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Simultaneous determination of epinephrine and norepinephrine in human blood plasma and urine samples using nanotubes modified edge plane pyrolytic graphite electrode

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ABSTRACT

The simultaneous determination of catecholamines – epinephrine and norepinephrine by square wave voltammetry (SWV) at physiological pH 7.2 is reported using multi-walled carbon nanotubes modified edge plane pyrolytic graphite electrode (MWNT/EPPGE). A broad bump at ~250 mV is appeared for the oxidation of epinephrine (EP) and norepinephrine (NE) at bare EPPGE whereas at MWNT/EPPGE two well-separated peaks at ~150 and ~215 mV are appeared for the oxidation of EP and NE, respectively. The oxidation peak current of both the neurotransmitters also increased significantly along with the negative shift of peak potentials using MWNT/EPPGE. The oxidation of both compounds occurred in a pH dependent, 2e and $2H^+$ process and the electrode reaction followed diffusion controlled pathway. Linear calibration curves were obtained for epinephrine and norepinephrine in the range 0.5–100 nM with limits of detection 0.15×10^{-9} and 0.90×10^{-10} M, respectively. The developed protocol is implemented for the simultaneous determination of epinephrine and norepinephrine in blood plasma and urine samples of smokers as well as in athletes.

1. Introduction

Catecholamines are produced by sympathetic nervous system activation and act as hormones and neurotransmitters to monitor heart rate, brain muscles activity, glycogenolysis, fatty acid mobilization and body temperature [1,2]. Catecholamine drugs are used to treat hypertension, bronchial asthma, organic heart disease and also in cardiac surgery and myocardial infarction [3]. Epinephrine (**I**) and norepinephrine (**II**) are two major endogenous catecholamines in human body having complementary actions [4]. The biosynthesis of both the neurotransmitters in human system occurs from tyrosine which is produced in the liver from phenylalanine [5]. Tyrosine then transported into catecholamine-secreting neurons and adrenal medullary cells where it converts into dopamine and finally to epinephrine via norepinephrine through a series of reactions [5]. Epinephrine (EP), known as the "fight or flight" hormone, energizes and speeds up the various systems within the body and plays an important role during the times of stress [6]. While norepinephrine (NE) increases the conversion of glycogen to glucose in the liver, helps in converting fats into fatty acids, and relaxing the bronchial muscles [7]. All these actions are related to calm down of the body. Owing to the fact that EP is a derivative of NE and both catecholamines have complementary actions in human body, it has been considered worthwhile to develop a selective and sensitive method for the simultaneous determination of epinephrine and norepinephrine in human body fluids. Several methods based on voltammetry have been developed for the individual determination of NE and its analogs EP [8–11]. But these reports have comparatively lesser importance to monitor physiological functions since; EP and NE have complementary actions in the body. Only single voltammetric method has been reported till date for the simultaneous determination of EP and NE however, analysis has not been carried out in human body fluids [12]. One of the reasons for this is due to oxidation of both the neurotransmitters at almost the same potential which results in overlapped voltammetric responses, and makes their discrimination very difficult in the same solution [13]. The carbon paste electrode reported for the simultaneous determination of epinephrine and norepinephrine having no real sample analysis although, the electrode modification procedure was enough complicated [12]. Hence, there is still an expanding demand for the development of a simple, selective and sensitive sensor that can resolve overlapped voltammetric signals of epinephrine and norepinephrine.

Literature survey reveals that the release of catecholamines in human system depends on smoking and exercise because these stimulants activates the sympathetic nervous system acting via

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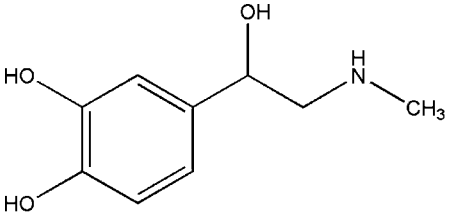
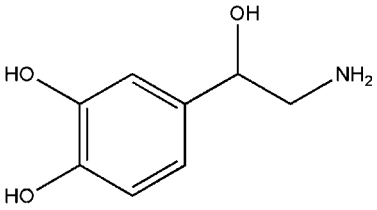
splanchnic nerves to the adrenal medulla and stimulates the release of catecholamines into the blood stream [14,15]. Since, in respect of neurotransmitters, only urine is not a sufficient indicator of central nervous system (CNS) activity [16] hence, considering the potential role of plasma catecholamines as a readily available measure of sympathoadrenal system activity [17], the developed protocol has been implemented for the simultaneous determination of EP and NE in urine as well as plasma samples of athletes and smokers. Further, most of the research focuses on biochemical and medical aspect of catecholamines and comparatively few studies are available regarding their analytical aspect [18–20] hence, it is believed that simultaneous sensing of EP and NE in human body fluids will be of great importance to monitor nerve physiology, doping cases, controlling medicine in biomedical, biopharmaceutical research and clinical diagnosis of some aforementioned disorders [21,22].

Carbon nanotubes are the kinds of porous nanostructure material which is currently in the forefront of materials research. Nanotubes have the ability to mediate electron transfer reactions with electroactive species in solution when used as an electrode surface modifier for designing new electrochemical sensors [23]. The carbon nanotubes modified edge plane pyrolytic graphite electrode (EPPGE) has been utilized successfully with adequate selectivity and sensitivity to determine number of biomolecules and drugs in human blood plasma and urine [24–27], hence, an attempt has been made to utilize multi-walled carbon nanotube modified EPPGE in the present investigation. Square wave voltammetry is a versatile technique for electroanalytical purposes as it has higher sensitivity and effectively suppresses background current. A novel approach using square wave voltammetry with multi-walled carbon nanotubes modified edge plane pyrolytic graphite electrode for the simultaneous assay of EP and NE in blood plasma and urine samples of athletes and smokers has been presented in proposed work.



(I)



(II)

2. Materials and methods

2.1. Instrumentation and chemicals

Epinephrine and norepinephrine were obtained from Sigma–Aldrich Inc., USA. Multi-walled carbon nanotubes (MWNTs) of purity >98% (5–15 nm diameter, 0.5–10 μm length) was purchased from Bucky, USA. Phosphate buffer solutions (PBS) were prepared according to the method of Christian and Purdy [28]. All other reagents and solvents used were of analytical grade. Bioanalytical System (BAS, West Lafayette, USA) CV-50 W voltammetric analyzer equipped with a three electrode system incorporating a bare or modified EPPGE (~6 mm^2) as the working electrode, an Ag/AgCl (3 M NaCl) as reference electrode (BAS Model MF-2052 RB-5B) and a platinum wire as counter electrode was used for square wave voltammetric experiments. The edge plane pyrolytic graphite piece was obtained from Pfizer Inc., New York, USA and the electrode was prepared as reported in the literature [29]. The surface morphology of bare and MWNT modified EPPGEs was characterized by recording FE-SEM images using Quanta 200 FE-SEM instrument. All potentials are reported with respect to Ag/AgCl electrode at an ambient temperature of 25 ± 2 °C.

2.2. Fabrication of edge plane pyrolytic graphite electrode

In order to remove adhered particles from the surface of bare EPPGE it was rubbed on the emery paper and then rinsed with double distilled water and dried. MWNTs suspension was prepared by dispersing 0.5 mg nanotubes in 1.0 ml N,N-dimethyl formamide (DMF) using ultrasonic bath. A known volume (40 μl) of this suspension was coated onto the surface of bare EPPGE and dried at room temperature. The MWNT modified EPPGE was then ready for use. The surface morphology of bare EPPGE and MWNT/EPPGE was observed by recording FE-SEM images and is presented in Fig. 1. The image presented in Fig. 1B for modified EPPGE clearly indicates the deposition of MWNT at the electrode surface.

2.3. Voltammetric procedure

Stock solutions of EP and NE (1 mM) were prepared by dissolving the required amount of the compounds in double distilled water. Optimized square wave voltammetric parameters used were: square wave amplitude (E_{sw}), 25 mV; potential step (E), 4 mV; square wave frequency (f), 15 Hz. Urine sample of nonsmoker healthy volunteer received from the laboratory personnel was used as control. The human urine and plasma samples of two smokers in resting stage were collected after 15 min of smoking from the institute hospital of Indian Institute of Technology, Roorkee. The anthropometric data of the smokers were, sample 1: male, age 35 yrs, height 154 cm, weight 66 kg; sample 2: male, age 46 yrs, height 170 cm, weight 76 kg; sample 3: male, age 42 yrs, height 156 cm, weight 55 kg. The blood plasma samples of two athletes (sample 1: female, age 24 yrs, height 152 cm, weight 52 kg; sample 2: female, age 28 yrs, height 156 cm, weight 56 kg) were collected soon after peak exercise of ~30 min. In order to minimize the matrix complexity urine and blood plasma samples were diluted four and two times, respectively with phosphate buffer solution of pH 7.2 prior to recording voltammograms.

3. Results and discussion

3.1. Cyclic voltammetry

Initially, cyclic voltammograms were recorded for 5 μM epinephrine or norepinephrine after bubbling high-purity nitrogen for 12–15 min using MWNT/EPPGE at pH 7.2. The cyclic voltammogram of EP exhibits an irreversible peak at 170 mV (I_a) when the sweep was initiated in positive direction. In the reverse sweep, a reduction peak (II_c) was noticed which formed a reversible couple with peak II_a, observed in second positive sweep. The peak potentials of the couple were −216 (II_c)/−194 (II_a) mV (Fig. 2). Cyclic voltammogram of NE also exhibited an oxidation peak at 220 mV (I_a) in the initial positive sweep. In the reverse sweep it exhibits two reduction peaks at 183 (I_c) and −182 (II_c) mV. Peak I_c formed a

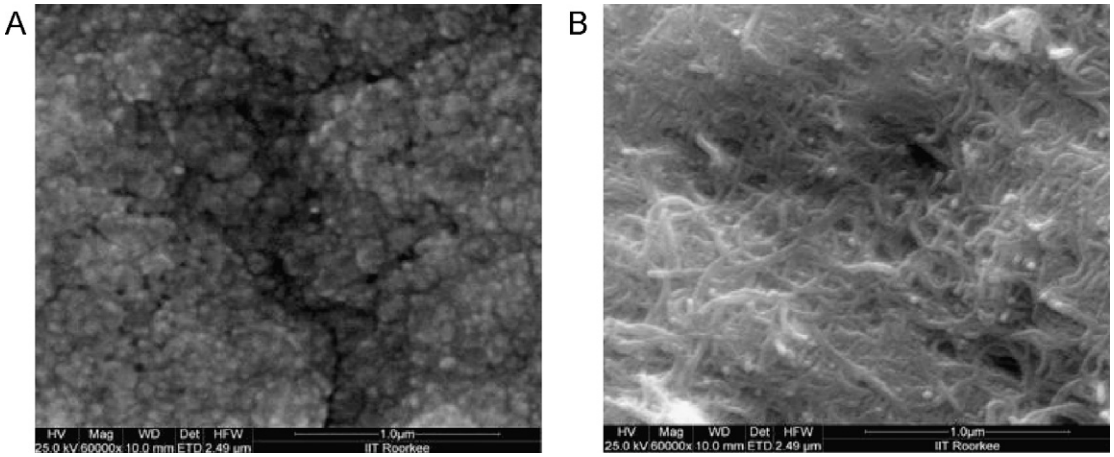


Fig. 1. FE-SEM images of (A) bare and (B) MWNT modified edge plane pyrolytic graphite electrodes.

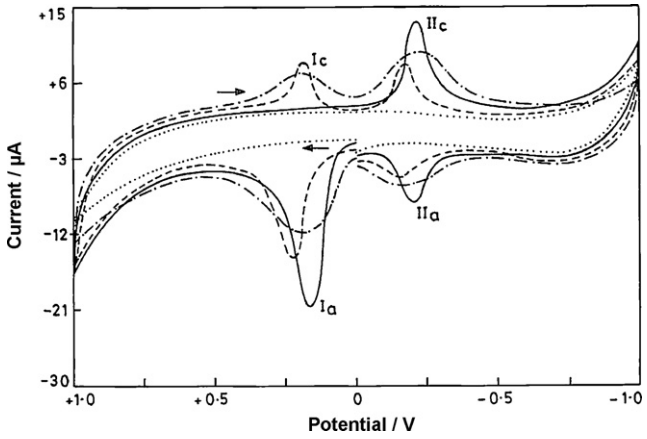


Fig. 2. Cyclic voltammograms of 5 μM epinephrine solution (—), 5 μM norepinephrine solution (- - -) and mixture of 5 μM EP and NE (–·–·–·) at 20 mV s^{-1} and response of MWNT/EPPGE in background PBS of pH 7.2 (. . .).

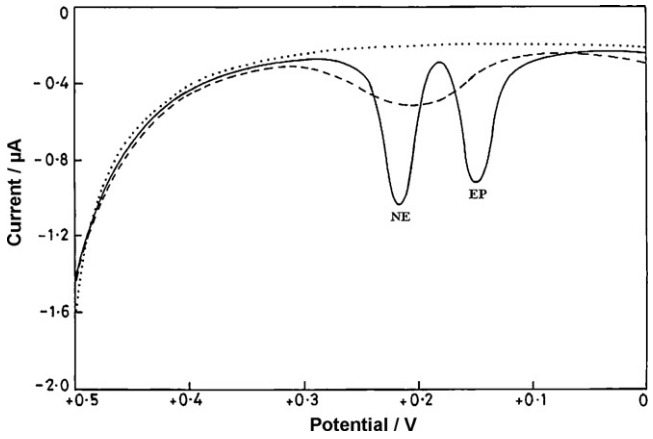


Fig. 3. Square wave voltammograms of binary mixture of epinephrine (80 nM) and norepinephrine (60 nM) using bare EPPGE (- - -) and MWNT modified EPPGE (—) at pH 7.20, and dotted line is the response of MWNT/EPPGE in blank PBS of pH 7.20.

quasi reversible couple with peak I_a. On further reversing the direction a peak at −154 mV (II_a) was observed, which formed a redox couple with peak (II_c) (Fig. 2). Finally, a cyclic voltammogram of binary mixture of 5 μM each EP and NE was recorded and oxidation peaks as well as reduction peaks of EP were found to merge with NE (Fig. 2). Thus, simple voltammetry was unsuccessful to resolve the two peaks of NE and EP. As square wave voltammetry is a more sensitive technique in comparison to cyclic voltammetry, hence it is used to resolve overlapped voltammetric response of epinephrine and norepinephrine.

3.2. Comparison of bare EPPGE and MWNT/EPPGE

Initially square wave voltammograms were recorded for a binary mixture of epinephrine and norepinephrine at bare and modified EPPGE working electrodes in phosphate buffer solution of pH 7.2 as shown in Fig. 3. At the bare edge plane pyrolytic graphite electrode, the voltammetric response is poor with a large voltammetric bump at ~250 mV. While the much improved voltammetric response having two well separated voltammetric peaks at ~150 and ~215 mV for epinephrine and norepinephrine, respectively was obtained at MWNT/EPPGE. Thus, Fig. 3 indicates that MWNT/EPPGE serves as a better substrate for the simultaneous determination of EP and NE neurotransmitters. The two well-separated peaks with shift of the peak potential towards less positive potential in conjunction with a significant increase in peak current at MWNT-modified EPPGE revealed that the proposed voltammetric sensor acts as a very efficient promoter to enhance the kinetics of the electrochemical process. Hence, MWNT/EPPGE has been utilized for further detailed studies of catecholamines.

3.3. Individual determination of epinephrine and norepinephrine

3.3.1. Effect of pH

The effect of pH on the oxidation of EP was studied in the pH range 2.4–8.8 using square wave voltammetry. It was found that the peak potential shifted towards less positive potentials with increase in pH and the variation of peak potential (E_p) with pH was linear. The dependence of E_p on pH obeys the relation:

$$E_p(\text{mV vs. Ag/AgCl}) = 616.7 - 63.46\,\text{pH}$$

having correlation coefficient 0.998. Similarly the oxidation potential of NE was also found to shift towards less positive potentials with increase in pH. The linear dependence of the peak potential on pH is represented by the following equation:

$$E_p(\text{mV vs. Ag/AgCl}) = 669.6 - 62.26\,\text{pH}$$

having correlation coefficient of 0.998. The slope of E_p vs. pH plots for EP and NE is close to 60 mV/pH and hence suggests that equal number of protons and electrons are involved in the electrode reaction [30].

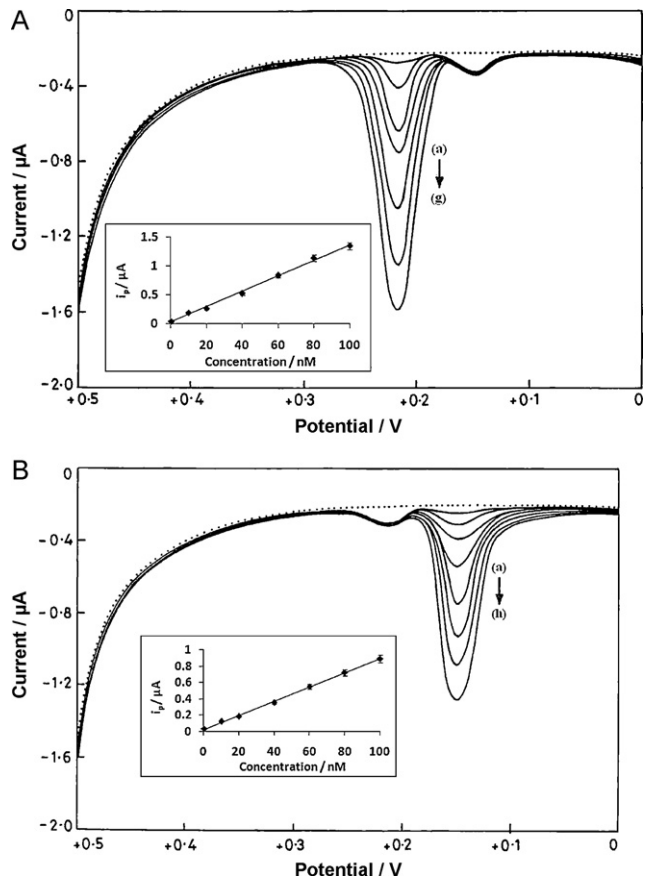


Fig. 4. (A) Voltammograms of a binary mixture of EP and NE, keeping the concentration of EP constant (10 nM) and concentration of NE was (a) 0.5, (b) 10, (c) 30, (d) 40, (e) 60, (f) 80 and (g) 100 nM at pH 7.2. Inset is calibration curve for norepinephrine. (B) Voltammograms of a binary mixture of EP and NE, keeping the concentration of NE constant (5 nM) and concentration of EP was (a) 0.5, (b) 5, (c) 20, (d) 40, (e) 60, (f) 80, (g) 100 and (h) 120 nM at pH 7.2. Inset is calibration curve for epinephrine.

3.3.2. Effect of square wave frequency

The dependence of oxidation peak current (i_p) on the square wave frequency (f) for EP or NE was studied in the range of 5–100 Hz. The peak current was found to increase linearly with square wave frequency (f) (Hz) for both the analytes. The linear relation between i_p and $f1/2$ for EP can be expressed by the equation:

$$i_p\,(\mu A) = 0.255(f)^{1/2} - 0.471$$

having correlation coefficient 0.998. On the same way the relation between i_p and $f1/2$ for NE can be represented by the following equation:

$$i_p\,(\mu A) = 0.406(f)^{1/2} - 0.693$$

having correlation coefficient 0.997. These observations indicate that the reactions occurred at the surface of MWNT/EPPGE are governed by the diffusion controlled process for both the catecholamines [31].

3.3.3. Concentration study

Square wave voltammograms for different concentrations of EP were recorded in phosphate buffer solution of pH 7.2 and the peak current increased with increase in concentration of EP. The peak current (i_p) was found to be linearly dependent on concentration in the range of 0.5–100 nM (inset Fig. 4A). The linear regression equation having correlation coefficient 0.998 is presented as:

$$i_p\,(\mu A) = 0.0087\,C\,(\mathrm{nM}) + 0.0198$$

where C is the concentration of EP. The detection limit was calculated by using the relation $3\sigma/b$, where σ is the standard deviation of the blank and b is the slope of the calibration curve. The detection limit and sensitivity of EP determination were calculated to be 0.15×10^{-9} M (S/N = 3) and 8.7 μA/μM, respectively. The limit of quantification was found to be 0.48×10^{-9} M. The peak current of NE was also found to increase linearly with increasing concentration (inset Fig. 4B) and the linear regression equation is expressed as:

$$i_p\,(\mu A) = 0.013\,C\,(\mathrm{nM}) + 0.024$$

where C is the concentration of NE. The correlation coefficient for the expression was 0.996. The detection limit and sensitivity of NE determination were calculated to be 0.90×10^{-10} M (S/N = 3) and 13.0 μA/μM, respectively and the limit of quantification for NE determination was calculated to be 0.28×10^{-9} M.

3.4. Simultaneous determination of epinephrine and norepinephrine

The main objective of the present investigation is to simultaneously determine the concentration of EP and NE. The MWNT modified EPPGE was utilized for this purpose and in the first set of experiments, concentration of EP was kept constant at 10 nM and NE was varied in the concentration range 0.5–100 nM as shown in Fig. 4A. It can be seen that the oxidation peak of epinephrine is unaltered by the addition of NE and the peak height of NE increased with increase in its concentration. Similarly, while varying the concentration of EP in the concentration range 0.5–120 nM and keeping the concentration of NE fixed at 5 nM, the oxidation peak current of EP increases as depicted in Fig. 4B. The current observed in both the cases for varied components were same as observed during the individual compound study and obeyed the calibration plot. Thus, the proposed method can be successfully used for the simultaneous determination of epinephrine and norepinephrine. These interesting and new results promoted us to use the proposed voltammetric sensor for the simultaneous determination of epinephrine and norepinephrine in human body fluids which is still aforementioned and also of great importance to monitor nerve physiology, doping cases, controlling medicine in biomedical and biopharmaceutical research [21,22].

3.5. Interference study

Catecholamines often exist together with high concentration of electroactive biomolecules like uric acid in natural environments that can interfere with each other. Hence, in order to examine the selectivity of MWNT/EPPGE influence of major interferents such as uric acid, ascorbic acid and dopamine was evaluated. For this purpose square wave voltammograms of a solution having mixture of standard ascorbic acid (AA), dopamine (DP), epinephrine (EP), norepinephrine (NE) and uric acid (UA) were recorded at pH 7.20 using MWNT/EPPGE. It was found that five well separated peaks at ∼−50, 80, 150, 215 and 300 mV were observed corresponding to the oxidation of ascorbic acid, dopamine, epinephrine, norepinephrine and uric acid, respectively. In order to further confirm the selectivity of modified sensor concentration of each interfering substance increased from 5 to 1000 fold by keeping the epinephrine and norepinephrine concentration constant. The experimental results show that no substantial changes in peak current response of epinephrine and norepinephrine were observed for entire concentration range of uric and ascorbic acid. However,

Table 1
Simultaneous determination of epinephrine and norepinephrine in plasma samples of smokers and athletes using MWNT/EPPGE.

Epinephrine (nM)				Norepinephrine (nM)		
Added	Found[a]	Actual	Recovery	Found[a]	Actual	Recovery
Smoker's sample 1						
0.00	0.25	0.25	–	0.90	0.90	–
0.50	0.76	0.26	101.33	1.36	0.86	97.14
1.00	1.74	0.24	99.43	2.38	0.88	99.16
Smoker's sample 2						
0.00	0.22	0.22	–	0.85	0.85	–
0.50	0.74	0.24	102.78	1.39	0.89	102.96
1.00	1.70	0.20	98.84	2.40	0.90	102.13
Athlete's sample 1						
0.00	1.50	1.50	–	4.50	4.50	–
0.50	1.98	1.48	99.00	4.95	4.45	99.00
1.00	3.02	1.52	100.67	5.98	4.48	99.67
Athlete's sample 2						
0.00	1.48	1.48	–	4.40	4.40	–
0.50	2.02	1.52	102.02	5.10	4.60	104.08
1.00	3.00	1.50	100.67	6.00	4.50	101.69

[a] The R.S.D. value for the determination of epinephrine and norepinephrine was less than ±3.2% for $n = 3$.

dopamine interferes severely when its concentration is 100 fold of epinephrine and norepinephrine. Therefore, it is concluded that MWNT/EPPGE can be safely used for simultaneous determination of epinephrine and norepinephrine in pharmaceutical preparations as well as in biological samples.

3.6. Analytical utility of proposed sensor

3.6.1. Simultaneous determination of epinephrine and norepinephrine in urine

The release of catecholamines in human system depends on smoking and exercise [14,15]. Therefore, the proposed sensor has been examined for the simultaneous determination of EP and NE in body fluids of athletes and smokers. The square wave voltammograms of urine samples of three smokers and nonsmokers (control) in resting stage were recorded under optimized parameters. To overcome the interference of major urinary metabolite viz. uric acid and ascorbic acid, an optimized potential region 0–250 mV was selected for the determination of catecholamines. The authentic sample of uric acid oxidized at ~300 mV, whereas, ascorbic acid was found to oxidize at ~−50 mV using MWNT/EPPGE. Square wave voltammogram of urine sample 1 of nonsmoker healthy volunteer in resting stage (used as control) clearly shows three well separated peaks at ~80, ~150 and ~214 mV as shown in (solid line curve) Fig. 5. The two well-separated peaks at ~150 and ~214 mV correspond to the oxidation of epinephrine and norepinephrine respectively. This was further confirmed by the standard addition method. Third peak at ~80 mV was found to be due to the oxidation of dopamine (DP) which was also confirmed by standard addition method although; no attempt was made to determine its actual concentration. Further, square wave voltammogram of urine sample of smokers was recorded and three peaks exactly at the same potentials as that observed in the case of control urine were observed. However, the peak height of all the three peaks increased significantly as shown in (dashed line curve) Fig. 5. The actual concentrations of EP and NE were detected by using regression equation and in control subjects were found to be 65×10^{-9} and 12×10^{-8} M respectively, whereas, the concentrations of EP and NE in urine of smokers were found to be 105×10^{-9} and 18×10^{-8} M, respectively.

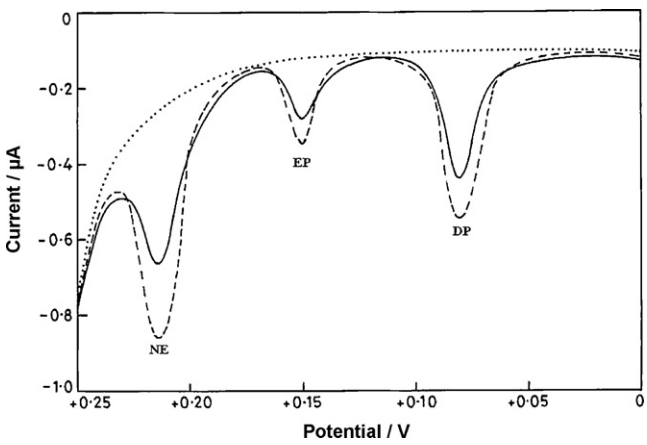


Fig. 5. Square wave voltammograms of (i) phosphate buffer solution (. . .), (ii) urine sample of healthy nonsmoker as control (—), and (iii) urine sample of healthy smoker (- - -) at pH 7.2 using MWNT/EPPGE.

3.6.2. Simultaneous determination of epinephrine and norepinephrine in blood plasma

In the case of neurotransmitters, analysis of only urine sample has been considered not a sufficient indicator of CNS activity [16]. Therefore, considering the potential role of plasma catecholamines as a readily available measure of sympathoadrenal system activity the analysis of plasma samples is recommended [17]. Hence, the developed protocol has also been implemented for the determination of EP and NE level in blood plasma samples. For this purpose, square wave voltammograms of plasma samples of two smokers in resting stage and two athletes at peak exercise stage were recorded. These samples were then spiked with known concentrations of standard epinephrine and norepinephrine and voltammograms were recorded. The actual concentrations of EP and NE in plasma samples of smokers and athletes were determined by using standard addition method and results obtained are tabulated in Table 1. The results show that recovery data are in the range of 97.14–104.08% and relative standard deviation (RSD) is less than ±3.2% hence, it can be concluded that proposed voltammetric sensor can be safely used for the simultaneous determination of EP and NE in human body fluids with excellent selectivity and sensitivity.

4. Conclusions

A novel approach using MWNT/EPPGE as a voltammetric sensor for the assay of catecholamines – epinephrine and norepinephrine in body fluids of normal subjects, athletes and smokers – is described. The modified electrode displayed strong catalytic function for the oxidation of EP and NE and resolved their overlapped

voltammetric response into two well-separated voltammetric peaks. EP and NE, both involve catechol redox centre and difference of ~60 mV at modified electrode clearly indicates that the side chain plays an important role. Thus, protonation of primary amino group in NE and secondary amino group in EP at pH 7.2 seems to play a significant role. The proposed voltammetric method offers several advantages over the methods described previously for the determination of EP and NE [32–34], such as fast response, high sensitivity, low detection limit, adequate stability, and determination of catecholamines in the presence of high concentrations of uric acid and ascorbic acid. Moreover, proposed voltammetric method can be used without tedious and time consuming sample preparation, derivatization and extraction steps which are essentially required for conventional methods [32–34]. Owing to its adequate stability, selectivity and sensitivity of the modified electrode could provide a promising tool for the simultaneous determination of EP and NE in complex biological samples. The developed method is also cost effective and fast tool for detecting various metabolic disorders and malfunctioning of various systems in human body.

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References

[1] F. Lechin, B. van der Dijs, A.E. Lechin, J. Appl. Res. 5 (2005) 605–621.
[2] B.S. McEwen, Metabolism 52 (2003) 10–16.
[3] M. Mazloum-Ardakani, H. Beitollahi, B. Ganjipour, H. Naeimi, Int. J. Electrochem. Sci. 5 (2010) 531–546.
[4] W.G. Meijer, S.C.V.M. Copray, H. Hollema, I.P. Kema, N. Zwart, I. Mantingh-Otter, T.P. Links, P.H.B. Willemse, E.G.E. de Vries, Clin. Chem. 49 (2003) 586–593.
[5] J.D. Fernstrom, M.H.J. Fernstrom, Nutrition 137 (2007) 1539S–1547S.
[6] D.L. Wong, Cell. Mol. Neurobiol. 26 (2006) 891–900.
[7] M.E. Gibbs, R.J. Summers, Prog. Neurobiol. 67 (2002) 345–391.
[8] F.C. Moraes, D.L.C. Golinelli, L.H. Mascaro, S.A.S. Machado, Sens. Actuators B 148 (2010) 492–497.
[9] T. Luczak, Electroanalysis 20 (2008) 1317–1322.
[10] C. Bian, Q. Zeng, H. Xiong, X. Zhang, S. Wang, Bioelectrochemistry 79 (2010) 1–5.
[11] J. Park, B.M. Kile, R.M. Wightman, Eur. J. Neurosci. 30 (2009) 2121–2133.
[12] H. Beitollahi, H. Karimi-Maleh, H. Khabazzadeh, Anal. Chem. 80 (2008) 9848–9851.
[13] S.-M. Chen, K.-T. Peng, J. Electroanal. Chem. 547 (2003) 179–189.
[14] J.H. Mendelson, M.B. Sholar, N. Goletiani, A.J. Siegel, N.K. Mello, Neuropsychopharmacology 30 (2005) 1751–1763.
[15] T. Tsuchida, N. Fukuma, K. Oikawa, K. Kato, Y. Kato, T. Takano, S.J. Kumita, Nippon Med. Sch. 74 (2007) 114–122.
[16] J. Ailts, D. Ailts, M. Bull, Urinary Neurotransmitter Testing: Myths and Misconceptions©, NeuroScience, Inc., 2007.
[17] M. Sofuoglu, D. Nelson, D.A. Babb, D.K. Hatsukami, Pharmacol. Biochem. Behav. 68 (2001) 455–459.
[18] G. Eisenhofer, T.T. Huynh, M. Hiroi, K. Pacak, Rev. Endocrinol. Metab. Disord. 2 (2001) 297–311.
[19] A. Bindoli, M.P. Rigobello, D.J. Deeble, Free Radical Biol. Med. 13 (1992) 391–405.
[20] A.M. Sawka, R. Jaeschke, R.J. Singh, W.F.J. Young Jr., Clin. Endocrinol. Metab. 88 (2003) 553–558.
[21] S.F. Morrison, W.-H. Cao, Am. J. Physiol. Reg. I 279 (2000) 1763–1775.
[22] Substance Classification Booklet, CCES, 2010, www.cces.ca/pdfs/CCES-PUB-SubstanceClassification-E.pdf.
[23] C. Hu, S. Hu, J. Sens. (2009) 1–40 (Article ID 187615).
[24] R.N. Goyal, S. Bishnoi, Biosens. Bioelectron. 26 (2010) 463–469.
[25] M. Ghalkhani, S. Shahrokhian, F. Ghorbani-Bidkorbeh, Talanta 80 (2009) 31–38.
[26] R. de, C.S. Luz, F.S. Damos, A.A. Tanaka, L.T. Kubota, Y. Gushikem, Electrochim. Acta 53 (2008) 4706–4714.
[27] R.N. Goyal, S. Chatterjee, A.R.S. Rana, Carbon 48 (2010) 4136–4144.
[28] G.D. Christian, W.C. Purdy, J. Electroanal. Chem. 3 (1962) 363–373.
[29] R.N. Goyal, S. Chatterjee, B. Agrawal, Sens. Actuators B 145 (2010) 743–748.
[30] J. Gong, X. Lin, Microchem. J. 75 (2003) 51–57.
[31] J.-M. Zen, J.-S. Tang, Anal. Chem. 67 (1995) 1892–1895.
[32] C. Ji, J. Walton, Y. Su, M. Tella, Anal. Chim. Acta 670 (2010) 84–91.
[33] W.-Y. Pyo, C.-H. Jo, S.-W. Myung, Chromatographia 64 (2006) 731–737.
[34] M. Suzuki, M. Mizoguchi, F. Yano, U. Hara, M. Yokoyama, N.Z. Watanabe, Naturforsch 58 c (2003) 220–224.