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In-line coupling of an aptamer based miniaturized monolithic affinity preconcentration unit with capillary electrophoresis and Laser Induced Fluorescence detection

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ABSTRACT

A composite 30-cm capillary was prepared. The head of the capillary was a 1.5-cm original and miniaturized aptamer-based monolithic affinity support that was in-line coupled to the end of the capillary used for capillary electrophoresis (CE) with laser induced fluorescence (LIF) detection. The device was used for the preconcentration, separation and quantification of ochratoxin A (OTA) as a test solute. The 1.5-cm preconcentration unit consists of a fritless affinity monolithic bonded with 5′-SH-modified oligonucleotide aptamers. A vinyl spacer was used for thiol-ene photoclick chemistry with a 5 min irradiation at 365 nm. Photografting allowed to confine the binding reaction to the desired silica monolithic segment, upstream the empty section of the CE capillary using an UV mask. The photografting procedure was optimized preparing 10-cm capillary monoliths for nano-LC. The retention factors of cationic solutes in ion-exchange nano-LC allowed to follow the aptamer binding on the monolith. The reproducibility of the photografting process was satisfactory with inter-capillary variation lower than 10%. The aptamer bonding density can be increased by successive graftings of 100 μM aptamer concentration solution (5 pmol/cm/grafting). The optimal conditions to successfully perform the in-line coupling (preconcentration, elution and separation of OTA) with the composite capillary were adjusted depending on individual requirements of each step but also insuring compatibility. Under optimized conditions, OTA was successfully preconcentrated and quantified down to 0.1 pg (percolation of 2.65 μL of a 40 ng/L OTA solution). A quantitative recovery of OTA (93 ± 2%) was achieved in a single elution of 30 pg percolated OTA amount. The reproducibility of the overall process was satisfactory with a relative standard deviation lower than 10% with negligible non-specific adsorption. This device was applied for the preconcentration and analysis of OTA in beer and wine at the ppb level within a total analysis time of 30 min.

1. Introduction

Affinity chromatography, introduced by Cuatrecasas more than 50 years ago [1,2], is a powerful technique for the purification and preconcentration of targeted compounds from a complex mixture. Antibodies were first used as ligands with high-affinity binding [3] and specific interactions [4–10] with target receptors. Recently, aptamers demonstrated their ability to recognize virtually any class of target molecules [11]. Aptamers are oligonucleotide or peptide molecules able to bind with high affinity and specificity to almost any target molecules. Aptamers were used in diverse fields of applications such as micro- and nanoscale biosensing [12], biomaterials [13,14], analytical [15], or medical techniques [16,17]. Compared to antibodies, aptamers are easily produced via reproducible chemistry not depending on living animals [18,19]. They are stable to long-term storage. Also, they are easily adaptable to introduce specific functional moieties or labeling molecules [19,20]. The great specificity and affinity of aptamers is due to their flexible specific folding allowing them to form complex three-dimensional structures with target molecules. Furthermore, this binding process can be reversible. Unlike antibodies, once denatured, functional aptamers can be regenerated easily [11]. Another big advantage of aptamers compared to antibodies is their small size, allowing to load significant amounts in separation devices such as those used for affinity chromatography.

Analysis of complex mixtures at trace levels has pushed forward the development of integrated and/or miniaturized separation systems coupling the preconcentration and separation steps. Besides the “off-line” classical approach [21], two different coupling approaches, so-called “on-line” or “in-line” couplings, have been

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proposed in the literature [22]. The distinction between on-line and in-line mode is often confusing. From our point of view, the on-line approach is referred as the physical coupling of columns through specific interfaces [23–25] like a 10-port valve. This approach is usually complex and band broadening, consecutive to the transfer of the sample from the preconcentration unit to the separation column, critical in miniaturized systems, may occur. With in-line coupling, the μ-SPE unit is part of the capillary column making its inlet [26–34]. In such integrated capillary electrophoresis (CE) system, the elution of the μ-SPE unit is achieved with few tens of nanoliters directly going to the separation zone without any dilution or band broadening effects. The scale shift from the injection volume (μL) to the elution one (nL) allows a high preconcentration factor highly valuable for sensitivity improvement. For example, a μ-SPE-CE-MS method using a monolithic sol–gel preconcentrator for in-line SPE for the analysis of enkephalin neurotransmitters in biological samples at the ng/mL level was presented [32].

The integration of affinity columns in microsystems is difficult [37–41]. The pioneering works of the Phillips's group presented the grafting of antibodies in the head section of open-tubular capillary columns with the upstream empty sections dedicated to electrokinetic separation [42,43]. In a previous work, we reported the in situ preparation of an antibody-based immunoaffinity monolithic capillary section coupling in line with CE [38]. Such affinity-based μSPE units could be greatly improved replacing antibodies by aptamers. To the best of our knowledge, setting in-line an aptamer-based μSPE in a CE capillary has not been investigated yet. If aptamers present interesting features, they have specific requirements: mandatory presence of bivalent cations for the recognition step, specific pH for the binding buffer, pH compatibility with the subsequent separation step, and electroosmotic flow constraints. The extraction and separation steps must be optimized to be compatible.

We recently focused on the development of an original photofunctionalization process for rapid, localized and versatile surface modification of silica, by thiol-ene photo-click reaction [44]. This process allowed us to prepare multimodal silica monolithic columns inside capillary tubing [45]. In the present work, we propose to take advantage of this functionalization process to develop an in-line μSPE-CE analytical system using aptamers as recognition molecules and laser induced fluorescence (LIF) as the detection method. Such system will be designed, optimized and evaluated for the purification, preconcentration, detection and quantification of ochratoxine A (OTA) in diverse matrices. The elaboration of a silica monolithic segment with appropriate bonded aptamer at the inlet of a 75 μm inner diameter fused silica capillary will be described. The μ-SPE monolith will be tested with OTA as a model compound in term of repeatability, linearity, overall recovery and limits of quantification. The fully automated method, obtained using the composite capillary, will be used to analyze OTA in spiked wines and beers.

2. Materials and methods

2.1. Reagents and chemicals

All the reagents used were of analytical grade. Tetramethoxysilane (TMOS), methyltrimethoxylsilane (MTMS), urea, polyethylene glycol (PEG, MW = 10,000), triethylamine (TEA), methanol (HPLC grade), thiourea, vinyltrimethoxysilane (VTMS), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AIBA), atenolol, propranolol, ochratoxin A ($C_{20}H_{18}ClNO_6$, MW = 403.8, N-{[(3*R*)-5-chloro-8-hydroxy-3-methyl-1-oxo-3,4-dihydro-1H-isochromen-7-yl]carbonyl}-L-phenylalanine, CAS 303-47-9), Tris–HCl, Tris–base ($C_4H_{11}NO_3$, MW = 121.1, tris-(hydroxymethyl)-aminomethane), KCl, NaCl, $CaCl_2$, Na_2HPO_4, NaH_2PO_4 NaH_2PO_4 were purchased from Sigma–Aldrich (Sigma–Aldrich, Saint-Quentin-Fallavier, France). Acetonitrile (ACN) (HPLC grade) was purchased from VWR (VWR, Fontenay-sous-bois, France). Acetic acid was from Riedel-de Haënn (Riedel-de Haënn, Seelze, Germany). All aqueous solutions were prepared using >18 MΩ cm water (Millipore, Molsheim, France). PTFE-coated fused-silica capillaries (TSU deep-UV Transparent Coating 75 μm i.d.) were purchased from Polymicro Technologies (Polymicro Technologies, Arizona, USA). Synthetic oligonucleotides were purchased from Eurogentec (Angers, France). The sequence of the OTA aptamer was 5′-GATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA-3′,5′-CY5 labeled poly(T)$_{10}$ oligonucleotide was used for the localization study. All oligonucleotides were modified at the 3′ end by a C6-thiol linker and purified by RP-HPLC. The oligonucleotides are received dried and solubilized in 200 mM phosphate buffer (pH 8) and 5 mM $MgCl_2$.

2.2. Preparation of vinyl pre-functionalized capillary with a 2 cm-lengh monolith

2.2.1. Synthesis of silica monoliths

The silica monolith preparation followed our reported procedure [36]. Briefly, a 18 mL mixture of TMOS/MTMS (85/15, v/v) was added to 40 mL 0.01 M acetic acid solution containing 1.9 g PEG and 4.05 g urea. The sol mixture was stirred at 0 °C for 30 min. Then, the temperature was raised to 40 °C and the mixture was loaded into the 75-μm fused-silica capillary. To prepare the μ-SPE unit at the head of the CE capillary, a 5-cm segment of a 40-cm capillary was filled by capillarity. Next, the partially filled and blocked capillary was kept at 40 °C overnight for gelification completion. Mesopores were formed by hydrolyzing urea gently raising the capillary temperature at 0.5 °C/min up to 120 °C maintained for 4 h. After cooling, the monolith was washed with methanol and the capillary was cut to desired lengths of the μ-SPE monolith side (about 1.5 cm) and the open tubular CE side (about 30 cm).

The 10-cm capillaries dedicated to SCX nano-LC characterization after photografting were prepared completely filling them with the sol mixture. The monolith preparation procedure was otherwise the same.

2.2.2. Silica surface activation by grafting of vinyltrimethoxysilane

Photo-click chemistry requires an anchor vinyl moiety onto the monolith surface. The silanization solution was composed of 5%, v/v, VTMS and 2.5%, v/v, triethylamine in a 95/5% (v/v) MeOH/water mixture. Silanization was performed at 80 °C for 3 h under hydrodynamic flow. After silanization, cooled monoliths were thoroughly rinsed with methanol using an LC pump (Shimadzu LC 10AD, Tokyo, Japan).

2.3. "Aptamer" photo-click functionalization of monoliths

The modified capillary with the vinyl-silanized monolith head and the open CE segment was filled with a 100 μM aptamer solution also containing Na_2HPO_4 200 mM, $MgCl_2$ 5 mM, pH 8. A nanobaume packing system (Western Fluids, Wildomae, CA, sold by CIL-Cluzeau Info Labo SA, Ste Foy la Grande, France) was used to fill the capillaries with the different mixtures under nitrogen pressure. Localization of the photochemical reaction is carried out by masking the open portion of the capillary with PEEK tubing as UV-mask. The irradiation system used for photo-click reaction was a BioLink cross linker (VWR International, Strasbourg, France) equipped with five 8 W 365 nm UV tubes.