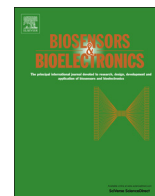




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Dopamine biosensor based on surface functionalized nanostructured nickel oxide platform

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ABSTRACT

A dopamine biosensor has been developed using nickel oxide nanoparticles (NPs) and tyrosinase enzyme conjugate. Nickel oxide (NiO) NPs were synthesized by sol-gel method using anionic surfactant, sodium dodecyl sulphate (SDS), as template to control the size of synthesized nanoparticles. The structural and morphological studies of the prepared NPs were carried out using X-ray diffraction (XRD), transmission electron microscopy (TEM) and dynamic light scattering (DLS) techniques. Afterwards, tyrosinase enzyme molecules were adsorbed on NiO NPs surface and enzyme coated NPs were deposited on indium tin oxide (ITO) coated flexible polyethylene terephthalate (PET) substrate by solution casting method. The formation of enzyme-NPs conjugate was investigated by atomic force microscopy (AFM) and Fourier transform infrared spectroscopy (FTIR) techniques and used in selective detection and estimation of neurochemical dopamine by electrochemical method. The fabricated Tyrosinase/NiO/ITO electrode exhibits high sensitivity of 60.2 nA/μM in linear detection range (2–100 μM) with a detection limit of 1.038 μM. The proposed sensor had a response time of 45 s, long shelf life (45 days) with good reproducibility and selectivity in presence of interfering substances and was validated with real samples. The tyrosinase enzyme functionalized NiO platform has good bio-sensing efficacy and can be used in detection of other catecholamines and phenolic neurochemicals.

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1. Introduction

Dopamine is known to be a significant catecholamine neurotransmitter that shows important role in the functions of central and peripheral nervous system, renal and hormonal systems of human and other mammals (Heien et al., 2005; Wightman et al., 1988). It is produced by decarboxylation of 3,4-dihydroxy phenylalanine and operated as a precursor in the synthesis of neurotransmitters epinephrine and norepinephrine. Dopamine also acts as a neuromodulator in brain circuitry and responsible for several physiological conditions such as mood, behavior, memory, attention and movement (Robinson et al., 2003). Abnormal metabolism and concentration of dopamine in body can lead to neurological diseases like Parkinson's disease, schizophrenia, epilepsy, senile dementia and attention deficit hyperactivity disorder (ADHD) (Cao et al., 2008; Huffman and Venton, 2009; Mo and Ogorevc, 2001; Robinson et al., 2003; Wightman et al., 1988). Moreover in emergency condition, dopamine is infused to the patients showing the symptoms of myocardial infraction, hypertension, bronchial

asthma and during acute heart surgery (Beitollahi et al., 2008). Due to such physiological and pathophysiological effects, it is essential to develop a quantitative method to accurately estimate dopamine for diagnosis and continuous monitoring of neurological disorders.

The currently available analytical methods for dopamine determination are high performance liquid chromatography (Carrera et al., 2007; Muzzi et al., 2008), UV spectrometry (Barreto et al., 2008), capillary electrophoresis (Kang et al., 2005; Li et al., 2010; Thabano et al., 2009), liquid chromatography-electrospray tandem mass spectrometry (El-Beqqali et al., 2007), flow injection analysis with spectrophotometric detection (Deftereos et al., 1993), coulometric (Myers et al., 1998), fluorescence (Chen et al., 2011; Khattar and Mathur, 2013; Nikolelis et al., 2004) and electrochemical detection (Chen and Peng, 2003; Matos et al., 2000; Mecker and Martin, 2008; Umasankar and Chen, 2008; Zhang et al., 2013). Apart from electrochemical detection, most of the procedures are complex, cumbersome, expensive, time consuming and requires lots of sample. Due to electrochemical activity, dopamine can be efficiently detected by applying appropriate potential across the electrodes (Njagi et al., 2010). With this in view, enzyme biosensor combined with electrochemical detection may be advantageous owing to rapid detection, simplicity and ease in miniaturization.

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Further, for reliable and rapid determination of dopamine and to increase sensitivity and selectivity of the biomedical devices, enzyme can be proficiently used as biorecognition element. Tyrosinase or polyphenol oxidase (EC 1.14.18.1) is binuclear copper containing enzyme that catalyzes oxidation of phenolic compounds in two steps to their respective *o*-quinones, which is further reduced at appropriate redox potential to form original phenols again (Tembe et al., 2008; Zhou et al., 2007). *o*-quinones are electroactive species and can be reduced at moderately negative potential (Tembe et al., 2006; Zhou et al., 2007) which is helpful in the prevention of the polymerization of phenols and interferences from oxidizable species (Koile and Johnson, 1979). Dopamine is a catechol like phenolic substance, can be detected with enhanced specificity using tyrosinase enzyme molecules through direct reduction of bio-catalytically liberated *o*-dopaquinone species (Njagi et al., 2010; Tsai and Chiu, 2007; Zhou et al., 2007).

For suitable immobilization of tyrosinase enzyme molecules, several materials such as graphite (Nistor et al., 1999), conducting polymer (Arslan et al., 2005; Rajesh et al., 2004), nafion membrane (Furbee Jr et al., 1993), carbon paste (Caruso et al., 1999), hydrogel (Daigle and Leech, 1997) and biopolymers (Liu et al., 2005) have been used as matrices to improve stability of the enzyme in sensor probe. Most of these matrices used to develop tyrosinase based system suffer from complexity and reduced sensitivity (Tembe et al., 2008, 2006). In this context, nanostructured metal oxides can be effectively used as matrices for biomolecule immobilization due to presence of fascinating electrochemical and optical properties, catalytic effects and efficient charge transfer abilities from biomolecules to a particular substrate (Doong and Shih, 2010; Thanh and Green, 2010). Nanostructured metal oxides have attracted plentiful attention of the researchers owing to their adsorption capabilities, effective surface area for biomolecule immobilization with desired orientation and better conformation that leads to high biological activity of the immobilized bio-sensing molecules (Caruso, 2001; Solanki et al., 2011). Among the various nanostructured metal oxides, nickel oxide nanoparticles have recently been used in bio-sensing application (Li et al., 2008) due to the presence of high electro-catalytic activity, fast electron transport property, high surface energy, chemical stability, low cost and biocompatibility (Ali et al., 2013; Kavitha and Yuvaraj, 2011; Salimi et al., 2007). In addition the existence of variable oxidation states in nickel oxide nanoparticles helps in easy mobility of the electrons (Mohan et al., 2011). Beside this, high isoelectric point (IEP 10.8) of NiO nanoparticles make it suitable for tyrosinase enzyme (IEP 4.7) immobilization. The NiO nanoparticles can be synthesized through sol-gel, reverse micelle, microwave irradiation and laser induced fragmentation method (Justin et al., 2010; Singh et al., 2011). In sol-gel method NiO nanoparticles are generally prepared by using non-ionic, anionic and cationic surfactants to control pore volume, surface area, crystallite size and organization of NiO nanocrystals (Justin et al., 2010). Among three types of surfactants, the anionic surfactant shows finest result in respect of higher surface redox activity, lower crystallite size, porous morphology and higher surface area (Justin et al., 2010). These properties are possibly most suitable for biomolecule immobilization and electrochemical measurement. Hence in our experiment sodium dodecyl sulphate (SDS) has been used as anionic surfactant during synthesis of NiO nanoparticles.

The electrochemical detection of dopamine is greatly affected by the presence of co-existing interfering substances ascorbic acid and uric acid because of similarity in oxidation potential of dopamine close to these interfering species (Liu et al., 2013; Zhang et al., 2005). Dopamine requires higher oxidation potential (between 0.5 and 0.7 V) which includes oxidation range of many other electrochemically active species (Njagi et al., 2010). To avoid the effect of interferents, tyrosinase based system has been used in

selective and specific detection of dopamine and the estimation of dopamine has been monitored through direct reduction of *o*-dopaquinone species at relatively lower potential (~ -0.15 V).

In the present study, sol-gel method with anionic surfactant SDS has been used to synthesize NiO nanoparticles (NPs) and tyrosinase enzyme has been immobilized on synthesized NiO NPs by physio-adsorption technique. The NiO NPs-tyrosinase conjugates, characterized by different methods, was then deposited on indium tin oxide (ITO) coated polyethylene terephthalate (PET) substrate by solution casting method. These enzyme strips were used for biosensor measurements and several optimization steps were performed to characterize the dopamine biosensor in synthetic samples, with interferents and in real sample.

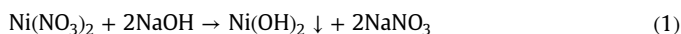
2. Experimental section

2.1. Materials and reagents

Tyrosinase (EC 1.14.18.1 from *Agaricus bisporus* with activity of 1000 U/mg of solid), dopamine hydrochloride ($C_8H_{11}NO_2 \cdot HCl$), Poly (vinyl alcohol) (PVA, MW 130000), ITO coated PET substrate and sodium hydroxide pellets (NaOH) were procured from Sigma-Aldrich (USA). Nickel nitrate hexahydrate [$Ni(NO_3)_2 \cdot 6H_2O$] and sodium dodecyl sulphate ($NaC_{12}H_{25}SO_4$) were purchased from Alfa Aesar (UK). The stock solution of tyrosinase (1 mg/mL) was freshly prepared in phosphate buffer (50 mM, pH 6.5). All solutions used in this study were prepared with deionized water of resistivity not less than 18 M Ω cm, obtained from Milli-Q water purification system (Milli-Q, USA).

2.2. Synthesis of nickel oxide (NiO) nanoparticles (NPs)

Nickel oxide nanoparticles (NiO NPs) were synthesized by coprecipitation method using sodium dodecyl sulphate (SDS) as anionic surfactant. For this purpose, 20 mM of [$Ni(NO_3)_2$]·6H₂O and 40 mM of NaOH were dissolved in deionized water (DW) individually and magnetically stirred for one hour to obtain homogeneous and transparent solutions. 10 mM of SDS was added in 20 mM of [$Ni(NO_3)_2$]·6H₂O solution and stirred for another one hour. The aqueous solution of NaOH was added drop wise into the resultant solution up to pH ~ 11.5 –12 and kept under constant stirring for 2 h at room temperature (25 °C). A light green precipitate of Ni(OH)₂ thus obtained was separated by centrifugation and washed 3–4 times with DW and ethanol. The precipitate was then oven-dried at 70 °C for 24 h and calcined at 300 °C for 3 h to obtain NiO NPs. The reaction mechanism involved in the synthesis of NiO NPs is shown in (Eqs. (1) and 2)



2.3. Nanoparticle characterization

The as-prepared NPs were characterized using various techniques to ascertain composition, homogeneity, particle sizes etc. The X-ray diffraction (XRD) spectra of the synthesized NiO NPs were obtained from X-ray diffractometer (Rigaku, MiniFlex 600) with CuK α radiation at $\lambda = 1.5406$ Å [range (20–80°) and scan rate 4°/min]. The structural and morphological properties of NiO NPs were investigated using transmission electron microscopy (TEM, FEI TECNAI G²S TWIN), dynamic light scattering (DLS, Malvern Zetasizer NanoZS90 particle size analyzer) and energy dispersive X-ray

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