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Molecularly imprinted polymers immobilized on 3D printed scaffolds as novel solid phase extraction sorbent for metergoline

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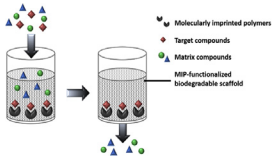
HIGHLIGHTS

- Molecularly imprinted polymers were produced by precipitation polymerization.
- Sub-micrometer sized MIP were obtained.
- MIP were immobilized by cross-linking polymer network building blocks.
- An optimal polymer network building block concentration of 7.5 w/w% was selected.
- Developed SPE sorbents rebound 44.87 ± 8.30% of a 1 μM metergoline solution.

GRAPHICAL ABSTRACT



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ABSTRACT

In the present work, a novel solid phase extraction (SPE) sorbent was developed based on molecularly imprinted polymers (MIPs) immobilized on 3D-printed scaffolds using polymer networks as MIP-immobilizing layer. MIPs were produced by precipitation polymerization in acetonitrile (ACN) using methacrylic acid (MAA) as functional monomer, trimethylolpropane trimethacrylate (TRIM) as cross-linker and metergoline as model template which allows final recognition of ergot alkaloid mycotoxins. Scanning electron microscopy (SEM) and dynamic light scattering (DLS) analyses showed an average MIP particle size of 457 ± 145 nm. Functional MIP analysis revealed dissociation constants (K_D) of 0.29 and 38.90 μM for high and low affinity binding sites respectively. Subsequently, crosslinking of polymer network building blocks was applied as MIP immobilization method on poly-ε-caprolactone (PCL) which was selected as polymer model. Methodology optimization and subsequent evaluation were first realized on 2D PCL surfaces. Based on analyses such as optical evaluation of MIP availability after immobilization through SEM and depth profilometry, an optimal polymer network building block concentration of 7.5 w/w% was selected. In a final part, transfer of MIP immobilization to 3D PCL scaffolds was successfully realized. Functional analysis showed that the newly developed SPE sorbents were able to rebind

Abbreviations: ACN, acetonitrile; AIBN, 2,2′-azobisisobutyronitrile; APS, ammonium persulfate; DLS, dynamic light scattering; IS, internal standard; LC-MS/MS, liquid chromatography tandem mass spectrometry; MAA, methacrylic acid; MeEm, methylergometrine; MeOH, methanol; microCT, micro computed tomography; MIP(s), molecularly imprinted polymer(s); MISPE, molecularly imprinted solid phase extraction; NIP(s), non-imprinted polymer(s); PCL, poly-ε-caprolactone; PF127, Pluronic® F127; PF127-BMA, Pluronic® F127 bismethacrylate; SCA, static contact angle; SEM, scanning electron microscopy; SPE, solid phase extraction; TEA, trimethylamine; TEMED, N,N,N′,N′-tetramethyl-ethylenediamine; THF, tetrahydrofuran; TRIM, trimethylolpropane trimethacrylate; VOI, volume of interest.

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44.87 ± 8.30% of a 1 μM metergoline solution. In conclusion, a new type of SPE sorbent was developed for the detection of metergoline by the use of MIP-functionalized polymer scaffolds. The applied technology opens up future possibilities for the extraction of a broad range of components such as other mycotoxins.

1. Introduction

Solid phase extraction (SPE) is widely used as sample cleanup method and exists in different chromatographic formats. On the one hand, reversed-phase chromatography is often applied and is based on hydrophobic interactions as illustrated by the use of C18 columns. On the other hand, affinity chromatography is also performed in which recognition elements can be applied to capture target analytes. However, the SPE technique does encounter some important disadvantages as can be illustrated for the use of SPE in mycotoxin analysis.

Mycotoxins are contaminants produced by various fungal species which can be present in diverse food and feed matrices. These secondary metabolites can elicit several toxic effects in both humans and animals upon contamination, although they are often present in low ppb-ppt concentrations. The most generally known mycotoxins are aflatoxins, trichothecenes, fumonisins and ergot alkaloids. Since fungi are able to infect a wide range of food and feed products, the economic consequences of mycotoxin contaminations should not be underestimated. Therefore, reducing the mycotoxin contamination risk by conducting rapid, sensitive and accurate analysis is highly needed [1]. Hereto, mycotoxin analysis includes rapid screening and confirmatory methods in which the general interest moved towards a multi-analyte approach [2–9]. Rapid screening tests are often based on qualitative immunochemical principles; whereas confirmation methods identify and quantify mycotoxins present in food and feed samples. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the preferred mycotoxin confirmation method since it establishes a high accuracy, reliability and robustness. Prior to LC-MS/MS analysis, sample preparation by methods such as SPE is an important step to remove potential interferences and to allow preconcentrating the analytes. Nevertheless, this process is time-consuming and often non-selective which indicates the need for more performant analytical tools [10]. Since very low detection limits with LC-MS/MS methods can only be achieved when selective extraction procedures are performed, the use of selective recognition elements is an important trend in sample preparation. Noteworthy, this approach is often difficult to combine with multi-analyte analysis. Therefore, selective recognition elements that bind with different target analytes are required [11]. In general, antibodies represent the most commonly used recognition elements in mycotoxin analysis [2,12]. However, since antibodies suffer from some disadvantages, amongst others stability problems [11], different alternative recognition elements such as molecularly imprinted polymers (MIPs) and aptamers are being developed which can be applied in affinity chromatography. Therefore, this study aimed to use MIPs as synthetic recognition elements in a new type of SPE application in order to fulfill the need for more performant analytical tools and methods for rapid, sensitive, robust and selective (multi-) mycotoxin analysis. In this respect, ergot alkaloids were selected as model compounds within the large and chemically diverse group of mycotoxins. As previously reported, metergoline (Fig. 1) can be used as a model template in MIP production for the detection of ergot alkaloid mycotoxins [13]. More specifically, MIPs produced by using metergoline as template are able to bind the six major ergot alkaloids and their epimers due to the similar structure of metergoline and the basic ergoline structure of these ergot alkaloids (Fig. 1). Consequently, this will result in a group specific MIP which allows multi-mycotoxin analysis.

For the development of an SPE application based on MIPs, spherical particles are often targeted to obtain an efficient column packing. Hereto, several polymerization techniques have been developed to date. In this regard, traditional solvent (*bulk*) polymerization is not suitable to obtain spherical MIP particles since this approach is characterized by some drawbacks such as low yields, irregular size and shape of the obtained particles, and destruction of interaction sites which can occur when the polymer is grinded to obtain particles [14–17]. On the contrary, in case porous monolithic MIP columns are targeted, solvent (*bulk*) polymerization remains a suitable method. However, the aim of this research is to obtain spherical particles for which other polymerization techniques such as suspension, emulsion and precipitation polymerization can be applied resulting in particles of diverse sizes [14,18]. As an example, particles obtained by precipitation polymerization are usually situated in the micrometer or even sub-micrometer size range [14,19–21]. Furthermore, MIPs obtained by precipitation polymerization have an increased rebinding capacity and more homogeneous distribution of binding sites than those obtained by bulk polymerization [16]. Next to the targeted spherical characteristics of MIPs when applied in SPE, it is also desired to obtain sub-micrometer sized MIPs since smaller particles are characterized by a higher surface-to-volume ratio which results in relatively more available binding surface and higher potential of binding target molecules [22]. Since precipitation polymerization is able to result in spherical sub-micrometer sized MIPs, this technique is preferential when production of MIPs with both characteristics is desired.

As indicated above, the application of spherical sub-micrometer sized MIP particles in SPE columns has several advantages with respect to packing and binding characteristics. MIPs used for sample preparation and detection purposes are often packed as such in molecularly imprinted solid phase extraction (MISPE) columns [23–27]. However, this can result in some complications when working with sub-micrometer sized MIPs since the possibility exists that these particles will pass through the typically applied FRITS in MISPE columns and will therefore not be retained inside the column due to their small size. Additionally, although working with spherical particles, previous in-house research demonstrated that MISPE columns in which particles are packed as such can suffer from important back pressure problems during SPE resulting in a more time-consuming sample preparation step [13]. To overcome the above described bottlenecks when working with spherical sub-micrometer sized MIPs in SPE, this research article proposes a novel SPE sorbent based on 3D-printed polymer scaffolds which will be applied as 3D supporting structures to immobilize MIPs using polymer networks as MIP-immobilizing layer.