Mixed metal oxide based electrochemical sensing of inhibitory neurotransmitter serotonin in the presence of dopamine

Rajasree G Krishnan, Beena Saraswathyamma, Sreedhar K M, Sofia David, Alisha P Prakash, and Greehma S

Department of Chemistry, Amrita School of Arts and Sciences, Amrita Vishwa Vidyapeetham, Amritapuri Campus, Clappana P. O. Kollam, India, 690525.

E-mail: *beenas@am.amrita.edu

Abstract: A mixed metal oxide modified electrode has been employed for the voltammetric determination of neurotransmitter serotonin in the presence of dopamine. The electrode was morphologically and electrochemically characterized using scanning electron microscope and cyclic voltammetry respectively. Differential pulse voltammetry were performed to measure the serotonin concentration at the electrode surface. A dynamic linear range of 0.2-52 µM was obtained. Viability of the sensor was tested in blood sample.

1. INTRODUCTION

5-hydroxytryptamine, widely recognized as serotonin is an essential biochemical in the human body that has an ability to act both as a neurotransmitter in the central nervous system as well as a hormone precursor in the pineal gland[1]. It has inhibitory effects on neuron, making it identified as inhibitory neurotransmitter like gamma-aminobutyric acid and glycine. It is synthesised through a dual step pathway in which an amino acid L-tryptophan is converted to L-5OH-tryptophan by an enzyme called tryptophan hydroxylase and this intermediate formed gets converted to serotonin by an aromatic L-amino acid decarboxylase [2]. It plays a vital role in regulating physiological functions such as mood, sleep, appetite and sexuality. Normal serotonin levels in the whole blood are in the range of 0.5-1.2 µM, 0.3–0.7 µM in urine, and less than 0.0591 nM in cerebrospinal fluid [3]. The reduced levels of serotonin may cause mood related disorders, depression, migraine, insomnia etc. whereas the increased levels of serotonin can range from mild shivering to muscle rigidity and also visible in carcinoid syndrome, Sudden Infant Death Syndrome (SIDS) etc. Serotonin cannot be directly taken from food but tryptophan rich foods can be taken through diet and gets converted to serotonin in the brain. Therefore the selective detection and measurement of 5-HT level is of great importance and can help in understanding the serotonin levels inside the body. Serotonin being electroactive can be used in electrochemical systems which possess high sensitivity, low cost, fewer reagents etc. compare to other analytical techniques [4-6].

Herein we report an electrochemical sensor for the determination of serotonin in the presence of another common neurotransmitter dopamine. The electrode modification employed here is a mixed metal oxide thin film comprising manganese and molybdenum as the metals used.
2. EXPERIMENTAL

2.1. Chemicals
Serotonin hydrochloride and Dopamine hydrochloride was obtained from Sigma Aldrich. Pencil lead with a diameter of 0.7 mm (Cello Fine Leads Pvt. Ltd.) was purchased from a stationary store. All other chemicals are of analytical grade and used without further purification. Millipore water (18.2 MΩ cm) was used to prepare all solutions. Phosphate buffer solution (PBS) used in this study was prepared from Na₂HPO₄ and NaH₂PO₄. All experiments were carried out at room temperature.

2.2. Instrumentation
Electrochemical studies such as cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed with electrochemical analyzer (CHI 610E, CH Instruments, USA). Typical three electrode cell setup was used. Mixed metal oxide modified pencil lead was chosen as the working electrode with platinum wire as counter and silver/silver chloride (Ag/AgCl (1 M KCl)) as the reference electrode. All the potentials referenced here are with respect to Ag/AgCl (1M KCl). Morphology analysis was performed with scanning electron microscope JEOL JSM-7610F Plus.

2.3. Fabrication of the electrode
The electrode for serotonin measurement was fabricated using electrodeposition procedure. The procedure for the electrodeposition was taken from a previously published article by Nakayama et al.[7]. The deposition bath contains aqueous solutions of 2mM MnSO₄.7H₂O with 10 mM (NH₄)₂MoO₄ and 120mM Na₂SO₄. CV was used to electrodeposit mixed metal oxide thin film with potential cycling between 0 and +1.0 V vs Ag/AgCl at a scan rate of 20 mV/s. Electrodeposition was carried out on a pencil lead electrode (PLE) with 0.5 cm length exposed and the rest covered up with a teflon tape. The resultant electrode was designated as Mn-Mo mixed oxide thin films modified pencil lead electrode (Mn-Mo mixed oxide/PLE) and used for further studies.

3. RESULTS

3.1. Morphological analysis of Mn-Mo mixed oxide/PLE
Figure 1 describes the SEM image of PLE and Mn-Mo mixed oxide/PLE. Here the PLE surface was found to be smooth and as the mixed metal oxide was deposited the surface morphology was turned to be rough with a dense morphology.

![SEM image of PLE and Mn-Mo mixed oxide/PLE at different magnifications.](image-url)

**Figure 1** SEM image of PLE and Mn-Mo mixed oxide/PLE at different magnifications.
3.2. Electrochemical characterization of Mn-Mo mixed oxide/PLE

Figure 2 A shows the cyclic voltammogram obtained for the Mn-Mo mixed oxide/PLE in the presence and absence of serotonin in 0.1 M PBS 8. CV was performed by scanning between -0.3 - 0.6 V with a scan rate of 120 mV/s. While anodic scan, no distinct peaks were observed for the Mn-Mo mixed oxide/PLE under the studied potential window. Upon addition of 2 µM serotonin into the PBS containing electrochemical cell, three new anodic peaks were found to arise at potentials -0.191 V (I1), -0.027 V (I2), +0.287 V (I3). These three anodic peaks were found to undergo a reduction reaction upon the reverse scan on the Mn-Mo mixed oxide/PLE surface. Thus serotonin was found to exhibit a redox response over the modified electrode surface in 0.1 M PBS 8. Even though we observed three anodic peaks, the well-defined peak at +0.287 V (I3) was chosen for further studies.

Further linear sweep voltammetry (LSV) was carried out to showcase the electrocatalytic ability of the Mn-Mo mixed oxide/PLE over the unmodified PLE. Figure 2 B corresponds to the LSV profile of PLE and Mn-Mo mixed oxide/PLE in 0.1 M PBS 8 with 1 µM serotonin. The current response obtained for 1 µM serotonin was 0.127 µA for unmodified PLE and 0.4085 µA at the modified electrode surface. Almost 3.2 fold times current increment was observed at the Mn-Mo mixed oxide/PLE over unmodified PLE. Also the oxidation overpotential was found to be shifted to less positive potential on the modified electrode surface compared to unmodified PLE. Thus Mn-Mo mixed oxide/PLE was found to possess electrocatalytic activity for serotonin oxidation than unmodified PLE. The mechanism of Mn-Mo mixed oxide film formation is explained as follows [7].

First electrodeposition of Mn^{2+} ions with H_{2}O yields Mn oxide and protons.

Mn^{2+} + 2H_{2}O → MnO_{2} + 4H^{+} + 2e^{-}

Then, the protons accumulated near the electrode surface react with MoO_{4}^{2-} to form polyoxomolybdate through a dehydrated condensation reaction.

7MoO_{4}^{2-} + 8H^{+} → Mo_{7}O_{24}^{6-} + 4H_{2}O

Thus condensed product thus co-precipitated with Mn oxide to form the final film.

![Figure 2](image)

**Figure 2** (A)CV of Mn-Mo mixed oxide/PLE in the presence and absence of 2 µM serotonin (B) LSV of unmodified PLE and Mn-Mo mixed oxide/PLE with 1 µM serotonin in 0.1 M PBS 8.

3.3. Selection of electrolyte

Selection of suitable supporting electrolyte was performed with DPV analysis. Thus obtained current response for 1 µM serotonin electroxidation at the Mn-Mo mixed oxide/PLE indifferent electrolytes like 0.1 M PBS 7, 0.1 M NaOH, 0.1 M H_{2}SO_{4}, 0.1 M acetate buffer solution pH 4 (ABS 4) are tabulated in table 1. Results show that best output in terms of response current was obtained from 0.1 M PBS 7 and hence it was used for further studies.
Table 1. Selection of electrolyte solution.

| Electrolyte   | Current (µA) | Potential (V) |
|---------------|--------------|---------------|
| 0.1 M PBS 7   | 0.174        | 0.311         |
| 0.1 M H₂SO₄  | 0.0804       | 0.571         |
| 0.1 M ABS 4   | 0.1706       | 0.475         |
| 0.1 M NaOH    | 0.0381       | 0.002         |

3.4. Selection of buffer pH

After selecting PBS as the suitable electrolyte for serotonin electroxidation at Mn-Mo mixed oxide/PLE surface, the influence of different pH of PBS was studied. Results are tabulated in table 2. Optimum results in terms of both anodic current response and over potential were observed with PBS pH 8. As the pH was increased from 6-8, the current was found to be increasing and then decreased after 8. Hence we have chosen 0.1 M PBS pH 8 as the optimal buffer pH.

Table 2. Selection of buffer pH.

| Different pH of PBS | Current (µA) | Potential (V) |
|---------------------|--------------|---------------|
| 0.1 M PBS 6         | 0.1077       | 0.363         |
| 0.1 M PBS 7         | 0.174        | 0.311         |
| **0.1 M PBS 8**     | **0.258**    | **0.235**     |
| 0.1 M PBS 9         | 0.153        | 0.284         |

3.5. Optimization of Mn:Mo ratio

Further the ratios of Mn to Mo and Mo to Mn was optimized. Mn:Mo ratios were varied as 1:5, 1:4 and 1:6. As the response current for 1:5 Mn:Mo was higher that was opted. Then a reverse study of the ratios was also performed. Mo:Mn ratios of 5:1, 5:3 and 1:1. The current for 5:1 Mo:Mn was obtained. So for the ratio of final mixed metal deposition bath, we fixed the Mo:Mn ratio as 5:1. Table 3 shows the results obtained for the optimization parameters of metal concentrations in deposition bath.

Table 3. Optimization of metal ratio in deposition bath.

| Metal ratios | Current (µA) | Potential (V) |
|--------------|--------------|---------------|
| Mn:Mo        |              |               |
| 1:4          | 0.215        | 0.244         |
| **1:5**      | **0.258**    | **0.235**     |
| 1:6          | 0.194        | 0.236         |
| Mo:Mn        |              |               |
| 5:1          | **0.258**    | **0.235**     |
| 5:3          | 0.126        | 0.241         |
| 5:5 (1:1)    | 0.03         | 0.243         |

3.6. Scan rate study

Figure 3A represents CV of Mn-Mo mixed oxide/PLE in 0.1 M PBS 8 containing 2 µM serotonin at different scan rates ranging from 20-120 mV/s. As the scan rates were increased from 20-120 mV/s, the peak current associated to the serotonin electroxidation also increased. A linear plot between current and square root of scan rate gives a linear fit depicting a diffusion-controlled process (figure 3B). The corresponding linear equation is \( I_{pa} = -0.00139 + 0.0162 \times v^{1/2} \) with \( R^2 = 0.9927 \).
3.7. Electrochemical measurement of serotonin

Figure 4A depicts the DPV response of Mn-Mo mixed oxide/PLE in 0.1 M PBS 8 containing varying concentrations of serotonin from 0.2-52 µM. It was found that the newly formed peak at +0.255 V corresponding to serotonin electroxidation at the modified electrode surface was found to be increasing as the concentration of serotonin in the solution increased. A calibration plot was generated between current vs. concentration of serotonin and a linear dynamic range was obtained from 0.2-52 µM (figure 4B). As normal blood serotonin levels are in the range of 0.47 µM-1.33 µM, the sensor is an effective candidate to measure the serotonin levels in human blood samples. The linear regression equation obtained was $I_{pa} (\mu A) = 0.7304 + 0.2355 \times C (\mu M)$ with regression coefficient $R^2$ as 0.996.

3.8. Selectivity analysis of the sensor

Selectivity study of the sensor was carried out using DPV. The electrochemical analysis of serotonin was conducted in the presence of dopamine, a coexisting molecule in the blood bearing a similar structure. DPV response of the same is shown as figure 5 A and 5 B shows the DPV of 0.2 µM serotonin in the presence of 100 µM dopamine etc.
Figure 5 (A) Selectivity study of the developed serotonin sensor (B) serotonin response over Mn-Mo mixed oxide/PLE in the presence of 100 µM dopamine.

3.9. Real sample analysis
Real sample analysis of serotonin was carried out in real blood matrix with spiked serotonin into it. DPV analysis were performed and obtained a recovery of 111.9 %. Table 4 shows the results observed for the real sample analysis of the fabricated sensor for the determination of serotonin using standard addition method.

| Added (µM) | Found (µM) | Recovery (%) |
|------------|------------|--------------|
| 1.6        | 1.8        | 111.9        |

4. CONCLUSION
We have employed a mixed metal oxide modified pencil lead electrode for the facile determination of neurotransmitter serotonin in phosphate buffer medium. The employed electrode possesses good linearity (0.2-52 µM) which satisfies the blood range. Also the sensor can be used to detect serotonin in the presence of another important neurotransmitter dopamine. Functionality of the sensor was ensured by testing in real blood sample matrix and obtained a recovery of 111.9 %.

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