Journal of Colloid and Interface Science 396 (2013) 234–241

Contents lists available at SciVerse ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Highly selective and sensitive analysis of dopamine by molecularly imprinted stir bar sorptive extraction technique coupled with complementary molecularly imprinted polymer sensor

Bhim Bali Prasad *, Amrita Srivastava, Mahavir Prasad Tiwari

Analytical Division, Department of Chemistry, Faculty of Science, Banaras Hindu University, Varanasi 221 005, India

ARTICLE INFO

Article history:
Received 16 November 2012
Accepted 24 December 2012
Available online 1 February 2013

Keywords:
Molecularly imprinted stir bar sorptive extraction
Dopamine
"Surface grafting-from" approach
Differential pulse anodic stripping voltammetry
Real sample analysis

ABSTRACT

This paper reports a combination of molecularly imprinted stir bar sorptive extraction and complementary molecularly imprinted polymer-sensor for the analysis of dopamine as a biomarker of several neurodegenerative diseases occurred at ultra trace level. This exploited iniferter initiated polymerization via "surface grafting-from" approach onto magnetic stir bar (for sorptive extraction) and multiwalled carbon nanotubes-ceramic electrode (for detection). Such hyphenation helped dual pre-concentration of dopamine in aqueous, biological and pharmaceutical samples. This enabled high sensitivity to achieve the stringent limit [limit of detection: 4.9 ng L^{-1}, RSD = 2.4%, S/N = 3, cerebrospinal fluid] of clinical detection, without any problems of non-specific contributions and cross-reactivity.

1. Introduction

Unequivocal identification, confirmation, and quantification of analytes warrant a viable sample preparation in terms of high selectivity, cost, and eco-friendly nature [1]. With these concerns, stir bar sorptive extraction (SBSE) could be considered a very elegant enrichment technique for complex samples [2]. SBSE is a simple and less-solvent procedure based on the partitioning of target analytes between liquid sample and stationary phase coated stir bar [3]. Since the volume and surface area of the extraction phase are respectively 50–200 and 400 times larger than those of solid phase microextraction (SPME), SBSE could be handled better to obtain more sensitive results [4]. Customarily used SBSE coating materials viz., polydimethylsiloxane (PDMS) [5,6], sol–gels [7], and a composite composed of PDMS/cyclodextrin [8] were often thick films and that could easily be crippled down upon long-term exposure to the solvent. Moreover, applications of these materials were limited to only non-polar species. The mechanical and solvent stabilities, however, can be improved by the sol–gel approach of coating molecularly imprinted materials on stir bars. Molecularly imprinted polymers (MIPs) are known for their unique characteristics such as predetermined selectivity and simple preparation. Since MIP monoliths often have limited mechanical stability, we have exploited the direct polymer growth on the surface of stir bar, implicating covalent bondings in between stir bar and polymeric network. Herein, the accompanying sol–gel approach provides a direct chemical bond between the stationary phase and the silica substrate that may also impart a better mechanical stability to the MIP coating. As reported in a recent review [9], MIPs have widely been utilized as the kernel coating materials for SBSE technique. The attendant problems of MIP integration to the surface of stir bar such as the robust connection, high stability, and desired functionality could be resolved by adopting a surface-initiated iniferter (initiator-transfer agent-terminator) technique for "living" controlled radical polymerization [10]. Iniferters have extensively been used in the preparation of surface modified multifunctional materials for polymer chain growth [11,12]. The surface grafted MIP has rather higher separation efficiency than to the totally porous MIP material, because more homogenous thin polymeric film could be formed with less mass-transfer resistance [13].

In this study, the iniferter-initiated surface polymerization was carried out with a silane- bonded iniferter [3-(aminopropyl)-trimethoxy silane-initiator derivative (APTMS II)], grafted on a stir bar, to obtain a thin molecularly imprinted stir bar (MISB). With dopamine (DA) as model analyte, the prepared MISB was

Abbreviations: SBSE, stir bar sorptive extraction; SPME, solid phase microextraction; APTMS, 3-(aminopropyl)-trimethoxy silane; APTMS II, 3-(aminopropyl)-trimethoxy silane-initiator derivative; DA, dopamine; MIP, molecularly imprinted polymer; MIP–MWCNTs–CE, MIP–modified multiwalled carbon nanotubes–ceramic electrode; MISBSE, molecularly imprinted stir bar sorptive extraction; SPE, solid phase extraction; LOD, limit of detection; LOQ, limit of quantification.

* Corresponding author. Fax: +91 542 22368127.
E-mail address: prof.bbpd@yahoo.com (B.B. Prasad).

0021-9797/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jcis.2012.12.073

characterized in terms of coating morphology, composition, stability, kinetics, and extraction performance such as selectivity and capacity. MIP–modified multiwalled carbon nanotubes–ceramic electrode (MIP–MWCNTs–CE) used in this work for analyte detection is same as reported earlier [14]. However, for the first time, we have combined the molecular imprinted stir bar sorptive extraction (MISBSE) technique with complementary MIP-modified sensor to achieve the stringent detection limit in dilute biological samples. In this study, any pretreatment of biological fluids was avoided because this might lead inaccuracies in the final results insofar as ultra-analysis is concerned. Instead, their dilution with water was recommended by several folds not only to mitigate the matrix effect but also to move the analysis in the ultra trace ranges. As a consequence, the sensitivity in this work has been attained as high as parts per billion (ppb) levels, without any cross-reactivity and matrix complications from real samples (biological fluids and pharmaceuticals).

DA, a member of the catecholamine class of compounds, plays a crucial role as a neurotransmitter in normal homeostasis [15]. Detection and quantification of DA is important in diagnosis, monitoring, prevention, and treatment of certain neurodegenerative diseases, for example Parkinson's disease, in which DA concentration varies from 1.22 ± 1.81 ng mL^{-1} to almost complete depletion, and Schizophrenia and Alzheimer's disorder, with low DA levels (1.89–189 ng mL^{-1}) in serum [16]. Extremely lower DA concentrations in the detection limit (0.007–0.015 ng mL^{-1}) in cerebrospinal fluids (CSFs) could be a possible sign of disorders like pure autonomic failure (PAF) and multiple system atrophy (MSA) [17]. Literature survey revealed various analytical methods for the qualitative and quantitative determination of DA in aqueous samples such as high performance liquid chromatography (HPLC) [18], optical absorption spectrometry in the micro-fluidic system [19], flow-injection chemiluminescence [20], fluorometry [21], mass spectrometry [22,23], and electrochemical detection (ECD) [24]. All these techniques are time-consuming, laborious and expensive; HPLC with ECD was the most preferred one because of its high selectivity [25,26]. It should be noted that sensitivities realized with above techniques were not enough to diagnose, particularly PAF and MSA disorders, in hospitalized patients. Several non-electrochemical and electrochemical MIP-based devices have also been proposed [27–29], but majority of them were reportedly not validated with real samples. There are few reports on MIPs applications in solid phase extraction (SPE) [30,31] and SPME [16] of DA. However, none of these techniques (either used alone or coupled with complementary sensor) were able to determine the stringent detection limit (0.007–0.015 ng mL^{-1}) of biomarker (DA) associated with PAF and MSA disorders, in CSF samples. Notably, our earlier work [14] related to MIP–modified MWCNTs–CE was also not efficient to detect DA at the stringent limit of above diseases, when used alone. Thus, the MISBSE hyphenation with complementary MIP–modified MWCNTs–CE was inevitable to ensure an ultra trace analysis of DA, with the limit of detection (LOD) as low as 4.9 ng L^{-1} and the limit of quantification (LOQ) is 16.2 ng L^{-1} of diluted CSF sample of a healthy volunteer [normal, 1.89–189 ng mL^{-1} (serum); 0.29–2.55 ng mL^{-1} (CSF)], by means of twice successive pre-concentrations during extraction and sensing experiments. The proposed method is free from any cross-reactivity and false-positives and can be designated as a practical biomedical device for neurodegenerative diseases.

2. Experimental

2.1. Reagents

Acryloyl chloride (AC), p-nitrophenol (NP), N,N-diethyldithiocarbamate, was purchased from Loba Chemie (Mumbai, India) and Spectrochem Pvt. Ltd. (Mumbai, India). Ethylene glycol dimethacrylate (EGDMA), 3-(aminopropyl)-trimethoxy silane (APTMS), 4-(chloromethyl) benzoyl chloride (CMBC), MWCNTs (internal diameter 2–6 nm, outer diameter 10–15 nm, length 0.2–10 μm; and purity >90%), DA and its interferents, were obtained from Aldrich (Steinheim, Germany) and Fluka (Steinheim, Germany). Solvents dimethylsulphoxide (DMSO), dimethylformamide (DMF), methanol, ethanol, ether, acetone, and triethylamine (TEA) were procured from Spectrochem Pvt. Ltd. (Mumbai, India) and used as received. Phosphate buffer solution (PBS) (pH 4.8, ionic strength 0.1 M), was used as a supporting electrolyte. Standard stock solution of DA (1.0 μg mL^{-1}) was prepared using demineralized triple distilled water (TDW) (conducting range 0.06–0.07 × 10^{-6} S cm^{-1}). This solution was stored in a refrigerator and protected from light. All working solutions of DA were prepared daily by an appropriate dilution with TDW. The pH values of test solutions were adjusted by addition of a few drops of either 0.1 M HCl or 0.1 M NaOH.

Pharmaceutical sample analyzed was dopamine hydrochloride injection USP (certified value 40 mg mL^{-1}). Human blood serum and CSF were obtained from the Institute of Medical Science, Banaras Hindu University (Varanasi, India) and stored in a refrigerator at ~4 °C until analysis performed. Glass capillaries were purchased from Top-Tech Biomedical (Varanasi, India).

2.2. Equipments

Extracts, desorbed from MISB, were analyzed using a voltammetric analyzer/stripping voltammeter [Model 264 EG and G Princeton Applied Research (PAR), USA] in conjunction with an electrode assembly X–Y recorder (PAR Model RE 0089), following differential pulse anodic stripping voltammetric (DPASV) technique. In the three electrode system, an MIP–MWCNTs–CE electrode, standard Ag/AgCl electrode with porous vicor frit, and a platinum electrode were used as working, reference and auxiliary electrode, respectively. FT-IR characterizations were performed with Varian 3100 FT-IR (USA). Morphological images of MISB were recorded on a scanning electron microscope (SEM) [JEOL, JSM, Netherlands, Model 840 A]. All experiments were carried out at 25 ± 1 °C.

2.3. Molecular modeling

Molecular modeling was performed in Gaussian 09 software. To examine the properties of MIPs at the molecular level, a model of template-monomer complexes was set with the help of Gauss view 5.0. For making selection, few monomers namely, p-nitrophenol acrylate (p-NPA), p-aminophenyl acrylate (p-APA), and itaconic acid (IA) were arbitrarily selected which could be a prospective monomer from the structural point of view. As many as two possible configurations of DA-monomer (1:*n*) molecular systems were optimized by applying relatively accurate Hartree–Fock (HF) computations at the second order Moller–Plesset perturbation (MP2) level using 6-31+G(d,p) basis set. The minimum binding energies between the optimized conformations of 1:*n* DA-monomer complexes are shown in Table S1. Through conformation optimization, interaction energies, ΔE, for all complexes were calculated using the following equation:

$$\Delta E = E(\text{template-monomer}) - E(template) - \Sigma E(monomer) \quad (1)$$

2.4. Preparation of stir bars

The "dumbbell-shaped" stir bars (1000 μm thickness) were prepared following a known procedure [32]. In brief, first a glass capillary (1 mm diameter, 20 mm length) consisting of 0.8 mm