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Molecularly imprinted micro solid-phase extraction technique coupled with complementary molecularly imprinted polymer-sensor for ultra trace analysis of epinephrine in real samples

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ABSTRACT

A simple hyphenation approach was adopted to obtain a new molecularly imprinted micro solid-phase extraction fiber (as a selective extraction tool) and complementary molecularly imprinted polymer coated pencil graphite electrode (as a detection tool) for the selective and sensitive analysis of epinephrine, which is a disease biomarker prevalent at ultra trace level in biological fluids. In both extraction and detection processes, the functionalized multiwalled carbon nanotubes (CNT-mers) were preferred to multiwalled carbon nanotubes (unmodified) in order to obtain a stable homogeneously dispersed imprinted polymer matrix of better electroconductivity and adsorptive characteristics. The hyphenation of both tools helped dual pre-concentration of epinephrine so as to achieve the stringent limit [limit of detection: 0.002 ng mL^{-1}, S/N = 3] of clinical detection, without any problems of non-specific contributions and cross-reactivity.

1. Introduction

Epinephrine (Ep) or adrenaline acts as a hormone as well as an important catecholamine neurotransmitter. Its concentration in blood affects contraction of smooth muscles, heart rate, blood pressure, glycogenolysis in liver and muscle, and lipolysis in adipose tissue [1,2]. The unbalances of Ep concentration in blood plasma and cerebrospinal fluids (CSF) [normal, 0.09–0.69 ng mL^{-1} (plasma) [3] and 0.02–0.16 ng mL^{-1} (CSF) [4]] are associated with several diseases viz. tachycardia (plasma Ep level 0.11 ng mL^{-1}) [5], Guillain-Barre syndrome (plasma Ep level 0.081 ng mL^{-1}) [6], renovascular hypertension (plasma Ep level 0.14 ng mL^{-1}) [6], Parkinson's disease (CSF Ep level 0.005–0.144 ng mL^{-1}) [4], pheochromatocytoma (plasma Ep level 0.21 ng mL^{-1}, CSF Ep level 0.057 ng mL^{-1}) [6], and hypertension (plasma Ep level 0.091–0.027 ng mL^{-1}; CSF Ep level 0.010–0.001 ng mL^{-1}) [6]. Therefore, it is very important to develop selective and sensitive analytical method for the determination of Ep manifesting aforesaid diseases at different stringent limits. Many techniques have been developed for this purpose; for instance, electrochemical detection [7–11], chemiluminescence [12,13], high performance liquid chromatography [14–20], flow injection [21], capillary electrophoresis [22], fluorimetry [23], and spectrophotometry [24]. These methods were although used for the accurate determination of Ep in aqueous samples, some of them actually involved expensive instrumentations, complex procedures of the sample pretreatment, and insufficient selectivity.

Micro solid-phase extraction (MSPE) can be considered as a very elegant sample preparation technique for complex samples. This extraction procedure, unlike routine solid-phase microextraction (SPME), involved analyte adsorption on a much smaller nanostructured solid-phase matrix followed by an exhaustive recovery [25,26]. Insofar as selectivity is concerned, molecular imprinting can be considered as a most burgeoning technology for making predetermined selective binding sites in synthetic polymers using a molecular template (analyte) [27–29]. Thus, the molecularly imprinted polymer (MIP)-based MSPE technique has been widely exploited in the last decade for the selective and exhaustive recovery [30–34]. Although several MIP-sensors have been reported for the Ep determination [35–40], there is no report on MIP applications in SPME/MSPE of Ep. Furthermore, none of MIP-sensors was found to be effective to determine the stringent concentrations of Ep manifesting several chronic diseases, in dilute biological fluids. Dilution of biological samples is essentially required to mitigate matrix complications.

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The aim of present work is to improve our previous work [40] in terms of selectivity and sensitivity of Ep measurement for the disease diagnosis. This could be feasible by combining molecularly imprinted micro solid-phase extraction (MIMSPE) technique with complementary MIP-modified pencil graphite electrode (PGE)-sensor. Note that the MIP-sensor earlier used was miserably failed to estimate Ep ultra trace level essentially required for the disease diagnosis (the limit of detection (*LOD*) realized by the sensor was 0.020 ng mL^{-1} [40]). In this work, we have adopted the similar approach of MIP development as has been utilized for the fabrication of sensor [40]. Herein, MIP was developed directly as monolith fiber or on the surface of PGE, in the presence of multiwalled carbon nanotubes (MWCNTs) covalently attached with N-hydroxy phenyl maleimide (MWCNTs-NHPM) (hereafter, this monomer is represented by the word coined as "CNT-mer"). The CNT-mer served as a potential monomer in the synthesis of a low cross-linked imprinted polymeric artificial receptor for Ep. Functionalization of MWCNTs was particularly aimed to ensure homogeneous dispersion of MWCNTs in the MIP motif (one MWCNTs per monomer unit) and inculcate better electroconductivity and intermolecular stability, in comparison to that prepared with randomly distributed MWCNTs dispersant involving non-covalent physical adsorption in maleimide monomer [41]. The strong van der Waals forces among MWCNTs and their maximum aspect ratio (length/outer diameter = 666) often result in self-agglomeration to interrupt a fine dispersion of MWCNTs into matrix. On the other hand, functionalizations of MWCNTs not only favor easy processing, but at the same time their adsorption properties with organic chemicals can also be altered greatly. Functional groups can change the wettability of MWCNTs surfaces and consequently make them suitable for the adsorption of relatively low molecular weight polar compounds [42]. Furthermore, when CNT-mer is used as MIP supporting material, the resultant stationary phase has higher mechanical strength and chemical stability, with the binding sites located at the outer layer of composite. This fact is clearly opposed to that of traditional use of randomly dispersed MWCNTs in MIP texture where the generated cavities may not be on the surface or in its proximity. In fact, the use of non-functionalized MWCNTs (or carbon), in the development of monolithic MIPs, may generate molecular cavities in the deep intrinsic core of fiber texture [43–46]. Therefore, CNT-mer was preferred to MWCNTs in the present work to reduce prolonged binding time owing to increased diffusional resistance and to improve site accessibility resulting in enhanced extraction efficiency [42]. It should be also born in mind that CNT-mer based MIP molded as MIMSPE fiber (without any solid support) with sample-volume to solid-phase matrix ratio 1.73×10^3 always renders a smaller micro solid-phase for the extraction (cf. SPME), without any surface crippling. Contrarily, the detection tool using complementary MIP-sensor necessarily required a solid support (pencil graphite) for the MIP modification in which CNT-mer along polymer backbone served as a "metallic wire" to facilitate the channelized electron transport and thereby the improved electroconductivity. To the best of our knowledge, no MIMSPE fiber has been, hitherto, reported utilizing the unique characteristics of CNT-mer. The proposed combination of MIMSPE with complementary MIP sensor could be advantageous to achieve ultra trace level detection of Ep in dilute biological samples by means of dual pre-concentrations (first on MIMSPE fiber and second on sensor), without any cross-reactivity and false-positives.

2. Experimental

2.1. Reagents

Maleic anhydride and *p*-amino phenol were purchased from Loba Chemie (Mumbai, India). All Solvents, dimethylsulphoxide (DMSO), triethylamine (TEA), tetrahydrofuran (THF), dimethylformamide (DMF), acetic acid (HOAc), chloroform, ethanol, and acetone, were purchased from Spectrochem Pvt. Ltd. (Mumbai, India). Cupric chloride ($CuCl_2$) and 2,2′-bipyridyl (bpy) were purchased from BDH chemicals (London, U.K.). Ethylene glycol dimethylacrylate (EGDMA), MWCNTs (internal diameter 2–6 nm, outer diameter (*d*) 10–15 nm, length (*l*) 0.2–10 μm, aspect ratio (*l*/*d* = 20–666) and purity >90%), Ep and its interferents, were obtained from Aldrich (Steinheim, Germany) and Fluka (Steinheim, Germany). All other chemicals were of analytical grade and used as such without further purification. Phosphate buffer solution (PBS, pH 6.8, ionic strength 0.1 M) was used as a supporting electrolyte. Standard stock solution of Ep (1.00 μg mL^{-1}) was prepared using deionized triple-distilled water (TDW) (conductivity range $0.06–0.07 \times 10^{-6}$ S cm^{-1}). All working solutions of different Ep concentrations were prepared by diluting stock solution with requisite volume of TDW. The pharmaceutical sample analyzed was Vasocon (1.0 mg mL^{-1} Ep) from Neon Laboratories Ltd. (Mumbai, India). Human blood plasma and CSF were procured from the Institute of Medical Sciences, Banaras Hindu University (Varanasi, India), and stored in a refrigerator at ~4 °C until analysis was performed. The endogenous (original) concentrations of test analytes in real matrices were first determined by the proposed hyphenation method (MIMSPE–MIP-sensor), after requisite maximum dilution of each sample. These diluted samples could be fortified (if required) by the standard addition of test analyte so as to explore the linear quantitation range of detection of the proposed technique. Glass capillaries of different internal diameters (0.4, 0.5, 0.6, 0.7, 0.8, and 1.0 mm) and pencil rods (2B, 0.5 mm in diameter and 5.0 cm in length), were procured from Top-Tech biomedical (Varanasi, India) and Hi Par, Camlin Ltd. (Mumbai, India), respectively.

2.2. Equipments

MIMSPE fiber of 1.5 cm exposed length was used for the preconcentration of template under study. Extracts, desorbed from this fiber, were analyzed using a voltammetric analyzer/stripping voltammeter [Model 264 EG and G Princeton Applied Research (PAR), USA] in conjunction with an electrode assembly and X-Y recorder (PAR Model RE 0089), following differential pulse anodic stripping voltammetric (DPASV) technique. In the three electrode assembly, a PGE [duly modified with the same MIP film as used for MIMSPE fiber, hereafter referred as MIP-sensor], a 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 MIMSPE fibers 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. Synthesis of monomer

All precursors, N-hydroxy phenyl maleimic acid (HPMA), its derivative N-hydroxy phenyl maleimide (NHPM), and MWCNTs-COCl, were obtained as described elsewhere [40]. The CNT-mer was obtained by the reaction of NHPM (150 mg) and MWCNTs-COCl (50 mg) following the known recipe [47] [for details, vide Supplementary Data section S1].

2.4. MIMSPE fiber preparation

For MIMSPE fiber preparation, normally in a single batch, the pre-polymer mixture containing monomer (CNT-mer, 15 mg, 1.0 mL DMSO), cross-linker (EGDMA, 0.2 mmol, 1.0 mL), and template (Ep, 0.08 mmol, 1.0 mL DMSO) was reacted with a Cu (II)/bpy complex (0.02 mmol) in the presence of TEA (2.0 mmol, 280.0 μL)