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Dispersive magnetic immunoaffinity extraction. Anatoxin-a determination☆

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Specific monoclonal antibodies were coupled with magnetic Sepharose-based beads and used, for the first time. The methodology was applied to preconcentrate anatoxin-a from water and the later determination by ion mobility spectrometry (IMS). Dispersive magnetic immunoaffinity (d-MagIA) extraction methodology provided a limit of detection of 0.02 $\mu g\,L^{-1}$ and a satisfactory precision with a relative standard deviation lower than 15%. Recoveries were evaluated at 0.5, 1.0 and 5.0 $\mu g\,L^{-1}$ anatoxin-a with quantitative values from 91 to 115%. Additionally, isobaric interferences with phenylalanine were completely avoided by the use of the developed d-MagIA extraction coupled to IMS determinations.

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1. Introduction

Sample pretreatment is usually the weakest link of the analytical chain, providing till 60% of error sources of the whole procedure and consuming around 60–70% of the analysis time [1]. Solid phase extraction (SPE) is the most popular sample pretreatment method for liquid samples due to its many significant advantages, such as easiness for automation, reproducibility, and high-throughput capability [2,3]. Since its introduction, there have been significant improvements in the sorbents employed in SPE [4] and in the available formats [5] including the development of related extraction techniques such as micro-extraction by packed sorbent [6], stir-bar sorptive extraction [7], solid-phase micro-extraction [8], and dispersive-SPE [9], among others. The role of the sorbent in dispersive-SPE is the retention of matrix components while the analytes remain in the liquid phase, or alternatively, the retention of the analytes in the particles and the later separation from the matrix by centrifugation or filtration, which involves several minutes [10]. This drawback can be overcome using paramagnetic sorbents dispersed in the solution to adsorb the target analytes, which can be easily separated from the solution by an external magnet. This method is usually known as dispersive magnetic SPE (d-MSPE) and it provides an easy and fast extraction of the analytes from the liquid sample [10].

The development of novel sorbents for d-MSPE with improved capabilities such as high sorption capacity, high surface area, stability, or selectivity is an active research field. In this sense, magnetic particles have been coated with silica [11], octadecylsilane [12], carbon nanotubes [13], graphene [14], molecularly imprinted polymers [15], and metal-organic-frameworks [16] among others.

Immunosorbents have been previously used for the selective extraction and enrichment of organic compounds by SPE [17], in the so-called immunoaffinity chromatography (IAC). Antibody-immobilized magnetic beads have been previously employed in the immunoprecipitation of chromatin or proteins, but in our knowledge the use of antibodies in magnetic-like formats for sample extraction has not been explored yet. Thus, the aim of this paper is the synthesis of magnetic particles coated with antibodies and their use for the first time as dispersive magnetic immunoaffinity

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(d-MagIA) extraction of organic compounds from liquid samples using the analysis of the cyanotoxin anatoxin-a as proof of concept.

Anatoxin-a is an alkaloid produced by a variety of cyanobacteria species, showing a high toxicity for humans and animals [18]. In spite of anatoxin-a half-life in sunlight ranges from 1 to several hours depending on environmental factors [19], it has long been recognized as a potential source of contamination for drinking and recreational waters [20]. Concentration levels of anatoxin-a in surface freshwater, reported in the US, ranged from 0.05 to 1929 $\mu g\,L^{-1}$, determined by different techniques like liquid chromatography-tandem mass spectrometry (LC–MS-MS) [21,22]; LC with fluorescence detection [23], gas chromatography (GC) with electron capture detection [24], and GC–MS [25]. Several US states have established regulations for anatoxin-a in drinking water proposing values from 0.7 to 20 $\mu g\,L^{-1}$ [19]. The World Health Organization (WHO), Australia, New Zealand, and Denmark also have specific regulations for anatoxin-a, with provisional maximum acceptable values from 1 to 6 $\mu g\,L^{-1}$ [26]. Thus, the determination of anatoxin-a is a challenging issue.

In summary, the primary purposes of this work were (i) to produce magnetic particles coated with antibodies for the selective d-MagIA extraction of anatoxin-a and (ii) to develop an immunoanalytical procedure for the determination of anatoxin-a, combining the selectivity provided by the d-MagIA treatment and the sensitivity and speed of the ion mobility spectrometry (IMS).

2. Experimental section

2.1. Material and reagents

NHS Mag Sepharose magnetic beads and MagRack™ 6 magnetic rack for 1.5 mL microcentrifuge tubes were obtained from Merck (Darmstadt, Germany). Specific monoclonal antibodies against anatoxin-a were produced by our research group. Amicon Ultra 30 K NMWL centrifugal filter devices, obtained from Millipore (Billerica, MA, USA), were employed in the dialysis and concentration of antibody solutions.

Anatoxin-a ((+)-2-acetyl-9-azbicyclo[4:2:1]non-2-ene) standard was acquired from National Research Council Canada (Otawa, Canada). Stock solutions were prepared in 2-propanol and kept at 4 °C in amber glass vials. Organic solvents and buffer constituents were obtained from Scharlab (Barcelona, Spain).

2.2. Preparation of magnetic immunosorbents

Specific monoclonal antibodies used in this study were produced in our laboratory following well-established procedures for hybridoma generation with immunized mice. Antibody solutions were purified by ammonium sulfate precipitation and protein G affinity chromatography, and stored at 4 °C after precipitation using ammonium sulfate for long-term storage. Prior to be used, the antibody was dialyzed using an Amicon Ultra 30 K centrifugal filter device and dissolved in 0.2 M $NaHCO_3$ buffer, 0.5 M NaCl, pH 8.3 to obtain an antibody concentrated solution of 500 $mg\,L^{-1}$.

A volume of 50 μL of NHS Mag Sepharose slurry, corresponding to 10 μL magnetic particles, was placed into an Eppendorf tube. Storage solution was removed using a magnetic rack and the particles were equilibrated with 500 μL ice cold 1 mM HCl. Magnetic particles were resuspended and the liquid was removed again. Immediately after the equilibration step, 200 μL of the antibody solution (100 μg) dissolved in 0.2 M $NaHCO_3$ buffer, 0.5 M NaCl, pH 8.3 was added to the magnetic particles. The mixture was incubated with slow end-over-end mixing for 2 h to allow the coupling of antibodies with magnetic particles. After that, the liquid was removed and the residual active groups of the NHS Mag Sepharose particles were blocked with 500 μL of 50 mM Tris buffer, 1 M NaCl, pH 8.0 for 30 min, and later with 3 cycles of successive washing steps with 500 μL of 0.1 M sodium acetate buffer, 0.5 M NaCl, pH 4.0 and 500 μL of 50 mM Tris buffer, 1 M NaCl, pH 8.0. Finally, the antibody-NHS magnetic sepharose particles were washed and stored with 500 μL of PBS (50 mM phosphate buffer, 150 mM NaCl, pH 7.4), being ready to be used for anatoxin-a extraction.

2.3. Characterization of the magnetic immunosorbent

Microscopy images of MagIA sorbents were recorded using a M165C stereoscope microscope with an integrated IC80 HD digital camera from Leica Microsystems (Wetzlar, Germany).

A FTIR spectrometer, model Tensor 27 from Bruker (Bremen, Germany), equipped with a DLaTGS detector, was employed for Fourier transform infrared spectroscopy (FTIR) spectra measurements. Solutions and slurries were measured by attenuated total reflectance (ATR) mode using a dry air-purged in-compartment Dura Sample IR II accessory for liquids from Smiths Detection Inc. (Warrington, UK) equipped with a nine-reflection diamond/ZnSe Dura Disk plate. Measurements were done by dropping 5 μL of the solutions and slurries on the ATR crystal surface and letting the solvent to evaporate with the help of an air flow. Once the solvent was evaporated, spectra were registered. Data were collected in the region between 4000 and 550 cm^{-1}, with a resolution of 4 cm^{-1} averaging 25 scans, both for sample measurement and background setting.

2.4. Magnetic dispersive immunoaffinity extraction procedure

20 mL water sample was introduced in a 50 mL glass tube and pH was adjusted to 10 using 0.2 mL of 1 M phosphate buffer solution at pH10. The produced magnetic immunosorbent was completely transferred to the sample tube and the mixture was incubated with slow end-over-end mixing for 10 min, and then the magnetic particles were separated rapidly from the solution by an external magnet (see the video in the Supplementary Material) and water sample was gently removed. Then, anatoxin-a was completely eluted with 100 μL of 2-propanol in 5 min with agitation. The solution was separated from the magnetic particles by an external magnet and directly analyzed by IMS.

2.5. IMS determination

An IONSCAN-LS from Smiths Detection (Morristown, NJ, USA) equipped with a ^{63}Ni foil radioactive ionization source was used to separate and identify anatoxin-a. IM station software (version 5.389) was used for data acquisition and processing. Plasmagrams were acquired in positive ion mode using nicotinamide, with a reduced mobility, K_0, of 1.860 $cm^2\,V^{-1}\,s^{-1}$, as internal calibrant. The number of segments per analysis was 100, containing every plasmagram 379 data points. The shutter grid width was 0.2 ms (the value optimized by the manufacturer) and plasmagrams were collected with a scan period of 20 ms. A counterflow of dry air, set at 300 $mL\,min^{-1}$, was introduced as drift gas at the end of the drift region. The electric field strength was 251 $V\,cm^{-1}$ with a total drift voltage of 1759 V and a drift tube length of 7 cm.

Thermal desorption from a polytetrafluoroethylene (PTFE) membrane was used for sample introduction. In this strategy, 10 μL sample extract was placed onto the PTFE membrane and heated to vaporize the analyte, which was transferred to the ionization region. Before first analysis, PTFE membrane was introduced into the IMS instrument to remove any possible interference. Desorption, inlet, and drift tube temperatures were adjusted to 285, 290, and 236 °C, respectively. The sample tray, containing the PTFE

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