 100 times higher concentration of ascorbic acid was achieved by differential pulse voltammetry. The modified electrode offered a simple, selective and sensitive determination of low levels of dopamine. Also the oxidation over potential was reduced to an extent of 360 mV comparing with the bare graphite electrode. The proposed sensor has the advantage of easy fabrication, low cost, good sensitivity, reproducibility and stability.

Keywords: Dopamine, Ascorbic Acid, Nafion, Methylene blue, Graphite electrode.

INTRODUCTION

Biological amines such as dopamine and norepinephrine act as neuromodulators in the central nervous system and also act as a hormone in the blood circulation. As dopamine is one of the most important catecholamine neurotransmitters in mammalian central nervous system [1,2], it has attracted the attention of neuroscientists and chemists. A decrease in dopamine level is associated with painful symptoms that frequently occur in Parkinson′s disease [3,4]. Abnormalities in dopaminergic neurotransmission have also been demonstrated in painful clinical conditions, including burning mouth syndrome [5], fibromyalgia [6,7] and restless legs syndrome [8]. Realizing the importance of this neurotransmitter in brain and in blood, several methods like chemiluminiscence [9], chromatography [10] and electrochemical methods [11-16] have been described for its determination. Of these, electrochemical methods have gained more attention than the other methods, due to its fast and sensitive response. In electrochemical research, the selective determi-

nation of a substrate in the presence of its interfering compound is a challenging subject because both substrates can be oxidized at close potentials, which results in overlapping of voltammetric response. Similarly, a major problem in constructing a sensor for dopamine determination is the resolution between dopamine and the coexisting species namely ascorbic acid. As the oxidation potential for both compounds are nearer to each other, the selective determination of dopamine in the presence of ascorbic acid is the major electrochemical challenge. Among several solutions to this problem, one of the most convenient methods is to coat the electrochemical sensor with Nafion, a negatively charged perfluorosulfonate polymer, which repels the anionic compounds such as ascorbic acid [17]. Also, Nafion coated sensor possesses good selectivity and stability of the electrode. In the past few decades, a series of organic dye based sensors, methylene dye [18] thionine [19], Azure dyes [20] and toluidine blue [21] have been utilized in analytical chemistry. These dyes are very attractive because they are chemically reactive, show negative formal potential at neutral pH and exhibit excellent

mediating ability. Many sensors have been reported using these dyes that have been either adsorbed, electropolymerized [22] or covalently attached [23] to the electrode surface. Another area of research which attracted much attention is the fabrication of electrochemical sensor with high efficiency, low cost and easy operation. Recently chemically modified graphite has been used in electroanalysis [24] and it was mainly preferred due to its low cost, high electrical conductivity and suitable thermal stability. Compton *et al*. [25] has suggested that the carbon nanotubes (CNTs) and graphite have unique catalytic properties. Also, graphite has edge plane like defect sites at its surface similar to that of carbon nanotubes [26] and hence it is possible to functionalize graphite with some groups at its edge plane like defect sites. In this study, an attempt has been made to modify the graphite surface with methylene blue through carbodiimide coupling method. This methylene blue functionalized graphite film coated electrode is used as an electrochemical sensor for the selective determination of dopamine.

EXPERIMENTAL

Graphite of spectroscopic grade (1-2 μm) was purchased from Aldrich. Methylene blue, *N*-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were obtained from S.D. fine-Chem Limited. Dopamine and ascorbic acid were from Himedia. All chemicals and reagents used were of analytical grade. All solutions were prepared using double distilled water. A 0.1M phosphate buffer solution (PBS) of pH 7.0 was always employed as the supporting electrolyte. A phosphate buffered saline (PBS) was prepared by mixing 0.1M potassium dihydrogen orthophosphate and 0.1M dipotassium hydrogen orthophosphate. Solutions were deaerated by bubbling high purity nitrogen prior to the experiment.

All the electrochemical measurements were carried out in a conventional three electrode cell by a CHI 660B electrochemical workstation (CHI Instruments USA). The modified electrode was used as the working electrode, platinum electrode as the counter electrode and standard calomel electrode (SCE) as the reference electrode. Amperometric measurements were carried out under dynamic conditions.

Preparation of carboxylated graphite: The introduction of carboxyl group at the surface of graphite was carried out according to the procedure given for the oxidation of carbon nanotubes (CNTs) with slight modification [27]. Briefly, about 500 mg of graphite was sonicated in conc. HCl for 30 min and washed thoroughly with double distilled water. Then the graphite was treated with 250 mL of 5.6 M HNO_3 and refluxed for 12 h. The black solid obtained was then washed thoroughly with double distilled water and was dried at 200 ºC for 18 h. This treatment generates -COOH group at the edge plane like defect sites on the surface of graphite.

Preparation of methylene blue functionalized graphite: Methylene blue was covalently linked to -COOH group of graphite using water soluble coupling agents, 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS), through amide linkages between the primary amine of methylene blue and -COOH of graphite. This was done by dispersing carboxylated graphite (100 mg) in a freshly prepared 10 mL aqueous solution of EDC (100 mg/mL) followed by the addition of about 300 mg NHS and stirred for 2 h. The pH of solution was adjusted to 7 and then 100 mg of methylene blue was added to the solution and stirred for another 2 h at room temperature. Methylene blue functionalized graphite was separated by centrifugation and washed extensively with double distilled water and dried at room temperature.

Preparation of electrochemical sensor: The paraffin impregnated graphite electrode (PIGE) was prepared as reported method [28], by immersing soft graphite rods into molten paraffin wax under vacuum until air bubbles cease to evolve from the rods. After reestablishing atmospheric pressure, the rods were removed before the paraffin solidified. The lower end of electrode was carefully polished on smooth paper and that surface was used for the modification. About 0.5 mg of methylene blue functionalized graphite was dispersed in 5mL of methanol and kept under sonication for 15 min. About 15 μL of dispersed solution was dropcasted on an already mirror finished smooth surface of PIGE and allowed to dry. Finally, 5 μL of 0.5 % Nafion solution was dropcasted on the surface of electrode for stability and selectivity. This MB-G/Nf electrode was used for further experiments.

RESULTS AND DISCUSSION

Covalent immobilization of methylene blue: The first step for immobilization of methylene blue was the oxidation of graphite using conc. HNO_3, which results in the formation carboxylated graphite. In the second and third steps, EDC and NHS have been used. This EDC and NHS are classified as zero-length cross linking reagents because during cross linking, atoms are eliminated from reactants, thus shortening the distance between the two linked moieties [29]. In this reaction, EDC activates the terminal -COOH groups of graphite (step 2) and NHS react (step 3) with carboxyl groups of graphite to give amine reactive NHS ester. This amine reactive NHS ester hydrolyzes very slowly to give -COOH group but it reacts fast with the amino groups of dye. Thus NHS increases the coupling efficiencies of carbodiimide for conjugating carboxylated compounds with primary amine group of the mediator.

Surface morphology: The surface morphologies of graphite and MB-G were characterized by scanning electron microscopy (SEM) and the results are shown in Fig. 1. It can be observed that oxidized graphite (Fig. 1a) of 1-2 μm was homogeneously distributed on the PIGE. It was found that morphology of MB-G (B) was similar to that of oxidized graphite, *i.e.* MB-G was distributed homogenously at the PIGE surface. This result shows that the immobilization of methylene blue on the graphite doesn't create any morphological changes of graphite.

UV-visible spectroscopy: The UV-visible absorption measurements were employed to study the interactions between methylene blue and graphite. Fig. 2a shows the UV-visible absorption spectra of (a) graphite (b) methylene blue and (c) MB-G suspended solutions. It was found that UV-visible spectrum of graphite (a) alone doesn't shows any peak where as pure methylene blue (b) has two characteristics absorption peaks, one in visible region around 610 nm and the other in UV region around 280 nm. A very small red shift for the peak around 610 nm and a blue shift for the peak around 280 nm in MB-G
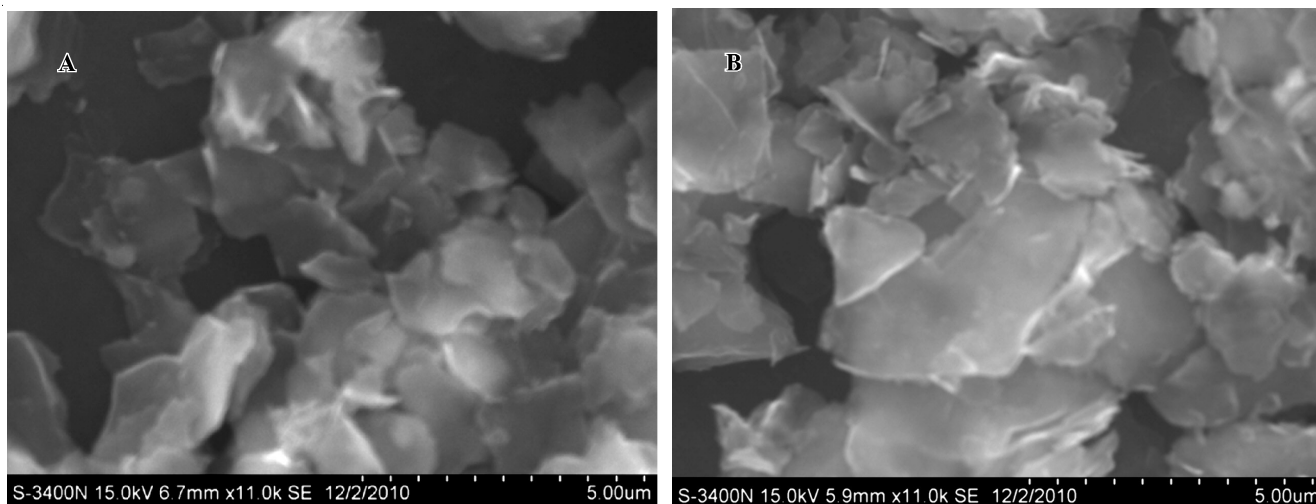
Fig. 1. Scanning electron microscopic images of (a) graphite and (b) methylene blue functionalized graphite
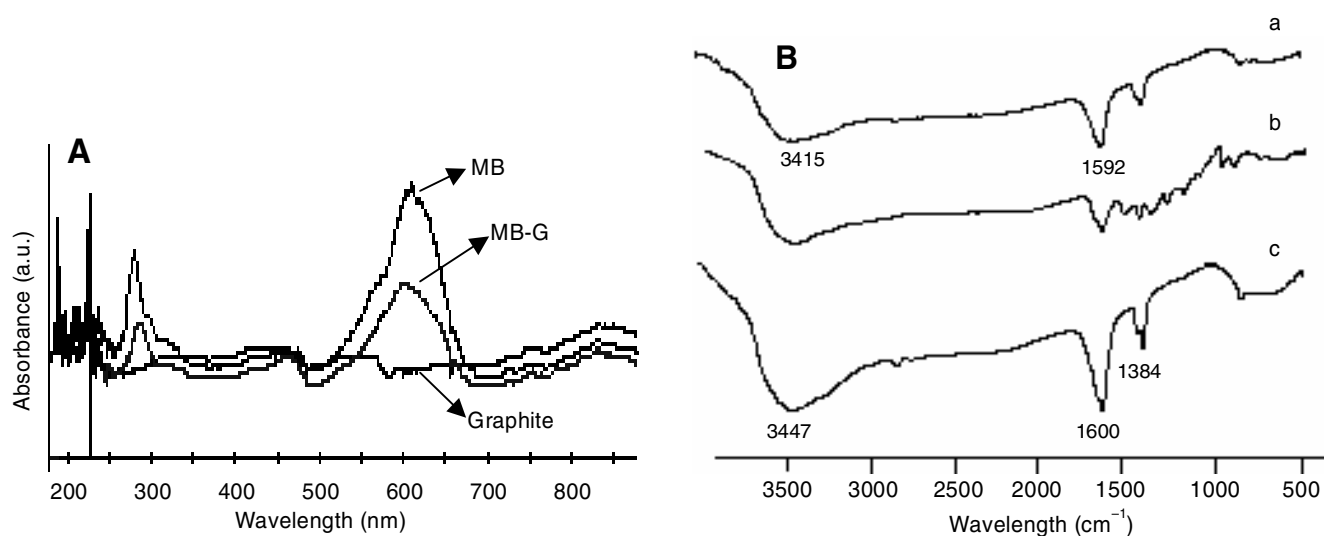

Fig. 2. (A) UV-visible spectra of (a) graphite, (b) methylene blue and (c) methylene blue functionalized graphite. (B) FTIR spectra of oxidised graphite (a), methylene blue (b) and methylene blue functionalized graphite (c)

spectrum (c) provide the evidence for the immobilization of methylene blue with graphite.

FTIR spectroscopy: The methylene blue functionalized graphite was also characterized by Fourier transform infrared spectroscopy. Fig 2b shows the FTIR spectra of (a) oxidized graphite, (b) pure methylene blue and (c) MB-G. The appearance of peaks at 1592 and 3415 cm^{-1} for the oxidized graphite (a) corresponds to -C=O and -O-H stretching vibrations of carboxylic groups, respectively. In the spectrum of MB-G (c), a peak at 3447 cm^{-1} corresponds to the N-H stretching frequencies of an amide bond and the new peaks at 1600 and 1384 cm^{-1} correspond to the aromatic ring of methylene blue. Thus, the functionalization of methylene blue with oxidized graphite is confirmed.

Electrochemical characterization: The electrochemical behaviour of MB-G/Nf electrode was characterized by cyclic voltammetry. The cyclic voltammograms of (a) bare PIGE and (b) MB-G/Nf electrode in 0.1M PBS at pH 7 in the potential range of −0.8 V to 0.8 V at a scan rate of 50 mV s^{-1} are represented in Fig. 3. The bare PIGE did not show any redox peaks. Conversely, MB-G/Nf electrode showed a distinct redox peaks
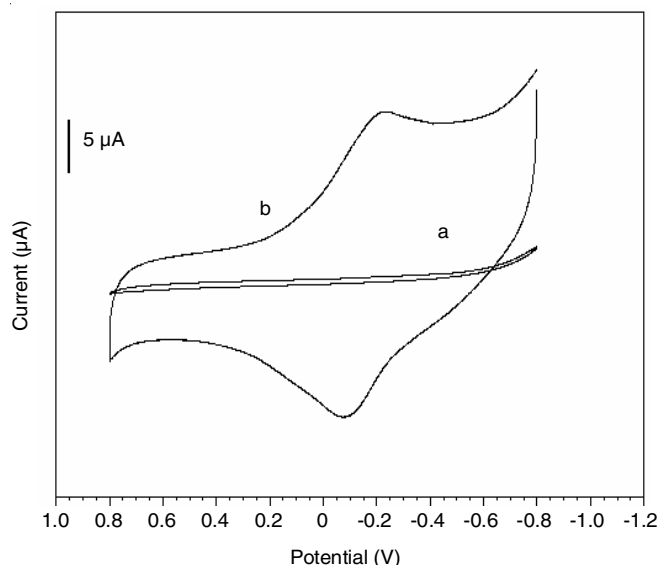

Fig. 3. Cyclic voltammograms of (a) bare PIGE and (b) MB-G/Nf modified PIGE in 0.1 M phosphate buffer solution (pH 7.0), scan rate 50 mV s^{-1}

with an anodic potential of –0.078V and a cathodic potential of –0.2492V. From this, the formal potential Ef0 was found to be –0.15 *vs.* SCE. This observation indicates that the mediator was immobilized on the surface of electrode.

In order to study the electrode reaction, the cyclic voltammograms of modified electrode at different scan rates were recorded (Fig. 4). As the anodic peak current and cathodic peak current were proportional to the scan rates, it was suggested that the redox reaction was surface confined process [30]. The plot of current *vs.* square root of scan rate has shown a linear plot with a regression coefficient of 0.9952 (for cathodic peak current) and 0.9966 (for anodic peak current) which indicates that the peak currents were proportional to the square root of scan rate (inset Fig. 4a).
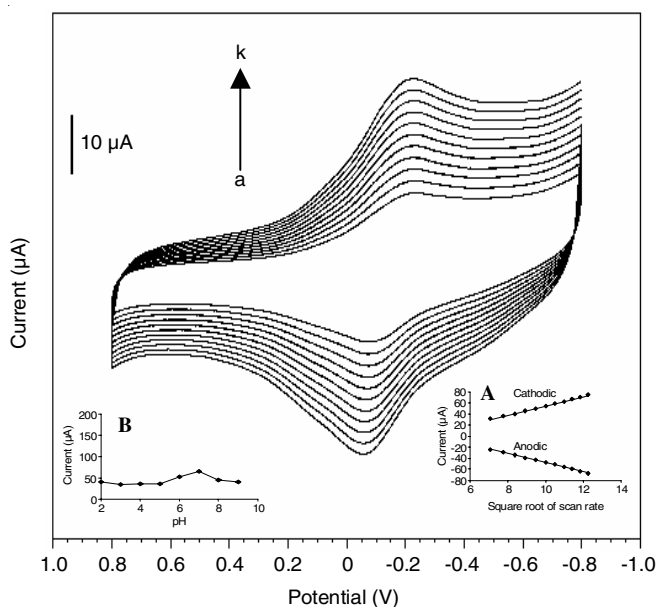

Fig. 4. Cyclic voltammograms of MB-G/Nf modified PIGE at various potential scan rates (a) 50, (b) 60, (c) 70, (d) 80, (e) 90, (f) 100, (g) 110, (h) 120, (i) 130, (j) 140 and (k) 150 mV s^{-1}, respectively. Inset: A) (bottom right): Dependence of anodic and cathodic peak currents on the square root of scan rate. B) (Bottom left): Effect of pH on MB-G/Nf modified PIGE

One of the important parameters which affect the response of both current and potential of the chemically modified electrodes in electroanalysis is pH. The solution pH and the type of buffer used for maintaining the pH are important to get maximum current for the modified electrode. For MB-G/Nf electrode, the effects of pH on the anodic and cathodic peak currents and on the potentials were investigated by cyclic voltammetry. The anodic and cathodic peak potentials shifted negatively with increasing pH using phosphate buffer. The plot of I_{pa} *vs.* pH (inset Fig. 4b) indicates that the maximum current is observed at pH 7. As the modified electrode has best response at neutral pH, this pH was chosen as optimum pH. The phosphate buffer is normally preferred to maintain pH around 7 and so this buffer was used for all the experiments.

Electrocatalytic oxidation of dopamine: The electrocatalytic oxidation of dopamine at the surface of MB-G/Nf electrode was studied by cyclic voltammetry. Fig. 5 shows the cyclic voltammogram for electrocatalytic oxidation of 0.2 mM
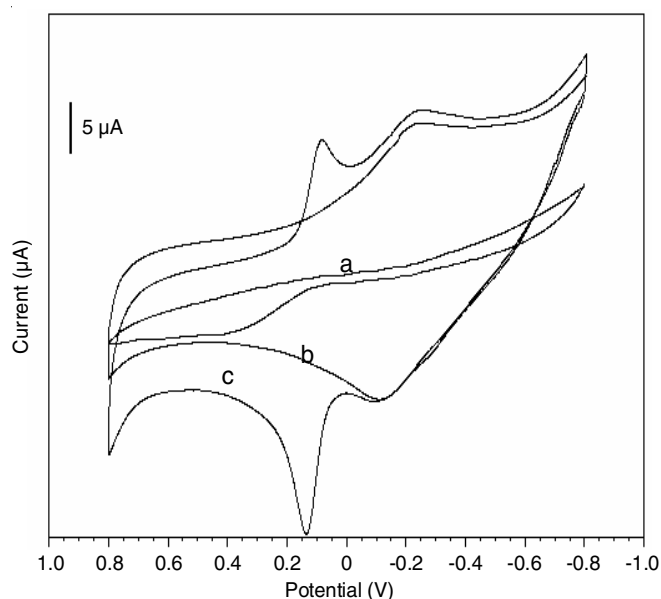

Fig. 5. Cyclic voltammogram of (a) MB-G/Nf modified PIGE and (b) bare electrode in 0.2 mM dopamine at a scan rate of 50 mV s^{-1}

dopamine using MB-G/Nf electrode. At bare PIGE electrode (Fig 5a), dopamine was oxidized around 500 mV where as at MB-G/Nf electrode it was oxidized at 160 mV (Fig 5c). Fig.5b is the cyclic voltammogram of MB-G/Nf modified electrode. From this, it was confirmed that the oxidation of dopamine was enhanced by the modified electrode and also it takes place with reduction in over potential. The MB-G enhanced the sensitivity of dopamine determination by increasing the current and decreasing the oxidation potential. The oxidation over-potential was reduced by 340 mV.

Effect of scan rate and solution pH on dopamine oxidation: The influences of scan rate on the catalytic oxidation peak potential (E_{pa}) and catalytic peak current (i_{pa}) of 0.2 mM dopamine at the MB-G/Nf modified electrode in pH 7.0 phosphate buffer was studied by cyclic voltammetry. As shown in Fig. 6, the peak currents of dopamine at the modified electrode were proportional to the scan rates (from 10-100 mV s^{-1}).
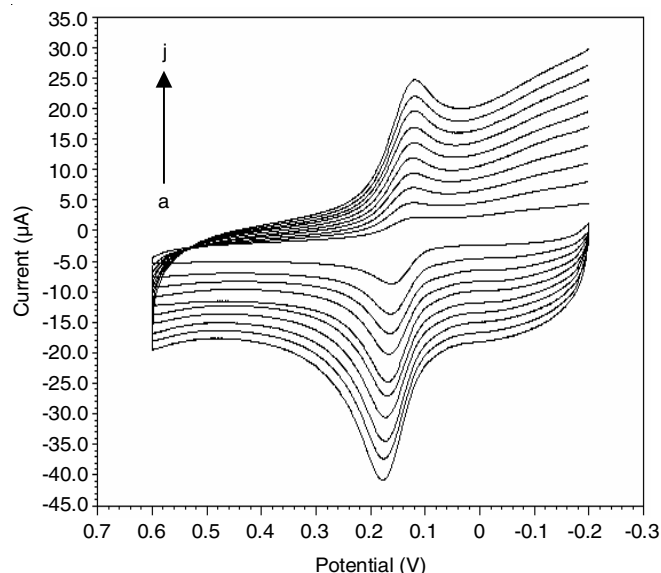

Fig. 6. Cyclic voltammogram of MB-G/Nf modified PIGE in 0.2 mM dopamine at different potential scan rate (10-100 mV)

The effect of solution pH on the electrocatalytic oxidation of 0.2 mM dopamine at the modified electrode was also studied by cyclic voltammetry in the pH range of 4.0 to 9.0 (Fig. 7). Acidity of solution has a large effect on the redox potentials of dopamine because proton is involved in the electrode reaction. It was also observed that the peak potentials shifted towards the negative potential with increasing pH of the solution. The peak current increases with increasing pH upto 7.0 and further increase of pH leads to decrease the peak currents. Thus phosphate buffer of pH 7.0 was chosen as supporting electrolyte since it is close to the pH of physiological condition.
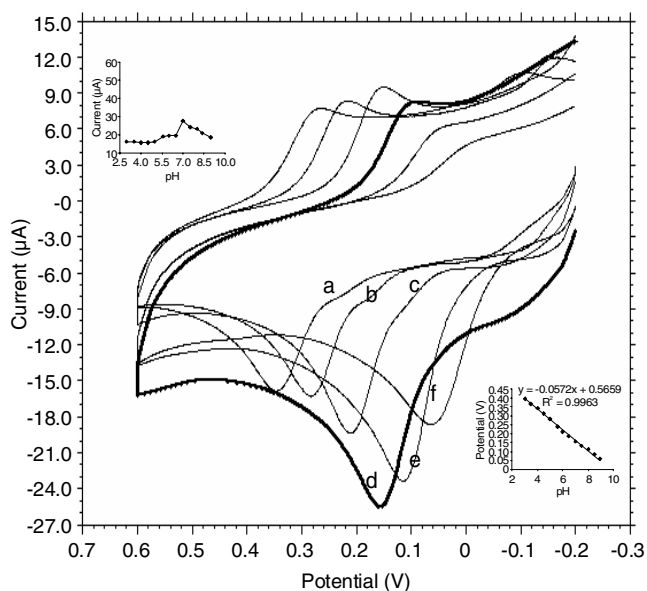

Fig. 7. Effect of solution pH of the MB-G/Nf modified PIGE on dopamine oxidation. pH range 4.0-9.0 [(a) 4.0 (b) 5.0 (c) 6.0 (d) 7.0 (e) 8.0 and (f) 0.9]. Inset I variation of current with respect to pH. II variation of oxidation potential with respect to pH

Determination of dopamine under dynamic condition: Determination of dopamine under dynamic condition has also been studied. Fig. 8A illustrates the hydrodynamic voltammo-grams of bare electrode in the absence (a) and presence of dopamine (b). The curves (c) and (d) show the hydrodynamic voltammogram of the modified electrode in absence and presence of dopamine, respectively. As shown in Fig. 8, the modified electrode (d) shows a good current response reaching maximum current around 140 mV which corresponds to the oxidation potential of dopamine, where as bare electrode (b) shows a maximum current response (which is very much less than modified electrode) around 500 mV. Using HDV, an operating potential of 200 mV was chosen to demonstrate the applicability of this sensor for the sensitive amperometric determination of dopamine. Fig. 8B is the chronoamperogram, which shows the catalytic current *vs.* time response of the modified electrode in PBS stirred with successive injection of 33 M dopamine at an applied potential of 200 mV. It was found that the sensor exhibits excellent electrocatalytic activity for the amperometric determination of dopamine at an applied potential of 200 mV. The modified electrode showed a rapid response to the concentration changes and a good linear relationship was observed between the peak current and the dopamine concentration over the range of 3.3×10^{-6} to 4.6×10^{-4} M with a correlation coefficient of 0.9978.

Selective determination of dopamine: The major interfering substrate in dopamine determination is ascorbic acid. As the differential pulse voltammetry was more sensitive, it was employed for the selective determination of dopamine in the presence of ascorbic acid. Fig. 9 shows the differential pulse voltammograms for the oxidation of different concentration of dopamine in the presence of 1 mM ascorbic acid at the modified electrode. At physiological pH, dopamine is positively charged while ascorbic acid is negatively charged. As the electrode is coated with Nafion, a negatively charged polymer, it repels the negatively charged ascorbic acid and allows dopamine alone to reach the electrode surface. The result indicates that the present sensor favours the selective determination of dopamine at lower oxidation potential, which is of great significant for the development of neurotransmitter sensors.
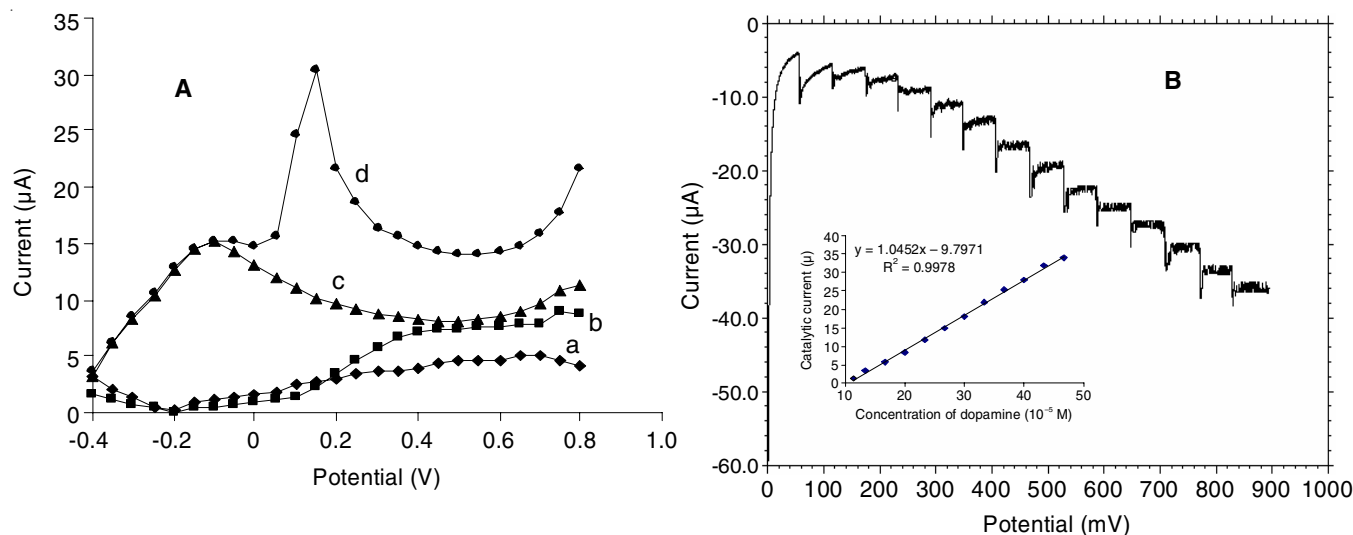

Fig. 8. (A) Hydrodynamic voltammogram of bare electrode in the presence (b) and absence (a) of dopamine and MB-G/Nf modified PIGE in the presence (d) and absence (c) of 0.2 mM dopamine. (B) Current response of MB-G/Nf modified PIGE in 0.1 M phosphate buffer solution (pH 7) for the successive addition of 49 mM of dopamine at 0.2 V. Stirring rate: 300 rpm
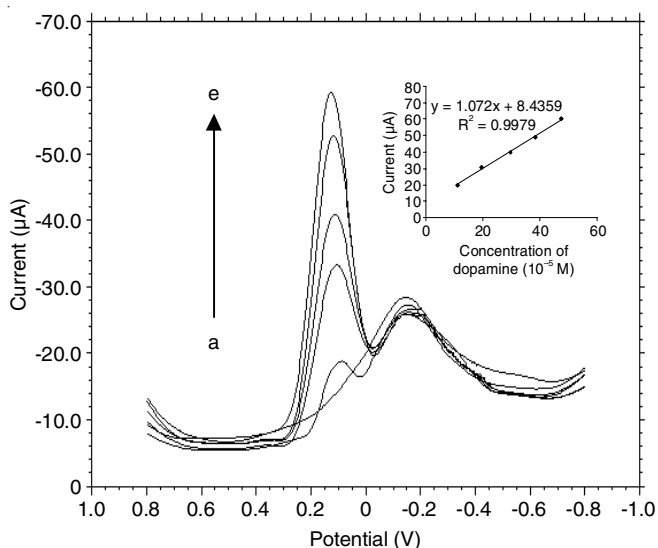
Fig. 9. Differential pulse voltammogram of dopamine oxidation at various concentrations in the presence of 1 mM ascorbic acid using MB-G/ Nf modified PIGE

Conclusion

The electrode fabrication in this method is based on the immobilization of methylene blue on the graphite surface through carbodiimide coupling method. The electrochemical properties of the modified electrode have been investigated by cyclic voltammetry. The developed sensor has been used for the selective determination of dopamine in the presence of ascorbic acid at lower oxidation potential. Determination of dopamine under dynamic condition has also been studied by hydrodynamic and chronoamperometric studies. Moreover, the modified electrode greatly reduced the oxidation over potential and has a great advantage of low cost, easy fabrication, good sensitivity and stability.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. P. Damier, E.C. Hirsch, Y. Agid and A.M. Graybiel, *Brain*, **122**, 1437 (1999); https://doi.org/10.1093/brain/122.8.1437.
2. A. Heinz, H. Przuntek, G. Winterer and A. Pietzcker, *J. Nervenarzt.*, **66**, 662 (1995).
3. E. Herlinger, R.F. Jameson and W. Linert, *J. Chem. Soc., Perkin Trans. II*, 259 (1995); https://doi.org/10.1039/p29950000259.
4. C.N. Lin, H.C. Liu, S.J. Tsai, T.Y. Liu and C.J. Hong, *J. Eur. Neurol.*, **48**, 207 (2002); https://doi.org/10.1159/000066162.
5. S.K. Jääskeläinen, J.O. Rinne, H. Forssell, O. Tenovuo, V. Kaasinen, P. Sonninen and J. Bergman, *J. Pain*, **90**, 257 (2001); https://doi.org/10.1016/S0304-3959(00)00409-7.
6. P.B. Wood, J.C. Patterson II, J.J. Sunderland, K.H. Tainter, M.F. Glabus and D.L. Lilien, *J. Pain*, **8**, 51 (2007); https://doi.org/10.1016/j.jpain.2006.05.014.
7. P.B. Wood, P. Schweinhardt, E. Jaeger, A. Dagher, H. Hakyemez, E.A. Rabiner, M.C. Bushnell and B.A. Chizh, *Eur. J. Neurosci.*, **25**, 3576 (2007); https://doi.org/10.1111/j.1460-9568.2007.05623.x.
8. S. Cervenka, S.E. Palhagen, R.A. Comley, G. Panagiotidis, Z. Cselényi, J.C. Matthews, R.Y. Lai, C. Halldin and L. Farde, *Brain*, **129**, 2017 (2006); https://doi.org/10.1093/brain/awl163.
9. L. Zhang, N. Teshima, T. Hasebe, M. Kurihara and T. Kawashima, *Talanta*, **50**, 677 (1999); https://doi.org/10.1016/S0039-9140(99)00164-2.
10. T.J. Panholzer, J. Beyer and K. Lictwald, *Clin. Chem.*, **45**, 262 (1999).
11. A. Liu, I. Honma and H. Zhou, *Electrochem. Commun.*, **7**, 233 (2005); https://doi.org/10.1016/j.elecom.2004.12.015.
12. P.R. Roy, T. Okajima and T. Ohsaka, *Bioelectrochemistry*, **59**, 11 (2003); https://doi.org/10.1016/S1567-5394(02)00156-1.
13. M.D.P.T. Sotomayar, A.A. Tanaka and L.T. Kubota, *Electroanalysis*, **15**, 787 (2003); https://doi.org/10.1002/elan.200390097.
14. T.J. Castilho, M.P.T. Sotomayor and L.T. Kubota, *J. Pharm. Biomed. Anal.*, **37**, 785 (2005); https://doi.org/10.1016/j.jpba.2004.11.043.
15. K. Miyazaki, G. Matsumoto, M. Yamada, S. Yasui and H. Kaneko, *Electrochim. Acta*, **44**, 3809 (1999); https://doi.org/10.1016/S0013-4686(99)00087-0.
16. J.M. Zen, W.M. Wang and G. Ilangovan, *Anal. Chim. Acta*, **372**, 315 (1998); https://doi.org/10.1016/S0003-2670(98)00375-4.
17. D.R. Shankaran, N. Uehara and T. Kato, *Anal. Chim. Acta*, **478**, 321 (2003); https://doi.org/10.1016/S0003-2670(02)01511-8.
18. M. Arvand, S. Sohrabnezhad, M.F. Mousavi, M. Shamsipur and M.A. Zanjanchi, *Anal. Chim. Acta*, **491**, 193 (2003); https://doi.org/10.1016/S0003-2670(03)00790-6.
19. S.A. John and R.J. Ramaraj, *J. Electroanal. Chem.*, **561**, 119 (2004); https://doi.org/10.1016/j.jelechem.2003.07.025.
20. Y. Sha, Q. Gao, B. Qi and X. Yang, *J. Microchim. Acta*, **148**, 335 (2004); https://doi.org/10.1007/s00604-004-0283-6.
21. D.R. Shobha Jeykumari and S.S. Narayanan, *J. Nanosci. Nanotechnol.*, **7**, 1824 (2007); https://doi.org/10.1166/jnn.2007.724.
22. Q. Gao, W. Wang, Y. Ma and X. Yang, *Talanta*, **62**, 477 (2004); https://doi.org/10.1016/j.talanta.2003.08.017.
23. M. Ohtani, S. Kuwabata and H. Yoneyama, *J. Electroanal. Chem.*, **422**, 45 (1997); https://doi.org/10.1016/S0022-0728(96)04888-7.
24. P. Abiman, G.G. Wildgoose, A. Crossley and R.G. Compton, *Electroanalysis*, **21**, 897 (2009); https://doi.org/10.1002/elan.200804519.
25. R.R. Moore, C.E. Banks and R.G. Compton, *Anal. Chem.*, **76**, 2677 (2004); https://doi.org/10.1021/ac040017q.
26. C.E. Banks, T.J. Davies, G.G. Wildgoose and R.G. Compton, *Chem. Commun.*, **27**, 829 (2005); https://doi.org/10.1039/b413177k.
27. D.R. Shobha Jeykumari, S. Ramaprabhu and S.S. Narayanan, *Carbon*, **45**, 1340 (2007); https://doi.org/10.1016/j.carbon.2007.01.006.
28. F. Scholz and B. Lange, *TrAc Trends Anal. Chem.*, **11**, 359 (1992); https://doi.org/10.1016/0165-9936(92)80025-2.
29. S.S. CRC Press, (1991).p.195-9[chapter 6].
30. E. Katz and I. Willner, *Sensors*, **6**, 420 (2006); https://doi.org/10.3390/s6040420.