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Fabrication of Carbon Nano Tube Based Amperometric Choline Biosensor for Detection of Neurological Disorders

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Abstract: An amperometric electrochemical biosensor is constructed for detection of important neurochemicalcholine based on choline oxidase enzyme catalyzed reaction. The electrochemical reactions produce current on platinum electrode surface, which is recorded by cyclic voltammetry. The carbon nanotube combined with choline oxidase biosensor produce higher sensitivity than simple choline oxidase biosensor. The sensor is optimized for various pH, temperature and substrate concentration.Detection limit of choline biosensor is $6x10^{-3}$M.
Keywords: Choline, Acetylcholine, Biosensor, Neurochemical.

Introduction

For the past few decades' biosensors play a major role in neurochemical detection. More than 100 neurochemicals present in brain's extracellular fluid, few chemicals play a major role in neurological disorders[1]. Choline is one of the essential neurochemical to be identified for Alzheimer's and Parkinson's disease. The enzyme based amperometric choline biosensors are simple, inexpensive, and highly sensitive compared to other conventional techniques like chromatography, magnetic resonance spectroscopy, electrochemiluminance and other electrochemical detection methods[2]. Amperometric biosensor consists of biological recognition element and detection element[3]. Biological recognition elements are based on enzymatic interaction or antigen-antibody interaction or protein interactions and detection methods are electrochemical or optical or mass based methods[4]. Enzyme interaction based biosensors have mainly used because they can offer simple and reliable operation[5]. The enzymatic reaction of choline with choline oxidase (ChOx) is given below. The choline reacts with choline oxidase in the presence of oxygen producesbetaine aldehyde and hydrogen peroxide[6,7]. The hydrogen peroxide releases two electrons on the electrode surface.

$$Ch + O_2 \xrightarrow{ChOx} Betaine\ aldehyde + 2H_2O_2$$

Researchers mainly concentrate on modification of electrodes to increase the sensitivity and selectivity of sensor[8].The nano particles such as carbon nanotubes, cadmium, zinc, gold, lead, and polymer showed higher electrochemical activity than their counterparts[9]. Carbon nanotubes have excellent electronic, chemical and mechanical properties.Generally, the two types of carbon nanotubes used in biosensors, they are single-walled carbon nanotubes (SWCNTs) and Multi-walled carbon nanotubes (MWCNTs). Most research articles show that carbon nanotubes can increase the electrochemical activity and cannot produce any current by carbon nanotubes itself [10,11].It improves the direct electron transfer reaction between hydrogen peroxide and platinum electrode

surface and quick shuttling of electrons[12,13,14]. In this study we designed a single wall carbon nano tubes and choline oxidase modified platinum electrode for choline detection.

Materials andMethods

Choline oxidase (from *Alcaligenes sp.*), choline chloride (>99%), MWCNTs (95% 20-60nm) are purchased from Sigma Aldrich and used without further purification. The electrolyte solution (0.1M of pH 7.8 phosphate buffer solution (PBS)) used for all experiments is prepared using potassium phosphate solution (KH_2PO_4 and K_2HPO_4; 0.1 M general phosphate). All the reagents used for experiments are of analytical grade. Double distilled water used for entire experiments. The MWCNTs are treated with 3:1 ratio of H_2SO_4 and $HNO_3$and then filtered and rinsed with double distilled water and then dried.

Surface Preparation and Modification

The platinum working electrode is washedwith H_2SO_4sonicated for 5 min in double distilled water. The 2µl of MWCNTs solution is added on the platinum electrode surface and dried at room temperature for 10 min. The 20 µl of glutaraldehyde (2.5%) is mixed with 80 µl of choline oxidase (0.25mg/dl) and mixer is applied on the MWCNTs modified electrode and dried at room temperature for 3 min. The electrode is stored at 4°C for experimental measurements.

Experimental Procedure

Cyclic voltammetry and time-based measurements areperformed with an electrochemical analyzer CHI 660B. Amperometric measurements are carried out using three electrode configurations, modified platinumelectrode as a working electrode, platinum electrode as counter electrode and Ag/AgCl as reference electrode.The various concentration of choline enzyme solution is prepared in PBS solution and stored in 4^0C.

Result andDiscussion

FT-IR Study

The Figure 1 shows the FT-IR spectrum of functionalized MWCNT to ascertain the presence of various functional groups.

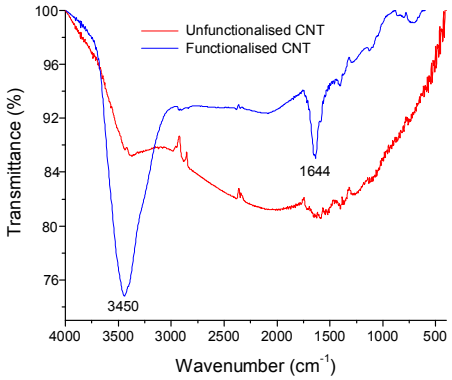

Figure 1: FT-IR of non-functionalized and functionalized MWCNTs

As seen from the spectrum of functionalized MWCNTs, it confirms the presence ofcarboxylic acid group bands at 1644 cm^{-1} and 3450 cm^{-1}. The presence of -COOH groups on the surface of the MWCNTs is advantageous for a better deposition.The absorption band at 1635 cm^{-1} is assignedto the stretching vibration mode of C=O in the amide group. TheNH_2 stretch band appeared at 3427 cm^{-1}[115].

Effect of Enzyme activity

Figure 2 shows the cyclic voltammograms of unmodified ChOx/Pt and MWCNTs modified ChOx/MWCNTs/Pt electrode. The response was taken for 4mM of choline chloride at the potential of 0.0V and 0.7V, pH of 7.8 and scanning rate of 100mVs^{-1}.

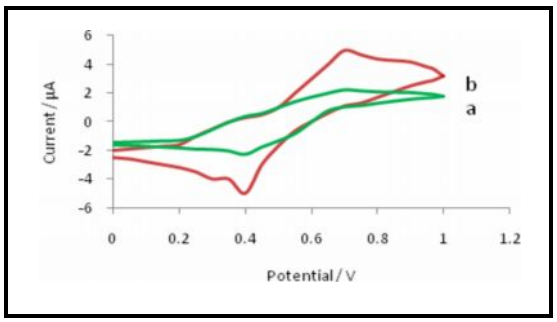

Figure 2: Cyclic voltammograms of (a) Unmodified ChOx/Pt electrode (b) ChOx/MWCNTs/Pt modified electrode

The MWCNTs modified electrode produces higher catalytic activity than the unmodified electrode.

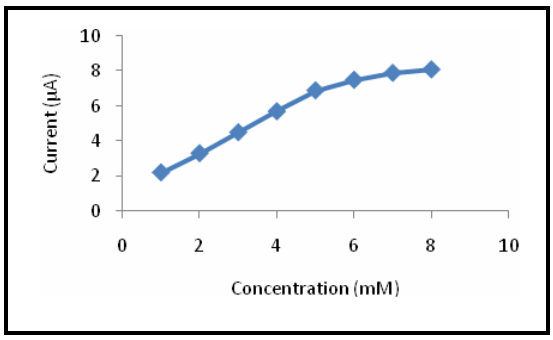

Figure 3: The activity of immobilized ChOx in different concentrations of Choline

Figure 3 shows the activity of immobilized ChOx in different concentrations of choline chloride (Michaelis–Menten plot) in 0.05 M phosphate buffer pH 7.8 at 37ºC and Fig. 4 shows the Lineweaver-Burkdouble reciprocal plot based on the experimental data shown in Figure 3.Figure 4 showthat the *Km* value is 5mM. It indicates that the diffusion of substrate and product into and out of the membrane is easy.

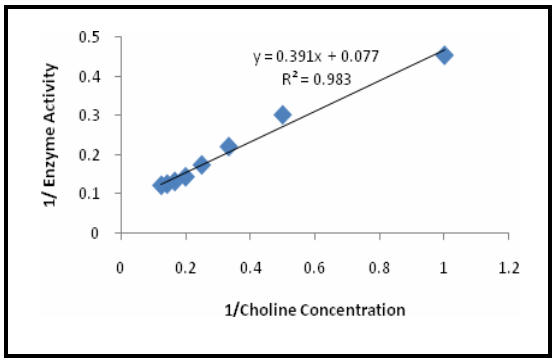

Figure 4: Lineweaver–Burk plot for immobilized Choline Oxidase

Effect of pH

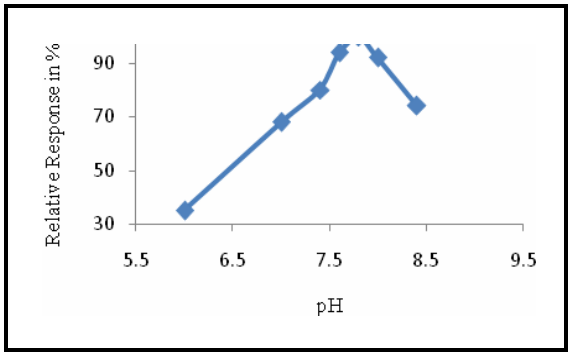

Figure 5: Response of choline sensor for various pH at 37^{0}C

The pH of the buffer solution mainly affects the enzyme activity. Figure 5 shows the effect of pH value on output current of the biosensor to 1mM of choline chloride at the potential of 0.7V. The optimum enzyme activity is obtained at pH 7.8.This is the optimal pH of ChOx reported in the literature [16].

Effect of Temperature

The temperature also influences the enzyme activity. The rate of an enzyme-catalyzed reaction increases as the temperature is raisedupto 37^{0}C and reduced beyond the optimum temperature.

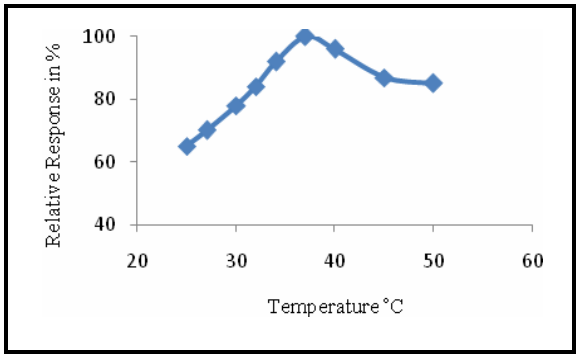

Figure 6: Response of choline sensor for various temperatures at pH 7.8

Figure 6 shows the effect of temperature on biosensor current at pH of 7.8 and 1mM of choline chloride at the potential of 0.7V. The optimum temperature is 37^{0}C.Above 40^{0}C temperature, enzymes are denatured[17]. The biosensor is stored at 4^{0}C for future use.

Conclusion

The ChOx biosensor is constructed on the platinum electrode with MWCNTs. The MWCNTs modified platinum electrode produces higher catalytic effect than unmodified electrode. The performance of choline biosensor is higher at temperature of 37^{0}C and pH of 7.8.

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