Electrochemical sensor for detection of neurotransmitter serotonin using platinum nanoparticle modified electrode

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Abstract

Impaired 5-HT function can lead to clinical depression, which is one of the most serious problems nowadays and the number of people suffering from it is growing worldwide. Given the (patho)physiological importance of serotonin, its analysis is necessary. It plays a major role in both neurological and metabolic diseases. In this research, a platinum wire modified with platinum nanoparticles (PtNPs) was used to detect serotonin. The structural and morphological characteristics of the electrode were extensively investigated using scanning electron microscopy (SEM). To characterize the electrochemical performance of the electrodes (before and after surface modification) cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) was employed. The CV results demonstrated that incorporating PtNPs significantly increased the current response of 5-HT and impedance studies revealed that charge transfer resistance decreases. Hence, the designed electrodes have potential applications in in-vivo neurotransmitter detection in future studies.

Keywords: Neurotransmitter, Serotonin, Electrode Modification, Electrochemical Techniques, Platinum Nanoparticle

1. Introduction

Serotonin (5-HT) is an ancient phylogenetic signaling molecule and the most broadly distributed neurotransmitter in the brain [1]. It is an evolutionary molecule that has remarkable effects on basic physiology and the central nervous system in regulating anxiety, mood, memory, stress, temperature regulation, aggression, sexual behavior, and numerous other physiological processes [2, 3]. It transmits messages between nerve cells and is the target of many physiological regulatory mechanisms and modulators, such as gene transcription, neurotrophic peptides, steroids, and psychotropic drugs [4]. It also acts as a crucial biogenic monoamine neurotransmitter, hormone, and mitogen in the body [5]. This neurotransmitter is present in platelets, smooth muscles, and the gastrointestinal, central, and peripheral nervous systems [6]. Serotonin is synthesized in a two-step process where the first step is the conversion of the essential amino acid tryptophan into 5-hydroxytryptophan (5-HTP) using tryptophan hydroxylase (TPH). Subsequently, 5-HTP undergoes decarboxylation to produce 5-HT using the enzyme aromatic amino acid decarboxylase (Fig. 1) [7]. 5-HT is further transformed into melatonin (N-acetyl-5-methoxytryptamine), a hormone released by the pineal gland that controls the sleep-wake cycle [8, 9]. Therefore, the synthesis of 5-HTP is essential as it acts as a precursor for the production of 5-HT and melatonin. 5-HTP is present across diverse life forms such as in animals and humans and is also produced by lower and higher plants, mushrooms, and microbes [10]. High levels of serotonin cause osteoporosis (bone-weakening disease), confusion, restlessness, and diarrhea, and low levels of 5-HT are linked to various diseases and disorders, including Alzheimer's disease, infantile autism, depression, anxiety, migraine, irregular hemostasis, blood clotting, sudden infant death syndrome, lack of attention, motivation, and learning [11]. The concentration of serotonin in the human biological system can be increased by exercising, high water intake, and eating serotonin-containing food such as bananas, eggs (particularly the yolks), cheese, pineapples, salmon, soy products, nuts, and seeds [12]. The normal concentration of serotonin in human body fluid is given in Table 1.

	Fluid	Range	Reference
1	Human serum	270 nM to 1490 nM	[13]
2	Urine	300–1650 nM	[14]
3	CSF	< 0.0568 nM	[15]
4	Blood	75.77 ng ml^{-1}	[16]

Table 1 - Normal concentration of serotonin in human body fluid

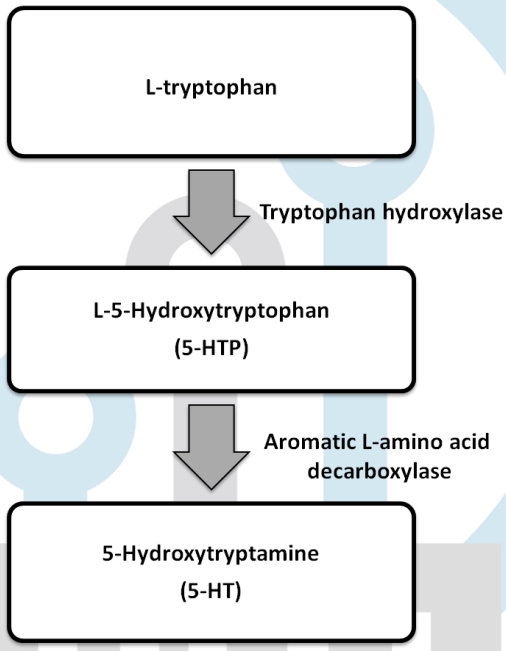

Fig 1- Biosynthetic pathway of serotonin

The IUPAC name of serotonin is 3-(2-aminoethyl)-1H-indol-5-ol, and it has a simple molecular structure containing an indole ring and two other functional groups: at the 5^{th} position an acidic OH group and at the 3^{rd} position a flexible ethylamine side chain. Both functional groups are ionizable and have pK_a values of approximately 9-10. Serotonin has a pK_a value of 10.2 [17]. It exhibits three charge variants: positive charge (cationic), unionized (uncharged), or negative charge (anionic). At pH lower than 10.2, both functional groups are protonated, and serotonin exists as positively charged molecules. At pH values higher than 10.2, both functional groups are unprotonated, and the hydroxyl group carries a negative charge. At physiological pH, the cationic form is predominant [18, 19]. Serotonin is unstable at very high or very low pH values and is susceptible to various biochemical and chemical oxidation reactions [20].

Considering the importance of 5-HT, several conventional techniques are used to analyze serotonin in humans, water, plants, and various other samples. These include high-performance liquid chromatography (HPLC), mass spectrometry (MS), chemiluminescence (CL), capillary electrophoresis, fluorescence, and immunoassay [21,22,23,24,25]. These techniques require long operational times, complicated processing, low spatial resolution, expensive instrumentation, a broad range of selectivity, and sample pretreatment; thus, they are not suitable for the rapid detection of 5-HT [26]. Electrochemical sensing methods are highly sensitive and selective, give fast responses, are selective, have low-cost attributes, are reproducible, are easily operated, and are portable, and that is why they are most preferred [27, 28, 29, 30]. The electrooxidation reaction of 5-HT occurs as a 2-electron-2-proton process as shown in fig. 2 [31].

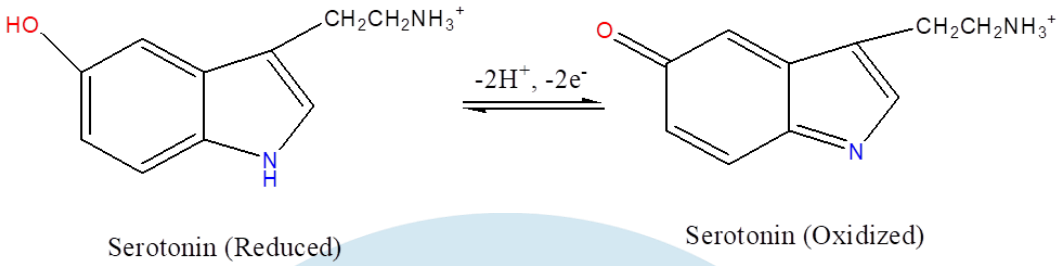

Fig 2- Electrochemical oxidation of serotonin

Currently the attention is on enhancing the electrode functionality for neurotransmitter detection. Coating the electrode surface using polymers, metal oxides, and metal nanoparticles is an extensive approach for improving the sensitivity of the electrode surface [32]. Earlier studies (literature) have shown that metal nanoparticle-modified electrodes have been used for the selective detection and determination of catecholamine neurotransmitters [33]. Studies on metal nanoparticle-modified electrodes have been significantly carried out nowadays due to their various outstanding features in the field of biosensor technology, drug delivery, and energy storage [34]. Among various noble metal nanoparticles such as silver (AgNPs), gold (AuNPs), palladium (PdNPs), and platinum (PtNPs), PtNPs are of great interest in the emergence of sensitive and selective materials in the field of sensors due to their reduced size, enhanced electrochemical properties, electron transfer, catalytic properties, high chemical stability, high surface-to-volume ratio, and fast electron transfer on the electrode surface, which increase the electroactive surface area available for the adsorption of neurotransmitters [35]. Previously, Platinum nanoparticle-modified electrodes have been used for various types of electrochemical sensors [36]. For example, there have been reports of Platinum nanoparticle-modified electrodes for glucose sensors [37], E. coli [38], and Hydrogen Peroxide [39]. In 2019, Mohanaraj developed a sensor by electrodeposition of AuNPs on a carbon fiber microelectrode and demonstrated its sensitivity on dopamine detection [40]. The sensitivity for dopamine detection on carbon fiber microelectrodes was enhanced after the electrodeposition of AuNPs.

In this work, for the first time we developed a relatively simple platinum wire sensor modified with platinum nanoparticles for the detection of serotonin. Platinum nanoparticles were electrodeposited on a platinum electrode using Chronoamperometry (CA), and the electrode was characterized using scanning electron microscopy (SEM). The results presented herein show that PtNPs-modified platinum wire electrodes exhibit much higher electrocatalytic activity of 5-HT than bare electrodes.

2. Experimental section

2.1 Chemicals

Serotonin was purchased from Sigma-Aldrich. Sodium dihydrogen phosphate (NaH_2PO_4), sodium hydrogen phosphate (Na_2HPO_4) were supplied by Merck. All solutions were prepared in ultra-pure water. 250 ml of 0.1 M PBS of pH 7 was prepared by mixing 2.99g of NaH_2PO_4 and 4.44g of $Na_2HPO_4.2H_2O$.

2.2 Electrochemical system

Experiments were conducted with Multi autolab M 204 equipped with Nova 2.1 software. All electrochemical experiments were carried out potentiostatically utilizing a conventional three electrode cell, consisting of a working electrode, a saturated calomel reference electrode (SCE) and a large platinum sheet counter electrode. All electrochemical measurements were performed at 25ºC. Cyclic voltammetry of 10^{-5} M 5-HT in the potential range -0.1 to 0.6 V vs. SCE and impedance study at potential 0.4 V in the frequency range of 0.1 Hz to 100 kHz was performed. Morphological images of PtNPs modified electrode were recorded using a scanning electron microscope (SEM), ZEISS, EVO 18.

2.3 Preparation of PtNPs modified electrode

Platinum wire sealed in soft glass was used as working electrode for the electrochemical measurements. The platinum working electrodes were mechanically roughened with emery paper then kept in ethanol for 48 hours. Rough electrode was sonicated in ethanol followed by sonication in triple distilled water. Thereafter Platinum working electrodes were boiled in nitric acid and were ultrasonicated in triple distilled water. Then it was cleaned electrochemically and Platinum nanoparticles were deposited onto the electrode using 5 mM $H_2Pt(OH)_6$]+0.5 M H_2SO_4 solution at 170 mV potential for 60 min using constant potential deposition.

2.4 Morphological characterization of the electrodes

Surface morphology of PtNPs modified electrode was characterized by SEM analysis and the SEM images are shown in Fig. 3.

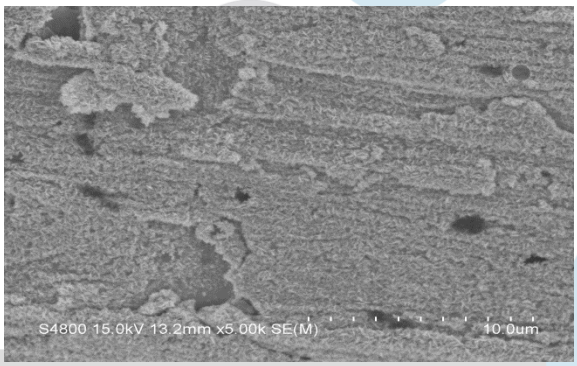

Fig 3- PtNPs deposited electrode

3. Results and discussion

3.1 Cyclic voltammetric studies of 5-HT

The electrochemical behavior of 5-HT was investigated using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). CV measurements of 10^{-5} M 5-HT in the potential range of -0.1 V to +0.6 V at scan rate of 100 mV s^{-1} was applied in a solution of 0.1 M phosphate buffer (pH 7) on bare and PtNPs modified electrode and the obtained voltammograms are given in fig 4. In PtNPs modified electrode, Current response (I_p) value is higher and E_p value is lower than bare electrode in CV. The high I_p value and low E_p value (CV; E_p: 0.32 V) was observed in PtNPs modified electrode than the bare electrode because PtNPs show an electrocatalytic effect in the electro-oxidation of 5-HT. In the electro-oxidation of 5-HT, the high electronic conductivity was observed in the PtNPs modified electrode due to the synergistic effect of PtNPs. From fig 4 it was observed that no peak appear in the buffer solution, while a low peak current was observed in the presence of 5-HT at bare electrode. I_p values of 5-HT on PtNPs modified electrodes increases compared to the bare electrode. PtNPs deposited electrode increases the current response due to synergistic effect and electronic effects of nanoparticles.

Randles-sevcik equation given in equation 1, is fundamental tool in cyclic voltammetry to obtain diffusion coefficient, number of electrons transferred, reversibility and active surface area of electrode.

$$I_p=2.69\times10^5\times n^{3/2}\times D^{1/2}\times A\times \nu^{1/2}\times C \qquad \text{(eqn…..1)}$$

Where I_p stands for current, n is the number of electrons transferred between electrode and electrolyte, D is the diffusion coefficient of electrolyte, A is the active surface area of electrode, ν is the scan rate, and C is the concentration of electrolyte. It is also used to describe the effect of the scan rate on the peak current.

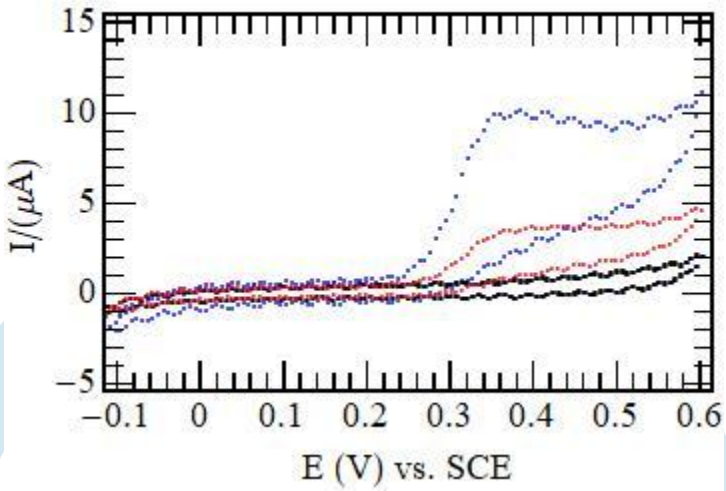

Fig 4 - CV voltammograms recorded in PBS (pH 7) in the absence of 5-HT (black) and in the presence of 10^{-5} M 5-HT on bare electrode (red) and on PtNPs modified electrode (blue) at scan rate 100 mV s^{-1}.

3.2 Electrochemical Impedance studies of 5-HT

Impedance studies was performed to investigate the electrocatalytic oxidation of 10^{-5} M 5-HT at the electrode-electrolyte interface at fixed potential of 0.4V in the frequency range of 0.10 Hz-100 kHz. The impedance spectra (Nyquist plots) obtained for the electrodes are presented in Fig. 5. Nyquist plot involve plot of real versus imaginary parts of frequency. Fig. 5 illustrates the Nyquist plots of the bare and modified electrodes obtained in the presence of serotonin. Charge transfer resistance (R_{ct}) is determined using the diameter of the semicircle formed and is responsible for the electron transfer kinetic reactions at the electrode-electrolyte interface. The R_{ct} value for bare, and PtNPs modified electrode was found to be 3310 **Ω**, 850 **Ω**, respectively. The R_{ct} for the modified electrode was relatively low compared to that of the bare electrode, indicating improved electron transfer kinetics between electrode and analyte during oxidation of serotonin compared to bare electrode.

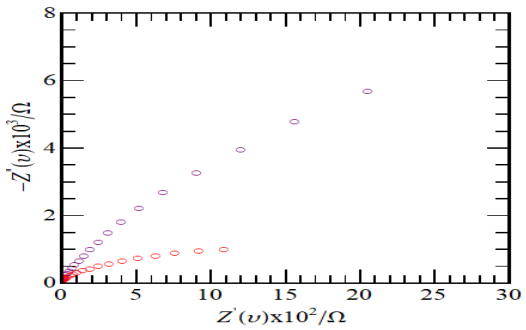

Fig 5 - Nyquist plots of bare electrode (purple), PtNPs modified electrode (red) at 0.4 V in the frequency range of 0.10 Hz-100 kHz

4. Summary and Conclusions

In summary, the serotonin concentration in plasma and whole blood are valuable biomarkers for estimating the risk of vascular complications and organ damage in patients at the early stages of diabetes mellitus (DM). The majority of clinical trials are on depression, anxiety, panic attacks, and sleep disorders. The progress in electrochemical 5-HT sensing strategies has demonstrated a potential for application in commercial strategies

of electrochemical sensors for the disease-related biomarker 5-HT. The Pt-nanoparticle deposited electrode were found to possess higher sensitivity of serotonin detection. The uniform coating of PtNPs onto the electrode surface via electrodeposition yields a higher electroactive surface area, thus increasing the adsorption of serotonin onto the modified electrode surface. This led to higher serotonin oxidative currents in cyclic voltammetric response and a decrease in charge transfer resistance in electrochemical impedance studies. In conclusion, the PtNPs electrode was successfully fabricated for voltammetric determination of 5-HT for higher sensitivity.

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