Electrochemical Detection of Dopamine in Presence of Serotonin and Ascorbic acid at Tetraoctyl ammonium bromide Modified Carbon Paste Electrode: A Voltammetric Study

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Abstract

An electrochemical simultaneous evaluation of dopamine (DA), serotonin (5-HT) and ascorbic acid (AA) in phosphate buffer solution (PBS) of pH 7.4 was attempted using a cationic surfactant, tetraoctylammonium bromide, modified carbon paste electrode (TOABMCPE). At the modified electrode, a well-defined redox peak with a great enhancement in current was witnessed. The oxidation of DA was found to be greatly pH dependent. The TOAB fabricated electrode overcomes the problem of resolution of DA with the coexisting species, AA and 5-HT. Simultaneous studies through cyclic voltammetric and differential voltammetric techniques gave excellent results with a great potential difference between DA-AA and DA-5-HT. The detection limit of the modified electrode was found to be 0.019 µM with the aid of amperometry. The developed sensor was applied for the detection of DA in injection samples.

Keywords: Surfactants; Modified electrode; Dopamine; Biosensor; Simultaneous

Introduction

The challenge of determination of dopamine (DA) and serotonin (5-HT) concentration in the presence of ascorbic acid (AA) has triggered the development of voltammetric sensors in the recent past. DA, chemically known as 4-(2-aminoethyl) benzene-1, 2-diol, widely distributed in the mammalian brain tissues, is one of the crucial catecholamine neurotransmitters [1] which plays a pivotal role in the function of the cardiovascular, hormonal, renal and central nervous systems [2-5]. Abnormal levels of DA are linked with various disorders including, Parkinson’s disease, Tourette's syndrome, Schizophrenia, attention deficit hyperactive disorder and generation of pituitary tumors [6-8]. Since the determination of DA in biological systems is informative in the diagnosis of aforementioned diseases, it is currently the subject of interest in biologically oriented research. Likewise, Serotonin (5-hydroxytryptamine or 5-HT), a monoamine neurotransmitter which is also extensively distributed in the brain, correspondingly plays significant roles in various pharmacological, physical and biological processes including temperature regulation, muscle contraction, liver regeneration, endocrine regulation and depression [9-13]. Copious studies revealed the influence that DA and 5-HT have on each other in their respective releasing. Consequently, simultaneous measurement of DA and 5-HT is a necessity, while they coexist in a biological system [14]. Conversely, a major obstacle encountered in the simultaneous determination of DA and 5-HT is the co-existence of high concentration of AA in vivo, of which the oxidation potential is adjacent to that of DA and 5-HT at the bare electrode, resulting in an overlapping voltammetric response. A great deal of effort has been directed towards the separation of anodic peaks of DA, 5-HT and AA with the assistance of several chemically modified electrodes [15-18]. Parallel determination of several neurotransmitters through CV, however, remains a challenge. The strategy now is to design electrodes that can allow simultaneous detection of plentiful neurotransmitters, while eliminating the interfering effects of AA.

Surfactants, a class of amphiphilic molecules with a hydrophilic head on one side and a long hydrophobic tail on the other, have marked extensive applications in the field of electroanalytical chemistry [19,20] and electrochemistry [21,22] because of the enhancement effect and its ability to improve the property of the electrode-solution interface. The usage of surfactants as modifiers to improve the electrode quality has been reported previously [19,23-26]. This electrode exhibited strong cation exchange property and improved electron transfer rate between the substrates and the electrode. Surfactants have also been in use for the immobilization of biomolecules on electrodes. Their applications are also extended successfully in the study of proteins. The electron transfer from electrode to redox proteins including, myoglobin, hemoglobin, was found to be facilitated in the surfactant films [27-30]. The modified carbon working electrode with Cationic surfactants including (CTAB) has proved significant improvement in the electrochemical response of various species [31]. Tetraoctylammonium bromide (TOAB), a quaternary ammonium compound with the chemical formula, [CH3(CH2)7]4N Br, finds its general application as a phase transfer catalyst between an aqueous and an organic solution.

The development of a sensitive electrochemical method for the simultaneous determination of DA, AA and 5-HT through a modified BCPE with a cationic surfactant (TOAB) was the core objective of this work. The electrochemical behavior of DA, AA and 5-HT were...
investigated. A great escalation in the oxidation peak currents of DA, AA and 5-HT was observed at the TOABMCPE, compared with those at BCPE. With this background, a novel, sensitive and convenient electrochemical method was developed for the individual and simultaneous determination of these compounds, which could find its successful application in the determination of dopamine in dopamine hydrochloride injection.

**Experimental Section**

**Chemical reagents**

DA stock solution was prepared in 0.1M perchloric acid, whereas 0.1 mM TOAB, 0.1 mM 5-HT and 1 mM AA in double distilled water. Aforementioned chemicals were of analytical grade and were used without any further purification. DA was purchased from Sterile Specialities India Pvt Ltd and Ajantha Pharma (Enikepadu, Vijayawada). Standard method was employed in the preparation of Phosphat buffers. The water used was double distilled. All the experiments were performed at room temperature.

**Apparatus and procedure**

A VSP-potentiotstat/galvanostat (Biologic Science Instruments) was utilized to conduct the experiments. All the experiments bore a conventional three-electrode system which consisted of a working carbon paste electrode with homemade cavity of 3 mm diameter, a platinum wire as counter electrode and a saturated calomel electrode as reference electrode. 70% graphite powder and 30% silicon oil was manually ground in an agitate mortar for about 30 minute to get a homogenous mixture for the preparation of a bare carbon paste electrode. The paste was packed into the cavity CPE and smoothened on weighing paper. 30 µl TOAB was immobilized on the surface of electrode. The paste was packed into the cavity CPE and smoothened on weighing paper. 30 µl TOAB was immobilized on the surface of carbon paste electrode for 15 min to prepare the modified electrode. After this, the fabricated TOAB modified CPE was washed with water and data were recorded in pH 7.4 PBS.

**Results and Discussion**

**Surface morphology of the TOABMCPE**

Scanning electron microscopy is apt to exemplify the electrode surface morphology. Figure S1 showed the surface morphology of TOABMCPE. Isolated and irregularly shaped graphite flakes of BCPE surface had completely transformed into smooth and regularly arranged particles throughout the surface of TOABMCPE. The significant differences in the surface morphology of both electrodes suggest that the TOAB were entrenched onto the surface of BCPE.

**Electrochemical behavior of potassium ferrocyanide at TOABMCPE**

Figure 1 showed the cyclic voltammograms of 1mM K₄Fe(CN)₆ in 1M KCl as supporting electrolyte at TOABMCPE. At TOABMCPE, the background current was greatly enlarged compared with that at bare CPE. This indicates the significant change in surface property of the modified electrode. In contrast to the poor response at bare CPE (dotted line), the electrochemical signal of K₄Fe(CN)₆ showed an enhancement at TOABMCPE (solid line), which is reflected in the improvement of both the shape of redox peaks and the magnification of peak currents. The oxidation and reduction peaks were located at 242 mV and 194 mV respectively at the bare CPE. Whereas, at the TOABMCPE, in the potential range of -200 to 600 mV, a pair of well-defined redox peaks appeared at 234 mV and 175 mV with ΔEp = 59 mV. These outcomes reveal the excellent catalytic activity of TOABMCPE towards the electro active species.

The TOABMCPE provides more surface area than the unmodified carbon paste electrode. Cyclic voltammetry of 1 mM K₄Fe(CN)₆ in 0.1M KCl solution at various scan rates gave information about the surface area. From Randles–Sewcik equation,

\[
Ip = 2.69 \times 10^{n/2}ACo D^{1/2}υ^{1/2}
\]

where \(I_p\) is the peak current, \(n\) is the number of electrons transferred, \(A\) is the surface area of the electrode (cm²), \(D\) is the diffusion coefficient of the molecule in solution (cm²/s), \(υ\) is the scan rate (V/s) and \(Co\) corresponds to the bulk concentration of the probe (mol/cm³). The surface area could be calculated from the slope of the plot of \(I_p\) Vs \(υ^{1/2}\)

(1mM K₄Fe(CN)₆, \(n=1\), \(D=7.6 \times 10^{-6}\) cm²/s) and was found to be 0.0024 cm² for BCPE and 0.0048 cm² for TOABMCPE.

**Optimization of concentration and immobilization time of TOAB**

The electrochemical response of K₄Fe(CN)₆ at the BCPE and TOABMCPE clearly illustrates that the TOABMCPE can remarkably improve the redox peak current of K₄Fe(CN)₆. However, further studies showed that the amount of TOAB in modified carbon paste electrode also affects the electrochemical response of K₄Fe(CN)₆. The influence of TOAB concentration towards the oxidation peak current of K₄Fe(CN)₆, Figure S2a. With the gradual increase in concentration of TOAB, the oxidation peak current first increased sharply up to 30 µl, and then steadily declined. The micellar effect of TOAB might be playing a significant role here, because the peak current changed abruptly around the critical micellar concentration (CMC) of the surfactants. With the aid of information obtained above, 30 µl of TOAB was employed for the fabrication of modified electrode in the work.

The influence of immobilization time on the oxidation peak current of K₄Fe(CN)₆ is exemplified in Figure S2b. When the immobilization time was raised from 0 to 15 min, the oxidation peak current of K₄Fe(CN)₆ also showed great improvement. The increase in immobilization time enhanced the accumulation of K₄Fe(CN)₆ at the TOABMCPE surface due to the strong adsorption ability of TAOB. Undoubtedly, the oxidation peak current of K₄Fe(CN)₆ remarkably increases. However, a very slight increase in the oxidation peak current of K₄Fe(CN)₆ was observed when the accumulation time was extended from 15 to 25 min. In order to achieve a quicker analysis with greater sensitivity, 15 min accumulation time was selected in this work.

**Electrochemical impedance spectroscopy**

The Electrochemical impedance spectroscopy was used for the
better understanding of electrochemical properties of the electrode-solution interface. Figure 2 showed Nyquist plot of both BCPE and MCPE in PBS at pH 7.4. Inset is the equivalent circuit used to analyze the impedance behavior. Large diameter of the semicircle at unmodified electrode with $R_{ct}$ value 3.623 kΩ indicates the higher electrode resistance towards the electron transfer processes. But after the modification with TOAB, diameter of the semicircle reduces ($R_{ct}$ 0.925 kΩ), which implies the lesser resistance by modified electrode towards the charge transfer processes.

**Electrocatalytic oxidation of DA at TOABMCPE**

Figure 3 displayed the cyclic voltammograms of 1 µM DA in pH 7.4 PBS at BCPE and TOABMCPE. At the BCPE, a pair of redox peak was observed with the oxidation peak potential at 193 mV and the reduction peak potential at 147 mV (dashed line). Whereas the TOABMCPE gave birth to significantly enhanced peak current and a more reversible electron transfer process to DA (solid line) under similar conditions. A well-defined redox wave of DA was observed with respective anodic and cathodic peak potentials at 200 and 152 mV. The separation of peak potentials at the TOABMCPE, $\Delta E_p (=E_{pa}−E_{pc})$, was 52 mV, which was in agreement with the Nernst reversible behavior and approximated the number of electrons involved in the reaction to be two. Intensive increase witnessed in peak current could be owed to the improvement in reversibility of electron transfer process and the larger real surface area of the TOAB film. The repulsion between cationic form of DA and tetraoctylammonium cations adsorbed layer on the surface of modified electrode paved the path to the over potential and increase in the current signals (Scheme S1). To ensure whether the redox peak appeared was for modifier or due to the presence of DA, a blank cyclic voltammogram was recorded only for phosphate buffer (dotted line). Consequently, there were no peaks obtained for the modifier, which suggests the redox peaks obtained were due to the redox behavior of DA. An efficient oxidation reaction toward DA at the TOABMCPE could be inferred through this.

**Effect of concentration of dopamine**

The electrocatalytic oxidation of DA was measured by varying its concentration at TOABMCPE. With the increasing concentration of DA from 1 µM to 6 µM, Figure S3 the electrochemical anodic and cathodic peak currents increases with a slight shift of $E_{pa}$ and $E_{pc}$ towards the positive direction. The graph of anodic peak current vs. concentration of DA displays a linear relationship within the range of 0.10 µM to 2 µM with the linear regression equation, $I_{pa} (\mu A)=0.00313 \times C_{DA} \mu M/L$, with a correlation coefficient of 0.997.

Chronoamperometry was devoted in the determination of lower detection limit. (Figure 4a) showed the chronoamperometric response of TOABMCPE towards DA oxidation in 0.1 M PBS of pH 7.4 at 1200 mV. The resultant calibration graph of current vs. DA concentration (Figure 4b) exhibited a linearity in the lower concentration region (4 × 10^{-6} M). Inset shows magnified chronoamperogram from 300 to 700 seconds of time.
10⁴ to 6 x 10⁻⁶) with R²=0.994 and one in higher concentration range (6 x 10⁻⁶ to 1 x 10⁻⁴) with R²=0.998. This proves the applicability of the modified electrode both in lower and higher concentration ranges of DA. Through calibration graph, the detection limit determined for DA was 0.019 μM, based on three times the standard deviation of blank method. The performance of TOABMCPE was compared with the reported surfactant modified electrodes and the results obtained were as denoted in the Table S1.

Effect of scan rate

The effect of scan rate on the anodic peak current of 1 μM DA at TOABMCPE was studied by cyclic voltammetry (Figure 5). The figure clearly depicts that both the anodic and cathodic peak current increases while the scan rate is increased from 50 to 350 mV. The excellent linearity obtained between the scan rate and Ipa within the range of 50-350 mV/s intimates a surface-controlled process on the modified electrode surface Figure S4. The linear equation was ip (µA)=-0.52+0.548υ (mV/s) (r=0.999). This result also denoted the great affinity that DA possessed towards the TAOBMCPE. This suggests the significant role of diffusion in the electrochemical process. Therefore the electrode process assumed to be controlled by diffusion process.

Effect of pH on the voltammetric response of DA

The oxidation of 1 μM DA was significantly influenced by the pH of the supporting electrolyte. The variation in peak current and peak potential of DA at TOABMCPE under the influence of change in p⁰ was investigated in the p⁰ range of 3.4 to 11.4 Figure S5b. As shown in Figure S5a, with a roughly linear increase in pH, the peak potential (Epc) for DA became more negative. The plot of Epc versus p⁰ was linear with the equation of best fit being: Epc=-0.52 + 0.027 p⁰ (R²=0.9986). The involvement of equal number of protons and electrons in the electrochemical process could be inferred from this result [32]. Likewise, the effect of p⁰ on the peak current is also depicted in Figure S5c. Although, a gradual increase in the peak current of DA with the increase of p⁰ from 4.0 to 7.4 was observed with a maximum value at p⁰ 7.4, it remarkably decreased with further increase in p⁰. Therefore, the phosphate buffer with p⁰ 7.4 was chosen as the supporting electrolyte for the subsequent experiments.

Electrocatalytic response of AA and 5-HT at TOABMCPE

The individual voltammetric response of AA and 5-HT at both BCPE and TOABMCPE are shown in Figures 6a and 6b respectively. The cyclic voltammogram of 0.1 mM AA in 0.1 M phosphate buffer solution at p⁰ 7.4 showed its anodic peak potential at 229 mV at BCPE (dotted line) with less current response, when compared to the anodic peak potential at TOABMCPE (solid line), under similar conditions, a highly enhanced anodic peak potential was obtained at around -14 mV, which presented a negative shift. This negative shift could be attributed to the presence of cationic monolayer which created the electrosstatic attraction of AA. This results in the large shift in the anodic peak potential of AA. The scan rate effect showed that reaction taking place at the electrode surface was diffusion controlled process for AA. The R² was found to be 0.9989. Correspondingly, in p⁰ 7.4 PBS, the TOABMCPE also possessed strongly electrocatalytic action for 5-HT. Regardless if it is at a BCPE or at the TOABMCPE, only could the oxidation peak be observed in p⁰ 7.4 PBS, which confirmed the irreversibility of the electrochemical reaction of 5-HT. At the BCPE the oxidation peak observed for 20 μM 5-HT was patulous with potential of about 301 mV. Meanwhile at the TOABMCPE, the anodic peak potential produced a little negative shift and the shape of the oxidation peak became sharp and symmetrical at the potential of 283 mV. It is certain that the TOABMCPE intensively catalyzed the electrochemical oxidation of 5-HT in p⁰ 7.4 PBS.

Electrocatalytic oxidation of mixture of AA, DA and 5HT

AA, 5-HT and DA coexist in the extra-cellular fluid of the central nervous system. The similar oxidation potential of these three at most solid electrodes is a great problem due to their overlapping signal, therefore separate determination of these species is necessitous. To
evaluate the sensitivity and selectivity of the present system for the quantification of these molecules, the electrochemical behavior of the mixture of the three at TOABMCPE was examined. Figure 7a showed the CVs of mixture of AA, 5-HT and DA at BCPE and at TOABMCPE in 0.1 M PBS, where the dotted line and solid line correspond to the oxidation of a mixture of 0.1 mM AA, 20 µM 5-HT and 1µM DA at TOABMCPE and BCPE, respectively. As envisaged, a poor current response was observed at the BCPE and three well-defined anodic peaks at the potential of -6.4 mV, 190 mV and 297 mV were observed for the oxidation of AA, DA and 5-HT respectively at the TOABMCPE. At the physiological pH of 7.4, DA, 5-HT and AA exists in different ionic forms, AA is in anionic form (pK_a=4.10) while DA (pK_a=8.87) and 5-HT are in cationic form Captivating the advantage of the opposite micelle effect of DA, 5-HT and AA, a simultaneous quantification of these bioactive compounds was done using TOABMCPE. The difference between the potentials of AA and DA peak was about 196.4 mV and that of DA and 5-HT difference was 107 mV, which is sufficient enough for the simultaneous quantification of AA, DA and 5-HT in a mixture. Merely a small oxidation peak shift for DA in the presence of AA indicates the non-interference of these species with each other. The reduction peak of DA in the modified electrode remains unchanged. The above results indicated the exhibition of TOABMCPE with excellent catalytic activity for simultaneous determination of AA, DA and 5HT at reduced and well-separated peak potentials with enhanced sensitivity.

The simultaneous investigation was also done using differential pulse voltammetry (Figure 7b). DPV was employed for its higher current sensitivity with better resolution. The corresponding anodic peak potentials for DA, AA and 5-HT were at 190, -6.4 and 312 mV respectively. The peak to peak separation for DA-AA and 5-HT-DA were 196.4 and 122 mV which were more in comparison to the separation achieved by CV.

The oxidation of DA and 5-HT in the presence of increasing concentrations of AA and vice versa were further studied to evaluate the analytical utility of the TOABMCPE. Figure 7c showed the DPVs recorded for the increasing concentration of DA in the presence of 0.1 mM AA and 20 µM 5-HT. The curves (a), (b), (c), (d) and (e) correspond to the presence of 1,2,3,4 and 5 µM, respectively. As perceived, an increase in the current response at the DA oxidation peak with the increase in the concentration of DA without any change in the AA and 5-HT peaks could be recognized. Similarly, increase in the concentration of AA in the presence of DA and 5-HT, did not give rise to any significant change in the oxidation current of DA and 5-HT. The curves (a), (b), (c), (d), (e) and (f), in Figure 7d, correspond to the oxidation of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mM AA in the presence of 1 µM DA and 20 µM 5-HT. An increase in current for AA with the increase in the concentration of AA without any significant change in the oxidation of DA and 5-HT could be clearly distinguished through
A DA level of 1 µM was 3.0%. When not in use, the TOABMCPE was stored in 0.1 M phosphate buffer of pI 7.4 [36,37]. After 15 days of storage at room temperature, 96% of the initial response was observed, indicating the stability of TOABMCPE. The TOABMCPE could be refreshed by simply immersing in the distilled water at the end of every experiment.

Conclusion

In this work, a TOABMCPE was fabricated and its characteristics were comprehensively studied. Both CV and DPV techniques were employed in the simultaneous determination of AA, DA and 5-HT in the PBS of pI 7.4 at the modified electrode. The modified electrode exhibited good stability and sensitivity. Well defined and discrete voltammetric oxidation peaks were observed. The detection limits (LOD) of DA at the TOABMCPE were found to be 0.019 µM. Moreover, the possible interference of AA and UA in the determination of DA was also studied using the modified electrode and the proposed method has been practically and successfully applied for the determination of DA in dopamine injection samples.

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