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Carbon Nanotube Paste Electrode for the Determination of Some Neurotransmitters: A Cyclic Voltammetric Study

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Abstract

A promising electrochemical sensor was developed by electro-polymerization of arginine on carbon nanotube paste electrode for the resolution of Dopamine in the presence of Uric acid and Ascorbic acid. The modified electrode shows excellent electrochemical catalytic activity towards the oxidation of dopamine. Cyclic voltammetry studies show the oxidation of dopamine at the poly-arginine modified carbon nanotube paste electrode surface is two electron Quasi-reversible process, diffusion-controlled process and showed improved current response compare to the bare carbon nanotube paste electrode. Under optimized conditions, the concentration range 8×10^{-6} to 5×10^{-5} M and 6×10^{-5} to 2×10^{-4} M and detection limit 10×10^{-7} M was observed. The sensor has also applied to the resolution of dopamine in real sample with a satisfactory recovery.

Keywords Ascorbic acid; Cyclic voltammetry; Dopamine; Electrochemical sensor; Uric acid

Introduction

Dopamine (DA) is an important neurotransmitter in mammalian central nervous system and plays important role in the functioning of central nervous, renal, hormonal and cardiovascular system. High concentration of DA is found in a particular region of brain called caudate nucleus (50 nmol/g) and very low concentration of DA is found in extracellular fluids (0.01-1mM). Excess level of DA leads to euphoria. Low level of DA may result in many neurological disorders, such as Parkinson's disease [1], Alzheimer's disease [2] and schizophrenia [3]. Uric acid (UA) is the primary product of purine metabolism. Abnormal concentration of UA in human blood serum and urine leads to various diseases such as gout, Leukemia, Pneumonia, hyperuricemia etc. [4,5]. Ascorbic acid (AA) is a water soluble vitamin present in many biological systems and food stuffs like vegetable and fruits. It acts as a powerful antioxidant against free radical induced diseases. Excess of AA can leads to gastric irritation and the metabolic product of vitamin (oxalic acid) can cause renal problems [6]. DA, UA and AA are oxidized at nearly the identical potential, which result in an overlapped voltammetric response. Therefore it is essential to develop simple and rapid method for the detection of these compounds not only for diagnostic studies but also for pathological research. Many analytical methods are used for the resolution of DA like spectroscopy [7], chromatography [8] etc. Since DA is an oxidisable compound, it can be easily determine by electro-analytical method because it provide simple, cost effective and quick way of analyzing biologically important molecules have been more of interest in recent years and was widely reported [9-15].

Now a day's amino acid modified electrode was widely used in the field of biosensor due to various advantages like biocompatibility, stability, easily availability [16,17]. CNTs are one of the most attractive nano-materials in nanotechnology. Whole research areas have attracted maximum attention to CNTs to biosensors application [18-25] due to their outstanding properties Among the researchers polymer modified electrodes [26-28] have obtained important attention in the field of for sensors and biosensors because Polymer film shows good stability and reproducibility.

The purpose of this study is to develop a stable, sensitive electrochemical sensor based on arginine and carbon nanotube for the resolution of DA in presence of UA and AA. The electrochemical behavior of these compounds was investigated using CV and DPV. The detection of DA injection is also demonstrated. In addition to that the stability and reproducibility were determined. Oxidation mechanism of DA, UA and AA were shown in Figure 1 [29].
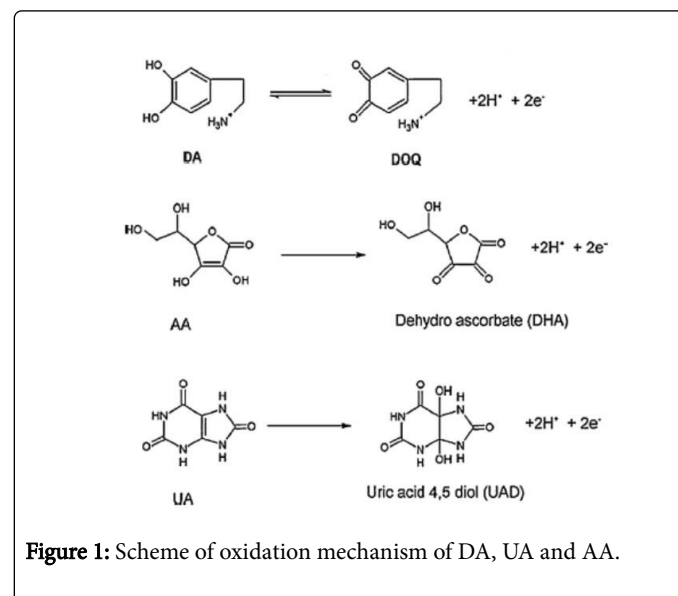

Figure 1: Scheme of oxidation mechanism of DA, UA and AA.

Experimental Methods

Apparatus

Electroanalyser (EA-201, Chemilink system, Mumbai, India) was used to obtain the cyclic voltammograms (CVs) and Differential pulse voltammograms (DPVs) with a three-electrode consists of Platinum wire, saturated calomel electrode (SCE), Bare carbon nanotubepaste electrode (BCNTPE) and Polyarginine modified carbon nanotube paste electrode (PAMCNTPE) as auxiliary electrode, reference electrode and working electrode, a computer for data storage and processing. All the experiments were achieved at room temperature. Field emission scanning electron microscopy (FESEM) was obtained using the instrument operating at 5.00 kV (DST-PURSE Laboratory, Mangalore University).

Materials

Multi walled carbon nano tube (MWCNT) was acquired from Sisco Research Laboratories Pvt. Ltd. Mumbai, Maharasthra (OD: 30-50 nm and Length: 10-30 μm). Silicone oil, sodium hydroxide, uric acid were obtained from Nice Chemicals Pvt. Ltd. Cochin. DA was purchased from Sigma Aldrich. Arginine was obtained from Molychem, Dihydrogen sodium phosphate and Disodium hydrogen phosphate was obtained from Merck 0.2 M phosphate buffer solution (PBS) used as a supporting electrolyte and was prepared by mixing appropriate amount of monosodium and disodium hydrogen phosphate solution. A stock solution of arginine (25×10^{-3} M) prepared by dissolving in distilled water, DA was prepared as 25×10^{-4} M by dissolving in distilled water, UA (25×10^{-4} M) prepared by dissolving in 0.1 M sodium hydroxide and AA was prepared as 25×10^{-3} M by dissolving in distilled water.

Preparation of BCNTPE/PAMCNTPE

BCNTPE was prepared by mixing 60: 40 (w/w%) carbon nanotube and silicone oil for 20 minutes in a mortar and pestle until to get a homogeneous paste. The paste was then packed into the cavity of Teflon tube (diameter 3 mm), was contacted by copper wire and smoothed out on a tissue paper. Thus BCNTPE was obtained. The BCNTPE was placed in 0.2 M PBS at pH 5.6 containing 1×10^{-3} M arginine solution. The poly (arginine) film was prepared by repetitive potential cycling between -200 to 1500 mV. After polymerization the electrode was washed thoroughly in order to remove the unreacted arginine monomer. The cyclic voltammogram was shown in Figure 2.
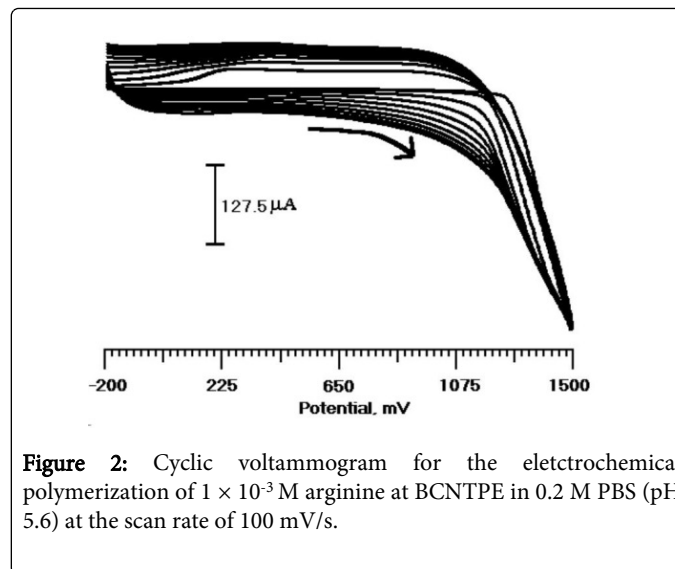

Figure 2: Cyclic voltammogram for the eletctrochemical polymerization of 1×10^{-3} M arginine at BCNTPE in 0.2 M PBS (pH 5.6) at the scan rate of 100 mV/s.

Results and Discussion

Characterization

Figure 3 shows the surface morphology of CNT (Figure 3A), BCNTPE (Figure 3B) and PAMCNTPE (Figure 3C).

It was observed that there was irregularly shaped nano-sized tubes of carbon nanotube at BCNTPE. However, the poly (arginine) film coated carbon nanotube has uniform arrangement of poly (arginine) film on the surface of BCNTPE. This indicates that the surface of BCNTPE was covered by poly (arginine) film.
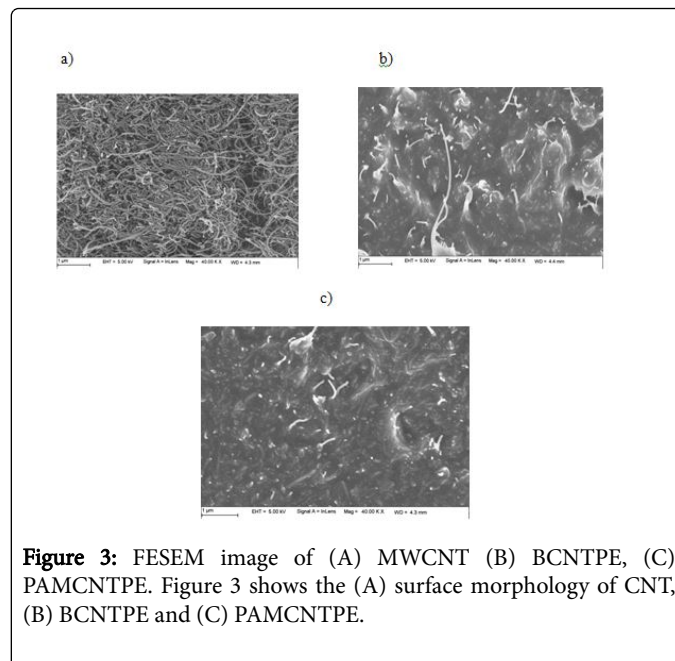

Figure 3: FESEM image of (A) MWCNT (B) BCNTPE, (C) PAMCNTPE. Figure 3 shows the (A) surface morphology of CNT, (B) BCNTPE and (C) PAMCNTPE.

Electrochemistry of potassium ferrocyanide at PAMCNTPE

Figure 4 shows the CVs of BCNTPE (solid line) and PAMCNTPE (dashed line) containing 1 mM $K_2Fe(CN)_6$ and 1 M KCl as a

supporting electrolyte at the scan rate of 100 mV/s. A pair of well-defined redox peak was observed at the surface of BCNTPE with a peak separation of 65 mV, on the other side, PAMCNTPE shows a good intense peak with peak separation of 35 mV which is smaller compared to bare electrode. It can be seen that compared to BCNTPE peak current at PAMCNTPE increased 5 time higher than those at BCNTPE. It is clear that due to the increased peak current at modified electrode which possesses the good electro-catalytic activity. In other word there is an improvement in good electron transfer kinetics and large surface area of modified electrode.
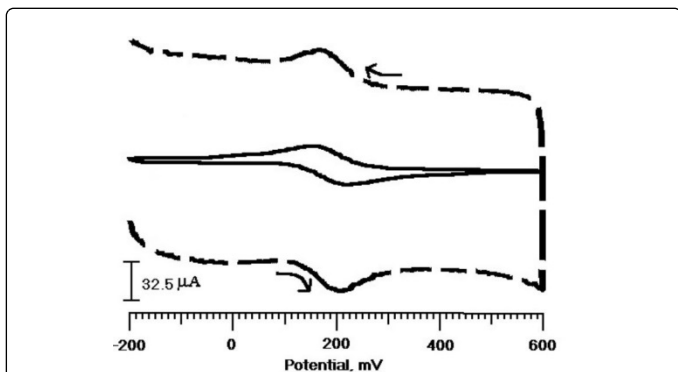

Figure 4: Cyclic voltammogram of 1 mM Potassium ferrocyanide/1 M KCl solution at BCNTPE (solid line) and PAMCNTPE (dashed line).

Electrochemical response of DA at the PAMCNTPE

Figure 5 show the CVs of blank solution (only PBS, pH 6.5). It was observed that there is no redox peak appears for blank solution. When 1×10^{-4} M DA was added to the solution, a well -defined redox peak was appeared at a potential of 181 mV (E_{pa}) and 113 mV (E_{pc}). It is due to the electrostatic interaction between positively charged modifier and DA and significantly increased the peak current and gives a clear evidence of electro-catalytic effect.
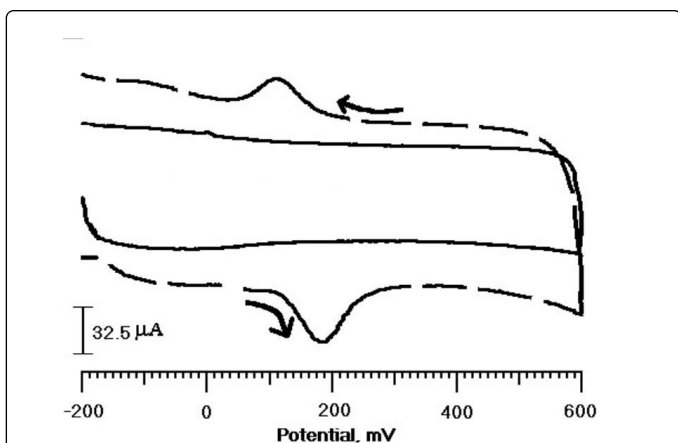

Figure 5: Cyclic voltammogram of DA at PAMCNTPE with (dashed line) and without (solid line) DA (1×10^{-4} M) in 0.2 M PBS, pH 6.5.

Figure 6 shows the CVs of DA (1×10^{-4}) in 0.2 M PBS of pH 6.5 BCNTPE (solid line) and PAMCNTPE (dashed line) with the scan rate of 100 mV/s. Comparing the peak current at modified electrode are much higher than those at bare electrode. It means the electrochemical oxidation of DA can be improved at PAMCNTPE.
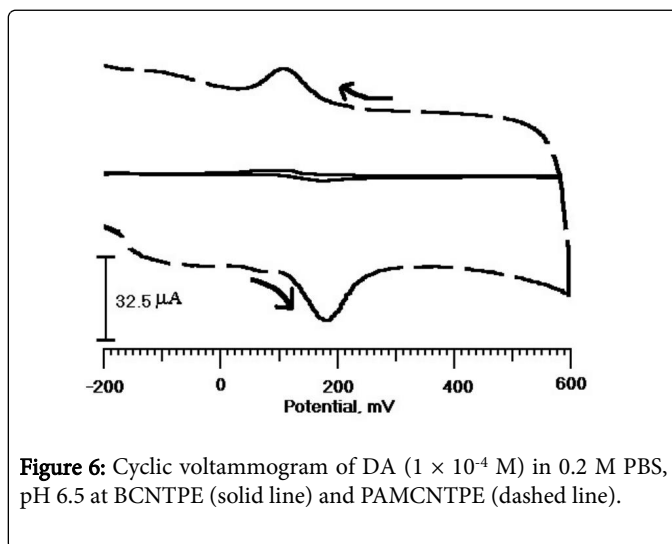

Figure 6: Cyclic voltammogram of DA (1×10^{-4} M) in 0.2 M PBS, pH 6.5 at BCNTPE (solid line) and PAMCNTPE (dashed line).

Effect of pH

Figure 7A shows the effect of pH on the resolution of DA at PAMCNTPE in the pH range 6.0 to 8.0. It was seen that the maximum peak current was obtained at pH 6.5 (Figure 7B). To get higher sensitivity and selectivity pH 6.5 was selected as optimum pH for the resolution of DA. Peak potential was moved to less positive side with higher buffer pH, indicating that the proton take part in the electrode reaction and obey the following equation $E_{pa}=625-67.8$ pH with correlation coefficient of 0.978 (Figure 7C). The slope of this equation was found to be -67.8 mV/pH. The obtained slope is very close to the expected theoretical value of -59 mV/pH; indicate that the number of electrons transferred is equal to the number of hydrogen ions taking part in the electrode reaction [30].
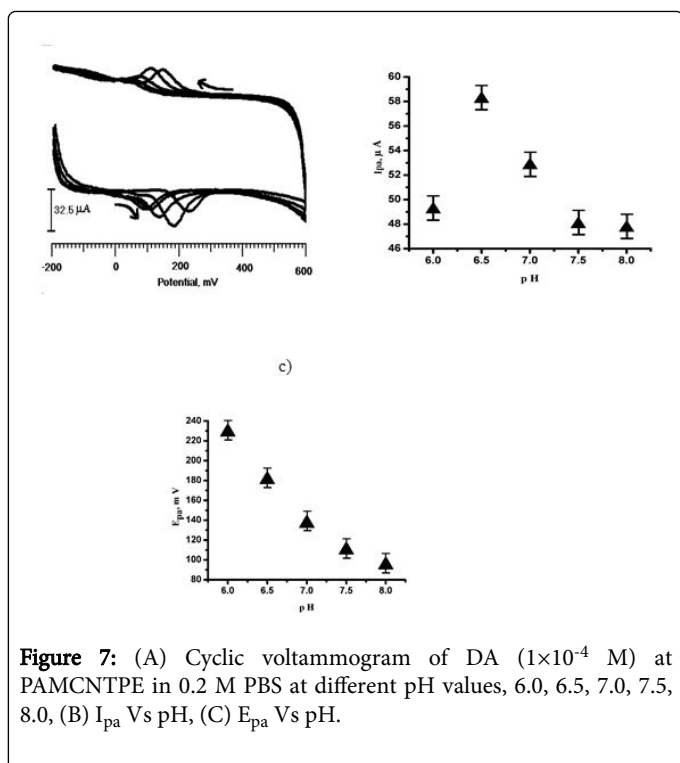
Figure 7: (A) Cyclic voltammogram of DA (1×10^{-4} M) at PAMCNTPE in 0.2 M PBS at different pH values, 6.0, 6.5, 7.0, 7.5, 8.0, (B) I_{pa} Vs pH, (C) E_{pa} Vs pH.

The effect of scan rate

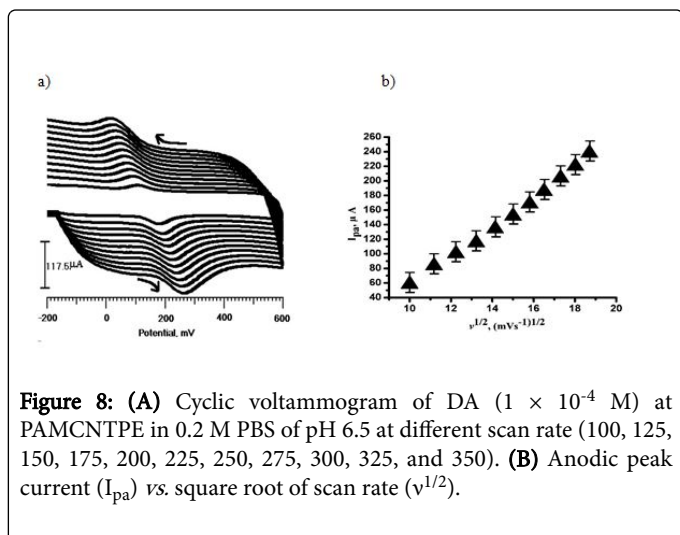Figure 8A shows the effect of scan rate on the electro oxidation of DA at PAMCNTPE in 0.2 M PBS (pH 6.5) by CV.

Figure 8: (A) Cyclic voltammogram of DA (1×10^{-4} M) at PAMCNTPE in 0.2 M PBS of pH 6.5 at different scan rate (100, 125, 150, 175, 200, 225, 250, 275, 300, 325, and 350). **(B)** Anodic peak current (I_{pa}) *vs.* square root of scan rate ($v^{1/2}$).

Peak current of DA shows a linear relationship with square root of scan rate in the range of 100-350 mV/s with the regression equation of I_{pa} (μA) = -49.15+20.35 $v^{1/2}$ (mV/s)$^{1/2}$ with the correlation coefficient of 0.99. (B) which is typical of diffusion controlled process [31]. Also observed that the oxidation peak potential is increased to more positive side with the increase of scan rate. The ratio of redox peak current (I_{pa}/I_{pc}) was 1.387 which were the feature of quasi-reversible electrode process [32].

Number of electrons transferred (Equation 1), charge transfer coefficient (Equation 2) and electron transfer rate constant (Equation 3) were calculated by using the following equation,

$$E_{pc} = E^0 - \frac{RT}{\alpha nF}\ln v \cdots (1)$$

$$E_{pa} = E^0 + \frac{RT}{(1-\alpha)nF}\ln v \cdots (2)$$

$$\log K_s = \alpha\log(1-\alpha) + (1-\alpha)\log\frac{RT}{nFv} - (1-\alpha)\frac{\alpha F \Delta E_p}{2.3RT} \cdots 3$$

Where α is the charge transfer coefficient, n is the number of electrons transferred, Ks is the electron transfer rate constant, E_0 is the formal potential, F is the faraday constant, Epc is the cathodic peak potential, Epa is the anodic peak potential. It was observed that charge transfer coefficient, number of electron transferred and electron transfer rate constant are in the value of 0.81, 2.23 ~ =1 and 1.66 S^{-1}.

Linear range and limit of detection of DA

The relationship between the peak current and the concentration of DA was examined by CV on the surface of PAMCNTPE. Figure 9 shows the calibration curve for DA in 0.2 M PBS, pH 6.5. With increasing the concentration of DA peak current increased linearly, using the optimum condition described above, linear calibration curve was obtained in the range 8×10^{-6} to 5×10^{-5} and 6×10^{-5} to 2×10^{-4} M with the linear equation of I_{pa} (A)=3.83×10^{-5}+0.20 C (M) with the correlation coefficient of 0.99824. Limit of detection (LOD) and limit of quantification (LOQ) were calculated using the following equation, LOD=3S/N and LOQ=10S/N, where S is the standard deviation of blank solution (Five runs), N is the slope of the calibration curve and were acquired as 10×10^{-7} M and 3.3×10^{-6} M [33-35]. The detection limit comparison with some other modified electrodes was tabulated in Table 1 [36-40].

Electrode	Method	Linear range (M)	Detection limit (M)	Reference
CILE	DPV	2.0×10^{-6} to 1.5×10^{-3}	1×10^{-6}	[36]
SDSMMWCNTPE	CV	1×10^{-6} to 2.8×10^{-5}	3.3×10^{-7}	[37]
Cu2O/ Graphene/GCE	CV	1×10^{-7} to 10×10^{-6}	10×10^{-8}	[38]
Au-CoP	DPV	10×10^{-6} to 50×10^{-6}	7.5×10^{-7}	[39]
SZP/MB composite electrode	DPV	6×10^{-6} to 100×10^{-6}	1.7×10^{-6}	[40]
PAMCNTPE	CV	8×10^{-6} to 5×10^{-5} and 6×10-5 to 2×10^{-4}	10×10^{-7}	This work

Table 1: Comparison of PAMCNTPE with some other modified electrode for the resolution of dopamine. CILE- Carbon Ionic Liquid Electrode, SDSMMWCNTPE- sodium dodecyl sulfate modified multiwalled carbon nanotube paste electrode, Cu2O/Graphene/GCE- Copper oxide/graphene/ glassy carbon electrode, Au-CoP - Gold-cobalt (II)-porphyrin, SZP/MB composite electrode- Zirconia-silica composite electrode/Methylene Blue.
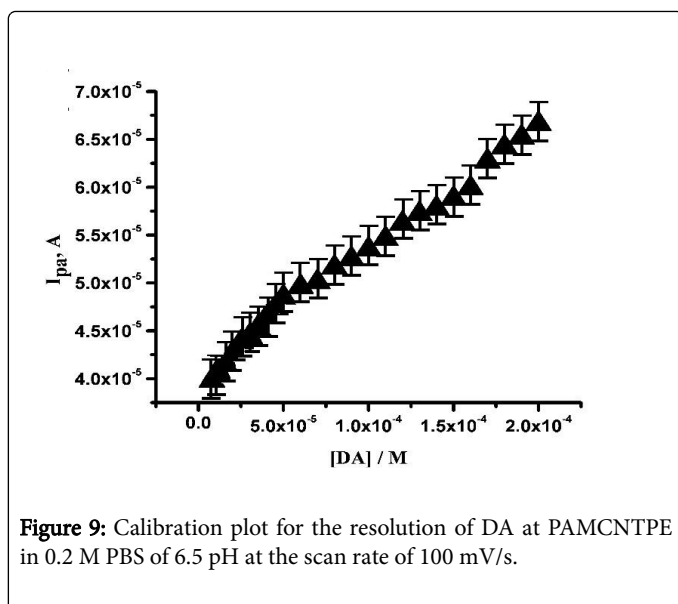
Figure 9: Calibration plot for the resolution of DA at PAMCNTPE in 0.2 M PBS of 6.5 pH at the scan rate of 100 mV/s.

Electrochemical behaviour of UA at PAMCNTPE

Figure 10A depicts the cyclic voltammetric response from the electrochemical oxidation of 1×10^{-4} M UA at BCNTPE (solid line) and PAMCNTPE (dashed line). It can be seen that anodic peak potential for the oxidation of UA is about 290 mV (BCNTPE), while PAMCNTPE peak current was observed at the peal potential of 305 mV. From these result it was concluded that the best electro catalytic effect for UA at modified electrode. Under the similar conditions, the PAMCNTPE produce significantly increased peak current and gives a clear evidence of the strong electro catalytic effect. In order to investigate the kinetic of electrode reaction, the experiment was carried for different scan rate and the CVs of 1×10^{-4} M UA at the PAMCNTPE was shown in Figure 10B with increase of the scan rate peak current also increased gradually, shows direct electron transfer between UA and modified electrode surface. (C) Anodic peak current (I_{pa}) *vs.* square root of scan rate ($v^{1/2}$). The peak current were proportional to the square root of scan rate in the range of 100 to 250 mV/s with a linear regression equation of I_{pa} (μA)$=-114.42+16.41\ v^{1/2}$ $(mV/s)^{1/2}$ (Figure 10C) with the correlation coefficient of 0.995, which indicates that the electrode reaction is diffusion controlled.

For irreversible electrode process E_p was given by the following equation,

$$Ep(V) = E^0 + \frac{RT}{\alpha nF}\ln\frac{RTK_s}{\alpha nF} + \ln v ...(4)$$ Where, α is the transfer coefficient, Ks is the standard heterogeneous rate constant, n is the number of electron transferred, v is the scan rate and E° is the formal standard potential. From the slope of Ep vs. lnv, the value of αn can be calculated. αn was calculated to be 0.955. For irreversible electrode process α value was assumed to be 0.5. Therefore the number of electrons was calculated and obtained as 1.91~ =2. Hence it is assumed to UA undergo two proton and two electron transfer in the electrode reaction (Figures 10A-10C).
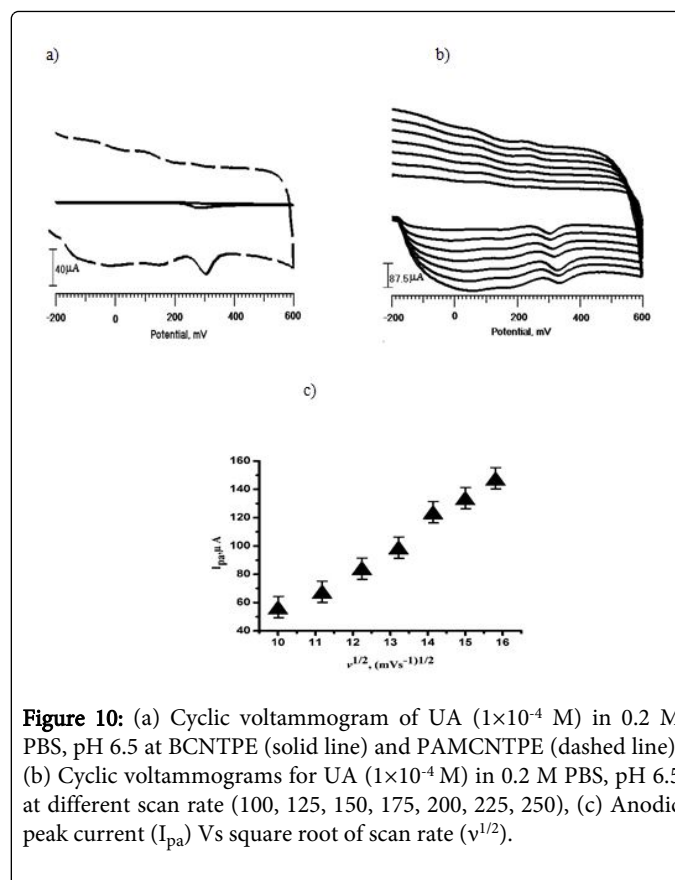

Figure 10: (a) Cyclic voltammogram of UA (1×10^{-4} M) in 0.2 M PBS, pH 6.5 at BCNTPE (solid line) and PAMCNTPE (dashed line), (b) Cyclic voltammograms for UA (1×10^{-4} M) in 0.2 M PBS, pH 6.5 at different scan rate (100, 125, 150, 175, 200, 225, 250), (c) Anodic peak current (I_{pa}) Vs square root of scan rate ($v^{1/2}$).

Sample	Added (µM)	Found (µM)	Recovery (%)
1	84.3	83.1 2 ± 0.65	98.6
2	168.7	171.36 ± 0.8	101.5
3	253	259.7 ± 1.4	102.6

Table 2: Recovery tests of DA in pharmaceutical sample using PAMCNTPE. ± represents the standard deviation.

Electrochemical oxidation of AA at PAMCNTPE

Figure 11A shows the CVs of 2×10^{-3} M AA on BCNTPE and PAMCNTPE in 0.2 M PBS (pH 6.5) at the scan rate of 100 mV/s. There is no oxidation peak was observed for BCNTPE. However, at PAMCNTPE peak current was observed at -71 mV, shows that the good electrocatalytic effect at modified electrode. (B) Cyclic voltammograms for AA (3×10^{-3} M) in 0.2 M PBS, pH 6.5 at different scan rate (100, 125, 150, 175, and 200). (C) Anodic peak current (Ipa) *vs.* square root of scan rate ($v^{1/2}$). 11C shows the CVs of AA at different scan rates, it was observed that with the increase of scan rate peak current also increased and observed a linear relationship between peak current and square root of scan rate in the range of 100 to 200 mV/s with a linear regression equation of $-114.76+18.03\ v^{1/2}(mV/s)^{1/2}$ (11C) with a correlation coefficient of 0.987. This reveals that the oxidation of AA at modified electrode is a diffusion controlled process.
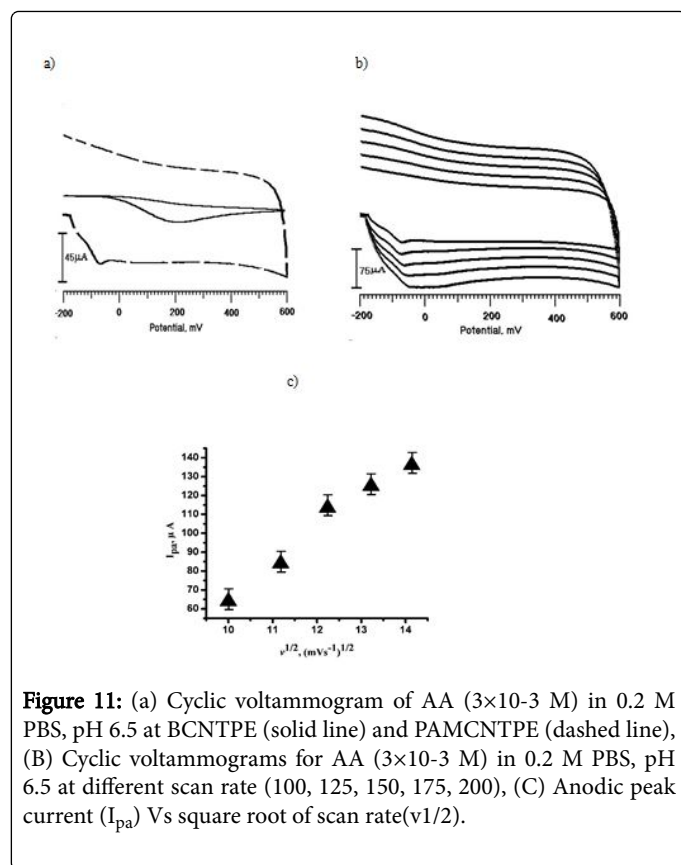
Figure 11: (a) Cyclic voltammogram of AA (3×10-3 M) in 0.2 M PBS, pH 6.5 at BCNTPE (solid line) and PAMCNTPE (dashed line), (B) Cyclic voltammograms for AA (3×10-3 M) in 0.2 M PBS, pH 6.5 at different scan rate (100, 125, 150, 175, 200), (C) Anodic peak current (I_{pa}) Vs square root of scan rate($v1/2$).

Voltammetric behavior of DA at PAMCNTPE by DPV

DPV will give better sensitivity compared to the CV. Figure 12 shows the DPVs of DA at BCNTPE and PAMCNTPE in 0.2 M PBS (6.5 pH) with the scan rate of 50 mV/s, Pulse width 60 msec and pulse amplitude of 20 mV. At PAMCNTPE the peak current was observed at the peak potential of 153 mV with a high current sensitivity compared to the BCNTPE, Showing that the strong electro-catalytic effect at PAMCNTPE compared to the BCNTPE.
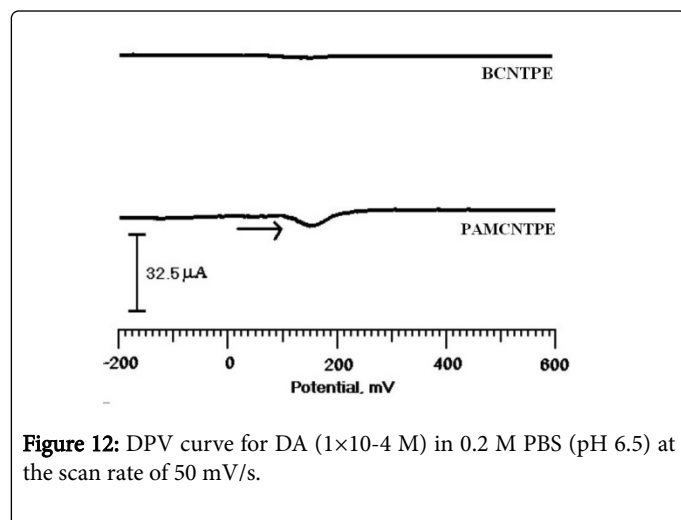

Figure 12: DPV curve for DA (1×10-4 M) in 0.2 M PBS (pH 6.5) at the scan rate of 50 mV/s.

Simultaneous resolutions of DA, UA and AA at PAMCNTPE by CV

DA, UA and AA coexist in the extracellular fluid of central nervous system and their oxidative potential are very close to each other. The main aim of this present study is the simultaneous resolution of DA, UA and AA. Figure 13A shows the CVs of DA (1×10^{-4} M), UA (1×10^{-4} M), and AA (2×10^{-4} M) in 0.2 M PBS (pH 6.5) at the scan rate of 100 mV/s. It was observed that a well-defined three oxidation peaks were acquired at -84 mV, 198 mV, 355 mV for AA, DA and UA. There for PAMCNTPE was effectively used to separate DA in the presence of UA and AA.

The electro-activity of DA, UA and AA in the mixture when the concentration of one species changed and those other two species was kept constant was also studied. (B) shows the CVs of DA (1×10^{-4} M to 1.5×10^{-4} M) by keeping the concentration of UA (1×10^{-4} M) in 0.2 M PBS (6.5 pH) same. It was noticed that with increase of concentration peak current also increased without change in the UA peak current and a linear relationship was observed with regression equation of I_{pa} (μA)=35.77+55.88 C_{DA} (M) with a correlation coefficient of 0.996 (C).
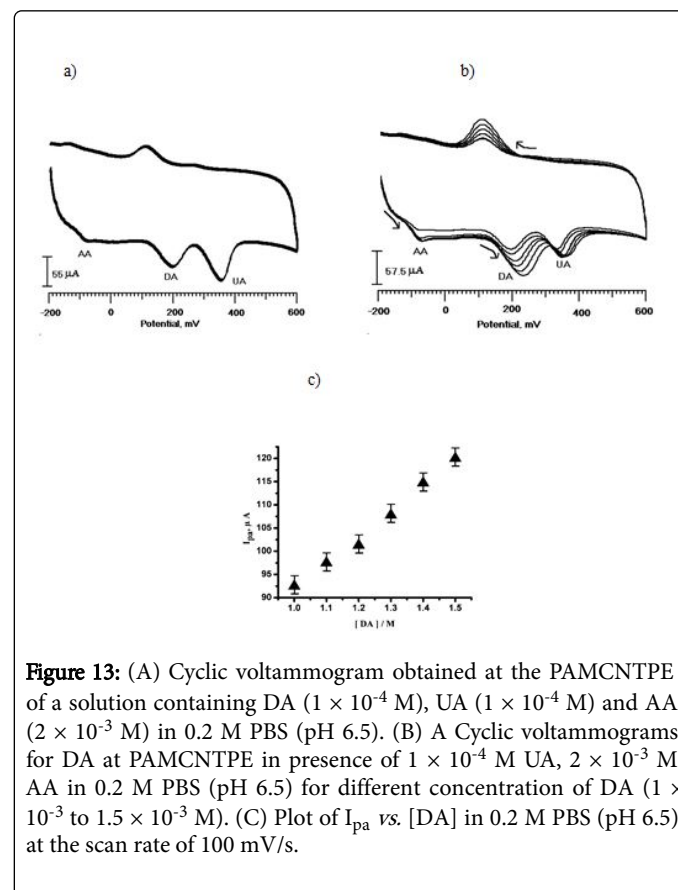

Figure 13: (A) Cyclic voltammogram obtained at the PAMCNTPE of a solution containing DA (1×10^{-4} M), UA (1×10^{-4} M) and AA (2×10^{-3} M) in 0.2 M PBS (pH 6.5). (B) A Cyclic voltammograms for DA at PAMCNTPE in presence of 1×10^{-4} M UA, 2×10^{-3} M AA in 0.2 M PBS (pH 6.5) for different concentration of DA (1×10^{-3} to 1.5×10^{-3} M). (C) Plot of I_{pa} *vs.* [DA] in 0.2 M PBS (pH 6.5), at the scan rate of 100 mV/s.

Stability, reproducibility

Stability and reproducibility of the modified electrode is carried out to estimate the sensing performance. Stability of the modified electrode was examined by detecting 1×10^{-4} M DA on 40 consecutive cycles. The developed sensor showed a good electrocatalytic response and retained 92% of initial current even after 40 cycles shows that the modified electrode having good stability. In the reproducibility, 5

independent developed electrode were used to detect DA, it was seen that the relative standard deviation of 0.54, showing that the modified electrode having good reproducibility.

Sample analysis

In order to study the practical application of DA at PAMCNTPE, DA injection was selected as a real sample for analysis and was collected from local medical store. The experiment was carried out by standard addition method. The result obtained is presented in Table 2. It was observed that the recovery in the range 98.6% to 102.6% for DA. Result shows that the prepared electrochemical sensor very effective for the resolution of DA in pharmaceutical sample with good recovery rate.

Conclusion

In this work, a voltammetric sensor was made using PA/CNT for the resolution of DA in presence of UA and AA. The method present is simple, sensitive and cost effective; moreover, it does not require sophisticate equipments. The electrochemical oxidation of DA is a quasi-reversible process and that is pH dependent. The modified electrode shows the effective sensor towards the investigation of DA in presence of UA and AA. Modified electrode showed a significant effect of redox peak current for DA and good electrochemical signal separation between UA and AA. Modified sensor shows a wide linear range from 8×10^{-6} to 5×10^{-5} M and 6×10^{-5} to 2×10^{-4} M and with a low detection limit (10×10^{-7} M) for the resolution of DA. Developed sensor shown to be useful for the resolution of DA in real sample.

Acknowledgement

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