Journal of Chromatography A, 1337 (2014) 22–31

Contents lists available at ScienceDirect

Journal of Chromatography A

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

An insulin monitoring device based on hyphenation between molecularly imprinted micro-solid phase extraction and complementary molecularly imprinted polymer-sensor

Mahavir Prasad Tiwari, Bhim Bali Prasad *

Analytical Division, Department of Chemistry, Faculty of Science, Banaras Hindu University, Varanasi 221005, India

ARTICLE INFO

Article history:
Received 15 December 2013
Received in revised form 8 February 2014
Accepted 14 February 2014
Available online 22 February 2014

Keywords:
Molecularly imprinted micro-solid phase extraction fiber
Grafting via surface attached monomer
Grafting via sol–gel
Insulin
Differential pulse anodic stripping voltammetry
Real sample analysis

ABSTRACT

Molecularly imprinted micro-solid phase extraction fiber was developed by modifying molecularly imprinted polymer film on the surface of silica fiber exploring "*grafting via surface attached monomer*" (method I) and "*grafting via sol–gel*" (method II) approaches. The latter approach was found to be inferior to the former one in terms of the sensitivity of insulin detection [method I, *LOD* = 0.009 ng mL^{-1}; method II, *LOD* = 0.064 ng mL^{-1}, *RSD* = 1.21%]. Notably, either of the techniques, molecularly imprinted micro-solid phase extraction or complementary sensor, was found to be incompetent to monitor the stringent level of insulin in the real samples. However, the combination of these techniques has been found quite suitable for achieving the high detection sensitivity of ultra-trace insulin in human blood serum and Huminsulin injection, without any non-specific (false-positives) contributions. The proposed hyphenated device could serve as a possible marker for risk of developing type 2 diabetes mellitus and diabetic coma due to insulin resistance in human beings.

1. Introduction

One of the tools that may create highly selective extraction process could be incorporation of bio-recognition materials in the extraction methodologies. However, in spite of the specificity, natural biomolecules are difficult to be procured, expensive, and chemically unstable. Alternatively, synthetic receptors such as molecularly imprinted polymers (MIPs) could be used due to their lower cost, high stability, easier preparation, and high sensitivity. Despite wide applications and numerous fabrication methods of MIPs, efforts to generate imprints of protein targets have so far been less successful [1–4]. This was due to the restricted mobility of protein molecules within the highly cross-linked polymer network and the poor reversibility of binding process. Furthermore, in some cases, grinding of bulk MIPs can cause the destruction of affinity sites. To overcome aforesaid attendant problems, surface imprinting is apparently more popular, in lieu of the bulk imprinting process of creating protein-imprints in the polymer network [5]. For this, the "*grafting from*" approaches are widely used to obtain polymer coating with high grafting density. However, a rigorous characterization of such polymer after grafting is necessarily required; and moreover, the high grafting density, as a consequence of polymer chain growth using the initiator on the surface, may involve analyte diffusion impediment particularly through pores of MIP network. On the other hand, in the "*grafting to*" procedure, the conventional free radical/living polymer chains are readily reacted with the suitable termination group on the surface. This might result in a low graft density simultaneously leading to the uncontrolled bulk polymerization owing to the presence of unreacted monomer in the solution. To avoid this constraint, the "*grafting via surface attached monomer*" approach could be more advantageous. Herein, the free-radical polymerization is carried out directly at the surface duly immobilized with functionalized monomer molecules [6]. At the initial stage, the polymerization rates for both the surface-attached monomer and the free monomer in solution are identical. At the later stage, the modified surface is crowded with permanently attached polymer core shells, and the concentration of free monomeric units become too small to exert any uncontrolled simultaneous polymerization in the bulk. In this way a highly dense polymeric film, with exposed MIP cavities, at the surface for micro-solid phase extraction (MSPE) fiber is obtained, in contrast to the "*grafting to*" approach. The non-specific protein adsorption, however, may be another constraint in physiological environment. In

* Corresponding author. Tel.: +91 9451954449; fax: +91 542 22368127.
E-mail addresses: prof.bbpd@yahoo.com, krineshtiwari@yahoo.com (B.B. Prasad).

http://dx.doi.org/10.1016/j.chroma.2014.02.041
0021-9673/

order to avoid non-specific adsorption and thereby obtaining a non-fouling polymer surface, we have typically used a monomer that contained phosphatidyl choline (PC) groups resembling natural phospholipids. This helped in synthesizing a biocompatible MIP that resists non-specific interactions with serum proteins and cells [7]. The water-compatibility of MIP constituting PC moieties could be fostered by the use of functionalized multi-walled carbon nanotubes (MWCNTs-COOH). The internal surface of homogeneously dispersed carbon nanotubes might augment protein-binding kinetics [8] and electro-conductivity in micro-extraction and detection, respectively.

Insulin is a polypeptide hormone synthesized by the β-cells of the islets of Langerhans in the pancrease. It regulates blood glucose by signaling when high levels of blood glucose present in the body. The normal level of the insulin concentration in human blood serum is between 2.8 and 28.0 ng mL^{-1} [9]. Insulin resistance is common finding in diabetes mellitus. This may serve as a measure of efficacy of therapies (exercise, exogenous insulin, sulfonyl ureas, and PPAR gamma agonists) for diabetes mellitus and as a possible marker for the risk of developing type 2 diabetes mellitus (1.32 ng mL^{-1}) [10] and diabetic coma (<0.44 ng mL^{-1}) [9]. These concentration levels can further decrease to pg mL^{-1}, if biological samples are inevitably diluted (20-fold) in order to mitigate matrix complications, and this warrants the development of a highly sensitive and selective device for insulin measurement in biological fluids.

Numerous methods have already been used for the determination of insulin including high performance liquid chromatography (HPLC) coupled with UV absorbance [11,12], mass-spectrometry [13–15], photolytic-electrochemical detection [16], fluorescence [17], bioassay [18], and immunoassay [19]. These methods are limited in their use as they can be time-consuming and slow, involving a costly set-up, a complex extraction, and purification procedure. The detection limits (*LODs*) obtained by these reported techniques are not enough to determine the stringent limit in insulin resistance patients. Various electrochemical methods [20–31] have also been developed for the detection of insulin. However, these methods still lacked the specificity, sensitivity, and stability, which are essential for an accurate and precise monitoring of trace-level insulin. Although MIPs selective for insulin have already been reported [32–36], their detection limits, linear concentration range, and analyte recovery were not satisfactory. We have recently reported an MIP-sensor, for recognition and detection of insulin with detection limit as low as 0.11 ng mL^{-1} [37], but this too failed to detect the stringent limit of insulin, particularly in the dilute blood serum samples. Molecularly imprinted micro-solid phase extraction (MIMSPE) can be considered as a very elegant sample preparation technique for complex samples. This extraction procedure is a renovated version of molecularly imprinted solid-phase microextraction (SPME) and it is a total extraction method that utilizes a reduced sample and sorbent volume (the ratio between the sample volume matrix and solid phase should be at least 10^4). Thus, the MIMSPE technique has been widely exploited in the last decade for the selective and exhaustive extraction [38–43]. Insofar as molecularly imprinted SPME fibers [44–47] are concerned, these were found to be suffered from several inherent problems of poor stability, lacking porosity, fragility, MIP crippling, low loading capacity, and massive chemical/matrix interferences. Therefore, in the present work, a new approach is demonstrated to attend these problems by developing a hyphenated tool MIMSPE coupled with MWCNTS-MIP composite sensor for ultra-trace analysis of insulin adopting "*grafting via surface attached monomer*" (method I) and "*grafting via sol-gel*" approaches (method II). To the best of our knowledge, neither SPME/MSPE nor MIMSPE/molecularly imprinted SPME, have been, hitherto, reported for the determination of insulin. The conventional MIMSPE approach [44], in which the bulk MIP was grafted onto silica surface using method II, was found inferior to the MIP-modified on the surface of Si-fiber using method I, in terms of sensitivity of the insulin detection, in the present work. We have thus resorted to adapt method I for the development of MIMSPE fiber and coupled with the complementary MIP-sensor for detection. This combination (MIMSPE–MIP-sensor, method I) is found superior and more practical in clinical settings for ultra-trace analysis of insulin, in dilute real samples, by means of double preconcentrations during extraction and detection processes.

2. Experimental

2.1. Chemicals and reagents

MWCNTs (internal diameter 2.6 nm, outer diameter 10–15 nm, length 0.1–10 μm, and purity >90%), *p*-aminophenol, 2-chloro-2-oxo-1,3,2-dioxaphospholane (COP), ethylene glycol dimethacrylate (EGDMA), 2,2′-azobis(isobutyronitrile) (AIBN), anilinediamine, 3-(trimethoxysilyl) propyl acrylate (TPA), tetraethoxysilane (TEOS), insulin, and its interferents, were purchased from Aldrich (Steinheim, Germany). Acryloyl chloride (AC), dimethylsulphoxide (DMSO), dimethylformamide (DMF), and other solvents were obtained from Loba Chemie (Mumbai, India) and Spectrochem Pvt. Ltd. (Mumbai, India) and used as received.

Standard stock solution of insulin (pH 7.2) was prepared by dissolving 10 mg of insulin in 100 mL demineralized triple distilled water [conductivity range $(6.0–7.0) \times 10^{-8}$ S cm^{-1}] and stored in a refrigerator to avoid any denature/destabilization of protein structure. All working solutions of insulin were prepared daily by appropriate dilution with water, and their pH was adjusted to 5.4 with the help of 0.1 M HCl.

Pharmaceutical preparation analyzed was Huminsulin injection (40 IU mL^{-1}, Sun Pharmaceutical Industries Ltd., Gujarat, India). Human blood serum samples 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. Test blood serum samples were analyzed, without deproteinization or filtration, after 20–100-fold dilutions with water, by the method of standard addition.

Silica (Si) fibers (140 μm in diameter) were taken from an optical cable normally used for the data transport and telecommunication (Polymicro, Phoenix, AZ). Thickness of the modified Si wire was measured with the help of Vernier Calipers (Mitutoyo, Japan).

2.2. Equipments

Extracts desorbed from MIMSPE-Si-fiber (1.5 cm exposed length), mounted in the plunger hole of insulin syringe (Hindustan syringes and medical devices, India) (Fig. S1, vide Supplementary material), were analyzed using a voltammetric analyzer/stripping voltammeter [Model 264 EG & G Princeton Applied Research (PAR), USA] in conjunction with a 303 A electrode assembly and X–Y recorder (PAR Model RE 0089) following differential pulse anodic stripping voltammetry (DPASV) technique. In the three electrode system, an MWCNTs-MIP composite modified pencil graphite electrode (PGE) (surface area 1.96×10^{-3} cm^2), a standard Ag/AgCl electrode with porous Vicor frit, and a platinum electrode were used as working, reference, and auxiliary electrodes, respectively (Fig. S1). FT-IR and FT-NMR characterizations were performed with Varian 3100 FT/IR (USA) and JEOL AL 300 FT/NMR (Japan), respectively. Exeter Analytical Inc. 'Model CE-400 Elemental Analyzer' (Mexico) was used for elemental analysis. Morphological images of MIP-grafted Si fibers were recorded on a Scanning Electron Microscope (SEM) (JEOL, JSM, Netherlands, Model 840 A).