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# Novel impedimetric dopamine biosensor based on boronic acid functional polythiophene modified electrodes

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ABSTRACT

In this study we report a new, simple and first impedimetric biosensor based on 3-Thienyl boronic acid for dopamine detection. Biosensor electrode preparation is 1 min long by simple electro-polymerization of 3-Thienyl boronic acid and copolymer Thiophene P($TBA_{0.50}Th_{0.50}$). Strong interaction between dopamine and thin layer of boronic acid has provided bio-sensing electrode high selectivity and stability, linear range of 7.8 to 125 μM, and detection limit of 0.3 μM. Characterization and optimization studies were conducted using electrochemical impedance spectroscopy (EIS) and cyclic voltammogram (CV). In order to test reliability of proposed biosensor real sample application study has been conducted using non-diluted human urine and it has been found that biosensor selectivity and recovery is excellent. As well P($TBA_{0.50}Th_{0.50}$) based electrode and dopamine interaction has been proven by single frequency impedance measurements. Biosensors acquired good reproducibility, stability, selectivity and very low interference.



## 1. Introduction

Dopamine (DA) is involved in the reward and pleasure centers of the brain as one of the most important catecholamine neurotransmitter in mammalian central nervous system [1,2,3], which also plays a role in the regulation of movement [4], having a stimulating effect on the heart and regulates a flow of information to different areas of brain [5]. Playing role as neuromodulator in the brain and influencing a variety of motivated behaviors [6], abnormal high or low levels of DA can cause several neurological diseases such as Alzheimer's disease for which DA concentration in urine is approximately 20.14 ng/ml (Mean $n = 14$) [7], for Parkinson's disease DA concentration 0.43 μg/g tissue [8] and in urine 0.139 μmol/mol creatinine [9], for Schizophrenia its found to be approximately 208.9 μg/3.5 h ($n = 11$) [10], for Huntington's disease ranges from 0.75 to 5.6 ng/mg tissue which depends on brain region [11], etc. Therefore there is strong need for selective and reliable method which can be used for precise determination of DA concentration in vitro and vivo. Various approaches such as spectrophotometry [12], mass spectrometry [13], high performance liquid chromatography (HPLC) [14], colorimetric detection [15], ion chromatography [16], fluorescent detection [17] have been developed and applied in DA detection. However these methods have some disadvantages such as requiring skilled personnel and highly specialized equipment, time consuming, high cost, complex sample preparations and pretreatments, etc. Where's detection of DA with electrochemical biosensor is straight forward method which does not requires highly skilled personal, takes short time for analysis and most important no need for sample pretreatment. In comparison to other techniques, EIS sensors have received considerable attention due to their remarkably strong operability, high sensitivity, and good selectivity [18].

Detection of DA by different types of electrochemical methods such as differential pulse voltammetry (DPV), cyclic voltammogram (CV) and very small amount of reports based on electrochemical impedance spectroscopy (EIS) has been shown in Table 2. All of them are concentrated on decreasing interference of ascorbic acid (AA) or detection of AA [19,20] which in human body can be as high as several millimolars and its overwhelming oxidation peak covers DA peak [21]. Various strategies based on fact that AA and DA are oppositely-charged at physical pH [22] has been developed to suppress or separate AA oxidation peak or by increasing selectivity towards DA, for example by incorporating Ion exchange membrane Nafion [23] or self-assembled monolayers (SAMs) [24]. In order to overcome the complex producers and try to develop EIS biosensor which will be able to apply in real sample without pretreatments and dilutions we employed 3-Thienyl boronic acid as and polymer surface on electrode. As already known boronic acids can covalently interact with cis-diol- containing molecules to form boronate esters [25,26] and on the other side ortho-quinols such as dopamine has strong interaction with boronic acids [27]. It is well documented that over the pH range of 6–8 two neighboring hydroxyl groups on dopamine undergo relatively fast reversible reaction with boronic acid [26,28]. It was found that dopamine combine with both boronic acid

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functionally attached to polymer back bone [28], and free phenylboronic acid in solution [26]. When dopamine binds to boronic acid moiety, resistance charge transfer (Rct) increases on the other hand the oxidation potential of dopamine shifts to anodic direction, thus avoiding the mask of redox peak of ascorbic acid in excess amount [29]. To the best of our knowledge, electrochemical polymerized film based on 3-thineyl boronic acid and dopamine interaction has not been reported yet. Which are sufficient evidence and reasons to select boronic acid based polymers for impedimetric DA biosensors. There are few works reported using boronic acids such as reported by Plesu et al., impedimetric DA biosensor based poly(aniline boronic acid) [30] and electrochemical DA biosensor based on molecularly imprinted poly(acrylamidophenylboronic acid) film reported by Hong et al. [29].

Herein we report impedimetric DA biosensors based on 3-Thienyl boronic acid. Impedimetric sensors was prepared by one minute single-step electropolymerization of 3-Thienyl boronic acid and copolymer Thiophene (Th) which is first DA biosensor reported based on this polymer. Strong interaction between DA and thin layer of boronic acid provided bio-sensing electrode high selectivity towards DA, which is also supported in literature [27]. Thin layer of P($TBA_{0.50}Th_{0.50}$) has provided ability to detect DA in non-diluted healthy human urine sample with excellent recovery values as well reliability. Interference studies has been conceded with very high concentrations of AA and uric acid (UA) and showed very low interference values and single frequency impedance clearly demonstrated successful interaction between DA and electrode surface. P($TBA_{0.50}Th_{0.50}$) electrode showed excellent selectivity and sensitivity towards DA as well high reliability.

## 2. Experimental

### 2.1. Materials

Dopamine hydrochloride, Thiophene 99% and 3-Thienyl boronic acid were obtained from Sigma-Aldrich, Natrium Fluoride (NaF) and Hydrochloric acid 37% (HCl) purchased from Merck. All other chemicals were of analytical grade and were used without further purification.

### 2.2. Preparation of bio-sensing electrode

Pencil graphite electrode (PGE) with dimensions of 10 mm, 2 mm, and 0.5 mm (length, thickness and height) which makes surface area of 51 $mm^2$ was cleaned by sonication in ethanol to remove adsorbed organic materials or any other impurity from the electrode surface. After cleaning, electrochemical polymerization of 3-Thienyl boronic acid was carried out in a solution of different monomer concentrations (Table 1) and 0.2 M NaF in 0.5 M HCl. Electro-polymerization was carried out in potential range of −1.0 V to 2.0 V in an unstirred solution with the scan rate of 100 mV $s^{-1}$. In the end electrode was washed with $dH_20$ and was ready for use in dopamine detection analysis.

### 2.3. Electrochemical analysis

Electrochemical impedance spectroscopy (EIS) and cyclic voltammogram (CV) measurements were performed via a three electrode system set composed of Ag/AgCl as reference, Platinum (Pt) wire as auxiliary, and electro-polymerized TBA on PGE as working electrode, all immersed in an electrochemical cell filled with 0.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) solution containing 0.1 M KCl which served as redox probe. For EIS studies impedance spectra was collected in the frequency range of 100 Hz to 1000 kHz with applied alternating wave of 10 mV amplitude. Where CV is used for characterization of electro-polymerized electrode surface and was recorded in the range of −0.2 V to 0.7 V with scan rate of 100 mV $s^{-1}$. Once EIS measurements were obtained Rct values were calculated by means of designed electrical equivalent circuit model. Results were analyzed and fit by CHI instruments and Ivium Technologies software's.

**Table 1**
Monomer concentration for electro-polymerized polymers.

| Electrode | 3-Thienyl boronic acid (mM) | Thiophene (mM) |
|---|---|---|
| TBA | 50.0 | 0 |
| P($TBA_{0.75}Th_{0.25}$) | 37.5 | 12.5 |
| P($TBA_{0.50}Th_{0.50}$) | 25.0 | 25.0 |
| P($TBA_{0.25}Th_{0.75}$) | 12.5 | 37.5 |

**Table 2**
Comparison of P($TBA_{0.50}Th_{0.50}$) electrode based DA biosensor with previous reported works.

| Electrode | DM | Linear range | DL | Real sample | Ref. |
|---|---|---|---|---|---|
| Fullerene-$C_{60}$Au | CV | 1 nM–5 μM | 0.26 nM | Urine, serum | [5] |
| Au-DT/MOA | AM | 0.01–5 μM | 20 nM | Urine | [32] |
| TCPP/CCG | DPV, CV | 0.01–70 μM | 0.01 μM | Urine, serum | [1] |
| MIP/GCE | DPV | 50 nM–10 μM | 33 nM | DHI | [25] |
| MWCNT/polv(AABA) | DPV | 50 nM–2 μM | 20 nM | No | [29] |
| PA-MWCNT/GCE | EIS | 10 μM–1 mM | 14.1 μM | No | [33] |
| EPPGE | CV | 0.2–25 μM | 90 nM | Horse blood serum | [34] |
| MWCNT/Nafion-GC | DPV | 10 nM–10 μM | 2.5 nM | No | [35] |
| Poly-Tiron/GCE | DPV | 0.2–45.8 μM | 0.07 μM | DHI | [36] |
| P($TBA_{0.50}Th_{0.50}$) | EIS | 7.8–125 μM | 0.3 μM | Urine | This work |

Detection method (DM); detection limit (DL); cyclic voltammogram (CV); electrochemical impedance spectroscopy (EIS); amperometric (AM), differential pulse voltammetry (DPV), dopamine hydrochloride injection (DHI).

### 2.4. Dopamine detection

After electro-polymerized electrode was assembled it was immersed into the dopamine solution with certain concentrations (Fig. 1). After which it was carefully removed from dopamine solution and gently rinsed with $dH_2O$. When electrode was ready for measurement it was immersed in electrochemical cell containing 0.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) solution from which the electrochemical impedance spectroscopy response value was recorded after 45 min incubation in dopamine solution. In the end, dopamine calibration curves were plot using impedance variations ($\Delta Rct = Rct_{(P(TBA0.50Th0.50)/DA)} - Rct_{(P(TBA0.50Th0.50))}$) with each measurement repeated 3 times ($n = 3$).

### 2.5. Instrumentations

Electrochemical measurements were performed using CompactSoft portable electrochemical interface and impedance analyzer (*Ivium Technologies*) for cyclic voltammogram recodes whereas for electrochemical impedance measurements were carried out using a CHI Model 6005 electrochemical analyzer. Measurements were recorded via a three electrode system set which was composed of Ag/AgCl as reference, Platinum (Pt) wire as auxiliary, and electro-polymerized TBA on PGE as working electrode, all immersed in an electrochemical cell filled with 0.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) solution containing 0.1 M KCl which served as redox probe. Conventional three-electrode electrochemical cell was purchased from CH Instruments. A scanning electron microscope (SEM) was employed to observe the surface of the samples. For SEM, pieces of the samples were mounted on stubs and coated with gold using a sputter coater. SEM micrographs of the samples were taken using a JEOL NeoScope scanning electron microscope.

## 3. Results and discussion

### 3.1. Electrochemical characterizations

Electro-polymerization of 3-Thienyl boronic acid and Thiophene was carried out in potential range of −1.0 V to 2.0 V in an unstirred solution with the scan rate of 100 mV $s^{-1}$ (Fig. 2A). The oxidation peak of Thiophene can be observed around 1.6 V as well peak current tends to decrease by increasing polymerization cycle which might be attributed to loss of electro-activity of P($TBA_{0.50}Th_{0.5}$) due to thickness increase. Cyclic voltammetry of electrodes having different monomer ratios were measured in 0.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) solution containing 0.1 M KCl between −0.2 V and 0.7 V shown in Fig. 2B. The decrease of the conductivity of the other polymer films were observed when the rate of increase in TBA and Th. It is clearly observed that the highest anodic and cathodic (Ipa and Ipc) redox peaks were obtained from the electrode having polymer ratio of 1:1, P($TBA_{0.5}coTh_{0.5}$). This may attributed be the orientation of the polymers affecting the electron transfer. Fig. 2C shows the cyclic voltammograms of the modified electrode with P($TBA_{0.5}coTh_{0.5}$) at various scan rates, ($n$ = 10–300 mV $s^{-1}$) in 0.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) solution containing 0.1 M KCl between −0.2 V and 0.7 V. These cyclic voltammograms were used to examine the variation of the peak currents vs. scan rates of the potential. The plot of the peak current inset of Fig. 2C was linearly dependent on $n^{1/2}$ with a correlation coefficient of 0.9983 for the scan rates of 50–500 mV $s^{-1}$. Well-defined cyclic voltammograms were obtained from a characteristic of a diffusion controlled redox process observed at the all electrodes.

Fig. 2D shows a typical Nyquist plot of electrochemical impedance spectroscopy measurements of bare TBA, P($TBA_{0.25}coTh_{0.75}$), P($TBA_{0.5}coTh_{0.5}$), and P($TBA_{0.75}coTh_{0.25}$). The plot of imaginary ($Z''$) vs. real ($Z'$) parts of the impedance spectrum was obtained as a function of frequency ($\omega$). The charge transfer resistance which increases from right to left due to increasing of the frequency was employed. From the Nyquist plots the diameter of the semicircles were equal to the charge transfer resistance ($R$ct), which controls the electron transfer kinetics [31]. The charge transfer resistance ($R$ct) data was obtained by fitting the EIS graph into a suitable equivalent circuit on each independently fabricated electrode. The $R$ct values for bare PGE, TBA, P($TBA_{0.25}coTh_{0.75}$), P($TBA_{0.5}coTh_{0.5}$), and P($TBA_{0.75}coTh_{0.25}$) electrodes were 5.78 kΩ, 13.5 kΩ, 17.8 kΩ, 7.34 kΩ and 10.93 kΩ, respectively. Fig. 2C shows the results obtained from the EIS graphs confirm the data which obtained from the CV graphs (Fig. 2B). The P($TBA_{0.5}coTh_{0.5}$) electrode gave the lowest charge transfer resistance compared to the other polymer films. The higher $R$ct of the polymer films can be attributed to poor charge conductors due to defect of electron transfer when the ratios of the polymers not equal to each other. It is clearly understood from the figures that 1:1 ratio of the TBA and Th in the polymeric film would increase the electron transfer efficiency and decrease the $R$ct value.

### 3.2. Morphological characterization

The interactions between boronic acid functional polymer and dopamine is monitored by using scanning electron microscopy (SEM). Fig. 3. shows SEM images of the P($TBA_{0.50}Th_{0.5}$) polymer coated electrode and dopamine dipped electrode. The surface of the electrochemically polymerized P($TBA_{0.50}Th_{0.5}$) coated PGE showed porous granular morphology which indicates the formation of polymer on PGE surface. After dipping polymer coated electrode into dopamine solution, the porous

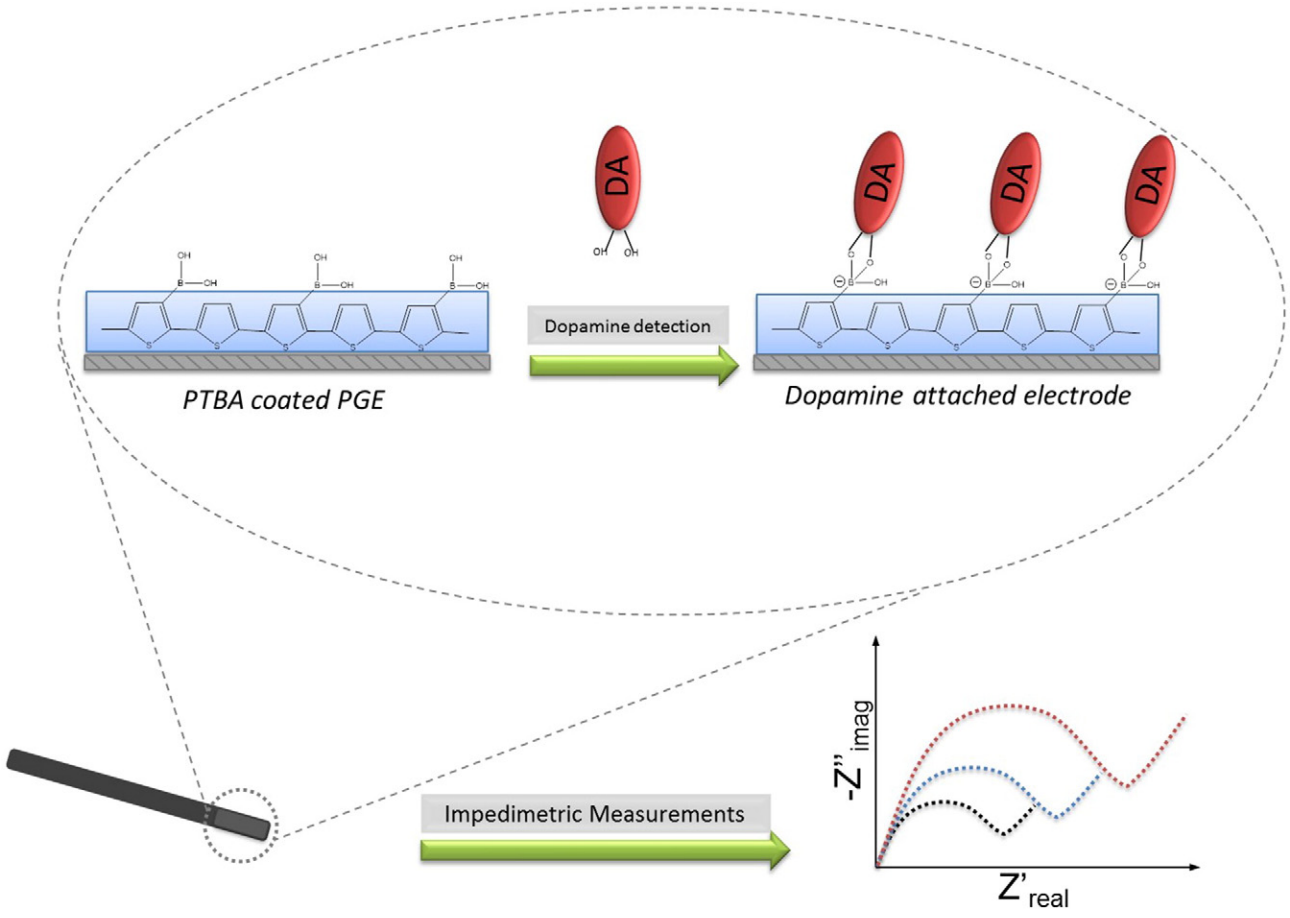


**Fig. 1.** Schematic illustration of dopamine detection of poly(3-Thienyl boronic acid) coated electrodes.

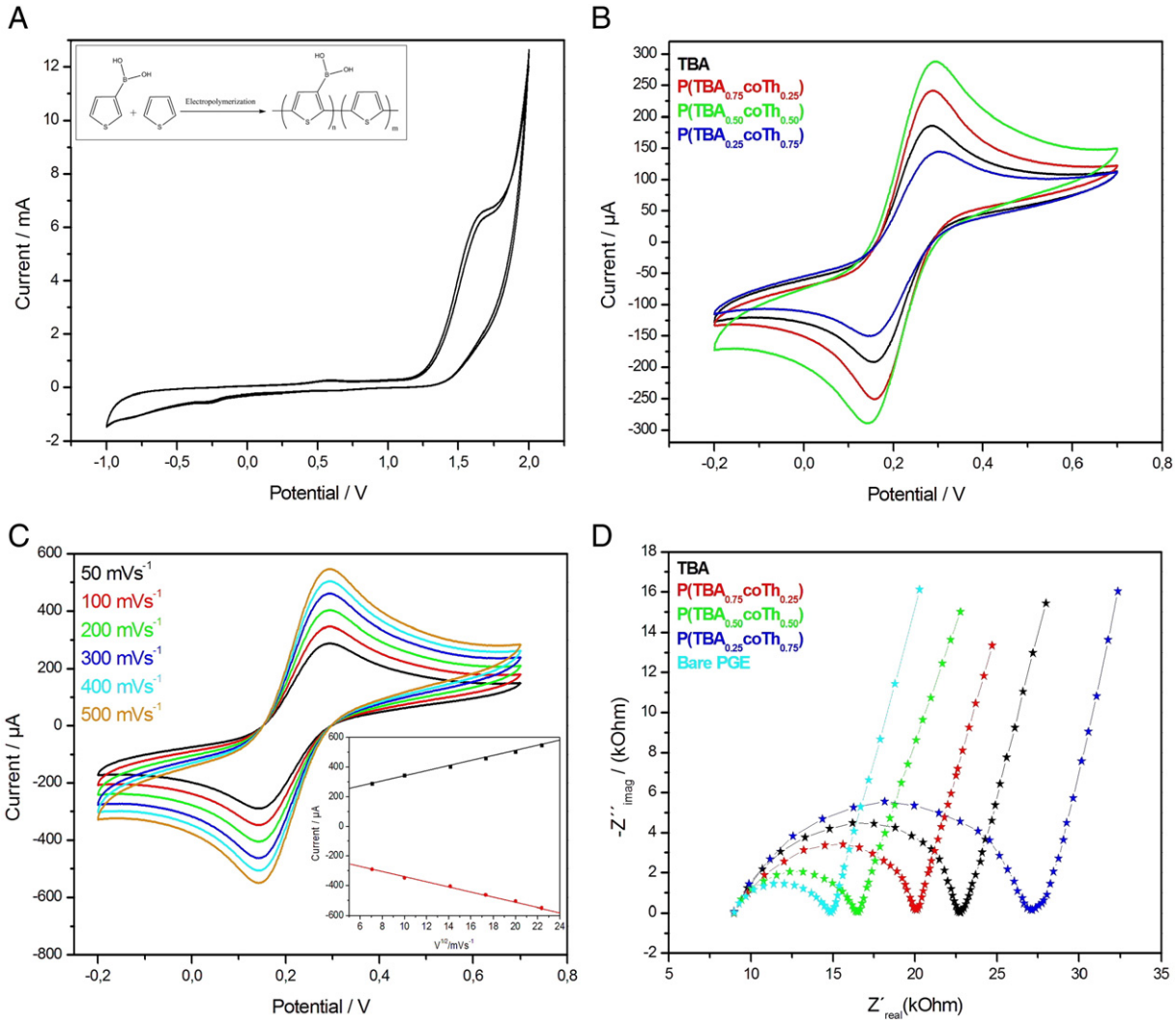


**Fig. 2.** A) Electro-polymerization of $P(TBA_{0.50}Th_{0.5})$ onto graphite pencil electrode. B) Cyclic voltammogram of different monomer ratios (3-Thienyl boronic acid:Thiophene). C) Cyclic voltammetric responses of $P(TBA_{0.50}Th_{0.5})$ coated electrode in 0.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) solution containing 0.1 M KCl at scan rates: (a to j) 50, 100, 200, 300, 400, and 500 mV $s^{-1}$. Inset: Plots of peak currents vs. scan rate. D) Electrochemical impedance spectroscopy of different monomer ratios (3-Thienyl boronic acid:Thiophene).

granular morphology changed to regular form indicating the successful interaction on the surface of the modified electrode.

### 3.3. Optimization studies of impedimetric dopamine biosensor

Polymer thickness of electro-polymerized $P(TBA_{0.50}Th_{0.5})$ electrode was investigated with both EIS and CV in presence and absence of dopamine. Experiments were performed by electro-polymerizing $P(TBA_{0.50}Th_{0.5})$ on electrode surface by increasing the number of scans used in electro-polymerization. After which electrodes were immersed in dopamine solution for 45 min CV and EIS were recorded. As it can observed from Fig. 4A (black), black lines represents CV values as a current versus number of scans of electro-polymerized $P(TBA_{0.50}Th_{0.5})$ where full black line represents $PGE/P(TBA_{0.50}Th_{0.5})$ and black dot line represents $PGE/P(TBA_{0.50}Th_{0.5})$-dopamine. EIS measurements were presented as resistance versus number of scans of electro-polymerized $P(TBA_{0.50}Th_{0.5})$ in red lines where full red line represents $PGE/P(TBA_{0.50}Th_{0.5})$ and red dot line represents $PGE/P(TBA_{0.50}Th_{0.5})$-dopamine (Fig. 4A (red)). The CV and EIS results of the polymer with different polymer thickness showed that the electrical conductivity of the polymer was decreased with increasing polymer thickness. That is due to the electron transfer diffusion barrier of the polymer film thickness. EIS measurements shows increase in resistance as amount of electro-polymerized polymer increases.

Same behavior can be noticed in CV and EIS measurements when $PGE/P(TBA_{0.50}Th_{0.5})$ electrode was immersed in dopamine solution, from which resistance increases as amount of electro-polymerized polymer increases and current decreases amount of electro-polymerized polymer increases. In the end, it can be clearly concluded that amount of dopamine in less thick polymer provides more clear and logical results as polymer thickness increases, as well it is clearly seen that amount of dopamine bound to low thickness polymer is more as compared to higher polymer thickness where dopamine binding is almost unnoticeable. Which might be due to easy interaction of dopamine acid functional groups and dopamine where in thicker polymer dopamine binding ability is decreased.

Incubation time study illustrated in Fig. 4B is conducted by incubation of $PGE/P(TBA_{0.50}Th_{0.50})$ electrode in dopamine solution in time intervals of 5, 15, 30, 45 and 60 min after which EIS measurements were recorded. It can be noticed resistance of electrode increases as time passes until 45 min where resistance starts to be same, from which it can be concluded that 45 min was enough for immobilization of the dopamine on the electrode surface and that there is no further

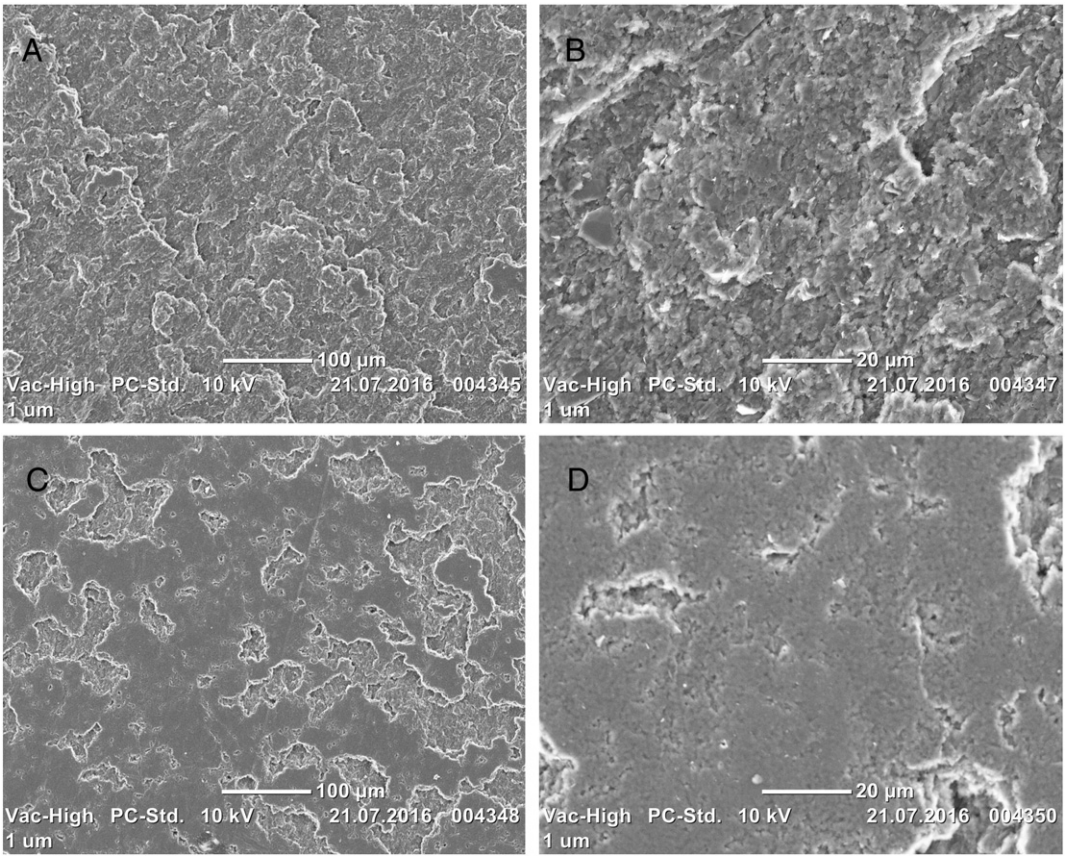


**Fig. 3.** SEM images of the (A & B) $P(TBA_{0.50}Th_{0.5})$ and (C) $P(TBA_{0.50}Th_{0.5})$-dopamine.

accumulation of dopamine. As a final result, 45 min was used as optimum incubation time for further experimental studies.

### 3.4. Analytical characteristics of biosensor

Fig. 5A–D represents the Nyquist plots of TBA, $P(TBA_{0.75}Th_{0.25})$, $P(TBA_{0.50}Th_{0.50})$, $P(TBA_{0.25}Th_{0.75})$ electrodes for the detection of dopamine samples in a concentration range of 7.8 μM, b-15.62 μM, c-31.25 μM, d-62.5 μM, e-125 μM. The charge transfer resistance values in the figures are obtained upon a gradual electrode incubation in different dopamine concentration. For each concentration, electrodes were incubated in dopamine solution for 45 min, followed by $dH_2O$ washing and EIS signals records. As illustrated in the Fig. 5A–D the Rct values of modified electrodes were increased with increasing dopamine concentrations. The increments of the semicircles exhibited to the binding of dopamine to the electrode which act as an insulating layer that decreases the electron diffusion. However the electrode modified with $P(TBA_{0.50}Th_{0.50})$ represented in Fig. 5C gave a better correlation to the dopamine concentrations. Rather than other ratios the 1:1 ratio of the monomers offered better electron diffusion which is attributed to the structure of the polymer. As can be noticed Fig. 5A and B do not have 7.8 μM concentration included and Fig. 5D do not have 7.8 and 15.62 μM DA concentration included. Reason for this is that the electrode modified with different monomer ratios demonstrated different detection abilities which also shows amount of DA bounding to electrodes with different monomer ratios. In this case Fig. 5A and B demonstrated electrodes which could not detect smaller amount of DA than 15.62 and Fig. 5D demonstrates electrode which could not detect DA concentration smaller than 31.25 μM. In this study comparison of electrode modified with different monomer ratios of TBA and Th resulted in that the best monomer ratio for DA detection using TBA and Th is 50:50 ($P(TBA_{0.5}Th_{0.5})$) which have ability to detect DA small as 7.8 μM while other electrodes are not able to do so, which might be due to amount of free active groups on electrode surface.

After EIS measurements were obtained Rct values were obtained by means of designed equivalent circuit model as well it represents perfect fit for dopamine biosensor (Fig. 5E). In equivalent circuit resistance of the all connections and electrolyte has been represented by Rs and capacitance of the bioactive layer is represented by C. Rct is describing the charge transfer resistance trough electrode surface and Zw stands for the Warburg impedance which represents redox probe diffusion through to biosensor surface. In this system Rs and Zw components have not been influenced or affected by immobilization of dopamine in different concentrations.

The analytical performances of impedimetric biosensors were tested under optimized conditions by using same dopamine standards in a range of 7.8 μM–125 μM. Fig. 6 shows the calibration graphs obtained from different polymeric film modified electrodes upon addition of different dopamine levels. Clearly understood from the figure the *Rct* signals were increased with increasing of the dopamine concentrations for each systems. Among the other biosensors $P(TBA_{0.50}Th_{0.50})$ modified system showed better linear range and lowest detection limit. The regression equation was calculated $Rct(kohm) = 0.0944 + 0.1006[DA\mu M]$ with a regression coefficient of (R) 0.9995. The $P(TBA_{0.75}Th_{0.25})$ and TBA modified electrodes showed results almost closed to each other the corresponding regression equations were recorded $Rct(kohm) = 2.179 + 0.0299[DA\mu M]$ and $Rct(kohm) = 0.0727 + 0.0509[DA\mu M]$ with a coefficient of (R) 0.9991 and 0.9984 respectively. The lowest biosensor performance according to the others was obtained from the $P(TBA_{0.25}Th_{0.75})$ based electrode which showed a linear range between 31 μM–63 μM and equation found to be $Rct(kohm) = -1.291 + 0.0446[DA\mu M]$ with a regression coefficient of (R) 0.9999. $P(TBA_{0.50}Th_{0.50})$ based bio-sensing electrode had the smallest detection limit, sensitivity and widest linear range in DA analyses which might be contributed to the low *Rct* value.

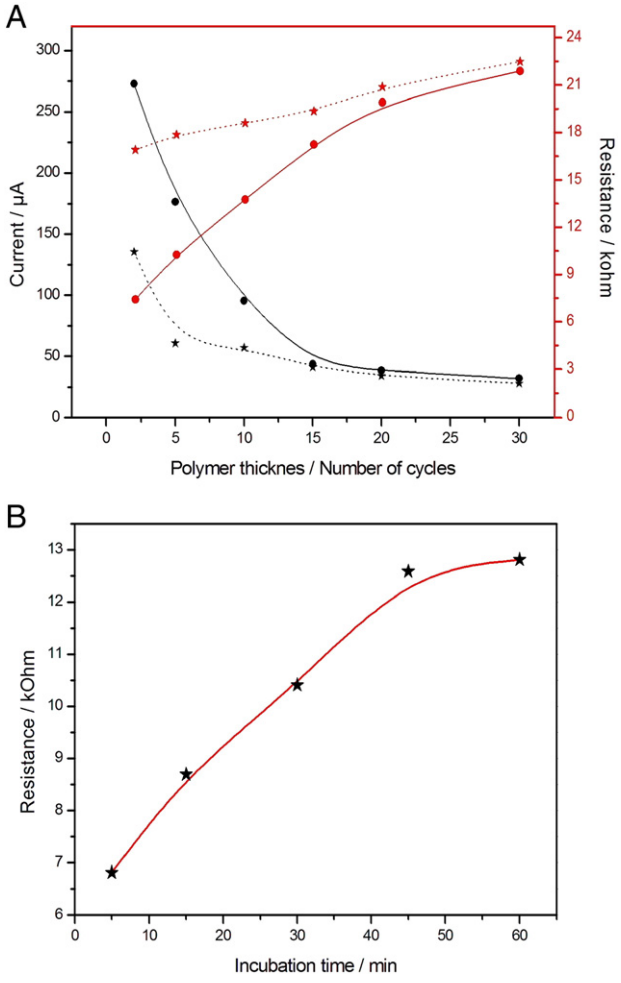


**Fig. 4.** A) Polymer thickness of electro-polymerized P($TBA_{0.50}Th_{0.5}$) by a) cyclic voltammogram of polymerized electrode in absence (black full) and presence (black dot) of dopamine and b) electrochemical impedance spectroscopy of polymerized electrode in absence (red full) and presence (red dot) of dopamine. B) Optimum incubation time of polymerized electrode in dopamine for 5, 15, 30, 45, 60 min.

In order to define the characteristics of these systems, obtained results were compared with the DA biosensors reported in literature (Table 1). The result in Table 1 shows biosensors prepared with different methods. In most cases P($TBA_{0.50}Th_{0.50}$) modified biosensor is able to detect DA in a wide range with low detection limit and as such can easily compete with reported biosensors. For Limit detection following formula might be used; LD = std/S, where std. stands for standard deviation and S stands for sensitivity. In this work for detection limit smallest amount which was detected by P($TBA_{0.5}Th_{0.5}$) modified electrode was chosen as LD, 7.8 μM.

### 3.5. Single frequency impedance (SFI)

Single frequency impedance (SFI) is method which enables simultaneous monitoring of variations in phase angle and impedance versus time at a fixed frequency which is obtained from frequency containing information plot or Bode plot. SFI method can be performed in DNA-protein, protein-protein interactions, or interactions among biomolecules [37]. SFI is preformed since it is already accepted method in biosensors applications [38] and it is usually applied for the investigation of molecular interactions occurring between modified electrode surface and dopamine. In this experiment SFI was carried out by using constant frequency of 5.5 Hz. As illustrated in Fig. 7A. SFI presents information related to dopamine and electrode surface molecular interaction from which red curve shows variations in phase angle in function of time and black curve stands for variations in the impedance value as a function of time. There has been noticed significant change in phase angle (Δphase angle) of 4.2° in 60 min and change in charge transfer resistance (ΔRct) of 1.9458 kΩ in 60 min. From this observation it can be concluded that SFI results agree with typical impedance measurement results and that SFI can be used to evaluate slow time dependent changes on bio-sensing electrode surface as well it proves 45 min incubation time since SFI results demonstrate almost no change after 45 min. Fig. 7B, represents Bode plots of electrodes with different monomer ratios obtained from measurements using 120 μM dopamine, in order to see direct relationship of logarithm of impedance and phase degree with logarithm of frequency. Solid lines represents log(Z) vs. log(frequency) where comparison of the binding ability of dopamine to electrode with different monomer ratios were represented, and from which it can be clearly seen that P($TBA_{0.50}Th_{0.50}$) base electrode highest Rct values as compared to other monomer ratios. Fig. 7B, dash lines illustrate phase angle vs. log (frequency) plot. As it can be seen in the intermediate frequency region the phase angle decreases with a log(frequency) increase which can be observed for all monomer ratios and sensitivity of low frequency region is higher than intermediate one. Low frequency region for P($TBA_{0.50}Th_{0.50}$) is almost 25% higher that P($TBA_{0.25}Th_{0.75}$).

### 3.6. Analytical recovery, interference and real sample

Analytical recovery experiment of P($TBA_{0.50}Th_{0.50}$) based electrode has been performed by EIS recording using 25, 50 and 100 μM pure dopamine solution prepared by PBS pH 7.4. (Table 3). Reason to perform this experiment is to determine the effectiveness of a bio-sensing electrode after addition of known amount of dopamine in solution. After obtaining Rct ($\Delta Rct = Rct_{(P(TBA0.50Th0.50)/DA)} - Rct_{(P(TBA0.50Th0.50))}$) values of EIS measurements dopamine amount was calculated using calibration curve (Fig. 6). In the end, biosensor effectiveness has been calculated in error rate (%) from which it can be observed that proposed electrode is of high reliability.

Interference studies on EIS measurements presented as Nyquist plot of P($TBA_{0.50}Th_{0.50}$) electrode have been conducted using 1 mM Ascorbic acid (AA) and 1 mM Uric acid (UA) and compared with 30 μM Dopamine EIS measurements. UA has been selected since real sample of biosensor has been conducted in human urine in which UA concentration is high and AA selection is based on its similar structure to dopamine as well its presence in urine. Fig. 8. represent Nyquist plot of EIS measurements in presence of potential interfering substances which shows no remarkable effect on response of P($TBA_{0.50}Th_{0.50}$)/DA electrode. Obtained Rct values have been compared to P($TBA_{0.50}Th_{0.50}$) electrode an it has been concluded that error caused by UA is 0.08% where's error caused by AA was 0.354%, from which it can be seen that interfering substances have no remarkable effect on EIS measurements of P($TBA_{0.50}Th_{0.50}$) based dopamine biosensor. Interfering results of AA are also supported by Plesu et al., where there was no interfering effect by AA on EIS measurements [30]. Zhong et al., reported in Biosensors and Bioelectronics journal results which include interference study using 100 times higher concentration of AA than DA to test electrode based on pyrrole-phenylboronic acid and as a result effect on electrode response was 10% [25] as well Fabre and Taillebois show response towards DA without AA interference in their study [28]. P($TBA_{0.50}Th_{0.50}$) electrode showed ability to detect small amount of DA concentration as well interference has no remarkable effect, and it has to be noted that AA concentration in human urine can be high as 5.7 mM and UA concentration in urine can be normal 3.9 mM and abnormal 9.39 [40]. Than looking at the results demonstrated in Table 4 (real sample) which shows detection of DA in health human urine sample where 30 μM of DA is successfully

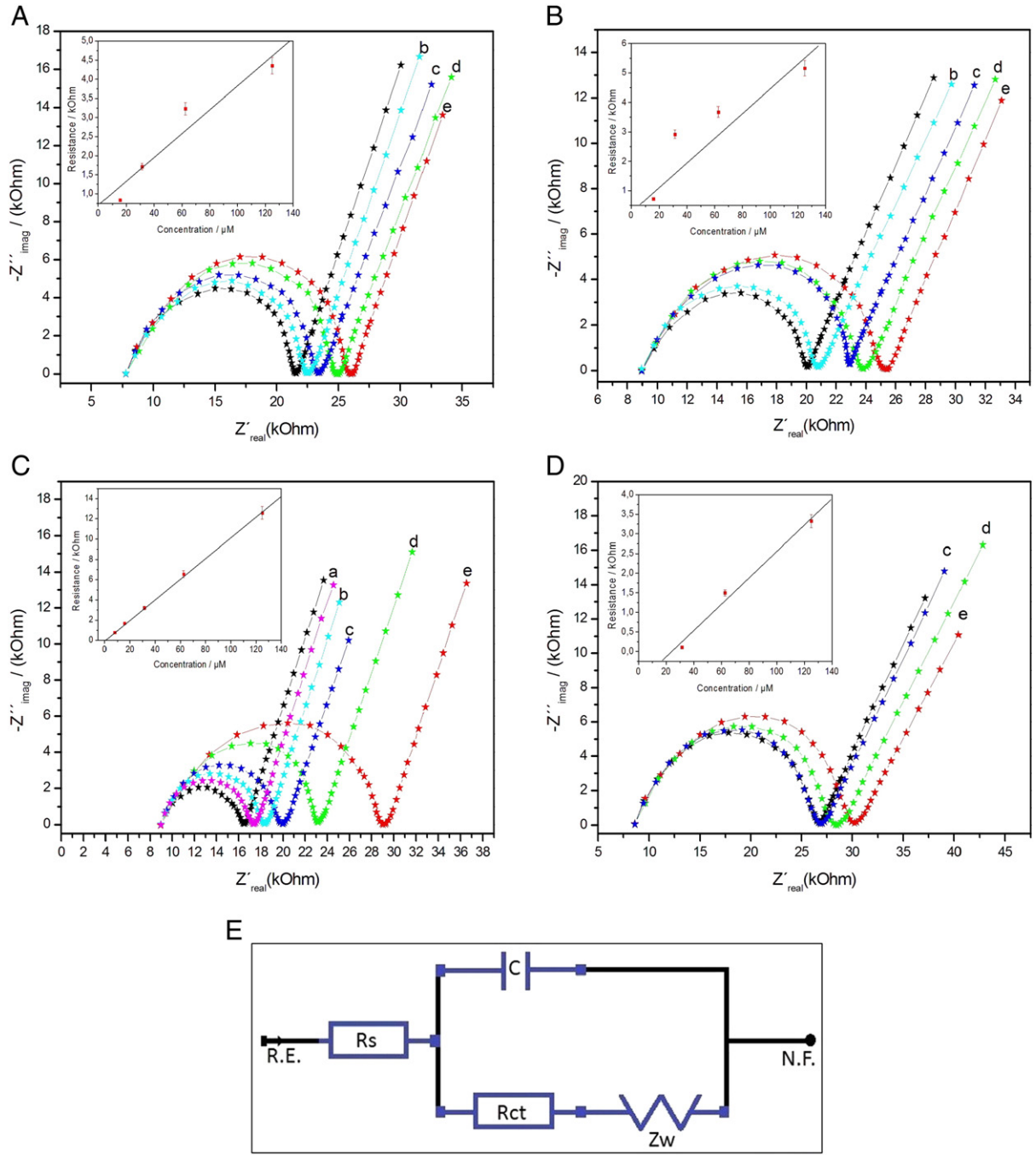


**Fig. 5.** Electrochemical impedance spectroscopy of polymerized electrodes with different monomer ratios A) TBA, B) P($TBA_{0.75}Th_{0.25}$), C) P($TBA_{0.50}Th_{0.50}$), D) P($TBA_{0.25}Th_{0.75}$) in presence of different dopamine concentrations; a-7.8 μM, b-15.62 μM, c-31.25 μM, d-62.5 μM, e-125 μM E) Developed electrical equivalent circuit model for the fabricated electrode.

detected. In conclusion 0.354% of AA interference has no remarkable effect on electrode response and P($TBA_{0.50}Th_{0.50}$) modified electrode has ability to detect small amounts of DA among huge amount of AA and UA interference molecules.

Real sample has been conducted after proving successful work and reliability of P($TBA_{0.5}Th_{0.5}$) based biosensor using healthy non-diluted human urine. Since normal level of dopamine in healthy human can vary from 0.3 to 2.6 μM/day [32,39] is low, non-diluted human urine samples were spiked with a known concentration of standard dopamine solution after which electrode recovery was investigated. Dopamine detection in human urine was conducted using proposed method and obtain results are listed in Table 4. Recovery of three different amount of DA solution added into urine sample contained 30, 60, 90 μM, was from 104.8 to 111.4% which indicates that proposed method is free from interferences in the urine. As well real sample has been tested with no dopamine added and concentration found was 2.4 μM ($n = 3$) which can be attributed normal concentration level of dopamine in normal human urine. In order to demonstrate real recovery of proposed biosensor from found DA concentration daily concentration of DA in human urine was subtracted and showed as "Theoretical Recovery" which varies from 104.8 to 111.4%, which once more shows proof for electrode selectivity towards DA. Obtained real sample results reliability can be attributed to electrode preparation method, minimum interference of AA and non for UA and specificity of boronic acid towards diols such as dopamine. It's important to note that urine samples were not diluted during sample preparation which increases the originality and better usage efficiency where's in most of dopamine studies have urine sample have been diluted 100 times [22]. In aqueous media the

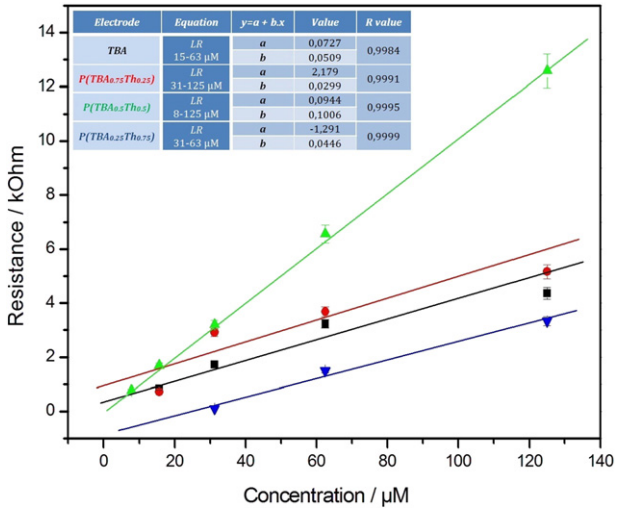


**Fig. 6.** Calibration curves of electro-polymerized electrodes.

formation of boronate ester from boronic acid and diol is dependent on the pH of the solution [29], as well it is well documented that over the pH range of 6–8 two neighboring hydroxyl groups on dopamine

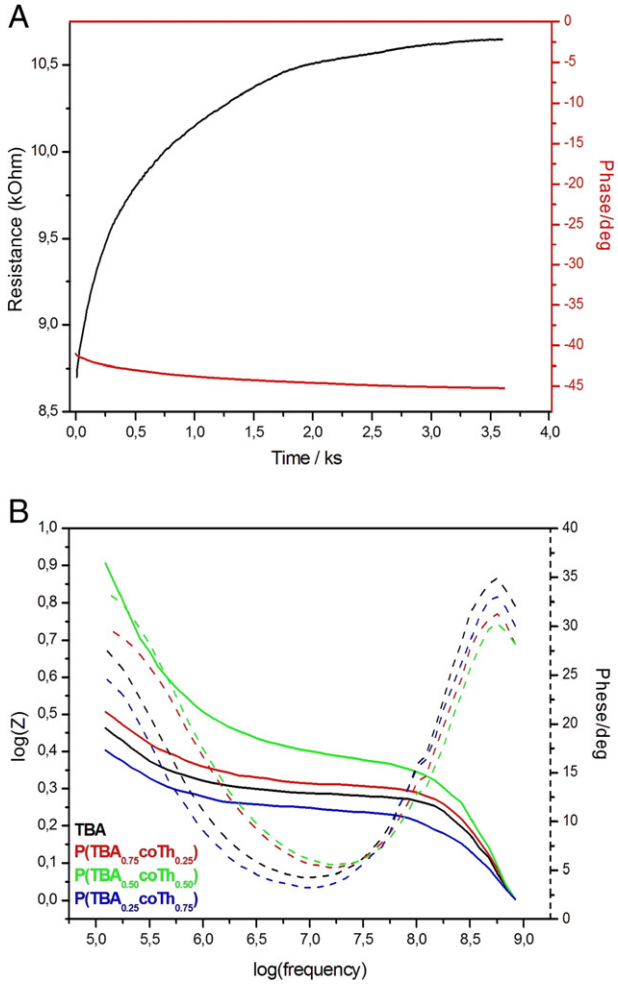


**Fig. 7.** A) Single frequency impedance (SFI) experiments of impedimetric biosensor, (red) variations in phase angle and (black) variations in the impedance value, B) Bode plot of electrodes with different monomer ratios, comparison in detection of 120 µM dopamine: log(Z) versus log(frequency) (solid lines) and phase/deg versus log(frequency) (dash lines).

**Table 3**
Analytical recovery of impedimetric dopamine biosensor.

| Added dopamine µM | Dopamine detected µM | RSD% | Recovery % |
|---|---|---|---|
| 25.0 | 26.2 | 0.35 | 104.8 |
| 50.0 | 48.35 | 0.5 | 96.7 |
| 100.0 | 103.5 | 0.8 | 103.5 |

undergo relatively fast reversible reaction with boronic acid [26,28]. Hong et al., reported in work that the amount of dopamine taken into the molecular imprinted polymer film as pH increased. Experiments have been analyzed in the pH solution with the range of 5 to 10. As seen from reported results difference in obtained striping current obtained at physiological pH (7.4) and at pH 9.0 is almost 9% which is relatively low [29]. According to the our results in Fig. S1, the optimal pH value of the incubation solution was pH 8.0. Since there is no big difference in dopamine binding at physiological and at higher pH and taking in consideration real application of bio-sensing electrode optimization in this work were done according to physiological pH in order to obtain real time results and by that reliability and accuracy in real samples is very high.

## 4. Conclusion

In the present work, a reliable biosensor was performed for the detection of dopamine from non-diluted urine samples with high sensitivity and specificity. Using the interaction between boronic acid and dopamine a selective systems was designed and best copolymer ratio was obtained. Electrochemical polymerization of two different monomers were conducted to fabricate electrodes in different ratios which lead to increase sensitivity. Based on the measurements the P($TBA_{0.50}Th_{0.50}$) electrodes showed a better performance for detections with a low detection limit of 0.3 µM and exhibited a wide linear range between 7.8–125 µM with a linear regression coefficient of (R) 0.9995. In order to exhibit the usability, the selectivity of the proposed biosensor was performed using different molecules. Moreover, real recovery analysis of the biosensor to the target DA was successfully performed in non-diluted urine samples with high selectivity and sensitivity. The comparison study was clarified the performances of these biosensors and results shows that the biosensor (P($TBA_{0.50}Th_{0.50}$)) can be used for the detection of DA in biological samples successfully.

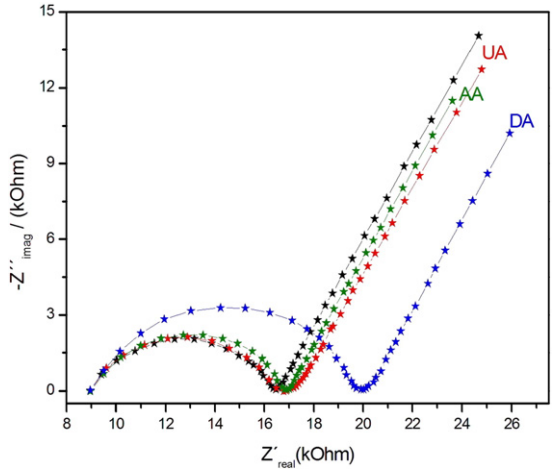


**Fig. 8.** Interference study on proposed impedimetric biosensors using of polymer coated electrode (PCE), uric acid (UA), of ascorbic acid (AA) and dopamine (DA).

**Table 4**
Determination of dopamine in human urine samples by P($TBA_{0.5}Th_{0.5}$) electrode ($n = 3$).

| Urine sample | DA μM | DA detected μM | Recovery (%) | Normal DA in human urine | Theoretical Recovery (%) |
|---|---|---|---|---|---|
| Sample 1 | 30.0 | 33.42 | 111.4 | 2.4 | 103.4 |
| Sample 2 | 60.0 | 65.2 | 108.6 | 2.4 | 104.6 |
| Sample 3 | 90.0 | 94.34 | 104.8 | 2.4 | 102.15 |

Note: Theoretical Recovery is calculated by subtracting DA concentration found in normal human urine (daily).
Recovery (%) $= [C_{(found)} / C_{(added)}] * 100$, $n = 3$.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.msec.2016.11.127.

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