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Monolithic molecularly imprinted polymeric capillary columns for isolation of aflatoxins

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ABSTRACT

Monolithic molecularly imprinted polymers extraction columns have been prepared in fused-silica capillaries by UV or thermal polymerization in a two-step process. First, a poly-(trimethylolpropane trimethacrylate) (polyTRIM) core monolith was synthesized either by UV or thermal polymerization. Then it was grafted with the mixture of methacrylic acid (MAA) as a functional monomer, ethylene dimethacrylate (EDMA) as a cross-linking agent, 5,7-dimethoxycoumarin (DMC) as an aflatoxin-mimicking template, toluene as a porogen solvent and 2,2-azobis-(2-methylpropionitrile) (AIBN) as an initiator of the polymerization reaction. Different thermal condition of the photografting and different concentrations of the grafting mixture were tested during polymerization. The extraction capillary columns were evaluated in the terms of their hydrodynamic and chromatographic properties. Retention coefficients for aflatoxin B1 and DMC were used for assessment of the selectivity and imprinting factor. The obtained results indicate that the temperature of photografting and concentration of the grafting mixture are key parameters that determine the quality of the prepared MIPs. From the MIP columns characterized by the highest permeability the column of the highest imprinting factor was applied for isolation of aflatoxins B1, B2, G1 and G2 from the model aqueous sample followed by on-line chromatographic separation. The process was performed using a micro-MISPE-microLC-LIF system of a novel design, which allowed for detection of the eluates from the sample preparation part as well as from the chromatographic separation.
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1. Introduction

Mycotoxins are plant pathogens mainly of agricultural origin. The contamination of food products corresponds to many species of lower fungi (molds) such as family of *Aspergillus*, *Penicillium* or *Fusarium* [1,2]. Fungi are saprophytes and develop on stored food and feed, or under field conditions as pathogens in crops. The wide range of matrices contaminated with mycotoxins includes both direct agricultural products such as: corn, peanuts, pistachio, coffee beans, corn, oilseeds, pepper and intermediate products: meat, milk, eggs, wine or beer [3,4]. Currently there are about 500 different mycotoxins known, among which the most common and of particular interest are aflatoxins, ochratoxin, patulin, and zearalenone [5]. These secondary metabolites of fungi are characterized by high and varied toxicity to humans and animals. For example, exposure to aflatoxins can cause acute and chronic toxic effects or death. They have a deleterious effect on the central nervous system, cardiovascular and respiratory systems, as well as the gastrointestinal tract. They can be carcinogenic, mutagenic, teratogenic and immunosuppressive [6]. International Agency for Research on Cancer (IARC) has classified aflatoxin B1 in group 1, as a human carcinogen, while the other aflatoxins are in group 2B, as compounds potentially carcinogenic to animals and humans [7].

A wide range of biological effects to the body is connected with different chemical structures of aflatoxins and their subsequent physical properties [8]. Currently there are known over 18 aflatoxins, but mostly four aflatoxins (B1, B2, G1 and G2) are a serious problem of contamination of plants [9]. Among the aflatoxins B1 is considered as the strongest carcinogen of natural origin. In human body the main destination of toxic exposure is the liver, and diet containing the contaminated products is considered to be most important risk factor for the development of primary hepatocellular carcinoma [4].

As about 20% of food products (mainly cereals) are contaminated with aflatoxins and because of their strong toxicity, the European Union Commission Regulation (EC) No 1881/2006 established a

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maximum concentration of aflatoxins in cereals and their derivatives: 2–5 μg/kg for B1, 4–15 μg/kg for a total concentration of B1, B2, G1 and G2, and 0.1 μg/kg for B1 in processed cereal-based foods for infants and young children [8,10,11].

Different analytical techniques can be employed for determination of aflatoxins in various samples with the most popular being HPLC with fluorescence or MS detection. Also thin layer chromatography, GC/MS or capillary electrophoresis have been used for this purpose. Although separation itself and quantitative determination of mycotoxins using the above mentioned techniques is not a major problem, the development of new, selective methods of their isolation (generally using solid phase extraction, SPE) is still the subject of some research [5,12]. Currently, best SPE cartridges for isolation of mycotoxins including aflatoxins are based on relatively expensive and basically disposable immuno-affinity adsorbents [13,14]. Thus, it would be beneficial to develop new selective materials for isolation of such analytes e.g., molecularly imprinted polymers (MIP). MIPs are polymeric materials possessing active sites (cavities) of the shape and arrangement of the functional groups that fit the molecules to which MIPs is selective [15–20]. To manufacture such materials the template (ideally the analyte of interest, but in many cases other molecules resembling the analyte can be used) and set of selected monomers are needed during the polymerization process [21]. There have been elaborated (with varied success) MIPs for solid phase extraction of several mycotoxins, for example: deoxynivalenol and zearalenone [22–25], moniliformin [26] or ochratoxin A [27,28]. As for aflatoxins the papers dealing with preparation of selective MIPs are extremely rare. One of the examples is the works of Sergeyeva et al. who have prepared aflatoxin B1 selective MIP in the form of the membrane [29,30]. In their work they used as a template ethyl-2-oxocyclopentanecarboxylate as a structural analogue of aflatoxin B1 and aflatoxin B1 itself. Based on their binding energies to the analyte the following functional monomers were tested: acrylamide, 2-acrylamido-2-methyl-1-propanesulfonic acid, allylamine, diethylaminoethylmethacrylate and N,N′-methylenebisacrylamide. It was shown that imprinted polymer membranes, synthesized using acrylamide and N,N′-methylenebisacrylamide were characterized by sufficient mechanical stability and high adsorption capability. Another example is the work of Batlokwa who in his doctoral thesis applied 19-nortestosterone as a structural analogue of aflatoxin B1 [31].

A very promising approach is molecularly imprinted polymer in a format of monolithic capillary column which can be used in miniaturized techniques like nano/microLC [32]. Monolithic materials which can be generally divided into inorganic (silica-based) and organic ones (polymeric) are a special form of a stationary phase which is a single piece of a porous rod created usually *in-situ* in a column [33–38]. In comparison to particle-based beds monoliths are regarded as much more permeable, often characterized by higher efficiency and easier to prepare stationary phases for miniaturized chromatographic techniques. However, one-step preparation of monolithic MIPs in fused silica capillaries can often be very problematic as it can be very hard to find a unique system of functional monomers, crosslinker, template, initiator and porogen to form a monophasic mixture resulting in good MIP monolith. For such a reason two-step procedures employing a grafting of a MIP layer onto core porous material seem to be much more effective [32].

In the present work, we focus on the preparation and characterization of monolithic capillary columns containing molecularly imprinted polymer selective to aflatoxin B1. Based on the previous theoretical work of Wyszomirski and Prus [39] we used 5,7-dimethoxycoumarin (DMC) as a template mimicking the target analyte. Both thermal and UV polymerization procedures have been compared and the effect of temperature of photopolymerization, different concentration of grafting mixture on permeability, imprinting factor and relative imprinting factor have been studied. Finally, the columns were used in a specially designed micro-MISPE-microLC-LIF on-line extraction–separation capillary system to isolate aflatoxins from the model water sample.

2. Experimental

2.1. Materials and chemicals

Benzoin methyl ether (BME), ethylene glycol dimethacrylate (EDMA), 2,2,4-trimethylpentane, trimethylolpropane trimethacrylate (TRIM), methacrylic acid (MAA), 5,7-dimethoxycoumarin (DMC) and aflatoxins B1, B2, G1 and G2 were purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). 3-(trimethoxysilyl)propyl methacrylate (γ-MAPS) and 2,2′-azoisobutyronitrile (AIBN) were from Fluka (Buchs, Switzerland). Toluene, methanol, acetone, sodium hydroxide (all of analytical grade) were purchased from Polskie Odczynniki Chemiczne (POCh, Gliwice, Poland). Acetonitrile (HPLC grade) was from J.T. Baker (Witko, Łódź, Poland). Deionized water was produced in our laboratory using a Milli-Q ultrapure water producing system (Millipore, Bedford, MA, USA). Classical polyimide-coated (TSP100375) and UV-transparent (TSU100375) fused silica capillaries of 100 μm ID and 375 μm OD were from Polymicro (CM Scientific, Silsden United Kingdom). Separate stock solutions of aflatoxins (100 μg/mL) were prepared in acetonitrile and stored at −20 °C in amber glass vials over a period of two months. Intermediate working solutions, which were prepared in ACN/H_2O, were used to prepare final working solutions in water. For the preparation of solutions only amber glass measuring flasks and vials were used.

2.2. Instrumentation

Temperature controlled photopolymeryzation was conducted in a home-made chamber equipped with four 15 W black light tubes (Sanyoko-Denki, Tokyo, Japan) which were a source of UV radiation of a predominant wavelength of λ = 365 nm. The system was thermostated using F25 thermostat (Julabo Labortechnik, Seelbach, Germany) and details of its construction are presented in our previous work [40].

The chromatographic measurements were performed on a lab-made capillary LC system consisting of a pump delivering the mobile phase (Rheos 2000, Flux Instruments, Reinach, Switzerland), 10-port nano-LC valve with a microelectric actuator (C72MX-4694EH, Vici Valco Instruments Inc. Co., Houston, TX, USA), fused silica 50 nL injection loop, a LIF detector (comprising a 355 nm laser, a photomultipier - TIDAS PMT IV, J&M, Germany); and an optical system of our own design. For extraction–separation experiments (micro-MISPE-microLC-LIF) the system was additionally equipped with the additional LC pump and the 6-port nanoLC valve (C72MFSX-6676, Vici Valco) equipped with a 5 μL sampling loop. As the separation column we used the 180 μm × 300 mm capillary column filled with the silica-based cholesterol stationary phase. The cholesterol stationary phase was a bonded-type phase synthesized and characterized by Dr. Szymon Bocian from our Department [41,42]. The capillary columns were prepared (balanced density slurry packing method) in our laboratory. Clarity software (DataApex, Prague, Czech Republic) was used for LC system control and data collection.

The pressure vs. flow relationship was measured using an air-driven constant pressure HPLC pump from Knauer (Knauer GmbH, Berlin, Germany). The SEM micrographs were taken using Leo 1430 VP apparatus (Leo Electronenmikroskopie GmbH, Oberkochen, Germany).

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