UNIVERSITY OF CALIFORNIA, SAN DIEGO

Electrochemical Detection of Epinephrine and Norepinephrine Using Unmodified Screen-Printed Carbon Electrode

A thesis submitted in partial satisfaction of the
requirements for the degree Master of Science

in

Chemical Engineering

by

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Professor Ying S. Meng

2015

The Thesis of Thomas Namwon Cho is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

Chair

University of California, San Diego

2015

DEDICATION

I would like to dedicate this work to my family and friends who helped me throughout my academic career.

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ABSTRACT OF THE THESIS

Electrochemical Detection of Epinephrine and Norepinephrine

Using Unmodified Screen-Printed Carbon Electrode

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Master of Sicence in Chemical Engineering

University of California, San Diego, 2015

Professor Joseph Wang, Chair

Detection of epinephrine and norepinephrine is a task that has been investigated thoroughly over past couple decades. The importance of epinephrine and norepinephrine is very significant for various applications. Unlike the conventional high performance and high throughput method that are used by the industry today, simple electrochemical

detection to achieve such task. For the electrode, unmodified screen-printed carbon electrode was utilized. Screen-printing technique is fairly new technology among electrode fabrication technology. This technology allows mass production of electrodes at very low cost. By looking at different electrochemical fingerprints generated by cyclic square wave voltammetry, detection of epinephrine and norepinephrine was investigated.

Chapter 1. Introduction

Detection of epinephrine and norepinephrine is the subject that been researched through various discipline over past couple decades. The importance of these molecules is significant since they govern many of the physiological function [1-4] and can be administered as a medicine for certain symptoms [5-7]. The knowledge of their concentration in the biological system can be also used to understand certain diseases [8-13]. The knowledge of them can be also used for pharmaceutical research. However, detection of epinephrine and norepinephrine has its challenge. Epinephrine and norepinephrine are found in biological system at very low concentration [14, 15], and they coexist with the molecules that interfere with their detection [16]. Ascorbic acid and uric acid are some of the common interfering molecules that coexist with epinephrine and norepinephrine. Their concentration is generally much higher than that of epinephrine and norepinephrine.

Due to limitation of detecting epinephrine and norepinephrine caused by the interference, mainly high quality and high performance procedure has been studied. Procedures like high performance liquid chromatography, gas chromatography, flow injection, and capillary electrophoresis are promising technique which allows epinephrine and norepinephrine detection [17-22]. However, these techniques are expansive and time consuming. Electrochemical procedure has been studied as well since it has an

advantage of simplicity and faster detection time. The study of these electrochemical procedures mainly focuses on modifying the working electrode to increase the selectivity and sensitivity so that the differentiation between the target molecule and interfering molecule is possible.

The modification of the working electrode involves functionalizing the electrode with enzyme immobilization, electropolymerization, or coating using organic compound [23-26]. These steps are minor compared to utilizing more high performance techniques; however, those modifications have low reproducibility and low chance of mass production. In this paper, screen-printing technology for electrode fabrication was studied. Screen-printing technology is fairly new technology when it comes to the electrode fabrication [27-31]. This technique allows mass production of electrodes with good reproducibility at low cost. With these advantages, this technique has been getting attention to those who wants to fabricate disposable electrode for various applications [32, 33].

In this paper, unmodified screen-printed carbon electrode was used for the detection of epinephrine and norepinephrine in presence of interference. This was done through looking at the unique fingerprint that epinephrine and norepinephrine mixture gives out through cyclic square wave voltammetry. Cyclic square wave voltammetry is given through overlaying oxidative square wave voltammetry and reductive square wave voltammetry. This procedure combines the advantages of two different electrochemical procedures: cyclic voltammetry and square wave voltammetry [34, 35]. Cyclic voltammetry shows both oxidative behavior and reductive behavior and square wave

voltammetry detect molecules at higher sensitivity [36, 37]. With this electrochemical procedure and unmodified screen-printed carbon electrode, detection of epinephrine and norepinephrine was done in presence of common interference molecules.

Chapter 2. Background and Motivation

2. 1. Epinephrine and Norepinephrine

Epinephrine and norepinephrine, which fall in a family of catecholamine, are very important medicine, hormone, and neurotransmitter. As a medication, they are mainly used to regulate the heart rate and blood pressure of the patient. Naturally, they are in biological system regulating physiological functions [1-4]. They get released from adrenal medulla as a response to various situations [38-40]. Their concentration is often correlated with memory and stress level. These molecules are widely known as part of fight-of-flight response of biological system [41, 42]. Since they are involved in wide spectrum of physiological function, the changes in concentrations of epinephrine and norepinephrine can correlate with diseases such as Alzheimer's disease and Parkinson's disease [8-10].

Due to their biological importance, epinephrine and norepinephrine detection been studied widely past couple decades. Despite of their importance, the concentrations of epinephrine and norepinephrine are very minimal compared to the other molecules that coexist with epinephrine and norepinephrine in the biological system. The most common interfering molecules that coexist with epinephrine and norepinephrine are ascorbic acid and uric acid [23-26]. To compensate for the large amount of interference molecules, high performance techniques been used to detect epinephrine and norepinephrine.

Typical procedures involved for the measurements are high performance liquid chromatography, gas chromatography, capillary electrophoresis, flow injection, and spectrophotometry [17-20, 43]. Although these techniques produce accurate with high precision, they are very complicated and cause long time for single detection.

In presented work, electrochemical method to detect epinephrine and norepinephrine has been investigated. Both epinephrine and norepinephrine are electro active molecules, which allows for electrochemical detection. However, electrochemical method been overlooked often since epinephrine and norepinephrine coexist with high concentration of ascorbic acid and uric acid, which are highly electro active as well. To countermeasure this disadvantage, different types of the modification on electrode have been studied in order to increase the sensitivity and selectivity [23-26]. Yet, the proposed method uses the bare electrode without any modification focusing on simplicity and low cost for the epinephrine and norepinephrine detection.

2. 2. Screen Printed Carbon Electrode

Screen-printing technology has been getting attention past couple decades as an electrode fabrication technique. Many of the electrodes are made through chemical vapor deposition technique or thermal deposition technique [44, 45]. These techniques produce electrodes with high quality and high performance, but they require large operation time with complex procedures as well as large cost. These limitations can be a hindrance when one wants to develop one-time use sensors.

A screen-printed electrode is usually a planar electrode based on multiple layers of conductive printed on different substrate materials. The materials of the electrode can be modified to increase the versatility. Also, screen-printed electrode is susceptible towards the further modification. The ability to design the electrode and low cost fabrication are the advantages of screen-printing technology allowing disposable sensor for various applications [32, 33]. It has been reported that using various substrates such as textile [46] and tattoo paper, wearable devices been developed using screen-printing technology.

In the presented work, simple carbon conductive ink and silver ink were used to produce screen-printed electrode. The produced electrodes didn't go through any modifications and were used for all the measurements. The use of unmodified simple screen-printed carbon electrode was to emphasize the possibility of epinephrine and norepinephrine detection at low cost.

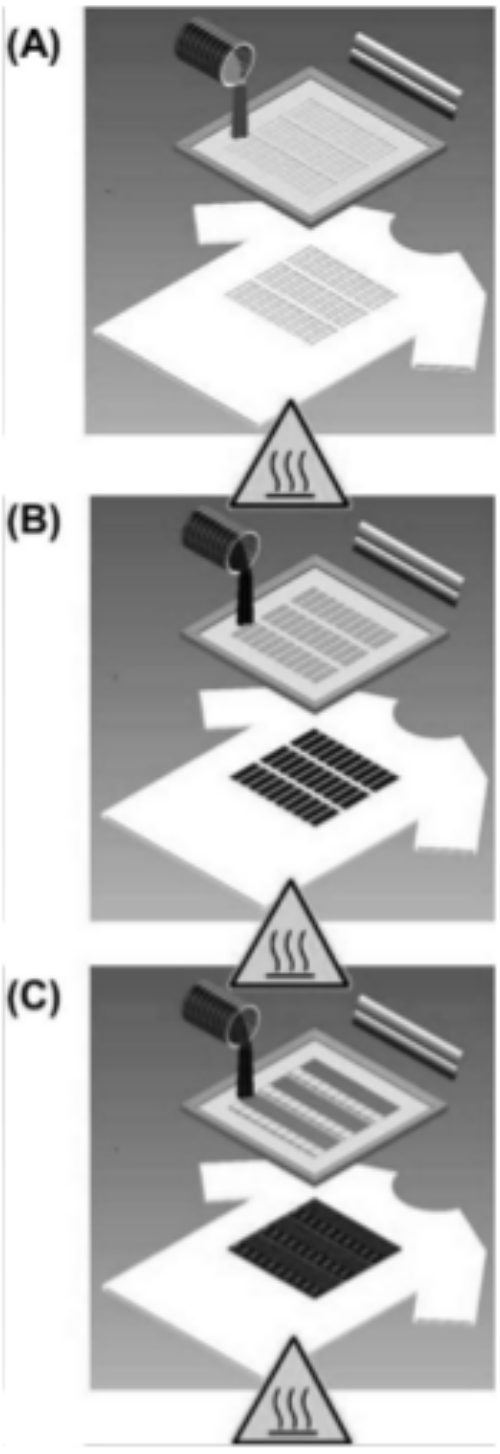

Figure 1. Fabrication steps of the screen-printed carbon electrode on the textile substrate [46]

2. 3. Square Wave Voltammetry

Square wave voltammetry is one of the major voltammetric techniques used for electrochemical procedures. The application of square wave voltammetry has been gaining lots of attention due to wide spread of potentiometric instruments as well as well-developed theory that supports the square wave voltammetry procedure [47]. Square wave voltammetry is a type of linear potential sweep; during the procedure, potential between the working and a reference electrode is swept linearly. The potential sweep can be set in two different directions to see either oxidative behavior of the molecule or the reductive behavior of the molecule. This is contrast to the cyclic voltammetry, which is another major voltammetric technique, where potential swept linearly in time cyclic manner in between set range of the procedure.

Cyclic voltammetry shows both oxidation potential and reduction potential in potential window set for the procedure if the analyte is electroactive within that potential window [48]. Thus, cyclic voltammetry shows both potential and reduction potential of the molecule and can be used to characterize the molecule. Despite of this analytical capability, sensitivity of the cyclic voltammetry isn't high enough compared to square wave voltammetry. This can be a limitation when it comes to detection of epinephrine and norepinephrine through cyclic voltammetry since their concentration is very low in biological system.

In the presented work, cyclic square wave voltammetry was utilized to compensate for limitation of cyclic voltammetry and square wave voltammetry. Cyclic square wave voltammetry is to run square wave voltammetry both directions in cyclic

manner to see both oxidative behavior and reductive behavior at higher sensitivity than regular cyclic voltammetry. Cyclic voltammetry is fairly new electrochemical protocol, which has been reported effective when it comes to measuring electrochemical behavior of the mixture. This newly introduced protocol creates unique fingerprints for the molecules so the target molecule can be identified in the mixture. Using this electrochemical procedure, epinephrine and norepinephrine detection in presence of interfering molecules was investigated.

Chapter 3. Experimental

3. 1. Chemicals and Reagents.

L-ascorbic acid (AA), uric acid (UA), (±)-epienphrine hydrochloride (EP), (-)-norepinephrine (NE), sodium phosphate monobasic (NaH_2PO_4), and sodium phosphate dibasic (Na_2HPO_4) were obtained from Sigma-Aldrich (St. Louis, MO). Graphite ink and silver/ silver chloride (Ag/ AgCl) inks were obtained through Ercon Inc. (Wareham, MA). The phosphate buffer solution was prepared by mixing 0.1M of NaH_2PO_4 and 0.1M of Na_2HPO_4 to reach pH 7.0. 10mM stock solution of AA and UA and 1mM stock solution of EP and NE were prepared with prepared PBS buffer solution freshly before every experiment.

3. 2. Preparation of screen-printed carbon electrode

Sensor patterns were designed in AutoCAD (Autodesk, San Rafael, CA) and outsourced for fabrication on stainless steel through-hole 12in. X 12 in. framed stencils (Metal Etch Services, San Marcos, CA). A semi-automatic screen printer (MPM-SPM, Speedline Technologies, Franklin, MA) was utilized for printing the three-electrode system. The sensor was patterned onto the polyethylene terephthalate substrate. The three-electrode system is consists of the pseudo reference (Ag/ AgCl ink) and the working electrode and counter electrode (graphite ink). The Ag/ AgCl ink was cured at

100^{o}C for 10min and graphite ink was cured at 90^{o}C for 10min in a convection oven. Fabricated screen-printed carbon electrode can be seen in Figure 2.

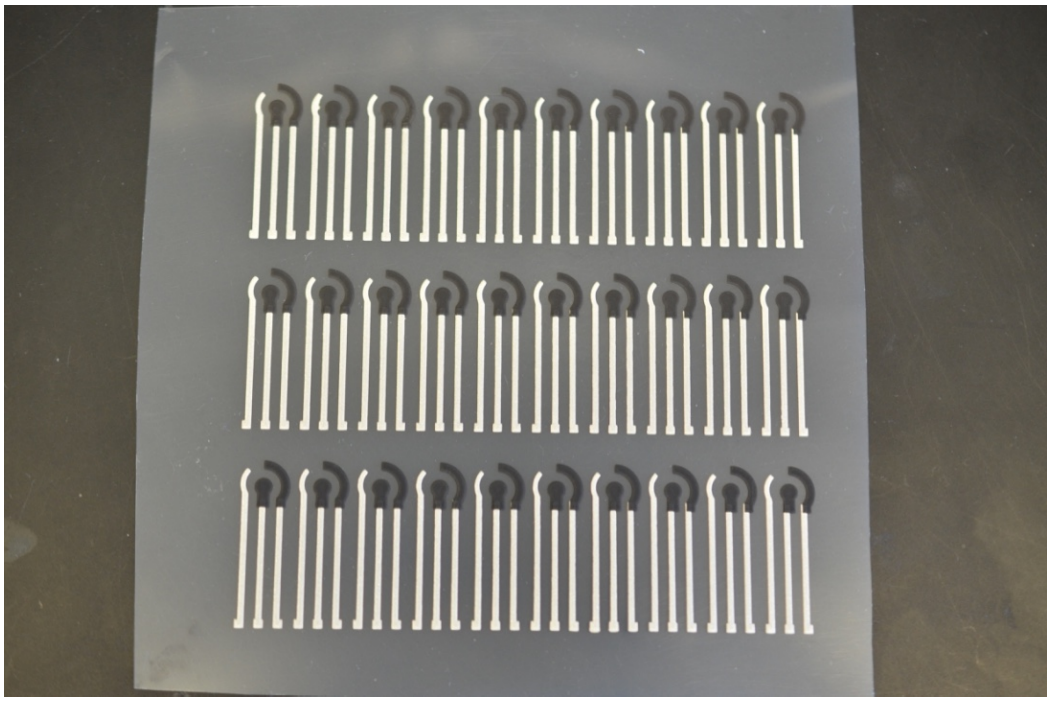

Figure 2. Fabricated screen-printed carbon electrode on PET substrate.

3. 3. Electrochemical procedures

Electrochemical characterization was performed at room temperature using a CH Instruments electrochemical analyzer (model 1440, Austin TX). Before any characterization of the molecule, electrodes were cleaned with deionized water and stabilized using cyclic voltammetry in PBS buffer. Cyclic voltammetry was carried out with range of -1.0V and 1.0V at a scan rate of 100mV s^{-1} for 50 cycles. The square-wave voltammetry (SWV) was used to characterize the molecules; the square-wave voltammetric parameters employed were a frequency of 15 Hz, a potential increment of 4mV, and amplitude of 25mV. To observe both oxidative behavior and reductive behavior of the target molecules, potential swept in range of -0.8V to 0.8V back and forth.

Chapter 4. Result and Discussion

4.1. Reproducibility of screen-printed carbon electrode

Due to simple method of electrode fabrication, screen-printing technology usually targets at creating disposable electrode. Since presented work utilizes the multiple screen-printed carbon electrodes, the reproducibility between the electrodes had to be tested. Figure 3 shows the cyclic voltammetry obtained from five different electrodes. The cyclic voltammetry procedure was carried out in pH 7.0 PBS buffer. The overlaid data suggest that the electrode fabricated using the screen-printing technique is indeed reproducible. This suggests the capability of mass production of electrodes using the screen-printing technology.

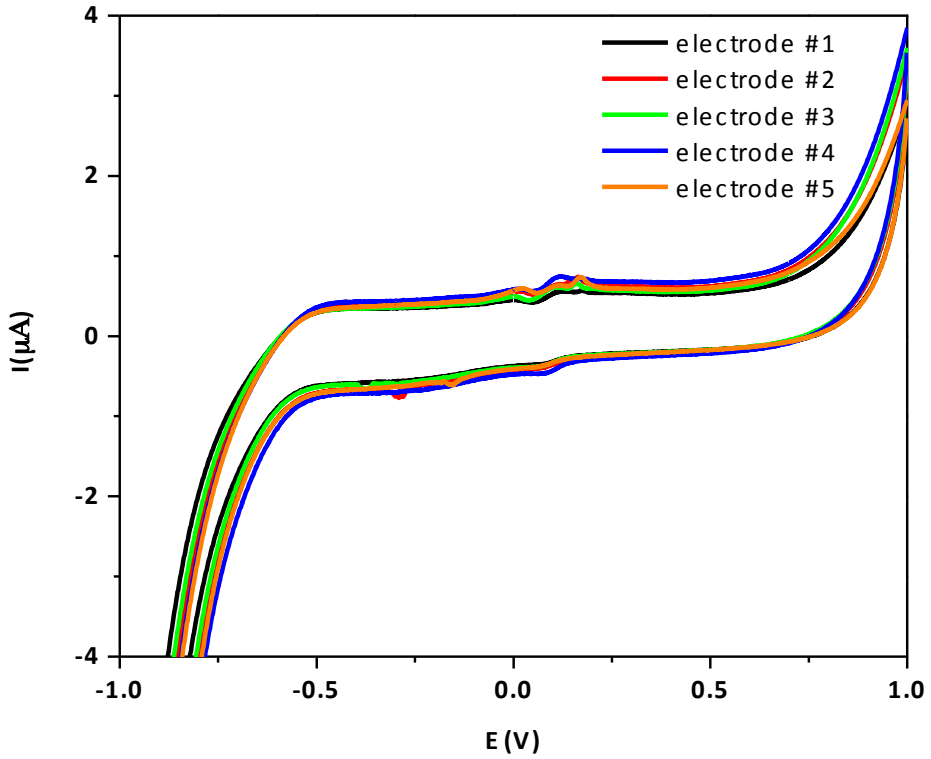

Figure 3. Overlaid cyclic voltammetry of five different electrodes in pH 7.0 PBS buffer solutions.

4.2. Detection through cyclic voltammetry

4.2.1. Cyclic voltammetry of molecules

As mentioned earlier cyclic voltammetry is a procedure to obtain an electrochemical behavior of the molecule. 100μM of each molecule were prepared in 100μL of pH 7.0 PBS buffer. Then, using the unmodified screen-printed carbon electrode and the potentiostat, cyclic voltammetry of the molecules of the interest was acquired. Looking at cyclic voltammetry of ascorbic acid and uric acid in Figure 4 and Figure 5, it can be noted that there is only oxidative peak presented. This suggests that oxidation behaviors of ascorbic acid and uric acid are irreversible. This is a clear difference from the cyclic voltammetry shown from epinephrine and norepinephrine, Figure 6 and Figure 7. Since both epinephrine and norepinephrine are of a family of catecholamine, they both have catechol structure in their molecular structure. By having this aromatic ring structure, both epinephrine and norepinephrine goes through reversible oxidation and reduction. These phenomena could be seen through cyclic voltammetry of epinephrine and norepinephrine respectively. Although cyclic voltammetry of all four molecules seems distinct, they have oxidative peak occurring at similar region. This overlaying oxidative peak can be a limitation when it comes to detecting target molecule in the mixture.

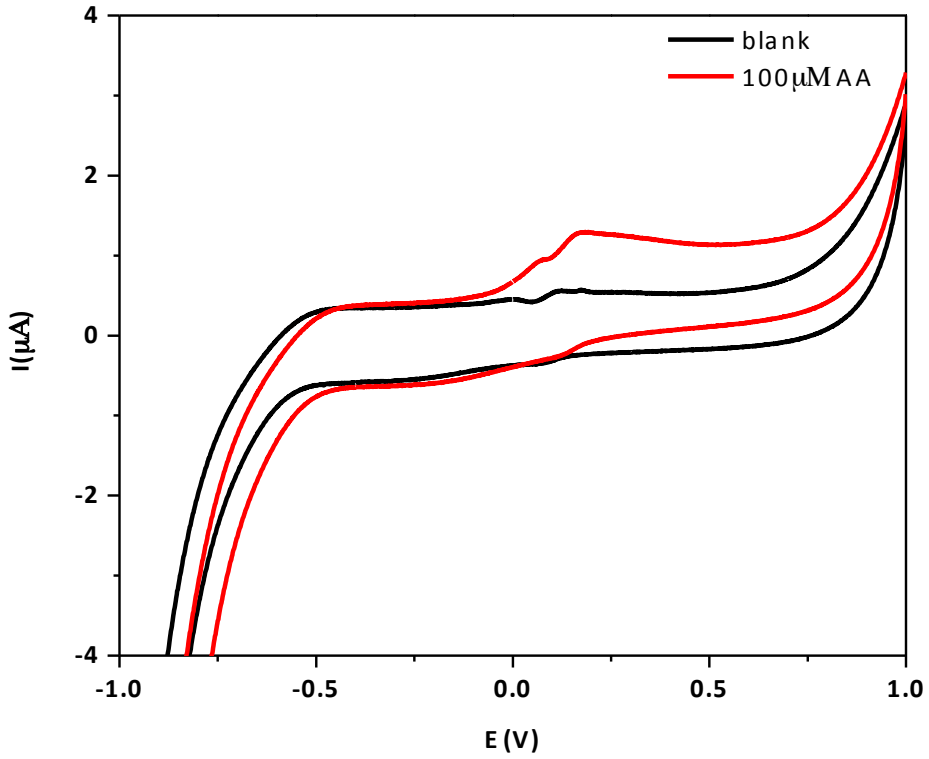

Figure 4. Cyclic voltammetry of 100μM ascorbic acid overlaid with blank response.

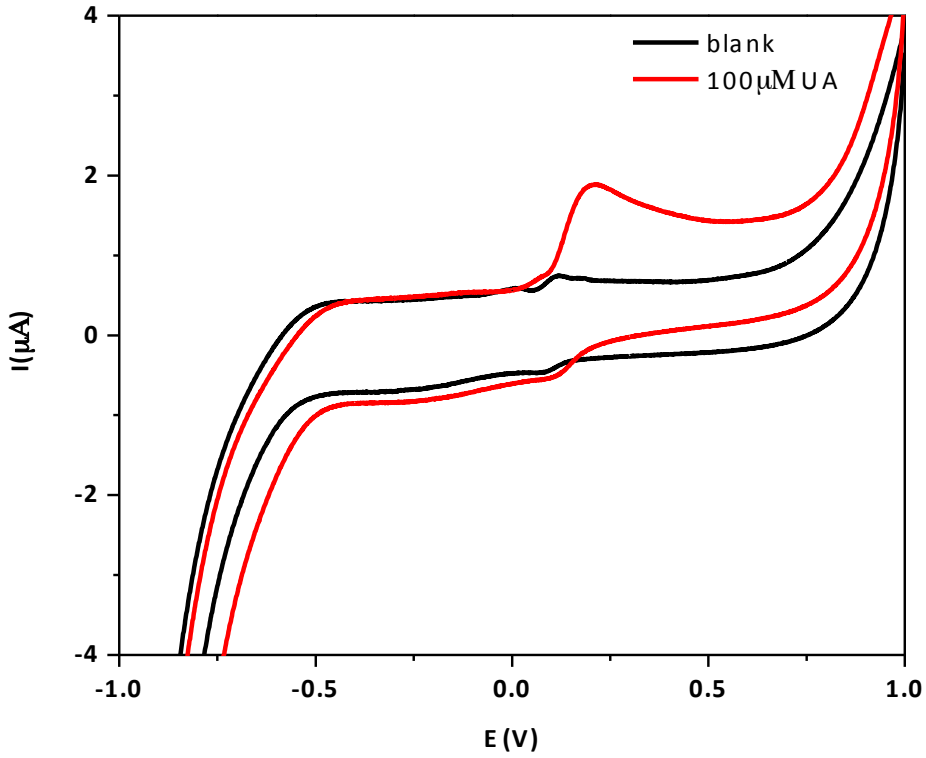

Figure 5. Cyclic voltammetry of 100μM uric acid overlaid with blank response.

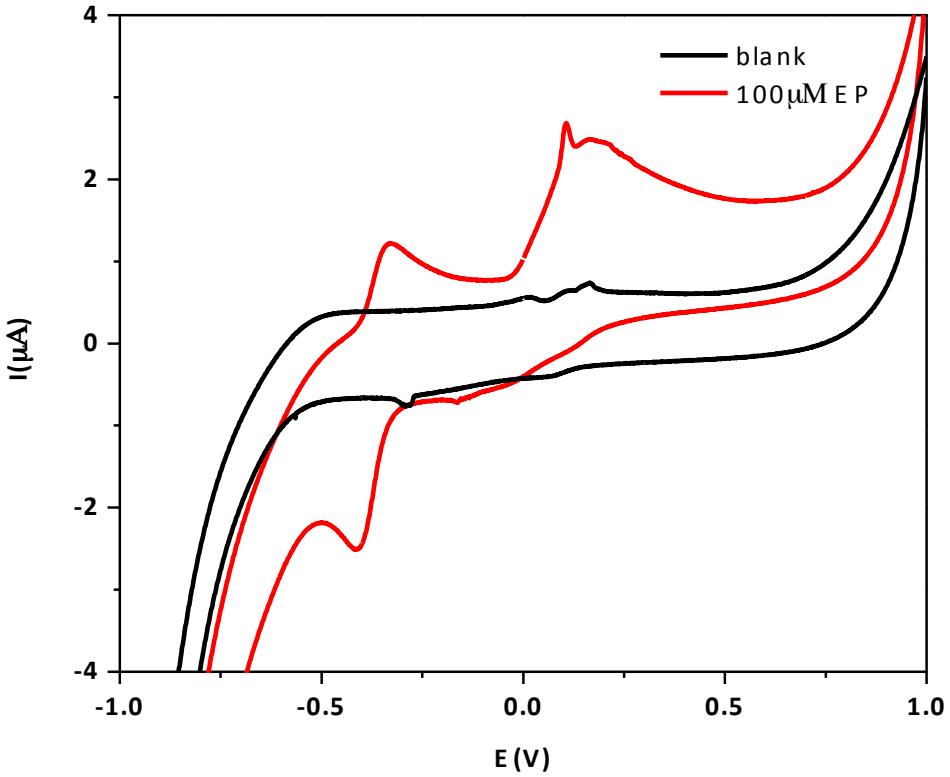

Figure 6. Cyclic voltammetry of 10µM epinephrine overlaid with blank response.

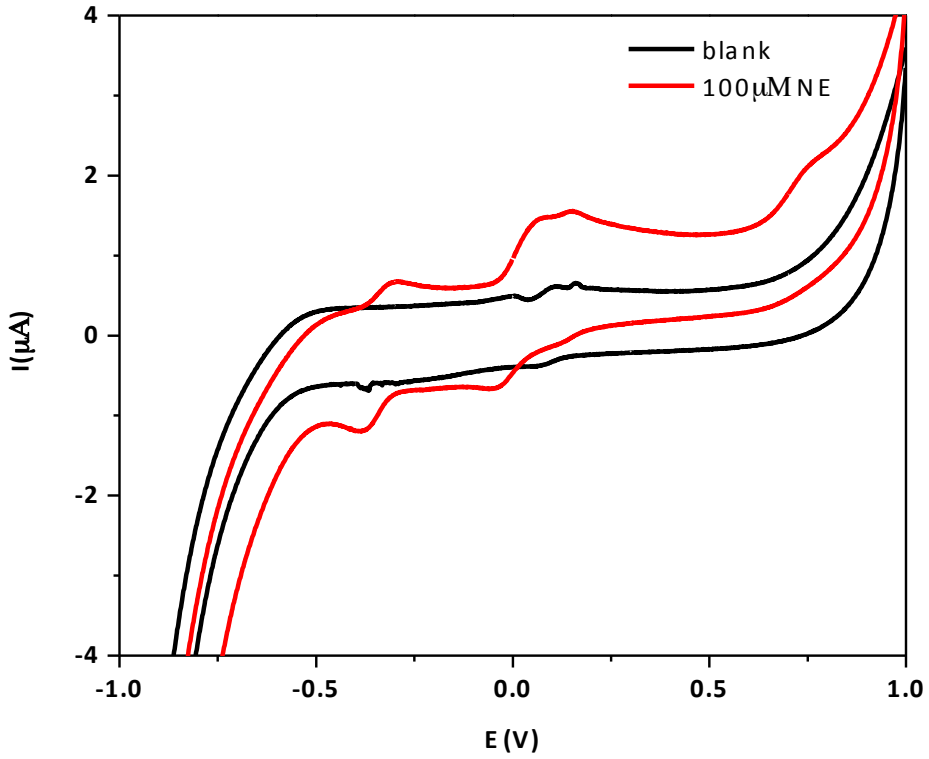

Figure 7. Cyclic voltammetry of 10μM norepinephrine overlaid with blank response.

4.2.2 Cyclic voltammetry of epinephrine and norepinephrine in presence of interference

Mixture of epinephrine and norepinephrine with interfering molecules, ascorbic acid and uric acid, to test the possibility of using cyclic voltammetry procedure as a detection method for epinephrine and norepinephrine in presence of interference. Preparation of the mixture was carried out though following procedure. First using the ascorbic acid and uric acid stock solution, 100μM of ascorbic acid and 100μM of uric acid in pH 7.0 PBS buffer solution was prepared. Subsequently, 10μM of epinephrine and norepinephrine was added for the respective mixtures. The reduced concentration of epinephrine and norepinephrine was to mimic the real biological system where concentration of ascorbic acid and uric acid is much higher than the concentration of epinephrine or norepinephrine. As shown in the Figure 8 and Figure 9, it is not possible to identify the presence of the epinephrine or norepinephrine. Epinephrine and norepinephrine showed reduction peak that wasn't shown by the ascorbic acid and uric acid from the individual cyclic voltammetry. However, when mixture was prepared sensitivity of the cyclic voltammetry wasn't high enough to show the reductive behavior of epinephrine and norepinephrine. This suggests that cyclic voltammetry cannot be the electrochemical procedure when it comes to detection of epinephrine and norepinephrine in presence of the interference.

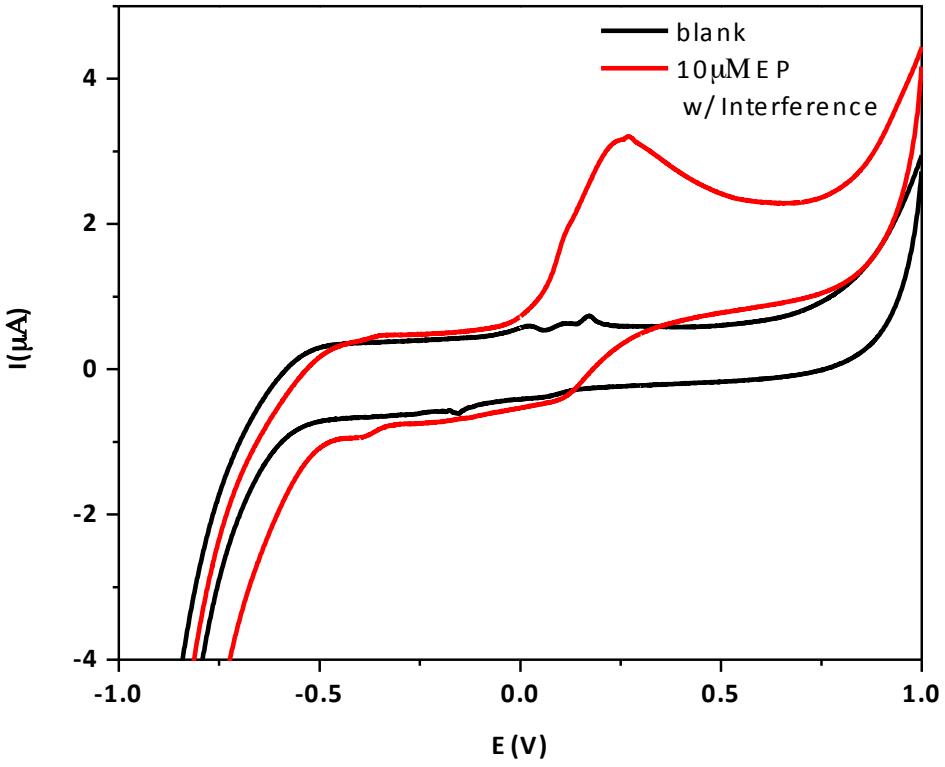

Figure 8. Cyclic voltammetry of mixture of 10μM epinephrine, 100μM ascorbic acid, and 100μM uric acid overlaid with blank response

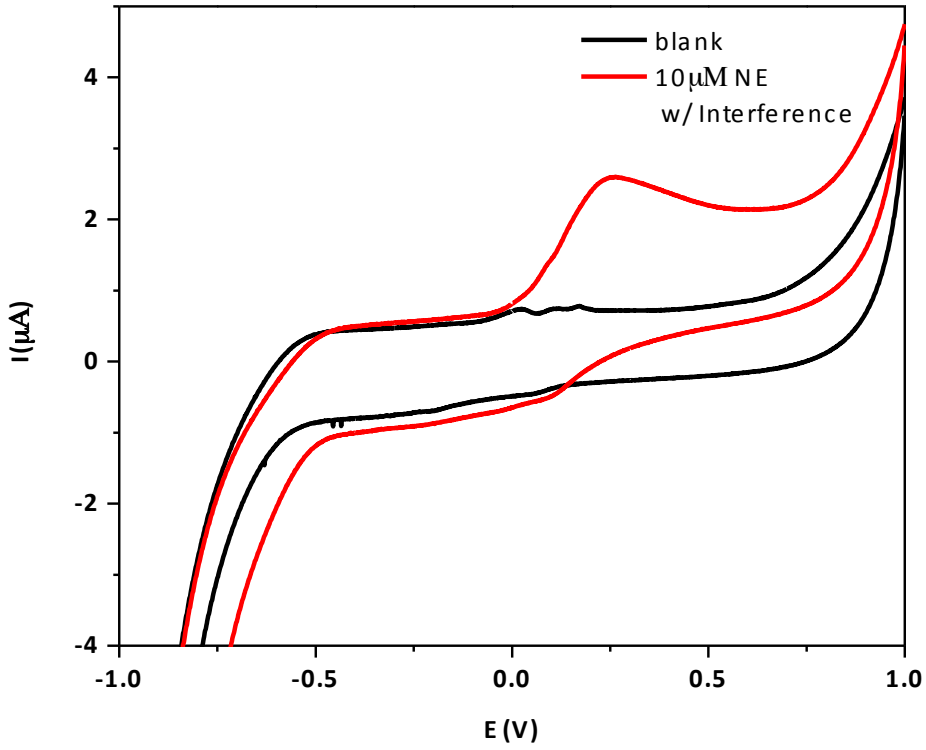

Figure 9. Cyclic voltammetry of mixture of 10μM epinephrine, 100μM ascorbic acid, and 100μM uric acid overlaid with blank response

4.3. Detection through square wave voltammetry

4.3.1. Square wave voltammetry of molecules

Square wave voltammetry is an electrochemical procedure that has higher sensitivity for sensing than cyclic voltammetry. Same reagents were tested by square wave voltammetry using the same sensing system including unmodified screen-printed carbon electrode and potentiostat. Individual molecules' square wave voltammetry for oxidative behavior and reductive behavior was overlaid in cyclic manner to show the unique fingerprints for each molecule. Figure 10, Figure 11, Figure 12, and Figure 13 shows unique fingerprints so that distinguishing the molecules is more convenient compared to the cyclic voltammetry. As shown through cyclic voltammetry, ascorbic acid and uric acid do not show reductive behavior. Using this information as an advantage, detection of epinephrine and norepinephrine can be investigated. As shown from the square wave voltammetry of epinephrine and norepinephrine, epinephrine and norepinephrine shows different reductive behavior, which can be a key to differentiate between them.

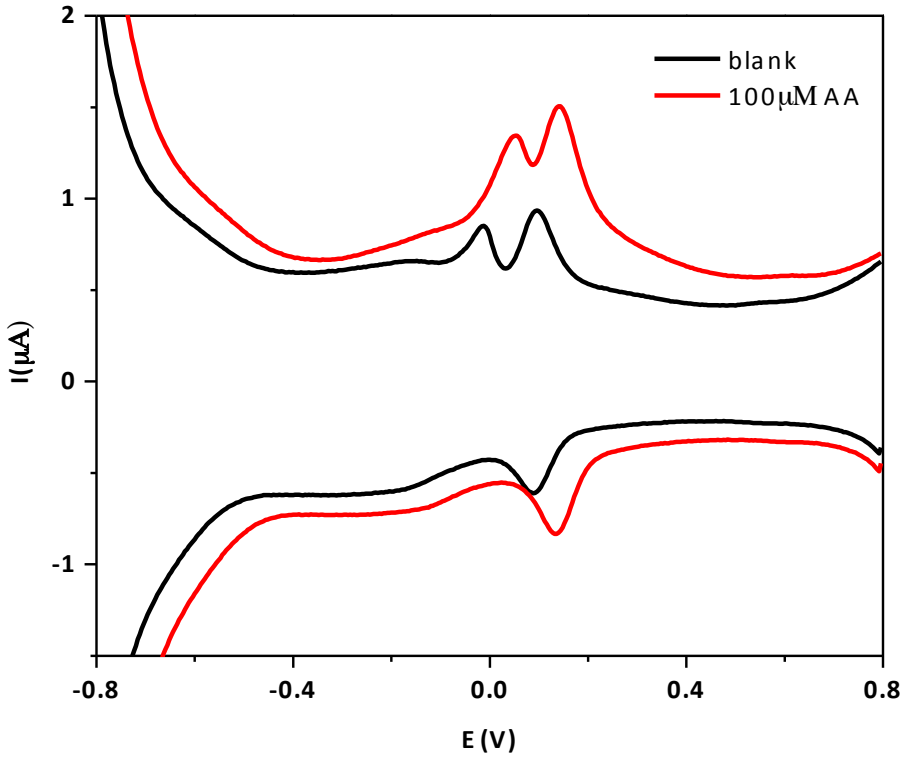

Figure 10. Square wave voltammetry of 100μM ascorbic acid overlaid with blank response.

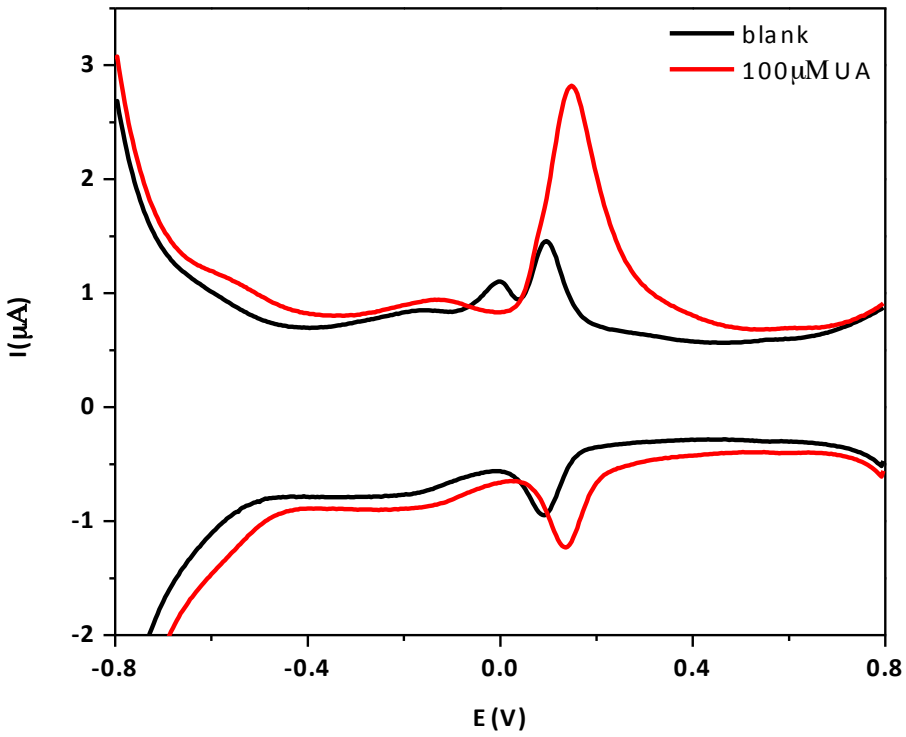

Figure 11. Square wave voltammetry of 100μM uric acid overlaid with blank response.

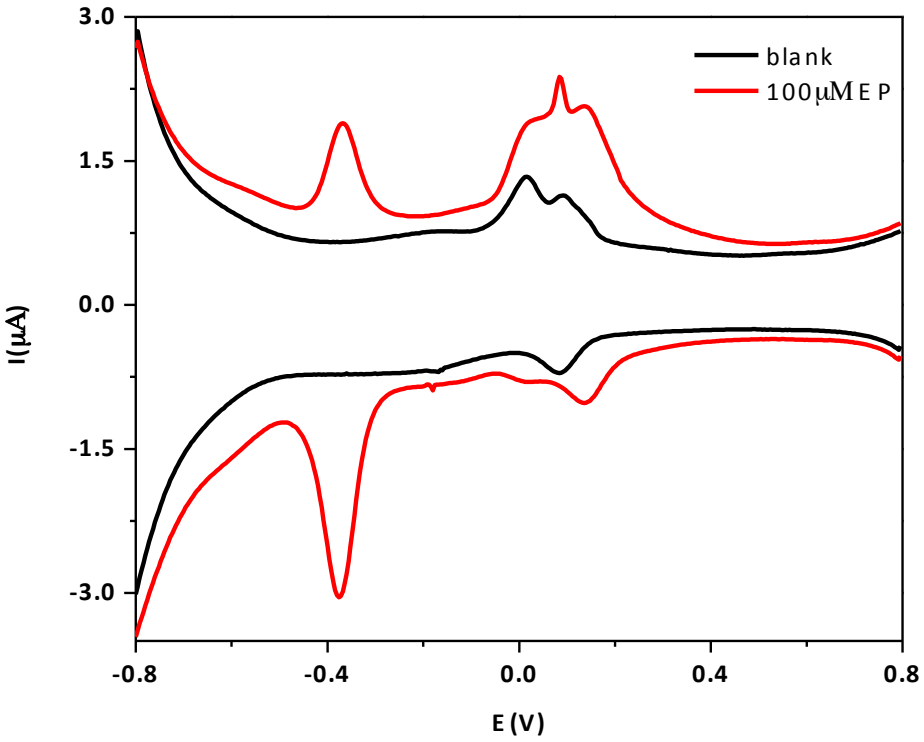

Figure 12. Square wave voltammetry of 100μM epinephrine overlaid with blank response.

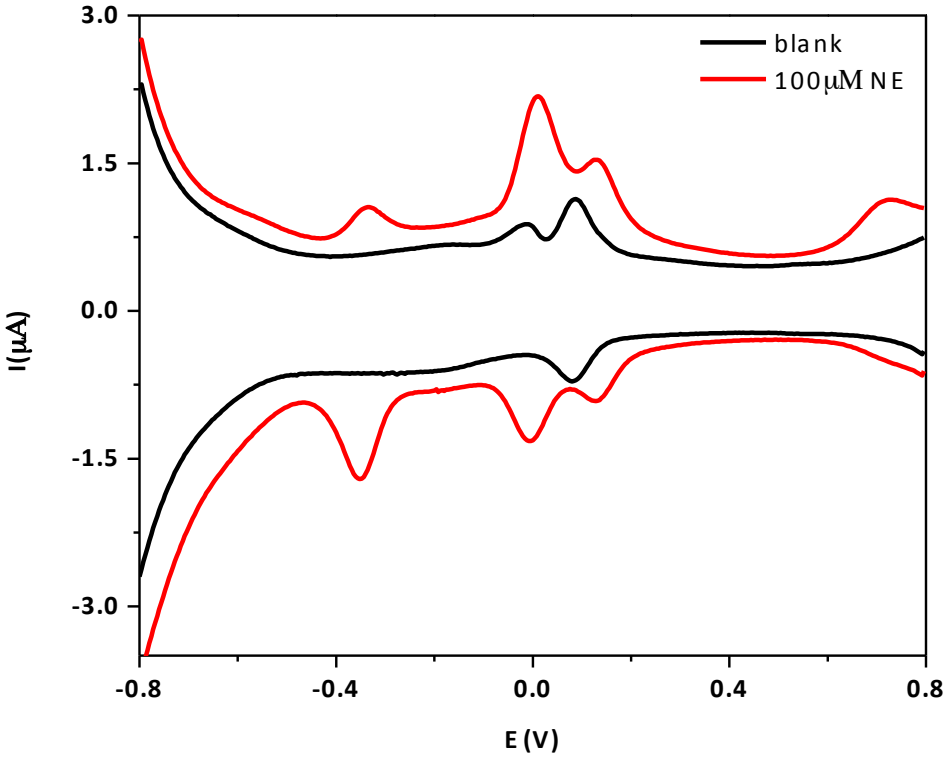

Figure 13. Square wave voltammetry of 100μM norepinephrine overlaid with blank response.

4.3.2 Square wave voltammetry of epinephrine and norepinephrine in presence of interference

The mixture of interference was prepared in similar manner as in cyclic voltammetry procedure. 100μM of ascorbic acid and 100μM of uric acid was prepared in 100μL of pH 7.0 PBS buffer. In the prepared interference mixture 5μM addition of epinephrine and norepinephrine was performed for the measurement respectively. The detection of epinephrine in presence of interference can be shown through Figure 14. The square wave voltammetry represents the unique fingerprint of ascorbic acid, uric acid, and epinephrine mixture. As shown from the magnified view from the Figure 15, epinephrine peak is not affected by the interference. The addition of interference wasn't shown in the area; however, addition of epinephrine showed clear linear response from the potential around -380mV. The linear response shown from the magnified view was visualized through the figure. From Figure 16, it is obvious that current response and the concentration of epinephrine have strong linear correlation at -380mV. The sensitivity was calculated to be 16.1 μA/mM. The Figure 17 shows different fingerprint from the fingerprint generated from epinephrine mixture. Due to different reduction behavior of norepinephrine from epinephrine, two different reduction peaks can be identified from the figure. Similarly from the Figure 14 the magnified view from Figure 18, it can be seen that addition of norepinephrine is not related with the existing interference in the mixture. The magnified view shows such characteristic clearly. The Figure 19 shows that both peaks' current response is linearly associated with the increasing concentration of the norepinephrine. The sensitivity of norepinephrine is calculated to be 18.5 μA/mM

and 15.6μA/mM from -364mV and -4mV respectively. From these results, it can be inferred that detection of epinephrine and norepinephrine in presence of interference is possible using unmodified carbon electrode.

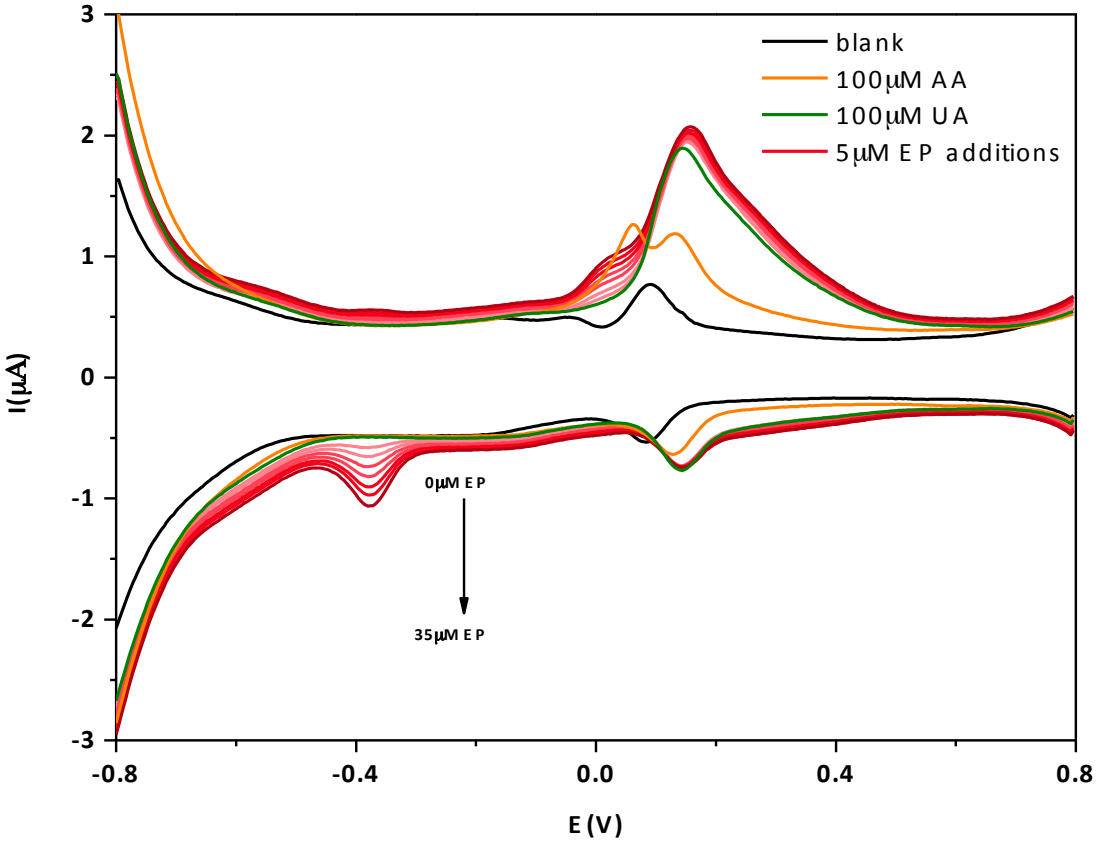

Figure 14. Detection of epinephrine from 5μM to 35μM in 5μM interval in presence of 100μM ascorbic acid and 100μM uric acid.

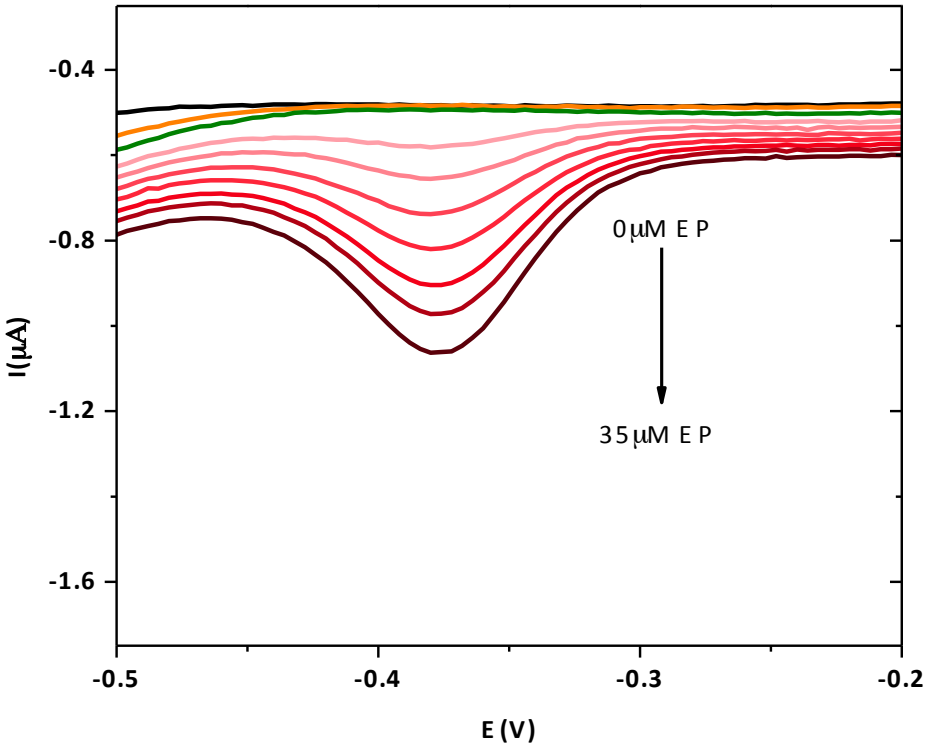

Figure 15. Magnified view from Figure 14.

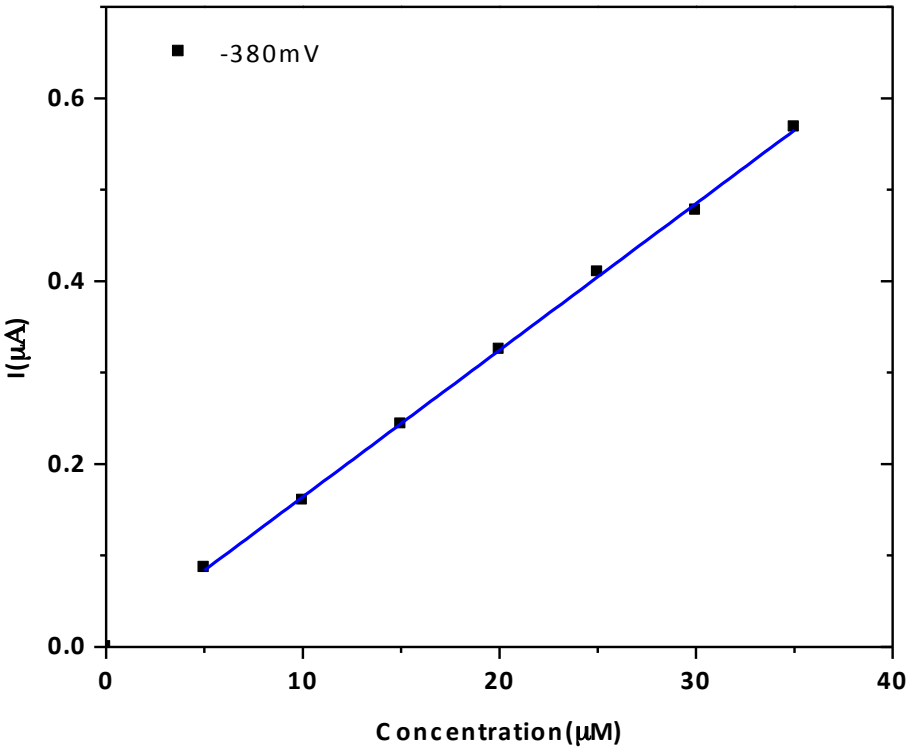

Figure 16. Current response vs concentration at -380mV from Figure 14.

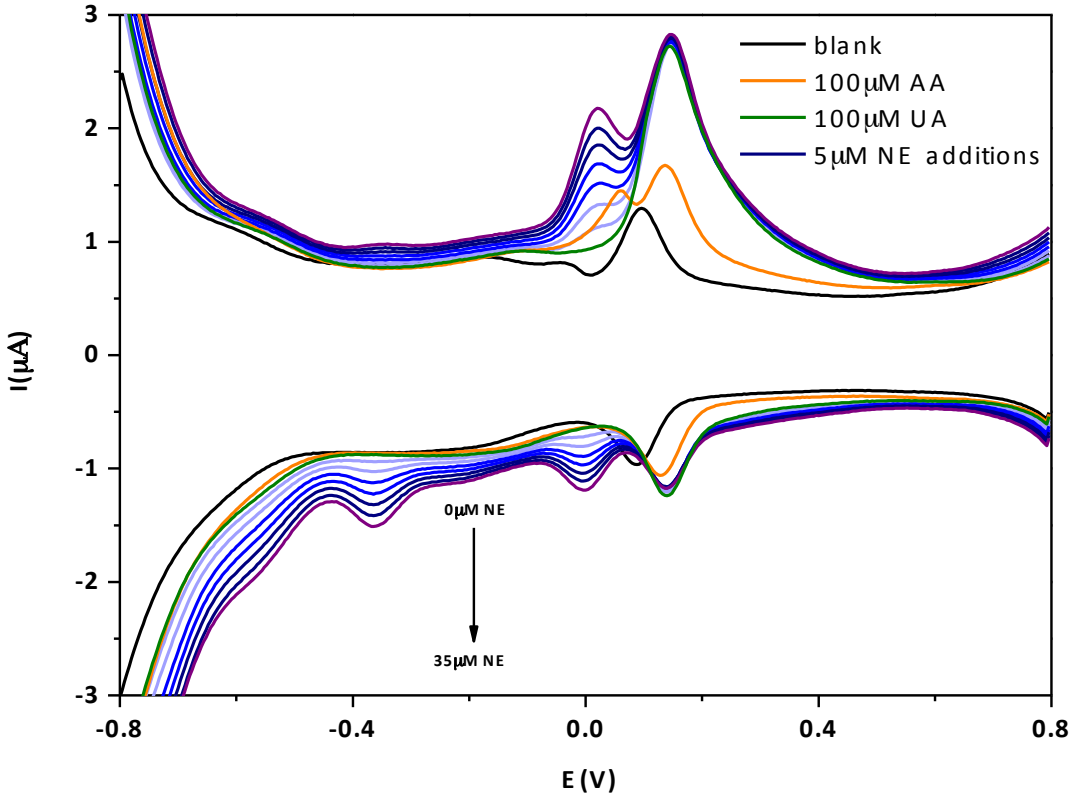

Figure 17. Detection of norepinephrine from 5μM to 35μM in 5μM interval in presence of 100μM ascorbic acid and 100μM uric acid.

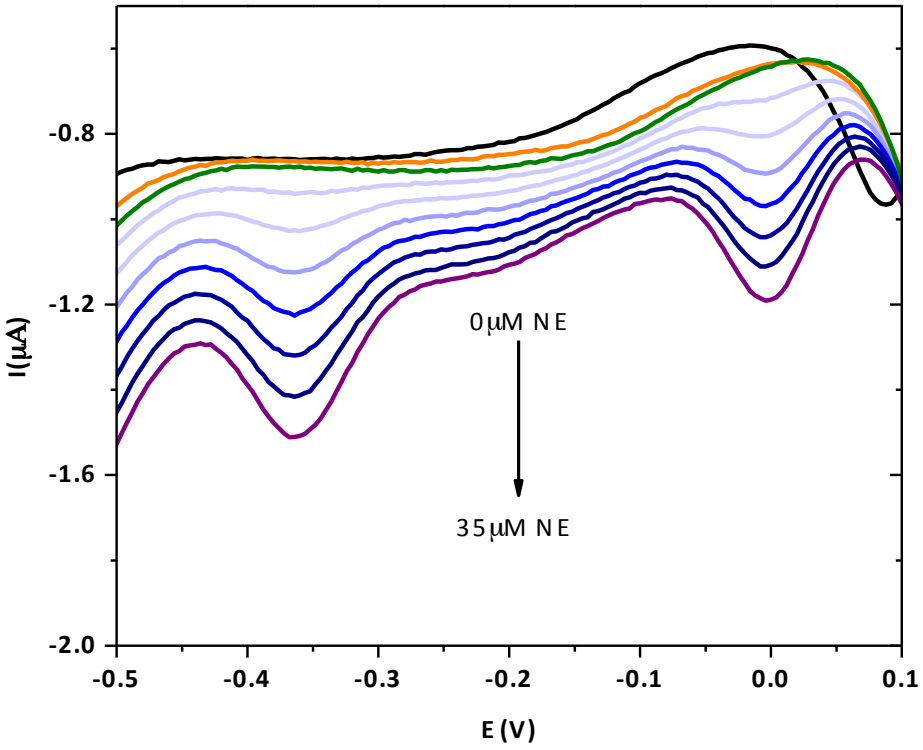

Figure 18. Magnified view from Figure 17.

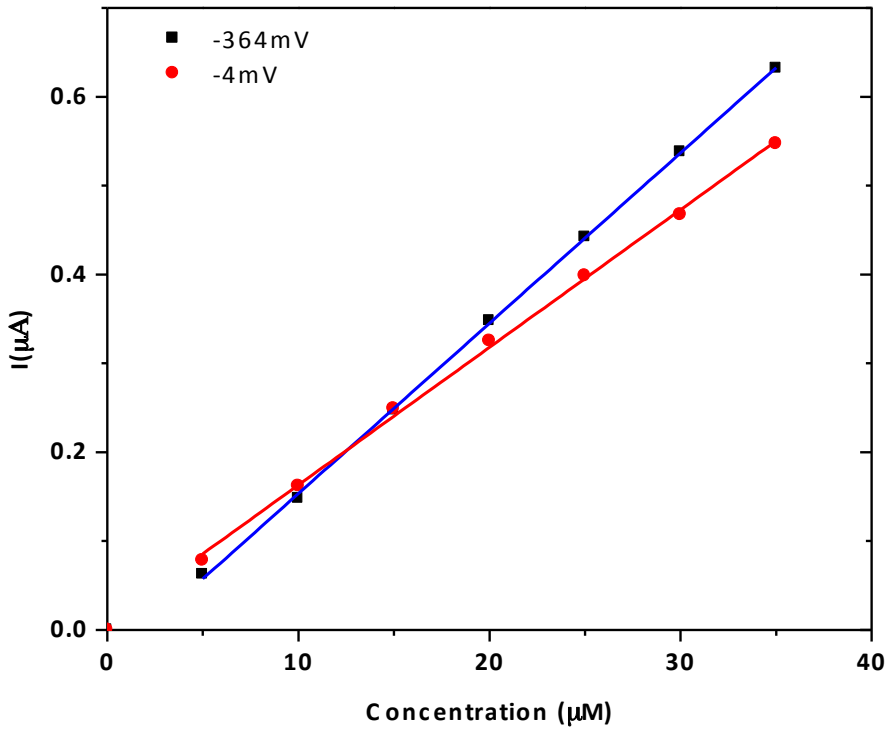

Figure 19. Current response vs concentration at -364mV and -4mV from Figure 17.

4.4 Alternate edition of epinephrine and norepinephrine

From the square wave voltammetry of epinephrine and norepinephrine, it can be seen that these molecule might interfere each other. This might be the limitation when it comes to detecting epinephrine and norepinephrine simultaneously using the unmodified screen-printed carbon electrode. To investigate this interference further, alternate addition of epinephrine and norepinephrine was carried out. After measuring the blank response of the screen-printed carbon electrode in 100μL of pH 7.0 PBS buffer, 5μL of epinephrine and norepinephrine was added alternatively measuring obtaining square wave voltammetry each time. From the Figure 20 it can be seen that one of the reduction peak is indeed overlapped by each molecule. The reduction peak at -380mV seems to be increasing each time regardless of epinephrine or norepinephrine addition. Unlike the peak from -380mV, peak near 0V shows increase only after the addition of norepinephrine. Using this information it is possible to detect epinephrine and norepinephrine with unmodified screen-printed carbon electrode with minor difficulty.

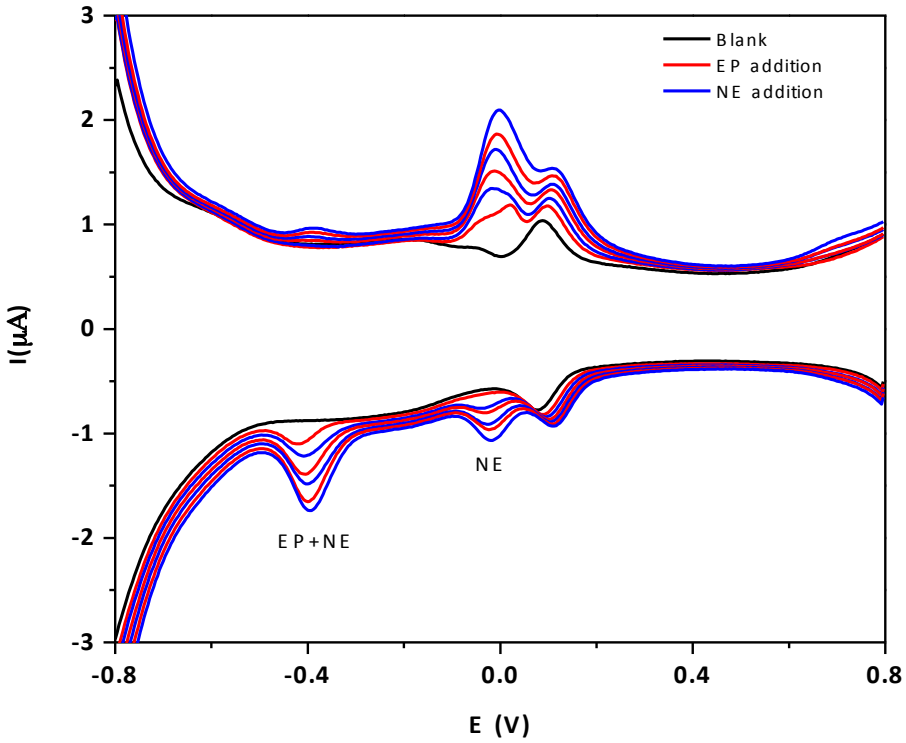

Figure 20. Square wave voltammetry of alternate addition of 5μM epinephrine and 5μM norepinephrine.

4.5 Epinephrine detection in presence of greater interference

In biological system, ascorbic acid and norepinephrine concentrations are actually more than 10 folds than epinephrine and norepinephrine concentration. To mimic this occurrence slightly more, measurement of epinephrine in presence of greater interference was investigated as well. The interference of this procedure was prepared similar to the interference preparation stated above. After getting the blank response in 100μL of pH 7.0 PBS buffer, response of 250μM of ascorbic acid and 250μM of uric acid was measured subsequently in the buffer solution. After interference preparation is complete, 10μM of epinephrine was added three times total obtaining square wave voltammetry after each addition. The obtained square wave voltammetry responses were overlaid as shown in Figure 21. It is clear that the pre-existing interfering molecules in the solution didn't affect the response from the epinephrine addition. This demonstration is significant considering the fact the total concentration of interfering molecules is 50 folds more than an epinephrine concentration. This result confirms the possibility of using unmodified screen-printed carbon electrode for detecting epinephrine and norepinephrine in presence of interference.

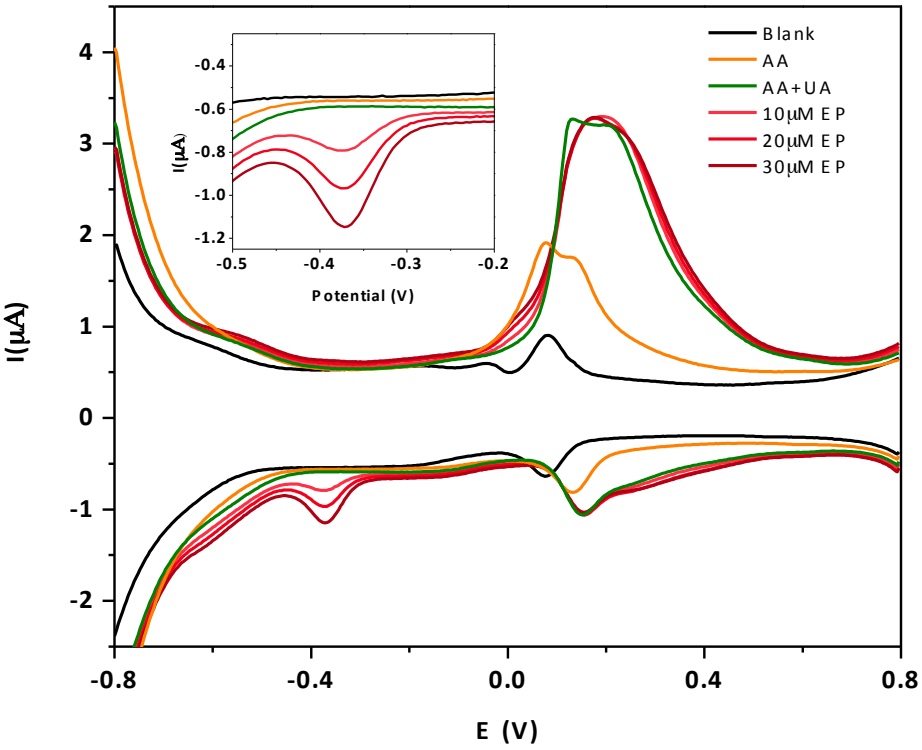

Figure 21. Detection of epinephrine from 10μM to 30μM in10μM interval in presence of 250μM ascorbic acid and 250μM uric acid.

Chapter 5. Conclusion

Epinephrine and norepinephrine are both significant molecule in biological system. The concentration of these molecules can be valuable information in diverse occasion. However, detection of epinephrine and norepinephrine has been challenged due to the presence of the interfering molecule in the biological system. To countermeasure this limitation, complicated and expensive procedures has been carried out to detect epinephrine and norepinephrine. The proposed method utilizes the unmodified screen-printed carbon electrode and electrochemical method to detect epinephrine and norepinephrine. This proposed method lowers the cost significantly as well as simplifies the procedure. By observing different reductive behavior of epinephrine and norepinephrine, detection of these molecules has been simplified.

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