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Approaches for coupling solid-phase microextraction to nanospray

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Abstract

Biocompatible solid-phase microextraction (SPME) devices were prepared using two restricted access materials (RAM) as the SPME coating. The restricted access materials were immobilized on steel and platinum wires. The selective coating eliminated most of the matrix interference, which allowed the coupling to mass spectrometry without further purification. The SPME devices were interfaced to mass spectrometry by electronanospray. Several experimental set-ups are described and discussed herein. For the in situ extraction of peptides from the tryptic digests, trypsin was immobilized both on steel wires and on the inside wall of a vial. The devices were incubated together with the RAM–SPME devices and a protein (casein) solution. After the protein digestion, the resulting peptides were analyzed by SPME/nanospray. The vial approach provided the best results; up to eight peptides could be identified which corresponds to a sequence coverage of 58%. The limit of detection of SPME/nanospray for the extraction of peptides from an aqueous solution was about 50 fmol/mL. The results demonstrate that the direct coupling of SPME to nanospray can reduce analysis time and is an attractive alternative to conventional approaches like Zip–Tip purification.

Keywords: Nanospray; Restricted access materials; Solid-phase microextraction

1. Introduction

Solid-phase microextraction (SPME) is a convenient and solventless extraction approach that offers many advantages over conventional analytical methods as it provides sampling, sample preparation and preconcentration in one step [1]. SPME reduces the time necessary for sample preparation, decreases the purchase and disposal cost of solvents and can improve detection limits. Therefore, the SPME technique is ideally suited for MS applications, combining a simple and efficient sample preparation method with good precision/accuracy and a very sensitive detection method [2]. It was previously demonstrated that by desorbing a SPME fiber directly inside an ion trap mass spectrometer, the sensitivity for the detection of toluene could be increased by two orders of magnitudes [3]. The direct coupling of SPME to mass spectrometry by fiber introduction mass spectrometry (FIMS) has also been reported for the analysis of volatile and semi-volatile compounds by Meurer et al. [4]. Using commercial fibers based on PDMS, this technique was suitable for the effective extraction of volatile and semi-volatile organic compounds (VOC, SVOC) and allowed simple introduction and thermal desorption directly into the ionisation region of a mass spectrometer [4].

Riter et al. demonstrated that SPME is suitable for direct coupling to a miniaturized portable mass spectrometer [5]. But with the current instrumental set-up, analysis is only suitable for on site measurement of VOCs in various matrices. To make the coupling of SPME to mass spectrometry suitable as a diagnostic tool in biomedicine, new approaches have to be developed for

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the analysis of drugs and peptides from body fluids, for example.

The combination of SPME and MALDI for the analysis of biomolecules was recently introduced [6,7]. The elimination of matrix interferences, especially for the bioanalysis of body fluids, is crucial for mass spectrometry as they can lead to analyte suppression [8]. Newly developed biocompatible, tailor-made SPME extraction coatings like restricted access materials (RAM) eliminate most of the matrix interferences from body fluids and allow for the direct coupling to mass spectrometry without the time-consuming chromatography [9,10] step.

Similar to MALDI, mass spectrometry with electrospray ionization (ESI) allows for the fast analysis of drugs and polar biological molecules [11]. Nanoelectrospray (nanospray) has emerged as a promising ionization technique for the analysis of drugs and biological molecules because of its high ionization efficiency, low flow rates and high tolerance of salts compared to conventional electrospray (ESI) [12]. In this study, the direct coupling of SPME to electrospray/nanospray for the analysis of drugs and biomolecules is demonstrated.

For the direct coupling of SPME to electrospray and nanospray, SPME devices were coated with biocompatible restricted access materials containing C18 extraction centres (ADS) or strong cation exchange properties (XDS). The properties of these materials have been described previously [13,14]. The biocompatible device was used to extract drugs and peptides from biofluids and tryptic digests. For the latter, immobilized trypsin particles were coated on a wire and on the inside wall of a plastic vial. The devices are used to digest the protein α-casein. The resulting peptides are extracted by the RAM SPME devices and desorbed inside the nanospray tip.

2. Materials and methods

2.1. Materials

Stainless steel wires (147 μm diameter) were purchased from Small Parts Inc. (Miami Lakes, FL, USA); high temperature epoxy glue was obtained from Isolation Technologies Inc. (Hopedale, MA, USA); UV Loctite glue was purchased from Henkel (Duesseldorf, Germany); LiChrospher ADS-RP18 (alkyl diol silica) and XDS (strong cation exchange) particles (25 μm) were supplied by Merck KGaA (Darmstadt, Germany) as research samples.

Platinum wires (150 μm diameter were purchased from Sigma–Aldrich (St. Louis, MO, USA) Eppendorf-tips from Eppendorf (Hamburg, Germany).

Controlled pore glass (CPG)-aminopropyl-glutaraldehyde-trypsin was supplied by Karen Waldron (University of Montreal, Montreal, Que.). Diazepam was purchased from Cerilliant (Austin, Texas, USA), leucine enkephalin and protein casein (bovine milk) were purchased from Sigma (St. Louis, MO, USA), Zip–Tips C18 were purchased from Millipore (Billerica, MA, USA), ammonium carbonate from Fisher Scientific (Fair Lawn, NJ, USA), formic acid from Merck KgaA (Darmstadt, Germany), and trifluoro acetic acid from Caledon Laboratories (Georgetown, Ont., Canada).

Nanopure deionised water from a Barnstedt/Thermodyne NANOpure Ultrapure water system was used (Dubuque, IA) to prepare all solutions.

An Intel Play QX3 (Santa Clara, CA) digital microscope was used to monitor the coating.

2.2. Preparation of SPME devices for bioanalysis

New SPME fibers were prepared by applying a uniform layer of RAM slurry on the surface end of the stainless steel and platinum wires. The wires were previously processed as follows: they were cut in 8.5 cm segments, etched for 2 min in concentrated hydrochloric acid, immediately washed with water, thoroughly cleaned by sonication (first in methanol and then in water) and finally dried at 120 °C. RAM (0.2 g) (ADS or XDS) particles were transformed into slurry by means of 0.8 mol of a 75:25 mixture of epoxy glue and chloroform. To create the extraction phase the wires were dipped 1.5 cm deep into the slurry, followed by 1 h of baking at 180 °C, to ensure complete polymerization of the epoxy glue.

Blood and urine were obtained with consent from healthy volunteers that did not receive any medical treatment prior to giving blood; samples were collected into blood collection tubes (BD Vacutainer) that contained of sodium heparin and the blood samples were stored at −20 °C until analysis.

For the extraction, the sample was placed on an orbital shaking platform and the extracting phase on the surface end of the wires was immersed in the sample for exactly 5 min. Afterwards, the SPME device was rinsed with water and inserted into the interfaces described in Section 2.5.

2.3. Preparation of SPME devices for extraction of peptides from tryptic digests

Two devices were prepared for the tryptic digestion experiments. (1) Trypsin particle fiber: CPG-aminopropyl-glutaraldehyde-trypsin particles were immobilized on a stainless steel wire as follows: the immobilized trypsin particles were prepared and reported by Waldron and co-workers [15]. The stainless steel wires were dipped into Loctite glue then stripped off by passing it right through a conventional HPLC vial septum to obtain a very thin layer of glue. Afterwards, the wires were dipped 1.5 cm deep into a 0.25 mL micro vial filled with 1 mg of trypsin particles to obtain a thin layer of particles (coating length 1.5 cm) on them, followed by UV-irradiation for 15 min using a Loctite UV-lamp. Weighing confirmed that 0.9 g of particles are bound to the surface of the wire. (2) Trypsin particle vials: 2 mg of CPG particles were immobilized onto the inside wall of a 0.25 mL Eppendorf vial, using the Loctite glue followed by 15 min curing under UV.