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Core–shell polydopamine magnetic nanoparticles as sorbent in micro-dispersive solid-phase extraction for the determination of estrogenic compounds in water samples prior to high-performance liquid chromatography–mass spectrometry analysis

Bárbara Socas-Rodríguez[a], Javier Hernández-Borges[a,*], Pedro Salazar[b], Miriam Martín[c], Miguel Ángel Rodríguez-Delgado[a,**]

[a] *Departamento de Química, Unidad Departamental de Química Analítica, Facultad de Ciencias, Universidad de La Laguna (ULL), Avda. Astrofísico Fco. Sánchez, s/n, 38206 La Laguna, Spain*
[b] *Laboratory of Nanotechnology on Surfaces, Institute of Materials Science of Seville (CSIC-Univ. Sevilla), Calle Américo Vespucio 49, 41092 Sevilla, Spain*
[c] *Neurochemistry and Neuroimaging Group, Laboratory of Sensors, Biosensors and Materials, Faculty of Medical Sciences, University of La Laguna, Campus de Ofra s/n,, 38071 La Laguna, Tenerife, Spain*

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ABSTRACT

In this work, core–shell Fe_3O_4@poly(dopamine) magnetic nanoparticles (m-NPs) were prepared and characterized in our laboratory and applied as sorbents for the magnetic-micro solid phase extraction (m-µSPE) of twelve estrogenic compounds of interest (i.e. 17α-estradiol, 17β-estradiol, estrone, hexestrol, 17α-ethynylestradiol, diethylstibestrol, dienestrol, zearalenone, α-zearalanol, β-zearalanol, α-zearalenol and β-zearalenol) from different water samples. Separation, determination and quantification were achieved by high-performance liquid chromatography coupled to ion trap mass spectrometry with electrospray ionization. NPs@poly(dopamine) were synthesized by a chemical coprecipitation procedure and characterized by different surface characterization techniques (X-ray diffraction, X-ray photoelectron spectroscopy, thermogravimetric analysis, transmission and scanning electron microscopy, infrared and Raman spectroscopy, vibrating sample magnetometry, microelectrophoresis and adsorption/desorption isotherms). Parameters affecting the extraction efficiency of m-µSPE (i.e. polymerization time, pH of the sample, extraction and elution conditions) were studied and optimized. The methodology was validated for Milli-Q, mineral, tap and wastewater using 2-methoxyestradiol as internal standard, obtaining recoveries ranging from 70 to 119% with relative standard deviation values lower than 20% and limits of quantification in the range 0.02–1.1 µg/L.

1. Introduction

Nanotechnology has gained important relevance in diverse scientific fields and also in Analytical Chemistry. The study and application of different nanomaterials are continuously increasing and among them, magnetic nanoparticles (m-NPs) have attracted special attention for their application in extraction procedures since they can be easily isolated from the matrix by using an external magnet without retaining residual magnetization. Consequently, large surface areas to volume ratio, rapid extraction kinetics, high extraction efficiencies, and reduction of sample amounts as well as toxic organic solvents are achieved [1,2].

Among the different magnetic materials commonly used, i.e. cobalt, chromium, magnetite or maghemite, the iron oxides magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) have been the ones most widely applied due to their good biocompatibility, particular physical and chemical properties and high potential of applications, not only in dispersive SPE (dSPE) but also in others such as cell separation, magnetically assisted drug delivery, enzyme immobilization, etc. [2–4]. NPs are usually grafted or coated with inorganic (silica, Al_2O_3, etc.) or organic (surfactants, polymers, etc.) layers, with the aim to increase their stability since they tend to form agglomerates and, in the case of m-NPs, to suffer easily oxidation

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* Corresponding author. Tel.: +34 922 31 89 90; fax: +34 922 31 80 05.
** Corresponding author. Tel.: +34 922 31 80 46; fax: +34 922 31 80 05.
E-mail addresses: bsocasro@ull.es (B. Socas-Rodríguez), jhborges@ull.edu.es (J. Hernández-Borges), psalazar@icmse.csic.es (P. Salazar), mmartinh@ull.edu.es (M. Martín), mrguez@ull.edu.es (M.Á. Rodríguez-Delgado).

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which involves loss of dispersability and magnetism [5]. Besides that, specific coatings not only offer a protector function to NPs but also allow their further functionalization or the establishment of successful interactions with the targets analytes.

Dopamine (DA) is a catecholamine neurotransmitter mimic of the adhesive proteins which has attracted wide interest due to its self-polymerization capacity in aqueous phase under weak alkaline conditions. It allows the formation of a surface-adhesive film onto a wide variety of organic and inorganic materials [6]. The nature of the film is not completely known yet; however, it is thought that the layer is generated by the reaction of the primary amino groups and quinone groups of the oxidized DA through the formation of Schiff bases and/or Michael addition adducts [3,6]. This type of coating allows developing a variety of reactions for active groups on the surface, provides conditions for further modifications [7], offers high biocompatibility and dispersability in water and also has amino and catechol groups that provide the establishment of hydrogen bonds as well as aromatic systems which establish π-π staking interactions with the target analytes [8,9]. To the best of our knowledge, poly(DA) (pDA) NPs, also designated as NPs@pDA, have only been applied in magnetic SPE (m-SPE) in very few occasions [2,8,10,11]. That is why there is still the need of demonstrating the full potential of these new materials for the extraction of a wide variety of analytes and matrices. In particular, they have been successfully applied to the determination of four aflatoxins in red wine [10], berberine in a Chinese medical plant (*Cortex Phellodendri*) [2], six polycyclic aromatic hydrocarbons in environmental water samples [8] and seven antibiotics, three perfluorinated compounds and benzo(a)pyrene in lake and tap water [11]. As a result, and despite their inherent advantages, they have not been used for the extraction of estrogenic compounds of interest like the so called natural estrogens estrone (E_1), estradiol (17β-E_2 and 17α-E_2) estriol (E_3), synthetic estrogenic compounds like 17α-ethinylestradiol (EE_2), dienestrol (DS) and diethylstilbestrol (DES), or the mycotoxins of the family of zearalenone (ZEN) that include the epymers α- and β-zearalanol (ZAL) and α- and β-zearalenol (ZEL). That is why the aim of this work is the preparation and characterization of core–shell Fe_3O_4@pDA m-NPs as well as their evaluation as sorbents for the m-μSPE of a group of three natural estrogens (17β-E_2, 17α-E_2 and E_1), four synthetic (EE_2, HEX, DES and DS) and five mycoestrogens (ZEN, α-ZAL, β-ZAL, α-ZEL and β-ZEL) from three types of water samples (mineral, tap and wastewater). To the best of our knowledge this is the first time that the performance of Fe_3O_4@pDA-m-μSPE is applied for the extraction of estrogenic compounds, also from water samples.

2. Experimental

2.1. Chemicals and materials

Estrogen analytical standards of 17β-E_2 (CAS 50-28-2), 17α-E_2 (CAS 57-91-0), E_1 (CAS 53-16-7), HEX (CAS 84-16-2), EE_2 (CAS 57-63-6), DES (CAS 56-53-1), DS (CAS 84-17-3), ZEN (CAS 17924-92-4), α-ZAL (CAS 26538-44-3), β-ZAL (CAS 42422-68-4), α-ZEL (CAS 36455-72-8), β-ZEL (CAS 71030-11-0) and 2-MeOE_2 (CAS 362-07-2) from Sigma-Aldrich Chemie (Madrid, Spain) were used without further purification (purity $\geq$ 95%).

Stock solutions of each analyte of about 1000 mg/L and 100 mg/L were precisely prepared in MeOH and stored in the darkness at 4 °C. Working analyte mixtures were daily prepared by dilution with the appropriate volume of mobile phase.

All chemicals were of analytical reagent grade (unless otherwise indicated) and used as received. ACN of HPLC-MS hypergrade, MeOH of HPLC-MS grade, hydrochloric acid (25%, w/w), sodium dihydrogen phosphate dihydrate and di-sodium hydrogen phosphate dehydrate were from Merck (Darmstadt, Germany), sodium hydroxide was from Panreac Química, DA hydrochloride and iron (II) sulfate hydrate from Sigma–Aldrich Chemie (Madrid, Spain) and iron (III) chloride hexahydrate from Scharlau (Barcelona, Spain). Water was deionized by a Milli-Q gradient system A10 from Millipore (Bedford, MA, USA).

2.2. Apparatus and software

Magnetic measurements were done using a LakeShore 7407 vibrating simple magnetometer (VSM) at room temperature. X-ray diffraction (XRD) spectrum was obtained using a Panalytical X'Pert Pro diffractometer. Field emission scanning electron micrographs (FE-SEMs) were taken using a Hitachi S 4800 microscope. Transmission electron micrographs (TEM) were acquired using a JEM-2000EX and a Jeol JEM 2100 microscopy. Nitrogen adsorption–desorption isotherms were measured at 77.3 K on a TriStar II analyzer, Micromeritics. The Z potential of the m-NPs@pDA at different pH values were obtained using a Zetasizer Nano ZS, Malvern Instruments. FT-IR spectrums were recorded using a Jasco FT/IR-6200 IRT-5000 spectrophotometer in the range of 400–4000 cm^{-1}. Raman spectrums were taken with a Horiba HR-800-UV microscope. Thermogravimetric analyses were performed with a Perkin Elmer Pyris Diamond TG/DTA apparatus. X-ray photoelectron spectroscopy (XPS) measurements were obtained using a VG-Escalab 210 spectrometer.

HPLC analyses were performed using a Waters HPLC system (Milford, MA, USA) equipped with a binary pump (model 1525), an autosampler (model 717 plus), working with the Empower 2 software from Waters. The HPLC system was coupled to an amaZon SL ion trap (IT) mass spectrometer from Bruker Daltonik GmbH (Bremen, Germany). MS parameters and spectra data were controlled, collected and processed by using the Esquire NT software from Bruker Daltonik. Separations were carried out at 30 °C in a X-Bridge C_{18} column (100 mm × 4.6 mm, 3.5 μm) using a X-Bridge C_{18} pre-column (3.5 μm), both from Waters.

Electrospray ionization (ESI) conditions were as follows: capillary voltage of 5500 V, an end plate offset voltage of −600 V, a nebulization gas pressure of 20 psi, and a dry gas flow and temperature of 8 L/min and 300 °C, respectively. Regarding IT parameters, the ion charge control (ICC) was set at 60,000, the maximum accumulation time at 200 ms with 10 average scans per experiment and a rolling averaging of 5.

MS/MS experiments were performed by fragmentation of the deprotonated molecule $[M-H]^-$ which was selected as the precursor ion. In these last experiments, the set mass range was 70–350 m/z, the m/z width was set at 1. The fragmentation amplitude and the precursor and product ions for each analyte are shown in Table 1 of the Supplementary material.

Considering ACN as mobile phase A and Milli-Q water as mobile phase B, the initial composition of the mobile phase was 50:50 (v/v) A/B. It was changed to 90:10 (v/v) A/B in 2 min, maintained at that composition for 4 min more, and in 1 min the initial composition was set up. The flow rate was 0.4 mL/min and the injection volume 20 μL.

2.3. Preparation of Fe_3O_4@pDA NPs

Firstly, Fe_3O_4 NPs were synthetized by the chemical coprecipitation approach [3,4] (see Fig. 1 of the Supplementary material). For this purpose, $FeCl_3 \cdot 6H_2O$ (5.41 g) and $FeSO_4$ (2.78 g), with a molar relation of 2:1, were dissolved in 200 mL of HCl 0.5 M by magnetic stirring. Then the solution was added drop by drop to a 1.25 M NaOH solution (300 mL) under vigorous stirring (850 rpm) at room temperature. After finishing the addition, the black precipitated Fe_3O_4 was stirred for 30 min more. Then, the NPs dispersion, which had