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Centri-Voltammetric Dopamine Detection

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Centri-voltammetry which includes the combination of centrifugal forces and voltammetry is a kind of new method. In this work, centri-voltammetry was applied for detection of very important biological molecule Dopamin (DA) for the first time. After the optimization of centrifugal parameters, linear range was obtained between $5x10^{-9}$M - $5x10^{-6}$M DA with the equation of y=7.8206x-0.3575 and correlation coefficient of R^2=0.997. RSD values were calculated for 10^{-7}M DA (n=5) and found as 1.37%. LOD (3s/m) and LOQ (10s/m) values were also calculated and found as $1.007x10^{-9}$M and $1.023x10^{-9}$M, respectively. Also the detection of DA in the presence of ascorbic acid (AA) has been conducted and it has been found that in the presence of 100 fold AA, it is possible to obtain accesible DA peaks.

1.Introduction:

Centri-voltammetry was developed by Huseyin TURAL and Ulku ANIK. Later the method was improved by Ullku ANIK's group and some other groups by applying the method fort he detection of variaty of analytes[1,2,3,4,5,6,7,8,9]. Centri-voltammetry is a two steps process. At the first step the analyte is accumulated onto the electrode surface and then in the second step, voltammetry is applied for analyzing the deposited analyte. A special centri-voltammetric cell is needed for conducting the centri-voltammetric experiments[1,2,3,4,5,6,7,8,9]. Since 2004 centri-voltammetric applications including heavy metal analysis[1,2,3,4,5] and biological molecule analyses[5,6] have been done. Then in 2011, the same group developed the method biocentri-voltammetry which includes the combination of biosensor system with centrifugal force[5,7,8,9].

Dopamine (DA) is a natural catecholamine formed by the decarboxylation of 3,4-dihydroxyphenylalanine and belongs to the family of excitatory chemical neurotransmitters[10]. DA plays a significant role in the renal, cardiovascular, central nervous and hormonal systems[11]. For central nervous system, DA is one of the important neurotransmitters and is widely distributed in the mammalian central nervous system for signaling. It is mainly responsible for the reward sensation, and transmits the information of excitement and fun, but at the same time, is related to the addiction[12]. Abnormalities in dopamine concentrations have been linked to several neurological disorders, for example, parkinson epilepsy and schizophrenia[13,14].For this reason, it is of great importance to monitor DA by applying sensitive and selective methods for biomedical chemistry, neurochemistry research and diagnostic and pathological purposes[15].

To date, several impressive techniques have demonstrated the feasibility for the assay of DA, including electrochemical[16,17], colorimetric[18,19], fluorescence spectrometric[20], liquid chromatographic/electrospray tandem mass spectrometric[21], and high performance liquid chromatographic methods[22]. Among these, electrochemical approaches have attracted most attention because of their simplicity, low instrumental cost, and capability in real-time and even in vivo measurements[15]. However, since uric acid or ascorbic acid (AA) in the biological samples has similar oxidation potentials close to that of DA, interferences are caused by these compounds in electrochemical DA detection[23]. To overcome these interferences several precautions, like modification of plain electrodes, have been taken. In some works, electrodes were modified by cation-exchange membranes[24,25], prussian blue[26] , ruthenium (III) diphenyldithio-carbamate[27] and polypyrrole(-dodecyl sulphate)[28] . Also nanomaterials like CNT[29] and graphen[30,31], cyclodextrin/graphene[32] , GO[33] , polypyrrole/graphene[31] have been used as modifiers.

In this study, a new approach was developed for DA determination. Centri-voltammetry was applied for the first time for DA detection. After the optimization of centrifugal parameters, a calibration graph was obtained. Also the effect of AA on DA voltammetric signals was investigated.

2. Experimental

2.1 Apparatus:

The oxidation signal of DA was investigated by applying cyclic voltammetry (CV) after centrifugation. For CVs µ-AUTOLAB Type III electrochemical analysis system and NOVA 1.10 software package were used. Sigma 3-16 pk centrifuge was used for centrifugation. The experiments were conducted in a 10 mL centri-voltammetric cell, using a three-electrode configuration (Figure 1). GCPE, Ag/AgCl and platinum wire were used as working, reference and counter electrode, respectively.

2.2 Reagents:

Dopamine hydrochloride, KCl, $CaCl_2$, Urea, Tris-HCl were purched from SIGMA. $Na_2HPO_4.12H_2O$, NaCl, D+Glucose monohydrate, $MgCl_2$ were purchased from MERCK. KH_2PO_4 was purchased from Riedel de Haen AG.

Synthetic plasma sample was prepared by incorporating the reagents into the Tris-HCl including 140 mM NaCl, 4.7 mM D+Glucose monohydrate, 0.8 mM $MgCl_2$ from Merck, 4.5 mM KCl, 2.5 mM $CaCl_2$, 2.5 mM Urea, 10 mM Tris HCl from Sigma.

2.3 Procedure

As can be followed from Fig 1, glassy carbon paste electrode (GCPE) was prepared by hand-mixing of 80:20 (%w/w) glassy carbon powder/ mineral oil and used as a working electrode where it was put at the end of centri-voltammetric cell. DA solution was placed into the centri-voltammetric cell and centrifugated for an amount of time at a proper rpm. Finally, the cell was carefully placed in the voltammetric stand where reference and counter electrodes were immersed into the working solution. CVs were recorded in the potantial range between -0.2 V and +0.8 V in phosphate buffer (pH 7.0).

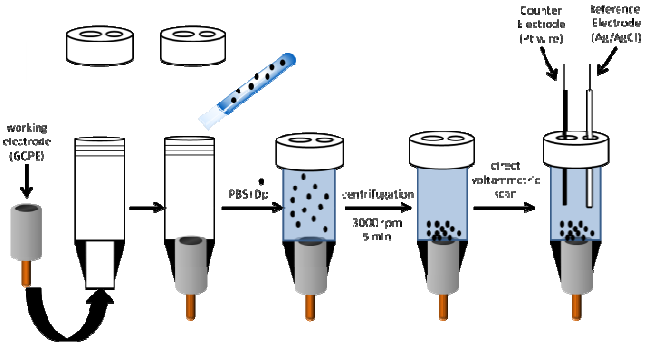

Figure 1. Schematic representation of centri-voltammetric procedure

3. Results and Discussions:

Centri-voltammetry can be accepted as a newly developed method. By applying this method, the analyte is preconcentrated onto electrode surface by centrifugal force. For this reason, more sensitive results were expected with centri-voltammetry. On the other hand, application of this technique for DA detection would extend the scope of this method.

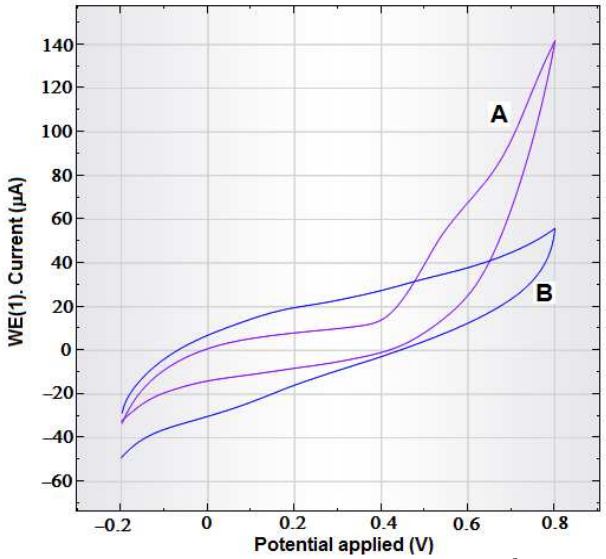

Figure 2. The effect of centri-voltammetry on 10^{-9} M DA. A) is centri-voltammetric, B) is plane voltammetric results.(centrifugation time is 5 min., centrifugation speed is 3000 rpm, scan rate is 100mV/s and step pot. is 24 mV/s.)

Figure 2 demonstrates the effect of centrifugation on the cyclic voltammetric results. It is obvious that centri-voltammetry improves the peak for 5 x 10^{-9} M DA (Fig 2 A and B). More significant peaks are observed. Also, by applying centri-voltammetry, higher current values (with centri-voltammetry 4 µA; without centri-voltammetry 2.01 µA) were obtained. Though it seems like with plain voltammetry smaller anodic potential (Ea) is obtained, the split in centri-voltammetric peak must be taken into account ($E_{a\ plain}$= 0.217 V; $E_{a\ centri}$= 0.542 V). The split in the peak can be attributed to the electron transfer from different layers around the GCPE which was placed at the bottom of the centri-voltammetric cell. Formation of different layers might be due to application of centrifugal forces. Also, with GCPE more reversible voltammogram was obtained compared to centri-voltammetric one (Fig 2 A and B). As a result it can be concluded that centrifugation changes the shape of the voltammogram and provides more sensitive results by increasing the obtained current value (Figure 2).

3.1 Optimization of Centrifugal Parameters:

The centrifugation parameters are very important in centri-voltammetry. Because centrifuge is the main force for the coprecipitation of analyte on electrode surface[1,2,3,4,5,6,7,8,9] . At the first series of experiments the effect of centrifugation time was investigated. For this purpose, centrifugation times of 1, 3, 5 and 7 min. were applied on centri-voltammetric cell at 3000 rpm (Figure 3a). As can clearly be seen from the graph best resullts were obtained at 5 min. For optimization of centrifugation speed, 1000, 2000, 3000 and 4000 rpm was applied to the centri-voltammetric cell in the presence of 2.5x10^{-6} M DA, 50 mM PBS (pH 7.0) for 5 min (Figure 3b). As a result, best current value was obtained at 3000 rpm.

The increase up to 3000 rpm can be related to the analyte amount increase on the electrode surface. The decrease after that value might be due to removal of some analyte on the electrode surface because of the higher speeds. Almost similar explanation could be reasonable for current increase and decrease at longer period of times. Up to 5 min., since the preconcentartion of analyte onto electrode surface

Electrode	LOD	Linear range	References
laccase/SiO_2-PA/GCE	0.26 ± 0.003 µM	0.99 – 103.10 µM	Wang et al.[34]
Au@PPy/GS /GCE	18.29 pM.	0.1–5000 nM	Qian et al.[35]
CS–ZnO/PANI /GCE	0.21 µM	20×10^{-5} to 180×10^{-5}M	Pandiselvi et al.[36]
DR/SWNT/GE	2.1 $x10^{-3}$ mM	1 to 40 mM	Yu et al.[15]
HRP–MWCNTs–SiSG/Poly (Gly)/CPE	6×10^{-7}M	15–865 µM	Raghu et al.[37]
HAu-G/GCE	0.05 µM	0.08 to 600 µM	Zhu et al.[38]
Au-DT/MOA/GE	20 nM	0.01–5 µM	Tsai et al.[39]
GCPE(centri-voltammetry)	1.007x10-9M	5x10-9M - 5x10-6M	Present Work

increases, the current value also increases. For longer times, because of analyte loss onto the electrode surface the current value decreases[8].

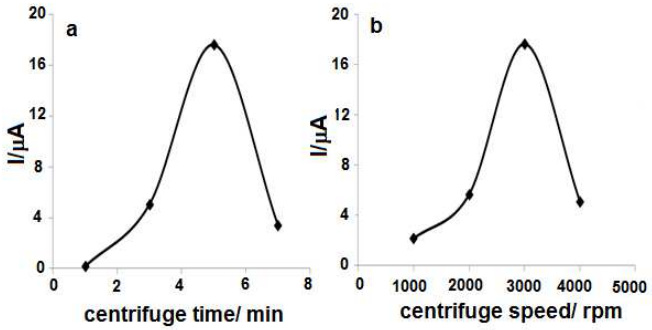

Figure 3. The effects of centrifugation parameters on the sensor response, a) centrifuge time, b) centrifuge speed for $2.5x10^{-6}$M DA. Conditions: in phosphate buffer solution (pH 7.0), working potential: -0.2V - +0.8V.

3.2 Analytical Characteristics:

After the optimization of centrifugal parameters, analytical characteristics were investigated. As can be seen from Figure 4., linear range was obtained between $5x10^{-9}$M - $5x10^{-6}$M DA with the equation of y=7.8206x-0.3575 and correlation coefficient of R^2=0.997. RSD values were calculated for 10^{-7}M DA (n=5) and found as 1.37%. LOD (3s/m) and LOQ (10s/m) values were also calculated and found as $1.007x10^{-9}$M and $1.023x10^{-9}$M, respectively. For comparison, DA detection studies with modified electrodes were examined and summerized in Table 1.

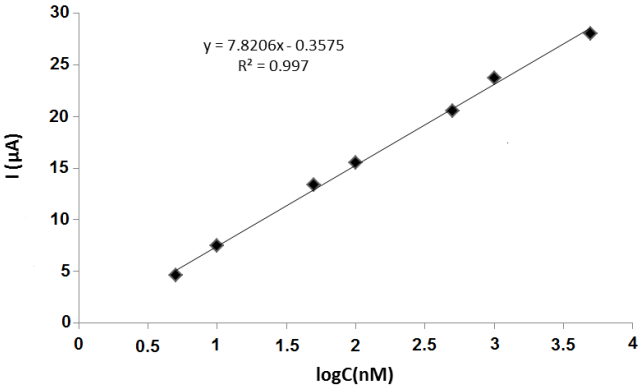

Figure 4. Calibration graph

Table 1. Comparison of some study about detection of DA.

GCE: Glassy carbon electrode, CPE: Carbon paste electrode, GE: Gold Electrode, PA: Phytic acid, PPy: Polypyrrole, GS: Graphene oxide hybrid sheets, CS: Chitosan, PANI: Polyaniline, DR: Diazoresin, SWNT: Single-walled carbon nanotube, MWCNT: Multiwalled carbon nanotubes, HRP: Horseradish peroxidase, SiSG: Silica sol–gel, Poly(Gly): Polyglycine, HAu-G: Gold-graphene, Au-DT: Three-dimensional gold nanodendrite, MOA: 8-mercaptooctanoic acid

Considering developed method's LOD value and linear range, it can be said that centri-voltammetry is a valid method for DA detection. On the other hand, compared to the modified electrodes mentioned in Table 1, centri-voltammetry provides practicality for detection of DA.

3.3 Sample Application and Interference Study:

As described in the experimental part, a synthetic serum sample solution was prepared. Then standard addition of 1 x 10^{-6}M DA was added into this solution. The experiment was repeated for five times. As a result, recovery value of 106%±2.169 was calculated.

For interference studies, the same concentration, ten fold more and a hundred fold more AA was added to the $1x10^{-6}$ M DA solution. The results were given in Table 2. As can be seen from the Table 2, acceptable values were obtained with centri-voltammetry.

Table 2. Resuts of interference study

Concentrations	Recovery Values
10^{-6}M DA	%100.00
10^{-6}M DA+10^{-6}M AA	%106.65
10^{-6}M DA+10^{-5}M AA	%105.26
10^{-6}M DA+10^{-4}M AA	%112.29

4. Conclusions:

In this work, centri-voltammetry was applied for the first time for the detection of DA. Obtained results demonstrated that

developed method is suitable for DA detection. In the presence of 1, 10 and 100 fold AA, it was possible to detect the analyte in acceptable limits. Considering modification of electrodes, centri-voltammetry provides practicality with the combination of electrochemistry and centrifugation. Further works are ongoing in our lab for improvement of centri-voltammetry

Notes

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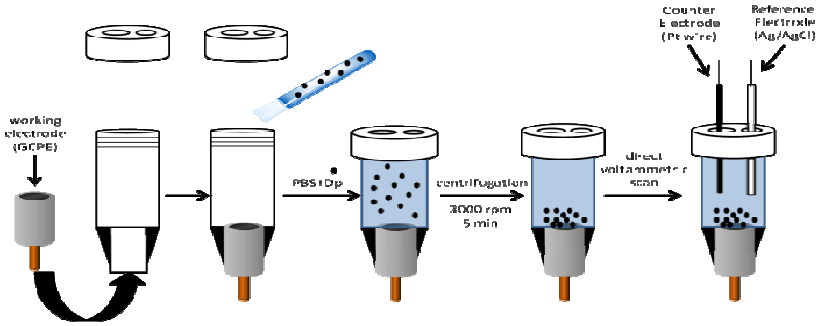

Centri-voltammetry is a novel method that combines centrifuge with voltammetry. In the present work centri-voltammetric detection of DA has been mad efor the first time.