



Short communication

Monolithic capillary column with an integrated electrochemical detector

Martina Komendová^a, Radovan Metelka^a, Jiří Urban^{a,b,*}^a Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Studentská 573, 532 10, Pardubice, Czech Republic^b Department of Chemistry, Faculty of Science, Masaryk University, 625 00, Brno, Czech Republic

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ABSTRACT

The carbon fiber and silver microwire were used as working and pseudoreference electrode, respectively, and inserted into the ending of capillary to prepare monolithic capillary column with an integrated electrochemical detector. Prepared capillary devices offered stable and robust results with relative standard deviations of retention, resolution, and detection signal lower than 1.5, 5.5, and 5.0%, respectively. To further increase sensitivity of developed electrochemical microdetector, multiple pulse amperometry detection mode has been used. Optimized integrated device provided reliable chromatographic separation of mixture of neurotransmitters with calibration curve for dopamine linear from 0.5 to 20.0 mg L⁻¹ and an instrumental limit of detection as low as 24 pg of injected dopamine. Finally, developed capillary column was applied to successful determination of dopamine in a human urine. By using both calibration curve and standard addition method, the dopamine level was determined to be 0.74 ± 0.03 mg L⁻¹ and 0.71 ± 0.02 mg L⁻¹, respectively. Triplicates of dopamine analysis provided relative standard deviations lower than 2.7% for intraday analyses, while interday relative standard deviations were lower than 3.6% for five consecutive days.

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1. Introduction

Electrochemical detection in capillary and micro high-performance liquid chromatography (HPLC-EC) of electroactive compounds is easily feasible nowadays [1]. Miniaturization of detection cell is required to suppress peak broadening of analyte, whereas enhanced current densities are necessary to achieve high sensitivity [2]. For that reasons, carbon fiber is often the electrode material of choice. Single carbon fibers [3–6] or strand of carbon fibers [7,8], microband electrodes [9] or interdigitated arrays [10–13] were utilized as working electrodes for capillary or micro HPLC of catecholamine neurotransmitters and related compounds.

Polymer monoliths were introduced in 1990s as an alternative stationary phases to spherical particles generally used in conventional chromatographic columns. Monoliths with dominant flow-through pores offer application mainly in a fast gradient elution of synthetic and natural polymers [14], although by using

various experimental protocols [15–19], monolithic stationary phases allowing separation of small molecules are being developed.

So far, only several attempts have been made to develop polymer-based monolithic stationary phase for the separation of neurotransmitters. Recently, we have prepared an online solid-phase extraction with liquid chromatography method based on polymer monoliths for the determination of dopamine [20] since undesired changes in its metabolism result in serious illnesses such as depression, schizophrenia, Parkinson disease, and tumors [21,22].

Herein, we describe novel and simple approach to prepare monolithic capillary column with an integrated on-column electrochemical detector. The carbon fiber as working electrode was inserted at the same time with silver microwire as pseudoreference electrode into the ending of monolithic capillary column. To prove the concept, chromatographic separation and amperometric detection of selected neurotransmitters is shown. Since urine is a suitable sample for high-throughput and low cost characterization of nervous system activity [23], the applicability of proposed setup was test on determination of dopamine in urine. On the other hand, proper optimization of experimental conditions allows determination of other electroactive small molecules including amino acids, (poly)phenols, and/or sugars.

Abbreviations: HPLC-EC, high performance liquid chromatography with electrochemical detection.

* Corresponding author at: Department of Chemistry, Faculty of Science, Masaryk University, Brno, 625 00 Czech Republic.

E-mail address: urban@chemi.muni.cz (J. Urban).

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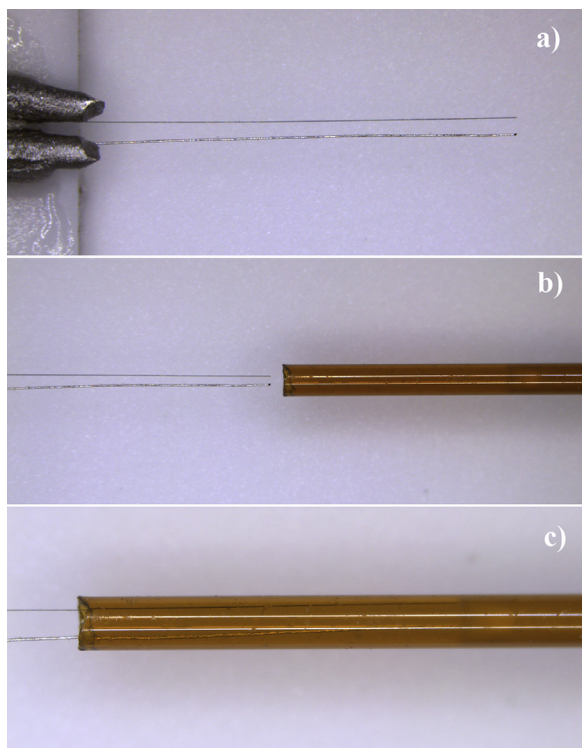


Fig. 1. Preparation of integrated electrochemical detector: a) working (carbon fiber, up) and pseudoreference (silver microwire, down) electrodes glued to the silver-plated wires, b) insertion of microelectrodes into the end of fused-silica capillary with monolithic stationary phase, and c) microelectrodes inside the end of the capillary. Diameter of carbon fiber and silver microwire is 7 μm and 25 μm , respectively. Internal diameter of fused-silica capillary is 320 μm .

2. Experimental part

2.1. Preparation of monolithic column with integrated electrochemical detector

Monolithic capillary columns based on di(ethylene glycol) dimethacrylate crosslinker were prepared according the protocol published previously [17]. Homogenous polymerization mixture was filled in capillaries and an air plug (2 mm) was left at the end of the capillary to allow space for further integration of microelectrodes. Then, both ends of the capillary were sealed with stoppers and the capillary was placed in a thermostated bath where polymerization reaction proceeded at 60 °C for 20 h. After that, monolithic capillary columns were flushed first with acetonitrile, followed by a particular mobile phase.

Carbon fiber as working electrode and silver microwire as pseudoreference electrode with diameter 7 μm and 25 μm , respectively, were attached to contact silver-plated wires using conductive silver paint and allowed to dry at room temperature. Afterwards, the microelectrodes were cut to desired length with a razor blade and fixed in parallel on ceramic slides using cyanoacrylate adhesive (Fig. 1a). The capillary monolithic column was carefully slid to required length onto the working and reference electrode with the aid of micromanipulator MNO-202ND (Narishige, Tokyo, Japan) under microscopic observation using S8APO optical stereomicroscope (Leica, Wetzlar, Germany) (Fig. 1b). The position of capillary with embedded microelectrodes on ceramic support was fixed with cyanoacrylate adhesive afterwards (Fig. 1c).

Detailed description of materials, instrumentation, and sample preparation is available in the Supporting Information.

3. Results and discussion

3.1. Working and reference microelectrodes

Carbon fibers are characterized by enhanced current densities due to a non-linear diffusion [24]. However, surface treatment applied during their production may have adverse effect on the resulting electrochemical properties of carbon fiber electrodes, therefore, we selected unsized carbon fibers, without any surface modification, as working electrodes for HPLC-EC of catecholamine neurotransmitters.

The prerequisite for electrochemical detection in general is the stability of reference part of the detector. A number of designs of reference electrodes were already presented in HPLC-EC with carbon fiber working electrodes. They mostly involve smaller variants of silver/silver chloride electrode of classical construction with a porous frit and a reference solution. Even when reference electrodes of reduced dimensions are used, they are still much larger than the carbon fibers and special flow cells have to be constructed to place them in the vicinity of working electrodes.

We present a completely different approach when the microwire reference electrode is an integral part of the detector directly inside the separation capillary together with the carbon fiber working electrode. We have utilized a solid silver microwire with diameter of 25 μm as a pseudoreference electrode. Its robust character allows to non-complicated insertion of the microwire into the capillary and the electrode surface is reproducible owing to manufacture process. Constant potential is properly maintained as confirmed by the cyclic voltammograms of dopamine in **Figure SI-1** using two-electrode arrangement with carbon fiber as working electrode and silver microwire as pseudoreference electrode. The course of limiting currents in voltammograms at higher concentration of dopamine is less stable, owing to the worse potential stability of silver wire pseudoreference electrode at currents approaching units of microamperes, where polarization of pseudoreference electrode might take place.

3.2. Integrated capillary electrochemical detector

At first, we have explored a repeatability of the microelectrodes integration inside prepared monolithic capillary columns. **Table 1** summarizes repeatability data for three independently prepared integrated monolithic capillary columns with electrochemical detectors. Chromatographic analysis provided variability of retention, resolution, and detection signal in the range of relative standard deviations of 0.5–1.4%, 3.9–5.5%, and 3.3–4.9%, respectively, and confirmed robust preparation of both monolithic stationary phase and an electrochemical detector.

Monolithic stationary phase used in this work provides dual-retention mechanism controlled by the composition of the mobile phase [17]. Hence, we have selected mobile phases with low concentrations of acetonitrile and high phosphate buffer concentrations allowing both chromatographic analysis in a reversed-phase retention mechanism and sensitive electrochemical detection. We have tested an effect of buffer molarity on the signal of dopamine and 3,4-dihydroxybenzylamine that is generally used as an internal standard. With increase in the buffer molarity from 0.1 to 0.2 and 0.5 M, the corresponding signal decreased from 1.7 to 1.4 and 0.2 nA for dopamine and from 1.1 to 1.0 and 0.9 nA for 3,4-dihydroxybenzylamine, respectively. Concentrations of buffer lower than 0.1 M did not have any significant effect on the electrochemical signal.

We have also determined an effect of mobile phase composition on the chromatographic resolution of dopamine and 3,4-dihydroxybenzylamine. Unfortunately, none of the mobile phases containing acetonitrile and phosphate buffer enabled

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