Polymer 55 (2014) 4820–4831

Contents lists available at ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

Surface modified multiwalled carbon nanotube based molecularly imprinted polymer for the sensing of dopamine in real samples using potentiometric method

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ARTICLE INFO

Article history:
Received 21 May 2014
Received in revised form
26 July 2014
Accepted 29 July 2014
Available online 12 August 2014

Keywords:
Acrylamide
Dopamine
Graft copolymerization

ABSTRACT

The aim of this work was to test the application of multi-walled carbon nanotube based molecularly imprinted polymer (MWCNTs-MIP) for the determination of dopamine (DA) in blood serum and urine samples. The acrylamide grafted MWCNTs with vinyl group (MWCNTs-g-AAm-CH=CH_2) was prepared by free radical graft copolymerization technique. MIP was synthesized by selective polymerization of MWCNTs-g-AAm-CH=CH_2 with itaconic acid (IA) as functional monomer in the presence of DA using ethylene glycol dimethacrylate (EGDMA) as a cross-linker and α,α'-azobisisobutyronitrile (AIBN) as the initiator. The response time was ~2 min and the detection limit was 1.0×10^{-9} mol L^{-1}, so the proposed sensor could be considered as a sensitive marker of DA depletion in Parkinson's disease. The MWCNTs-MIP sensor demonstrated a high selectivity, sensitivity and stability, in addition to that good reproducibility. The useful lifetime for the DA sensor was longer than 2 months and the sensor was successfully tested in real samples.

1. Introduction

Dopamine [2-(3,4-dihydroxyphenyl)ethylamine, DA], a monoamine neurotransmitter and hormone, is one of the biogenic catecholamines in the mammalian central nervous system. The concentration of DA in biological organisms is considerably low (0.01–1.0 μmol L^{-1}). Abnormal levels of DA leads to several diseases such as epilepsy, senile dementia, HIV infection, Parkinson's, Alzheimer's and Huntington's diseases [1]. In the patients of untreated Parkinson's disease most studies found that DA concentration decreases considerably and it varies 1.22 ± 1.81 ng mL^{-1}, to almost complete depletion [2]. Thus the quantification of DA in biological systems is an important for diagnostic and pathological research.

Commonly used method for DA analysis involved relatively laborious and expensive analytical techniques, such as mass spectrometry [3], high performance liquid chromatography [4], flow injection chemiluminescence [5] and optical absorption spectrophotometry in a microfluidic system [6] etc. Yet, these methods have limitations of large sample volumes, expensive instrumentation and environmental unfriendly solvents etc. Considering the above said factors along with the high electro activity of DA, electrochemical analytical technique for DA determination is an attractive method, owing to its low cost, ease of operation, fast response, high sensitivity and feasibility of miniaturization [7].

Molecular imprinting technique is a promising method for engineering three dimensional networks with a suitable recognition site complementary in size and shape to the template molecule for subsequent rebinding process [8]. They were used in a variety of applications such as drug separations, template-assisted synthesis and catalysis and as receptor mimics biomimetic sensors and antibody mimics [9]. Yet, the bulk MIPs manifest high selectivity, there are certain drawbacks such as heterogeneous distribution of binding sites, the diffusion of the analyte across MIPs and the slow binding kinetics along with poor site accessibility for template molecules [10]. Thus nanomaterials can be used as a supporting material to overcome the above mentioned problems that encountered with the use of MIPs. Multi-walled carbon nanotubes (MWCNTs) serve as an excellent support because it possesses large specific surface area, highly porous nature and it consist of hollow structures. This successful combination between MWCNTs and MIPs gives a superior route which is used for the large application of MIPs. Kan et al. [11] reported the immobilization of vinyl group on the surface of MWCNTs for the selective determination of DA. Komathi et al. [12] reported nanomolar detection of DA through the

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http://dx.doi.org/10.1016/j.polymer.2014.07.057
0032-3861/

use of a new nanocomposite made up of MWCNTs, a grafted silica network and gold nanoparticles. Modified electrodes based on MWCNTs, such as MWCNTs modified gold electrode [13] and layer-by-layer assembled MWCNTs modified electrode [14] have been employed for the study of DA.

The presence of large surface area, high van der Waals force and high aspect ratio, these features make the MWCNTs to go through self aggregation. A significant promising approach to recover these problems is the surface modification through graft polymerization technique. The method is used to introduce alternate chemical groups on the surface of MWCNTs [15]. The hydrogen bonding among the constituent acid groups present on the surface of MWCNTs could lead to compact stacking of the CNTs and cause bundling. Surface modifications of MWCNTs surface have been an important research area due to their hydrophobicity properties in aqueous systems. It also effectively improves the dispersibility in addition to reactivity of the MWCNTs for different applications. Chemical functionalization of MWCNTs during grafting can improve its properties along with the polymer matrix produced can be modified for specific applications. Acrylamide (AAm) is grafted on the surface of MWCNTs that increases its solubility. Zhang et al. (2010) [7] demonstrated the use of AAm for the grafting of MWCNTs. In the literature of Kan et al. (2008) [11] firstly the MWCNTs was treated with nitric acid to give MWCNTs-COOH. This will cause defects to the walls of the MWCNTs. But in this proposed method a direct grafting of AAm onto the surface of MWCNTs takes place, which will minimize the defect formation and also increases selectivity and sensitivity. The modification of the MWCNTs is a complicated task and time-consuming, but once the material is prepared it is stable for a long time. In addition to that a minute quantity is required for sensing purposes and it can be reused for several times.

Previous works highlighted the MIP reports on electrochemical sensors with capacitive [16], conductometric [17], amperometric and voltammetric [18] transduction. However, the above said methods are concerned in the use of more sophisticated instrumentation, more difficult procedures; in addition to that some of them suffer poor linearity of the calibration curve or extensive response times. The noteworthy feature of Potentiometric sensors are simple design, good selectivity and give promising results, which make it attractive [18]. Furthermore, for the generation of membrane potentials, the potentiometric sensors do not need the template molecules to diffuse through the electrode membranes [19]. Hence potentiometric technique is an excellent option to combine through MIP technique. Moreover in the proposed potentiometric method results a lower limit of detection of 1.0×10^{-9} M and good sensitivity compared to other methods.

In the present work, a vinyl functionalized MWCNTs were prepared as a substrate, above which DA imprinted polymer was synthesized by means of free radical polymerization. In the first part grafting of AAm onto the surface of MWCNTs was carried out using $(NH_4)_2S_2O_8$ as a radical initiator and trimethylolpropane trimethacrylate (TRIM) as a cross-linking agent. The double bonds on the surface of MWCNTs were opened by initiator molecules. In the presence of vinyl monomer AAm, the radical is added to the double bond of the monomer resulting in a covalent bond between the monomer and the MWCNTs to form MWCNTs-g-AAm [20]. The MWCNTs-g-AAm upon further reaction with allyl alcohol in the presence of $FeCl_3$ and CH_3NO_2 to form MWCNTs-CH=CH_2 [21]. The MWCNTs-CH=CH_2 undergoes polymerization reaction with IA in presence of EGDMA as the cross-linker, AIBN as the initiator and DA as the template molecule. The surface physical and chemical properties of the resulted materials were characterized and confirmed by SEM, FTIR, XRD, TEM and Raman spectroscopy.

The mechanism of the formation of MWCNTs-g-AAm is shown below.

2. Initiation

Creation of primary radicals.

$$S_2O_8^{2-} \rightarrow 2SO_4^{-}\bullet$$

$$2SO_4^{-}\bullet + H_2O \rightarrow HSO_4^{-} + \bullet OH$$

$SO_4^{-}\bullet$ and $\bullet OH$ are primary radicals.

Creation of secondary radicalic sites on the surface of MWCNT.

$$SO_4^{-}\bullet \Big/ \bullet OH + MWCNT{-}H \rightarrow MWCNT^{\bullet} + HSO_4^{-} \text{ or } H_2O$$

$MWCNT^{\bullet}$ is secondary radical.

3. Propagation and termination

$$MWCNT^{\bullet} + CH_2 = CH{-}CO{-}NH_2 \rightarrow MWCNT{-}CH_2{-}CH_2{-}CO{-}NH_2$$

4. Material and methods

4.1. Materials

MWCNTs were obtained from Shenzhen Carbon Nanotechnologies Co. Ltd. Dopamine hydrochloride, TRIM, EGDMA, IA, AAm, AIBN, allyl alcohol were purchased from Sigma–Aldrich, MO, USA. Tetrahydrofuran (THF), acetonitrile, toluene, $(NH_4)_2S_2O_8$ and dimethyl formamide (DMF) were obtained from E.Merck India Limited. Phosphate buffer solution (PBS, pH 7.0, ionic strength 0.1 mol L^{-1}) was used as a supporting electrolyte. Standard stock solution (0.1 mol L^{-1}) of DA was set via deionized water. For the analytical applications, human blood serum and urine samples were collected from a clinical laboratory located at Thiruvananthapuram City. These collected samples were stored in a refrigerator at −4 °C before use.

4.2. Instruments for characterization

The saturated calomel electrode (SCE) was used as reference electrode for potential measurements. Potentiometric measurements were made with 4 ½ Digit True RMS Multimeter (MODEL 1085). pH measurements studies were determined using Systronic (model μ pH system 362)-pH meter (Systronic India Ltd). Spectrophotometric determinations of DA were identified out using a JASCO UV-Visible (model V-530, Japan) spectrophotometer. Raman spectra were collected with a micro-Raman spectrometer Lab Ram UV HR, Jobin-Yvon and 10 mW intensity is used, in addition to that 784.9 nm excitation wavelength of a diode laser was focused onto the sample. FTIR spectra were recorded on a Perkin Elmer FTIR spectrophotometer. It was collected under room temperature/humidity control after background correction. Scans number was 32 for both samples as well as background and *X*-Axis was wave number, ranging from 0 to 4000 cm^{-1} and *Y*-axis was % transmittance. The surface morphology study of MWCNTs-MIP and MWCNTs-NIP were performed using a JEOL JSM 6390 LA scanning electron microscopy (SEM). SEM study was conducted at room temperature via using low vacuum mode. The transmission electron microscopy (TEM) images were taken by Philips CM12 instrument. X-ray diffraction (XRD) patterns were recorded using a Rigaku Dmax IC model (Japan) X-ray diffractometer. HPLC analysis was performed using Diomex Ultimate 3000 UHPLC and the