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Development of electrochemically controlled packed-in-tube solid phase microextraction method for sensitive analysis of acidic drugs in biological samples

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ABSTRACT

In the present research, for the first time, a novel "packed-in-tube" configuration has been applied to electrochemically controlled in-tube solid phase microextraction, followed by high performance liquid chromatography. In order to prepare a mini packed column, small beads of stainless steel were first placed into the stainless steel column. Then, a nanostructured polypyrrole film was prepared on the internal surface of a stainless steel tube and the surface of stainless steel particles through a facile in-situ electrodeposition method. Filling the column with tiny particles of stainless steel effectively reduces the dead volume of the extraction tube and increases the extraction phase volume. The column was used for separation and preconcentration of diclofenac and mefenamic acid as model analytes from biological samples. Several important factors affecting extraction efficiency, such as extraction and desorption times, flow rates of the sample solution and eluent, and extraction and desorption voltages were investigated and optimized. This method showed good linearity for the drugs in the range of 0.3–200.0 μg L^{-1}, 1.1–200.0 μg L^{-1}, and 1.8–200.0 μg L^{-1} with coefficients of determination better than 0.9986, 0.9973, and 0.9973 in water, urine, and plasma samples, respectively. Intra- and inter-assay precisions (RSD%, n = 3) were in the range of 2.6–4.8% and 2.9–5.1, respectively, at three concentration levels of 10, 25, and 75 μg L^{-1}. In addition, the limits of detection were in the range of 0.02–0.04 μg L^{-1}. The validated method was successfully applied to the analysis of diclofenac and mefenamic acid in some biological samples. Finally, it is concluded that this method can be a general and reliable alternative to the analysis of ionic compounds in biological matrices.

1. Introduction

In recent years, environmental pollution by chemical substances has received great attention. Thus, fast and precise determination of trace amounts of different analytes has received significant attention recently. Solid-phase microextraction (SPME) is an effective sample pretreatment method; therefore, it has been extensively used in environmental, medicine, food, and biological analyses [1–4]. This method is more rapid and less expensive than the traditional methods and can be easily combined off-line or on-line with various separation techniques, including gas chromatography (GC) [5], high-performance liquid chromatography (HPLC) [6], and capillary electrophoresis (CE) [7]. In-tube solid-phase microextraction (IT-SPME) is a form of SPME, which typically uses open tubular fused-silica capillary columns as extraction devices [8,9]. IT-SPME is valuable for miniaturization, rapidness, high-throughput performance, online coupling with analytical instruments, such as liquid chromatography/mass spectroscopy (LC/MS) and LC/MS/MS, and avoidance of solvent consumption [10]. In general, the majority of the commercially available IT-SPMEs are mostly devoted to fused silica capillaries with different polymeric coatings [11]. However, the utility of conventional capillary columns is limited by their low extraction efficiency because of the large breakthrough volume and the small amount of adsorbent phases, low stability, and, in some cases, the long extraction times involved due to the slow diffusion of the analytes from the sample to the capillary coating [12]. Considerable efforts have been focused on developing new adsorbent phases, such as fiber-packed, sorbent-packed, and rod-type monolith capillaries, to improve the extraction efficiency, stability, and selectivity [13–15]. For example, the extraction capacity can be improved by directly packing adsorbent particles into the polyether ether ketone (PEEK) tube [16,17]. However, the high column pressure, resulting from tightly packed micrometer particles, has limited the improvement of sample loading speed. In the other works, Saito *et al.* developed the fiber in-tube SPME method to reduce the internal volume of the extraction

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capillary by inserting the stainless steel wire [18] and also using polymer zylon fibers to pack into the capillary or PEEK tubes to increase the extraction phase volume [19]. Recently, in order to solve the problem of fragility of the conventional capillary columns and increase repeatability of coating preparation, electrochemically coated stainless steel capillaries have been developed [20,21]. Electrochemically coated tubes exhibit several advantages including higher extraction efficiency, better thermal and mechanical stability, and easily controllable film thickness and morphology by alteration of different parameters, such as electrolyte concentration, applied potential, and polymerization time [20]. However, the IT-SPME method showed poor selectivity and low extraction efficiency to ionic compounds. Recently, to overcome this difficulty, a new method, namely the electrochemically controlled in-tube SPME (EC-IT-SPME) method, has been developed for extraction of ionic components [22–24]. In this method, just the same as in the IT-SPME method, selection of a suitable adsorbent is necessary. In this method, some conductive polymers, such as polypyrrole and polyaniline, were electrochemically coated on the inner wall of the stainless steel tube and employed as the extraction medium. Intrinsically conducting polymers with conjugated double bonds have attracted much attention as advanced materials because of their potential applications [25]. Among those conducting polymers, polypyrrole (Ppy) and its derivatives have become one of the most widely studied coatings due to their facile polymerization from organic or aqueous media through electrochemical or chemical methods, good environmental and higher conductivity than many other polymers [26]. However, because of the large breakthrough volume and the small amount of adsorbent phases, the utility of inner wall coated capillary columns is limited by their low extraction efficiency. Developing low-cost EC-IT-SPME packed column sorbents with high specific surface area, high extraction efficiency, and low column pressure has been the main goal of our research.

In this work, tiny stainless steel particles were packed into a stainless steel column in order to increase the extraction phase volume. Then, the nanostructured Ppy coating was electrochemically deposited on the inner surface of a stainless steel tube and the surface of the stainless steel particles as the unbreakable substrate. After inserting the stainless steel particles, the free space of the extraction column reduced, while the surface area of the coating contacting the sample solution increased significantly. In addition, compared to conventional packed capillaries, the column pressure significantly decreased. The results also indicate that a further preconcentration effect, as opposed to the conventional EC-IT-SPME method, can be obtained with this modification. The characteristics of this packed column coating, such as mechanical stability, coating preparation reproducibility, and extraction efficiency were investigated. The developed packed column EC-IT-SPME method was then applied to the extraction of the diclofenac and mefenamic acid from urine and plasma samples.

2. Experimental

2.1. Chemicals and reagents

Standards of diclofenac (Dic, pKa = 4.15), mefenamic acid (Mef, pKa = 3.88), synthetic pyrrole (98% pure), and sodium dodecyl benzene sulfonate (SDBS) were obtained from Sigma-Aldrich (Milwaukee, WI, USA). Sodium hydroxide and hydrochloric acid (95% pure) were obtained from Merck (Darmstadt, Germany). Ultra-pure water was produced by using a Youngling ultrapure water purification system model Aqua MaxTM-ultra (Seoul, South Korea). Other chemicals applied were of analytical reagent grade or of the highest purity available.

2.2. Apparatus

The particle size and morphology of the synthesized nanoparticles were determined by a scanning electron microscope (SEM) model EM3200 from KYKY Zhongguancun (Beijing, China). GPFA1–380 peristaltic pump from Ultra-Voltammetry Company (Tehran, Iran) was applied to pass the solutions through the stainless steel packed columns. All the pH measurements were performed with a WTW Inolab pH meter (Weilheim, Germany). The chromatographic analysis was performed with a HPLC instrument, including a Varian 9012 HPLC pump (Walnut Creek, CA, USA) and a six-port Cheminert HPLC valve from Valco (Houston, TX, USA) with a 100-μL sample loop, equipped with a Varian 9050 UV–vis detector. The chromatographic data were recorded and analyzed using Chromana software (version 3.6.4). The separations were run under isocratic elution conditions on an ODS-3 column (250 mm × 4.6 mm, with a 5-μm particle size) from Hector Company (Daejeon, Korea). The chromatographic separation was performed using a mobile phase, consisting of 10 mmol L^{-1} phosphate buffer (pH = 4.5) and acetonitrile (40:60) for 15 min, flow rate of 1.0 mL min^{-1}. The detection of the analytes was achieved at 210 nm.

2.3. Preparation of polymer-coated packed tubes

In this work, stainless steel particles (0.2 mm) were inserted into the stainless steel tube (4 cm length and 0.6 cm diameter) and were used as a packed column (Fig. 1). The stainless steel particles were plugged with a small portion of cotton at the ends of the column to retain the particles in the column. Then, the packed column was used as the working electrode. Before the electrochemical deposition, the stainless steel packed column was cleaned by methanol and HPLC grade water and finally air dried at the room temperature. Then, a nanostructured polypyrrole-dodecyl benzene sulfonate (Ppy-DBS) was coated on the inner surface of a stainless steel tube and the surface of the stainless steel particles using cyclic voltammetry in the potential range of − 0.1 to + 0.8 V during 30 cycles (scan rate: 50 mV s^{-1}) in an aqueous solution containing 0.2 mol L^{-1} pyrrole and 0.01 mol L^{-1} SDBS as the supporting electrolyte. A platinum electrode and an Ag/AgCl electrode were used as the counter and reference electrodes, respectively. A peristaltic pump was used to deliver the monomer solution from the inner surface of the stainless steel tube. After the electrochemical deposition, the packed column coated with the Ppy-DBS film was washed with methanol, acetone, and water in sequence to remove all excess pyrrole and SDBS from the film. It was then dried under the nitrogen gas flow. Fig. 1 shows a schema of the packed column system and the stainless steel particles before and after the synthesis of the Ppy-DBS coating.

2.4. On-line packed column EC-IT-SPME procedure

A schema of the complete assembly and operation mode is shown in Fig. 2. The Ppy-DBS coated stainless steel packed column was mounted on valve 1 (V_1) in the position. According to Fig. 2, the packed column was inserted into the HPLC guard column holder and the connections were facilitated using 2.5 cm sleeve of 1/16-in PEEK tubing at each end of the column. Both V_1 and valve 2 (V_2) were initially set at the load position (red arrows). Pump A is on to direct the sample solution through the tube at 2.4 mL min^{-1}, and Pump B is off. The effluent of V_1 was poured again into the sample compartment after passing through the coated packed column. In other words, this procedure was carried out in a circulating path. The platinum electrode was connected to the negative potential and was used as the cathode electrode. By passing the sample solution through the Ppy-DBS electrode, the extraction of Dic and Mef occurred by applying the positive potential (+ 0.6 V) under the flow conditions. After extraction for a given time interval, the Pt electrode was connected to the positive potential and was used as the anode electrode. V_1 was directed to the inject position. Pump A was turned off, while the pump B was turned on to pass the desorption solvent (0.1 mol L^{-1} NaCl in methanol) through the tube at 1.5 mL min^{-1}. By passing 80 μL of the desorption solvent from the inner surface of the Ppy-DBS electrode, the desorption of the drugs occurred by applying the negative potential (− 0.5 V). Finally, after a given