Silica supported Fe$_3$O$_4$ magnetic nanoparticles for magnetic solid-phase extraction and magnetic in-tube solid-phase microextraction: application to organophosphorous compounds

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Reagents

Iron (III) acetylacetanoate (Fe(acac)$_3$), 1,2-hexadecandiol, oleyamine, oleic acid, chlorfenvinphos, chlorpyrifos, tetraethylorthosilicate (TEOS), polyethylene glycol (PEG), hexadecyltrimethylammonium bromide (CTAB), sodium dodecylsulphate (SDS) and phenyl ether were purchased from Sigma-Aldrich (Steinheim, Germany). Methanol and acetonitrile were purchased from Panreac (Barcelona, Spain). Stock standard solutions of chlorfenvinphos and chlorpyrifos (100 mg L$^{-1}$) were prepared in acetonitrile and stored at 4 °C. Working standard solutions were made by appropriate dilution of the stock standard solutions in water or water:methanol.

Instrument and chromatographic conditions

The capillary chromatographic system used consisted of a LC isocratic capillary pump (Jasco Corporation, Tokyo, Japan) and a UV-Vis diode array detector (Agilent, 1200 series) equipped with a 80 nL flow cell.
The extracts of the MSPE were analysed by IT-SPME utilizing a GC open tubular capillary column TRB-5 (35 cm, 0.32 mm i.d) coated with 5% diphenyl-95% polydimethylsiloxane. The capillary column was placed in the high pressure six port valve replacing the injection loop. Capillary connections to valve were facilitated by the use of 2.5 cm sleeve of 1/16 in. polyether ether ketone (PEEK) tubing; 1/16 in. PEEK nuts and ferrules. Chromatographic separation was carried out with a Onyx Monolith (150 mm x 0.2 mm i.d.) column (Phenomenex, USA). The mobile phase was a mixture of water and acetonitrile 70:30 at 15µL min⁻¹. Aliquots of 1.5 mL of the extracts were manually loaded into the TRB-5 capillary column followed by 50 µL of water to eliminate the remaining solution. Finally, the analytes were transferred to the analytical column for separation and detection.

The Magnetic-IT-SPME was carried out by replacing the injection loop of the high pressure valve with the SiO₂-Fe₃O₄ capillary column (60 cm, 0.075 mm i.d). The capillary column was placed inside the magnetic coil such that the magnetic field (B) could be adjusted by varying the current (Fig.S1). Capillary connections to the valve were facilitated by the use of a 2.5 cm sleeve of 1/32 in. polyether ether ketone (PEEK) tubing; 1/32 in PEEK nuts and ferrules were used to complete the connections. The analytical column utilized was a Zorbax SB C18 (35 mm x 0.5 mm i.d, 5 µm) column (Agilent), operating with a mobile phase methanol/water 95:5 (v/v) at flow rate 6 µL/min.

The absolute recoveries (extraction efficiencies) were calculated by comparing the amount of analyte extracted, which is the amount of the analyte transferred to the analytical column, with the total amount of analyte passed though the IT-SPME capillaries or contained in the water sample for MSPE. The amount of analyte extracted was established from the peak areas in the resulting chromatograms and from the calibration equations constructed through the direct injection of 2 µL of standard solutions of the analytes in the 1–100 µg/mL concentration range.
Absorbance of the analytes was registered at 230 nm. Spectra of the analytes were also registered. All solvent were filtered through a 0.45 mm nylon membranes (Teknokroma) before use. All the experiments were carried out in triplicate.

**Preparation and characterization of the silica supported Fe₃O₄ NPs and magnetic capillary column.**

Fe₃O₄ NPs were synthetized by previously reported approaches [1,2]. Briefly, 0.706 g of Fe(acac)₃, 2.013 g of 1,2-hexadecanodiol, 1.695 g of oleic acid, 1.605 g of oleyamine and 20 mL of phenyl ether (under Ar stream (20 min)) were refluxed at 263°C during 30 min. After cooled to room temperature, 80 mL of ethanol was added to the reaction mixture and centrifuged. Then, the mixture was redissolved in hexane (20 mL). Finally, water soluble Fe₃O₄ NPs (Fe₃O₄-CTAB) were obtained by adding 20 mL of 0.1 M CTAB to the hexane extracts and rotaevaporating the mixture until complete elimination of hexane.

The synthesis of the sorbent material was carried out according to the method reported by our group. Typically, 2.5 mL of a mixture of PEG (0.9 g) and urea (0.9 g) in 10 mM acetic acid (10 mL) was mixed with 1 mL of Fe₃O₄-CTAB water dispersion, adjusted to pH 11 with 1 M NaOH (7 mL) and 1 mL of TEOS, which polymerized hybrid sorbent. This mixture was stirred until it became a homogeneous gel. Then, 60
µL of this gel was injected into the fused silica capillary column previously treated with 1 M NaOH. According to the previously work, the ends of the capillary were sealed and placed into an oven 20 h at 43 °C. After, the temperature was raised to 120 °C at 0.5 °C/min and kept constant for 2 h, followed by water and methanol washes. After drying, the capillary was heated at 330 °C for 25 h. This capillary was used as injection loop for Magnetic-IT-SPME. The rest of the gel was isolated by centrifugation (10000 rpm, 10 min) and vacuum dried during 24 h to obtain the magnetic hybrid sorbent material for MSPE.

Characterization

SEM images were acquired using a HITACHI-S4100 equipment operated at 20 kV. Atom composition of the sorbent material was measured using a Philips SEM XL30 equipped with an EDAX microprobe. For HRTEM images a Philips Tecnai F20 equipment operating at 200 kV was employed. Samples for electron microscopy were prepared with a drop of the synthesised material suspension onto a lacey carbon/formvar-coated copper grid. A Quantum Design MPMS Squid Magnetometer was used to measure the magnetic properties. Magnetic measures were carried out with variable-temperature (T=2 K) and field-dependent (applied field: 150 Oe) dc measurements. The same device was used in the ac susceptibility measurements at different frequencies of the oscillating field of 17 G amplitude.

Fig. S2. A) HRTEM image SiO2 supported Fe3O4 NPs and B) SEM micrographs of the internal surface of the silica supported Fe3O4 capillary for Magnetic-IT-SPME
**Adsorption isotherms**

50 mg of sorbent were added to 20 mL of a mixture of chlorfenvinphos and chlorpyrifos and after 60 min as adsorption time, the magnetic sorbent was isolated from the solution with a Nd disk magnet and the residual analytes were determined by using a previously reported method proposed in [3].

**Table S1. Parameters of Langmuir and Freundlich isotherms**

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**Extraction conditions and Detoxification procedure**

In preliminary studies, the sorption capacity of the magnetic sorbent for MSPE was evaluated by adding 10, 20, 30 and 50 mg of sorbent to 2 mL of a mixture of chlorfenvinphos and chlorpyrifos (6 µg L⁻¹) with a contact time of 60 min (see Figure S3).

**Fig. S3.** Adsorption kinetics of chlorfenvinphos and chlorpyrifos (6 µg L⁻¹) in the silica support Fe₃O₄ magnetic material
After the adsorption time, the magnetic sorbent was isolated from the solution with a Nd disk magnet and the residual analytes in the solution were determined. Adsorption near to 100% was found when using 50 mg of sorbent. Water volumes between 2 and 100 mL were studied in order to evaluate the adsorption of the analytes as function of the volume of sample. In this study the amount of sorbent was 50 mg and 60 min as adsorption time. With volumes lower than 20 mL, the separation of the sorbent and the water samples is immediate, but with higher volumes it is necessary to establish a separation time. The use of the magnetic sorbent for magnetic solid phase extraction of chlorfenvinphos and chlorpyrifos was carried out by using 20 mL of sample taking into account the extraction and separation time and the detection limits that can be reached with the IT-SPME system. Previous to each extraction, a cleaning step of the magnetic NPs was carried out in order to obtain clean extracts. A mixture of water and methanol was used as cleaning solvent.

The elution of the target analytes from the magnetic sorbent was carried out with methanol. In this case 500 µL of methanol were necessary to completely elute chlorfenvinphos and chlorpyrifos from the magnetic sorbent. Lower volumes did not quantitative extract the analytes and higher volumes were disadvantageous due to the higher dilution of the sample. After that, water was added to that solution to achieve a mixture 1:3 methanol: water and finally, 1500 µL were processed in the chromatographic system. The application of the magnetic sorbent for removal procedures required to process high sample volumes, and so the separation time was studied. With 100 mL of water sample, we observed that 15 min applying the magnetic field was enough to achieve the complete separation of the sorbent material and from the water sample. Under these conditions, the results obtained showed that removal efficiency was not depending on the volume of sample. The separation time increased with and increment in the water volume processed, thus we selected 100 mL for further studies.

100 mL of wastewater samples collected in the effluents of different wastewater treatment plant were agitated during 60 min with 50 mg of magnetic sorbent. The detoxification efficiency was evaluated by analysing the residual concentration in the supernatant with the procedure described in [3]. In all cases, the concentration of chlorfenvinphos and chlorpyrifos in the wastewater samples were below the detection
limits. Thus, spiked wastewater samples (3 and 4 µg L⁻¹ for chlorfenvinphos and chlorpyrifos, respectively) were processed.

**Fig. S4.** Variation of the magnetic sorbent material composition as function of the number of uses. Xᵢ= Fe or Br

**Fig. S5.** Variation of the analytical response of chlorfenvinphos and chlorpyrifos (5 µg L⁻¹) as function of processed volume in the Magnetic-IT-SPME approach
References


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