Scientific paper

# Electrochemical Determination of Dopamine in the Presence of Ascorbic Acid Using a Poly(3-Acetylthiophene) Modified Glassy Carbon Electrode

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## Abstract

A glassy carbon electrode (GCE) was modified with electropolymerization of 3-acetylthiophene in acetonitrile using cyclic voltammetry. The modified electrode showed an excellent electrocatalytic effect on the oxidation of dopamine (DA). The poly(3-acetylthiophene) modified electrode also accelerated the rate of electron transfer reaction of DA. Compared with a bare GCE, the modified electrode exhibits a distinct shift of the oxidation potential of DA in the cathodic direction and a marked enhancement of the current response. The separation between anodic and cathodic peak potentials (ΔEp) for DA is 195 mV and 34 mV at bare and modified glassy carbon electrodes, respectively. The poly(3-acetylthiophene) modified GCE was used for the determination of dopamine (DA) in 0.1 M phosphate buffer solution (PBS) at pH 4.0. The peak current increases linearly with the concentration of DA in the range of $1.0 \cdot 10^{-6} \sim 1.0 \cdot 10^{-4}$ M. The detection limit was $3.8 \cdot 10^{-8}$ M by square wave voltammetry. The modified electrode has successfully been applied for the detection of DA in the presence ascorbic acid (AA). The proposed method also showed excellent stability and reproducibility.

**Keywords:** Chemically modified electrode, 3-acetylthiophene, dopamine, ascorbic acid, electropolymerization.

## 1. Introduction

Dopamine (DA), a naturally occurring catecholamine, is a neurotransmitter in the mammalian central nervous system. It has been reported that DA undergoes a protective reaction affecting the fast chemical repair of oxidative free-radical damage to DNA.[1] It is suggested that the fall of levels of DA caused Parkinson's disease in which DNA damage is not prevented.[1–3] However, ascorbic acid (AA) coexisted in the body fluids can be easily oxidized at a potential close to that of DA and always interfere with the detection of DA at the conventional electrodes. Therefore, many scientists have focused on the determination of DA in the presence of AA and to develop rapid and reproducible methods for the determination of DA.[4–8] To meet these needs, a number of analytical methods such as enzyme-based techniques,[9,10] adsorption/medium exchange,[11] electrochemically pre-treated electrodes,[12] and self-assembled monolayers[13,14] have been used to solve the interference from AA. Peak overlap in voltammetry poses challenges for the quantitative analysis of electroactive species.[4] DA and AA are typically challenging to determine voltammetrically because of their very similar oxidation peak potentials.[4] However, compared to other techniques, polymer film modified electrodes provide certain advantageous such as long term stability, sensitivity and homogeneity in electrochemical deposition.[5,15–18]

In this paper, we report voltammetric behaviour of DA at a bare and a poly(3-acetylthiophene) film modified GCE. Moreover, it has been shown that the oxidation potential of DA can be separated from than that of AA at the poly(3-acetylthiophene) modified glassy carbon electro-

de. The results showed that the modified electrode could be used to detect DA in presence of AA.

## 2. Experimental

### 2. 1. Chemicals

Dopamine obtained from Sigma (Germany) was used as received. 3-Acetylthiophene and ascorbic acid were purchased from Fluka (Germany). Solutions of 3-acetylthiophene was prepared in acetonitrile (Merck, Germany) containing $LiClO_4$ (Fluka, Germany) as supporting electrolyte. Dopmin and Dopamine Fresenius injections were purchased from the local pharmacy. Three blood serum samples were kindly provided from the Laboratory of Biochemistry of the State Hospital of Sanliurfa. All other reagents were of analytical grade or equivalent, and obtained from Merck or Fluka. Solutions of DA and AA were prepared in 0.1 M phosphate buffer solution (PBS) at pH 7.2 or pH 4.0. Aqueous solutions were prepared with doubly distilled water. Oxygen-free nitrogen was bubbled through the cell prior to each experiment. All experiments were carried out at *ca.* 25 °C.

### 2. 2. Apparatus

Electrochemical experiments were performed using an EcoChemie Autolab PGSTAT 12 potentiostat/galvanostat (Utrect, The Netherlands) with the electrochemical software package 4.9 or an Epsilon potentiostat (Bioanalytical Systems, Lafayette, USA) with the electrochemical software 1.6.70_XP. A three-electrode system was used: a bare or poly(3-acetylthiphene) modified glassy carbon electrode as working electrode [3 mm in diameter (Bioanalytical Systems, Lafayette, USA)], a Pt wire counter electrode and an Ag/AgCl reference electrode.

### 2. 3. Preparation of Modified Glassy Carbon Electrodes

Prior to electrochemical modification, the bare GCE was polished with 0.05 μm alumina slurry on a polishing pad. Then it was rinsed with water, and sonicated with 1 + 1 $HNO_3$ and acetone, and water for 10 min, respectively. After being cleaned, the electrode was activated by 5 cyclic sweepings from –0.6 to +0.8 V in PBS at pH 7.2. Then, the electrode was immersed in a solution of 10 mM 3-acetylthiophene dissolved in acetonitrile and containing 0.05 M $LiClO_4$ as the supporting electrolyte and was conditioned by cyclic sweepings from –1.5 to +1.8 V for 20 scans. Afterwards, the modified electrode was electroactivated by cyclic voltammetry from –0.6 to +0.8 V at 100 mV/s in 0.1 M PBS at pH 7.2. Then, the poly(3-acetylthiophene) modified electrode was rinsed with doubly distilled water to use for the determination of DA.

## 3. Results and Discussion

### 3. 1. Cyclic Voltammograms of DA at Bare and Poly(3-acetylthiophene) Modified Electrodes

Cyclic voltammograms of DA at bare GCE and poly(3-acetylthiophene) modified GCE in 0.1 M PBS at pH 7.2 are given in Fig. 1. The electrochemical response of DA has greatly been increased on the poly(3-acetylthiophene) GCE. At bare glassy carbon electrode, DA shows an oxidation peak at 0.301 V and a corresponding reduction peak at 0.106 V. The separation in peak potential (ΔEp) is about 195 mV. At the poly(3-acetylthiophene) modified GCE, the oxidation occurs at Epa = +0.183 V and reduction at Epc = +0.149 V with peak potential separation (ΔEp) of 34 mV. This has indicated that the poly(3-acetylthiophene) modified GCE has accelerated the electron transfer rate of DA. However, another reduction was observed at Epc = –0.253 V as the initial potential of scanning shifted negatively. Interestingly, an oxidation peak was also appeared at Epa = –0.216 V at second cycle. The electrochemical behaviour of DA at poly(3-acetylthiophene)/GCE might be represented as follows:[19–21] peak (1) results from the oxidation of DA, which is a two-electron transfer process to produce dopaminequinone (reaction 1). Peak (2) appears by the reduction of dopaminequinone to dopamine (reaction 1). Peak (3) corresponds to formation of leucodopaminechrome resulting from the ring closure of dopaminequinone which contains an electron-deficient ring (reaction 2). The resulting product, leucodopaminechrome, can undergo further a two-electron oxidation to produce dopachrome (peak 4) (reaction 3). The behaviour of DA at poly(3-acetylthiophene)/GCE is an electrochemical-chemical-electrochemical (ECE) process.[22] The proposed dopamine electrochemical reactions are given in Scheme 1.

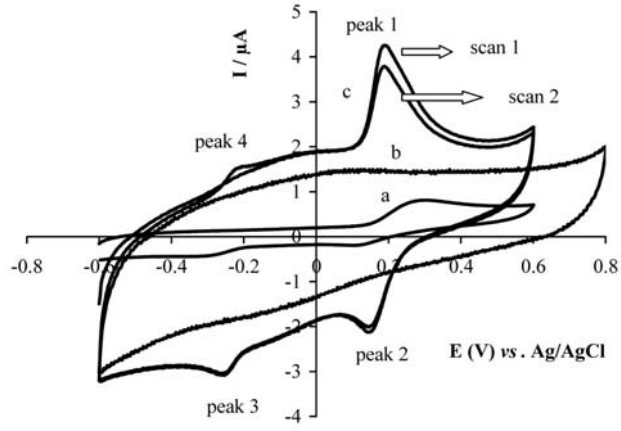
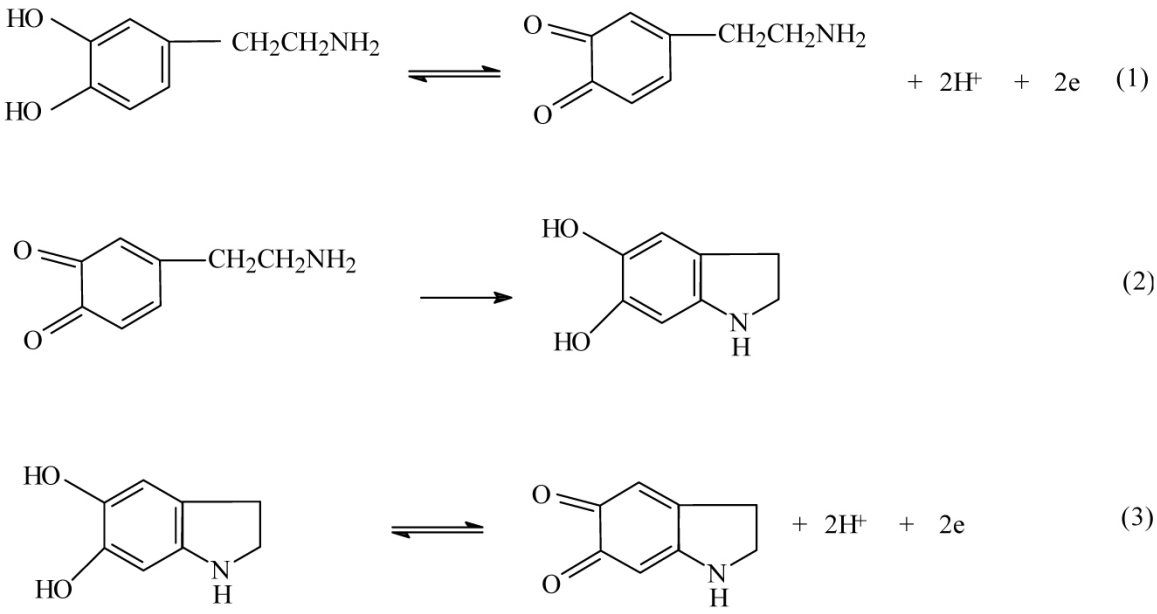



**Fig. 1.** Cyclic voltammograms of (a) $2.0 \cdot 10^{-5}$ M DA at bare GCE (b) poly(3-acetylthiophene)/GCE and (c) $2.0 \cdot 10^{-5}$ M DA at poly(3-acetylthiophene)/GCE. Supporting electrolyte: 0.1 M PBS at pH 7.2. Equilibrium time: 5 s, scan rate: 50 mV/s.




**Scheme 1.** Proposed DA reactions at poly(3-acetylthiophene)/GCE in 0.1 M PBS at pH 7.2.

Interestingly, only one redox couple was observed in 0.1 M PBS at pH 4.0 for DA at poly(3-acetylthiophene)/GCE as shown in Fig. 2. The oxidation of DA at poly(3-acetylthiophene)/GCE in 0.1 M PBS at pH 4.0 occurs at 0.376 V and a corresponding reduction peak at 0.340 V. The separation in peak potential (ΔEp) is about 36 mV. This reveals that DA undergoes only a reversible two-electron oxidation to form dopaminequinone at pH 4.0. The proposed DA reaction at pH 4.0 is given in Scheme 2.

However, electrochemical properties of DA at poly(3-acetylthiophene)/GCE were investigated in 0.1 M PBS at pH ranges between 4 and 10. It has been observed that the electrochemistry of DA at the modified GCE was electrochemical-chemical-electrochemical process except at pH 4.0.

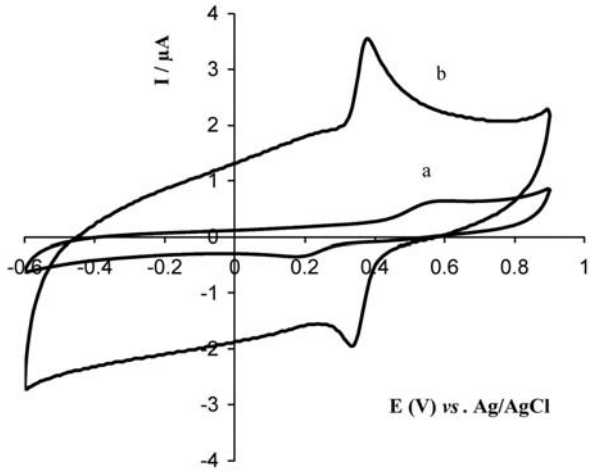
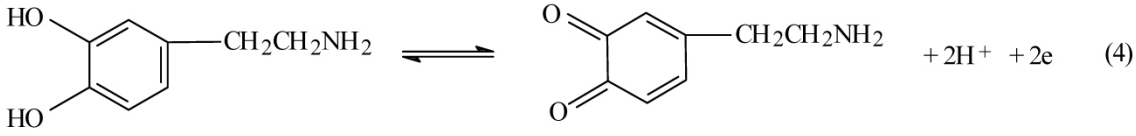



**Fig. 2.** Cyclic voltammograms of $1.5 \cdot 10^{-5}$ M DA at (a) bare GCE and (b) poly(3-acetylthiophene)/GCE in 0.1 M PBS at pH 4.0. Equilibrium time: 5 s, scan rate: 50 mV/s.

**Scheme 2.** Proposed DA reaction at poly(3-acetylthiophene)/GCE in 0.1 M PBS at pH 4.0.




## 3. 2. Influence of Scan Rate on the Peak Current and Peak Potential of DA

Fig. 3 shows the effect of the scan rate on the electrochemical response of DA at poly(3-acetylthiophene)/GCE using cyclic voltammetry in 0.1 M PBS at pH 4.0. The anodic peak current (I*pa*) was proportional to the scan rate (*v*) over the range of 50–250 mV/s. No shifts in the oxidation peak potential of DA were observed with increasing scan rate. The results indicated that the electrode process is controlled by adsorption of DA. Also, the slope of log I*pa vs.* log*v* is larger than 0.5 indicating that the process is adsorption controlled.

## 3. 3. Effect of pH on Peak Potential of DA

The influence of the pH value of the PBS buffer solution on peak potential of DA at poly(3-acetylthiophene)/GCE was also investigated. The relationship between oxidation peak potential of DA and pH value of PBS is given in Fig. 4. The oxidation peak potential of DA shifted in the negative direction with increasing pH. This shows that the redox couple of DA includes transfer of protons in the reduction and oxidation processes. The slope of Fig. 4 was about 58.1 mV/pH. This indicated that the proportion of the electron and proton involved in the reactions is 1 : 1. Since equal numbers of electrons and protons should be involved in the electrode reaction, the number of hydrogen ions involved in the whole electrode reaction is 2.

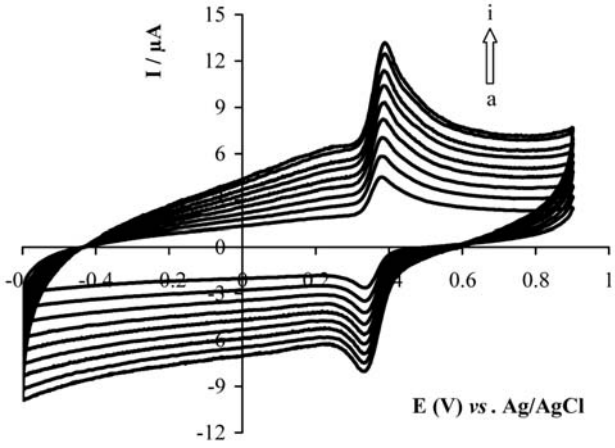



**Fig. 3.** Cyclic voltammograms of $2.5 \cdot 10^{-5}$ M DA at poly(3-acetylthiophene)/GCE in 0.1 M PBS at pH 4.0. Scan rates: (a) 50 mV/s (b) 75 mV/s (c) 100 mV/s (d) 125 mV/s (e) 150 mV/s (f) 175 mV/s (g) 200 mV/s (h) 225 mV/s (i) 250 mV/s. Equilibrium time: 5 s.

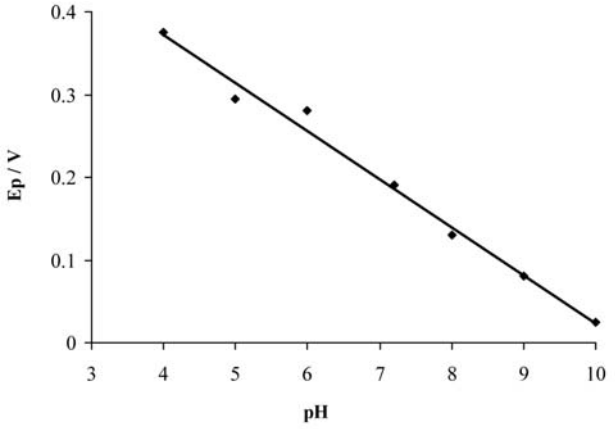



**Fig. 4.** A plot of oxidation peak potential of DA versus solution pH.

## 3. 4. Determination of DA

The determination of DA at poly(3-acetylthiophene)/GCE was performed using square wave voltammetry. SWVs of various concentrations of DA at poly(3-acetylthiophene) in 0.1 M PBS at pH 4.0 are shown in Fig. 5. The response of anodic peak currents of DA was proportional to the DA concentrations over a range of $1.0 \cdot 10^{-6}$ ~ $1.0 \cdot 10^{-4}$ M. The linear regression equation obtained is Ipa (µA) = 0.01285 + 0.28778 C (µM) with a correlation coefficient of 0.9994. The detection limit was $3.8 \cdot 10^{-8}$ M (S/N=3). The relative standard deviation (RSD) of 10 scans was 2.5% for $2.0 \cdot 10^{-5}$ M. This indicated that the reproducibility of the poly(3-acetylthiophene) modified GCE was excellent.

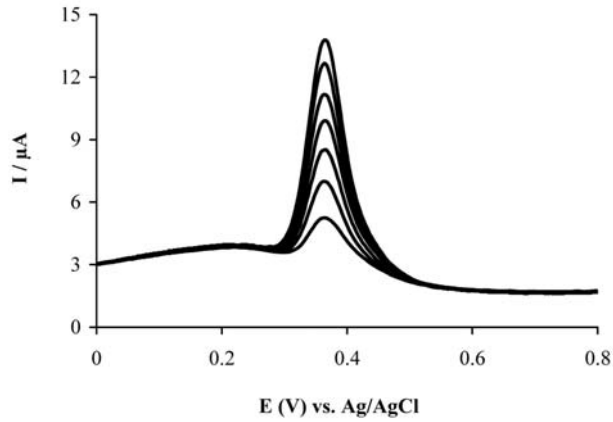



**Fig. 5**. Square wave voltammograms of increasing concentration of DA at poly(3-acetylthiophene)/GCE in 0.1 M PBS at pH 4.0. DA concentrations: $5.0 \cdot 10^{-6}$ M, $1.0 \cdot 10^{-5}$ M, $1.5 \cdot 10^{-5}$ M, $2.0 \cdot 10^{-5}$ M, $2.5 \cdot 10^{-5}$ M, $3.0 \cdot 10^{-5}$ M, $3.5 \cdot 10^{-5}$ M. Equilibrium time: 5 s, frequency: 10 Hz, step potential: 20 mV, amplitude: 25 mV.

## 3. 5. Detection of DA in the Presence of AA

It is known that AA coexisted in the body fluids can be easily oxidized at a potential rather close to that of DA and always interfere with the measurement of DA.[23,24] Thus, a sensitive determination of DA in the presence of AA is of great importance in DA measurements. The interference from AA has been studied in 0.1 M PBS at pH 4.0. The cyclic voltammograms of the mixture of DA and AA at bare GCE and the modified electrode are given in Fig. 6. At bare GCE only a single broad voltammetric peak at 0.480 V was observed. However, at poly(3-acetylthiophene)/GCE, two well-defined voltammetric peaks were obtained. The anodic peaks were appeared at about +0.247 V and +0.376 V for AA and DA, respectively. This shows that poly(3-acetylthiophene)/GCE could easily separate the oxidation peak potentials of AA and DA. Square wave voltammograms (SWV) of the mixture of AA and DA is also shown in Fig. 7. Peak current of increasing concentration of DA was not influenced by coexisted AA. It is remarkable that 50-fold excess of AA does not interfere with the determination of DA. This behaviour reveals that poly(3-acetylthiophene) modified GCE could enable the determination of DA in the presence of AA. This feature also indicates the high

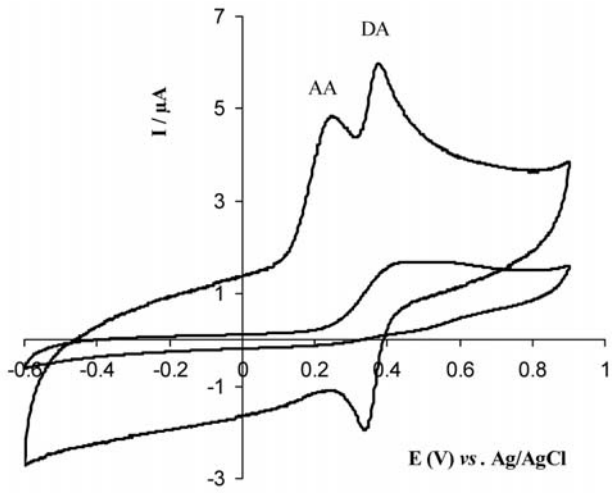



**Fig. 6.** Cyclic voltammograms of a mixture of $1.5 \cdot 10^{-4}$ M AA and $4.5 \cdot 10^{-5}$ M DA at (a) bare GCE and (b) poly(3-acetylthiophene)/GCE. Equilibrium time: 5 s, scan rate: 50 mV/s.

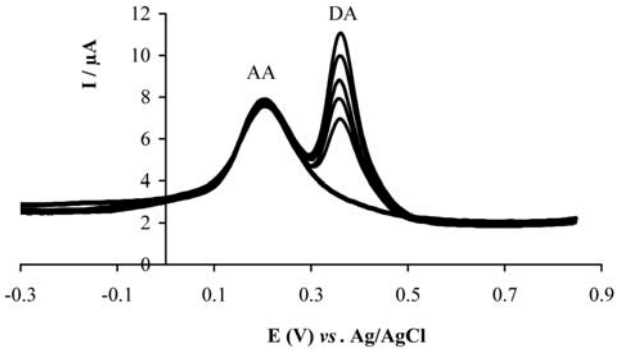



**Fig. 7.** Square wave voltammograms of the mixture of various concentrations of DA in the presence of $2.5 \cdot 10^{-4}$ M AA at poly(3-acetylthiophene)/GCE. DA concentrations: $1.25 \cdot 10^{-5}$ M, $1.5 \cdot 10^{-5}$ M, $2.0 \cdot 10^{-5}$ M, $2.25 \cdot 10^{-5}$ M and $2.75 \cdot 10^{-5}$ M. Equilibrium time: 5 s, frequency: 10 Hz, step potential: 20 mV, amplitude: 25 mV.

sensitivity of the poly(3-acetylthiophene)/GCE for the detection of DA in the presence of AA.

### 3. 6. Analytical Applications

The proposed method was utilized for the determination of DA in pharmaceuticals. Two different brands of dopamine injections named Dopmin and Dopamine Fresenius were analysed using the proposed method. Dopamine hydrochloride samples were diluted with 0.1 M PBS. The injections were analysed by the standard addition method. The data obtained at poly(3-acetylthiophene)/GCE are in close agreement with the claimed values and compare favourably with the standard methods of British Pharmacopoeia using non-aqueous titrimetry[25] and Pharmacopoeia of China.[26] The average recoveries of 97.0% and 97.6% with mean standard deviations of 0.19 and 0.17 were obtained employing the proposed method for the six different determinations of the two brands of injections, respectively. The data are also in good agreement with certified values obtained by the reported spectrophotometric method[27] (average recovery of 97.9%), and British Pharmacopoeia non-aqueous titrimetry[25] (average recovery of 98.0%). However, the data obtained by the proposed method are comparable with classical method of Chinese Pharmacopoeia[26] with an average recovery of 104.5%. The average relative standard deviation (RSD) of 1.86% obtained from the proposed method is well compared with the relative standard deviations of 2.96% and 2.60% obtained from the previous studies of dopamine analysis using different modified electrodes.[21,28] The validity of the proposed methods was also assured by the recovery of dopamine in blood samples. The mean recovery of the three blood serum samples was 98.2% with RSD of 2.05. The results indicated that the proposed method could be easily used for the determination of DA in pharmaceuticals and body fluids.

## 4. Conclusions

This study has indicated that poly(3-acetylthiophene) modified glassy carbon electrode exhibits highly electrocatalytic activity towards the oxidation of dopamine. The obtained results also showed that poly(3-acetylthiophene)/GCE has accelerated the electron transfer rate of dopamine. The results has also indicated that the poly(3-acetylthiophene)/GCE could be used for the determination of DA. The anodic peak current of DA is linear with the concentration range of $1.0 \cdot 10^{-6} \sim 1.0 \cdot 10^{-4}$ M with a detection limit of $3.8 \cdot 10^{-8}$ M. The results also proved that AA do not interfere with the detection of DA at the poly(3-acetylthiophene)/GCE. The poly(3-acetylthiophene) modified electrode has a good sensitivity and reproducibility. The method could also be utilized for the detection of DA injections and body fluids.

## 5. Acknowledgements

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## Povzetek

Z uporabo ciklične voltametrije smo na steklasti elektrodi (GCE) polimerizirali 3-acetiltiofen, pri čemer je modificirana elektroda izkazala veliko elektrokatalitsko učinkovitost pri oksidaciji dopamina (DA). V primerjavi z nemodificirano elektrodo tudi izkazuje premik oksidacijskega potenciala DA v katodni smeri an veliko povečanje toka. Razlika med potenciali anodnega in katodnega vrha za DA je 195 mV pri običajni in 34 mV pri modificirani elektrodi. Slednjo smo zato uporabili za določanje dopamina v 0,1 M fosfatnem pufru pri pH 4,0. Linearen odziv smo dobili v območju $1{,}0 \cdot 10^{-6}$ – $1{,}0 \cdot 10^{-4}$ M. Meja zaznavnosti je $3{,}8 \cdot 10^{-8}$ M. Metodo smo uporabili za določanje dopamina v prisotnosti askorbinske kisline, rezultati so ponovljivi in odziv stabilen.