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Rapid magnetic-mediated solid-phase extraction and pre-concentration of selected endocrine disrupting chemicals in natural waters by poly(divinylbenzene-*co*-methacrylic acid) coated Fe_3O_4 core-shell magnetite microspheres for their liquid chromatography–tandem mass spectrometry determination

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ABSTRACT

A new Fe_3O_4/poly(divinylbenzene-*co*-methacrylic acid) core-shell magnetite microspheric material have been successfully developed as magnetic-mediated solid-phase extraction micro-particle sorbent in dispersion mode (MM-SPE-MP) for the determination of selected estrogenic endocrine disrupting chemicals (EDCs), namely: estrone (E1), 17β-estradiol (E2), estriol (E3), 17α-ethynylestradiol (EE2) and bisphenol-A (BPA), in natural water, via quantification by HPLC tandem mass spectrometry. The magnetite Fe_3O_4 core of this MM-SPE-MP sorbent was fabricated by a solvothermal approach and the thin layer of amphipolar poly(divinylbenzene-*co*-methacrylic acid) (pDVB-MAA) coating was established via suspension polymerization. The resultant core-shell MM-SPE-MP sorbent material was characterized by electron microscopy, X-ray diffraction and Fourier-transformed infrared spectroscopy. Particle size distribution of the core-shell microspheres was within the range 300–700 nm in diameter and the thickness of the pDVB-MAA coating was ca. 10 nm. This magnetite microspheric material can be easily dispersed in aqueous samples and retrieved by the application of external magnetic field via a small piece of permanent magnet. The MM-SPE-MP process for the selected estrogenic EDCs involved the dispersion of the core-shell microspheric sorbent in water samples with sonication, followed by magnetic aided retrieval of the sorbent and solvent (methanol) desorption of extracted EDCs for LC–MS/MS analysis. Partition equilibrium for all the selected EDCs onto this MM-SPE-MP sorbent was achieved within 15 min. Recoveries of the EDCs were in ranges of 56–111%. Analytes with smaller K_{OW} value showed relatively lower recovery (and relatively longer equilibration time for partitioning). Method detection limits achieved were found to be 1–36 pg ml^{-1} ($n = 3$), while the repeatability was 6–34% ($p < 0.05$, $n = 3$). This work demonstrates the usefulness of MM-SPE-MP in the rapid and highly sensitive monitoring of trace organic contaminants in natural waters.

1. Introduction

Advanced solventless solid–liquid and headspace extractions, such as solid-phase extraction (SPE) and solid-phase microextraction (SPME), have become standard methodologies in environmental, food and biomedical analyses [1–13]. Solid sorbents of various physiochemical properties have been successfully applied to extract and pre-concentrate a wide range of analytes from complex solution matrices or enclosed headspace of samples. Advantages of these solid-sorbent based solid–liquid/headspace extraction techniques include the drastic reduction in the use of environmental and health hazardous organic solvents, the omission of tedious clean-up procedures, the ability to achieve high pre-concentration factor, and the compatibility with chromatographic analytical instruments (especially for SPME) [14–17]. On the other hand, due to the limited rate of diffusion and mass transfer of analytes in the bulk sorbent phases, equilibration time of ordinary SPE and SPME processes is usually long [18,19]. Thus, SPME measurements are often performed under non-equilibrium conditions and relatively large amount of solid sorbents are required in solid-phase extraction to avoid analyte breakthrough due to the slow partitioning equilibration. One of the ways to overcome this

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bottleneck is the adoption of micro-/nano-phase extraction where micro-/nano-scaled solid-sorbent particles are dispersed in the sample matrix to facilitate mass transfer of analytes by drastically increasing the sample–sorbent interfacial area [20–25]. While there are a number of simple methods, such as ultra-filtration and ultra-centrifugation, for the collection of the dispersed sorbent particles after sample extraction, the most efficient way is magnetic separation [26,27]. Magnetite particles can be reversibly agglomerated and re-dispersed in solution suspensions by the application and removal of a moderate external magnetic field [28]. This attractive feature of magnetic separation have led to its numerous applications in the fields of biomedicals and molecular biology, such as, drug delivery [29,30], cell imaging [31], proteomics and nucleic acid sequencing [32–35], and bio-/immunomagnetic separations [36–38].

Comparing to their popularity in biological applications, potentials of magnetic-mediated solid-phase extraction with the use of micro-/nano-scale sorbents in dispersion mode in environmental monitoring and treatment have only been recognized in recent years. Song et al. used naked Fe_3O_4 nanoparticles as an extracting agent for the determination of triazine-based pesticides and herbicides [39]. Recovery of the extracted chemicals was achieved by the dissolution of the nanoparticles in an acidic medium. Yang et al. applied magnetic Fe_3O_4-activated carbon nanocomposite materials to remove methylene blue from aqueous solutions [40]. Magnetite core-shell nanoparticles with various surface modifications by chemical functional moieties, surfactants, polymeric coatings and biosorption thin-films have also been developed for the removal/extraction of heavy metals, phenolic compounds and plant sterols in natural waters, industrial effluents and plant tissues [41–49]. All of these pioneering works have divulged the usefulness of magnetite materials as solid sorbents for the efficient and high capacity extraction and pre-concentration of trace metals and organics.

Endocrine disrupting chemicals (EDCs) are those compounds that can mimic or block the actions of natural hormones in living organisms, including human, and impair their normal functioning, such as growth, metabolism and reproduction. Many synthetic chemicals released into the environment, such as synthetic pesticides and herbicides, antifouling paints, plasticizers, raw materials for polymer and resin fabrication and hormonal pharmaceuticals, have been identified to be potent EDCs and their environmental occurrence and ecotoxicological effects are being actively studied in recent years [50,51]. Amongst the various types of EDCs, estrogen mimics have perhaps captured the most research and public attentions. These estrogenic EDCs have been related to behavioural changes, reproduction difficulty and cancer development in a wide range of biota, both vertebrates and invertebrates [52–54]. Their quantification in the various environmental compartments is, therefore, vital to the assessment of their ecological potential impacts [55–58]. In this work, we developed a new magnetite Fe_3O_4/poly(divinylbenzene-*co*-methacrylic acid) (pDVB-MAA) core-shell micro-particle sorbent material for the rapid solid-phase extraction of selected natural and synthetic estrogens, namely estrone (E1), 17β-estradiol (E2), estriol (E3), 17α-ethynylestradiol (EE2) and bisphenol-A (BPA), in natural waters for liquid chromatography tandem mass spectrometry (LC–MS/MS) determination using the highly analyte-specific multiple reaction monitoring (MRM) mode of detection. Relatively small amount of sorbent (in milligram scale), compared to conventional SPE, was enough for this magnetic-mediated solid-phase extraction with micro-particle sorbent (MM-SPE-MP) process and the sorbent possessed good reusability. Partitioning equilibrium was achieved within 15 min, and the MM-SPE-MP process was free of the purging problem faced by conventional solid-phase extraction. This MM-SPE-MP coupled LC–MS/MS technique represents a rapid, efficient and highly sensitive analytical method for the monitoring of potent estrogenic EDCs in natural waters.

2. Materials and methods

2.1. Chemical and equipment

HPLC grade methanol was purchased from RDH. Ferric chloride, sodium chloride, analytical grade toluene, divinylbenzene, methacrylic acid, 1,1′-azobis(cyclohexanecarbonitrile), poly(ethylene glycol) and ethylene glycol were obtained from Aldrich. Sodium acetate was obtained from Riedel-de Haen. Authentic standards of estrone (E1), 17β-estradiol (E2), estriol (E3), 17α-ethynylestradiol (EE2) and bisphenol-A (BPA) were obtained from Sigma. Stock solutions (1000 μg ml^{-1}) of the EDCs were prepared in methanol. Milli-Q water (Millipore, Milli-Q system) was used in the preparation of samples.

Electron microscopy images of the magnetite micro-particles and core-shell microspheres were recorded by a Philips XL30 ESEM-FEG Environmental Scanning Electron Microscope and a Philips Tecnal 12 Bio TWIN Transmission Electron Microscope. X-ray diffraction (XRD) patterns of the micro-particles were recorded at room temperature on a Siemens D500 powder diffractometer.

An Aglient 1100 series liquid chromatography (Waldbronn, Germany), consisting of a vacuum solvent degassing unit, a quaternary high-pressure gradient pump and an automatic sample injector was used for the LC–MS/MS analysis. Chromatographic separation was performed using a C_{18} ODS column (Beckman Coulter, 5 μm × 4.6 mm × 25 cm). An XTerra MS C_{18} 5 μm (3.9 mm × 20 mm) guard column was placed in front of the analytical column. Tandem MS detection was performed by a triple stage quadrupole/linear ion-trap mass spectrometer (Q-TRAP™, Applied Biosystems, Darmstadt, Germany) equipped with the Analyst 1.4.1 software and an electrospray ionization (Turboionspray) ion source. In order to achieve good specificity and sensitivity of detection, analytes were detected in the Multiple Reaction Monitoring (MRM) mode with a dwell time of 50 ms. The ionization source parameters were as follow: ion spray voltage, −4500 kV; curtain gas (N_2) setting = 10; collision gas (N_2) set at high; temperature of heater gas = 650 °C; nebulizer gas setting = 45; heater gas setting = 65. Declustering potential (DP), entrance potential (EP), collision energy (CE) and collision cell exit potential (CXP) of all analytes were also optimized (as tabulated in Table 1) to obtain maximum sensitivity.

Sonication of samples during MM-SPE-MP extraction was performed by a BRANSON 2510 sonicator.

2.2. Preparation of the Fe_3O_4 magnetite microspheres

The Fe_3O_4 magnetite microspheres were prepared in accordance with the literature method reported by Deng et al. [59]. In a typical fabrication run, 0.17 g of $FeCl_3$, 0.7 g of poly(ethylene glycol) and 0.72 g of sodium acetate were dissolved in 10 ml of ethylene glycol under magnetic stirring. The resultant homogeneous yellow solution was sealed in a Teflon-lined stainless-steel autoclave and heat to 200 °C for 12 h. The autoclave was then cooled to room temperature. The black magnetite microspheres obtained were collected by the application of an external magnetic field via a small piece of permanent magnet, thoroughly washed with ethanol and water and dried in vacuum at 60 °C for 12 h.

2.3. Coating of the Fe_3O_4 magnetite microspheres with poly(divinylbenzene-co-methacrylic acid) (pDVB-MAA)

The poly(divinylbenzene-*co*-methacrylic acid) coating on the magnetite microspheres was fabricated by a suspension

Table 1
MS/MS transitions and MS parameters adopted in the analysis.

EDCs	Retention time (min)	MRM traces (m/z)[a]	Declustering potential (V)	Entrance potential (V)	Collision energy (V)	Collision cell entrance potential (V)	Collision cell exit potential (V)
Estrone (E1)	24.60	269 → 145	−66	−10	−57	−16	−1
		269 → 183	−66	−10	−57	−16	−1
17β-Estradiol (E2)	24.50	271 → 145	−70	−10	−57	−15	−1
		271 → 183	−70	−10	−57	−15	−1
Estriol (E3)	15.16	287 → 145	−72	−10	−60	−15	−1
		287 → 171	−72	−10	−55	−15	−1
17α-Ethynylestradiol (EE2)	22.85	295 → 145	−70	−10	−60	−18	−1
		295 → 183	−70	−10	−60	−18	−1
Bisphenol-A (BPA)	18.28	227 → 133	−54	−9	−36	−16	−0.6
		227 → 212	−54	−9	−30	−16	−1

[a] Primary MRM traces for quantitative analysis are listed first, followed by the MRM transitions for qualitative identification of the analytes.

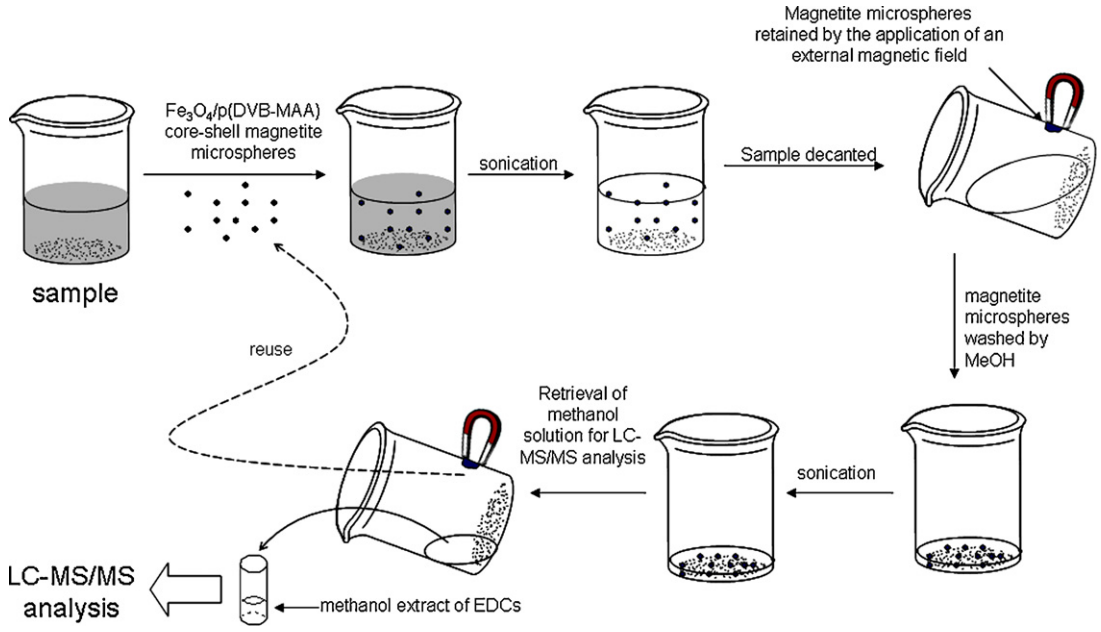
copolymerization approach using toluene as the porogen. Divinylbenzene (9 ml), methacrylic acid (1.5 ml) and 1,1′-azobis(cyclohexanecarbonitrile) (0.1 g) were added to a suspension of 0.2 g of the Fe_3O_4 microspheres in 25 ml of toluene. The organic suspension was then mixed with 140 ml of an aqueous solution containing 0.7 g of poly(ethylene glycol) and 1.4 g of NaCl. The resultant mixture was stirred for 10 min to form an even emulsion, and was purged with nitrogen for 20 min. The mixture was then heated to 70 °C over a water bath for 24 h. The polymer-coated microspheres obtained were collected by the application of an external magnetic field via a small permanent magnet, washed with hot water and thoroughly soxhlet extracted by acetone for 24 h. The pDVB-MAA coated Fe_3O_4 microspheres were dried at 70 °C overnight.

2.4. Environmental water samples

Tap water was obtained from a tap in the laboratory at the City University of Hong Kong. Seawater was retrieved from Victoria Harbor, Hong Kong. Sewage influents were collected from the Shatin Waste Water Treatment Plant of Hong Kong which was located in a satellite town known as Shatin in the New Territories of Hong Kong. Population of that satellite town was ca. 0.6 million. All water samples were filtered through 0.45 μm membrane filters before use.

2.5. Magnetic-mediated solid-phase extraction with use of micro-particle sorbent in dispersion mode (MM-SPE-MP)

MM-SPE-MP extraction of all the samples involved in this study was carried out in 500 ml conical flasks at room temperature. Sample volume of 200 ml was adopted throughout the study. The pDVB-MAA coated Fe_3O_4 microspheres were added into the sample followed by sonication for a fixed duration. The micro-particle MM-SPE-MP sorbent was then collected at the bottom of the flask by the application of external magnetic field on the outside of the flask via a piece of permanent magnet, and the sample was decanted. Methanol (1 ml) was then added and the permanent magnet was removed. The methanol suspension of microspheres was sonicated for 5 min before the permanent magnet was re-applied to collect the microspheres at the bottom of the flask. The methanol desorption solvent was then transferred out of the flask for LC–MS/MS analysis. The MM-SPE-MP extraction process is illustrated in Scheme 1.



Scheme 1. Schematic illustration of the MM-SPE-MP extraction process using the Fe_3O_4/pDVB-MAA core-shell magnetite micro-particle sorbent.

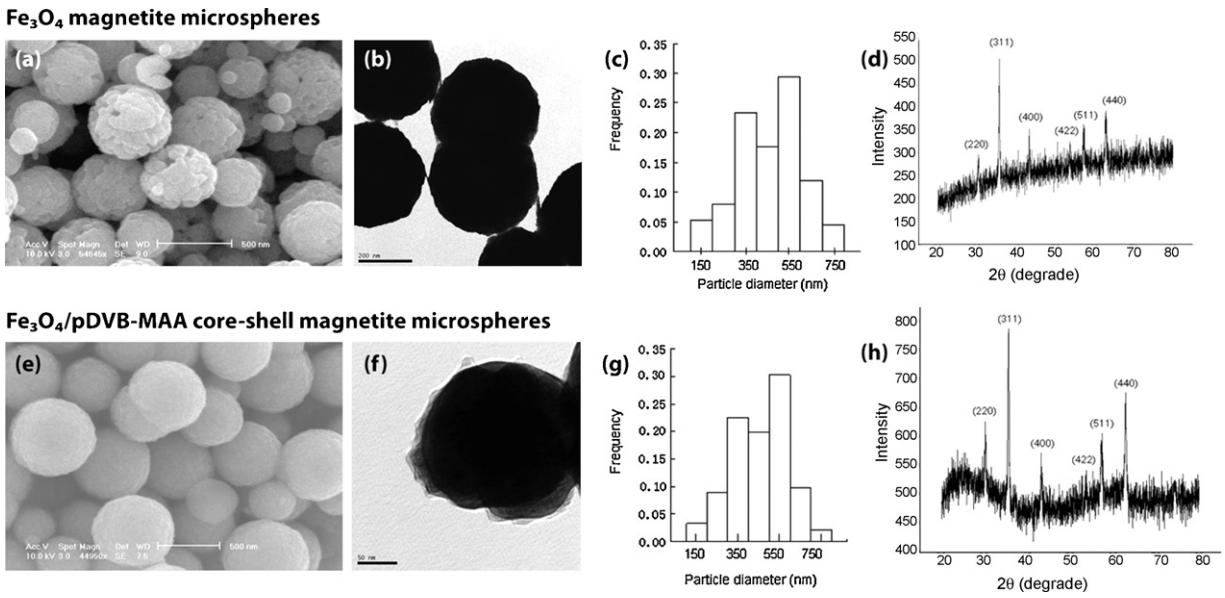


Fig. 1. SEM and TEM images, particle size distributions and XRD patterns of the Fe_3O_4 magnetite microspheres and the Fe_3O_4/pDVB-MAA core-shell magnetite microspheres: (a and e) SEM images; (b and f) TEM images; (c and g) particle size distribution (obtained from TEM photomicrographs); (d and h) XRD patterns, of the Fe_3O_4 and Fe_3O_4/pDVB-MAA core-shell microspheres, respectively.

2.6. LC–MS/MS analysis

An isocratic LC condition was adopted for the separation and quantification of the various estrogenic EDCs in samples. The mobile phase composition was methanol: 0.15% aqueous ammonia at a ratio of 75:25 (v/v). The mobile phase flow rate was 0.2 ml min^{-1} and the column temperature was maintained at 25 °C throughout the run. Injection volume was 5 μl. The LC–MS/MS was operated in the negative electrospray ionization mode and the multiple reactions monitoring (MRM) detection mode.

2.7. Determination of sorbent extraction capacity

Extraction capacity of the pDVB-MAA coated Fe_3O_4 microspheres for the five EDCs was estimated according to the methodology outlined by Dou et al. [49]. A known amount of the MM-SPE-MP sorbent was added to concentrated solutions (100 μg-EDC ml^{-1}) of individual EDC in Milli-Q water at 25 °C. Duration of the MM-SPE-MP extraction was 15 min. Amounts of EDC extracted by the sorbent were determined by LC–MS/MS and were taken as the saturated capacity of the MM-SPE-MP sorbent for the EDCs. For each EDC, at least three trials were carried out.

3. Results and discussion

3.1. Characterization of the Fe_3O_4 magnetite microspheres and the pDVB-MAA coated core-shell magnetite microspheres

Characterization of the solvothermally synthesized Fe_3O_4 microspheres was carried out by X-ray diffraction (XRD) and electron microscopy (Fig. 1a–d). The XRD pattern of the microspheres demonstrated that their crystal structure was similar

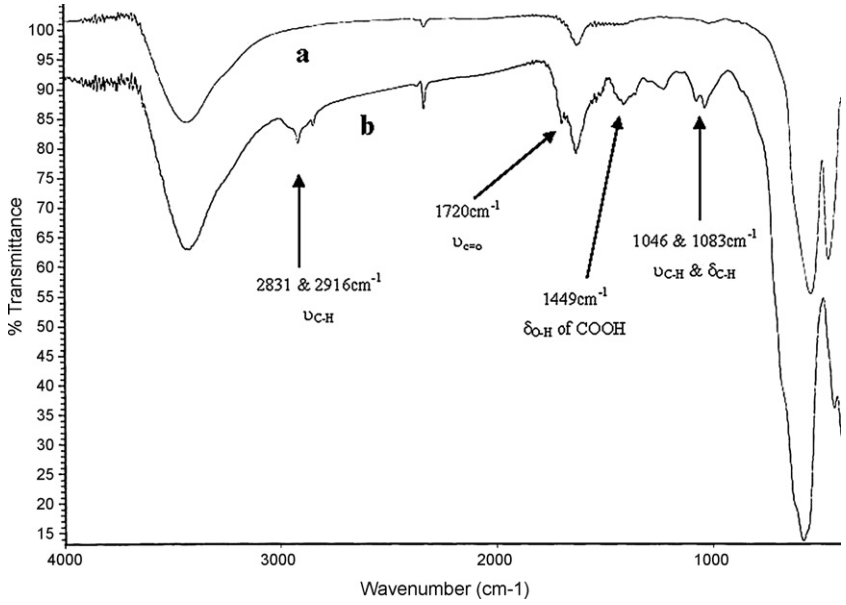


Fig. 2. FT-IR spectra of the Fe_3O_4 magnetite microspheres (a), and the pDVB-MAA coated core-shell microspheres (b).

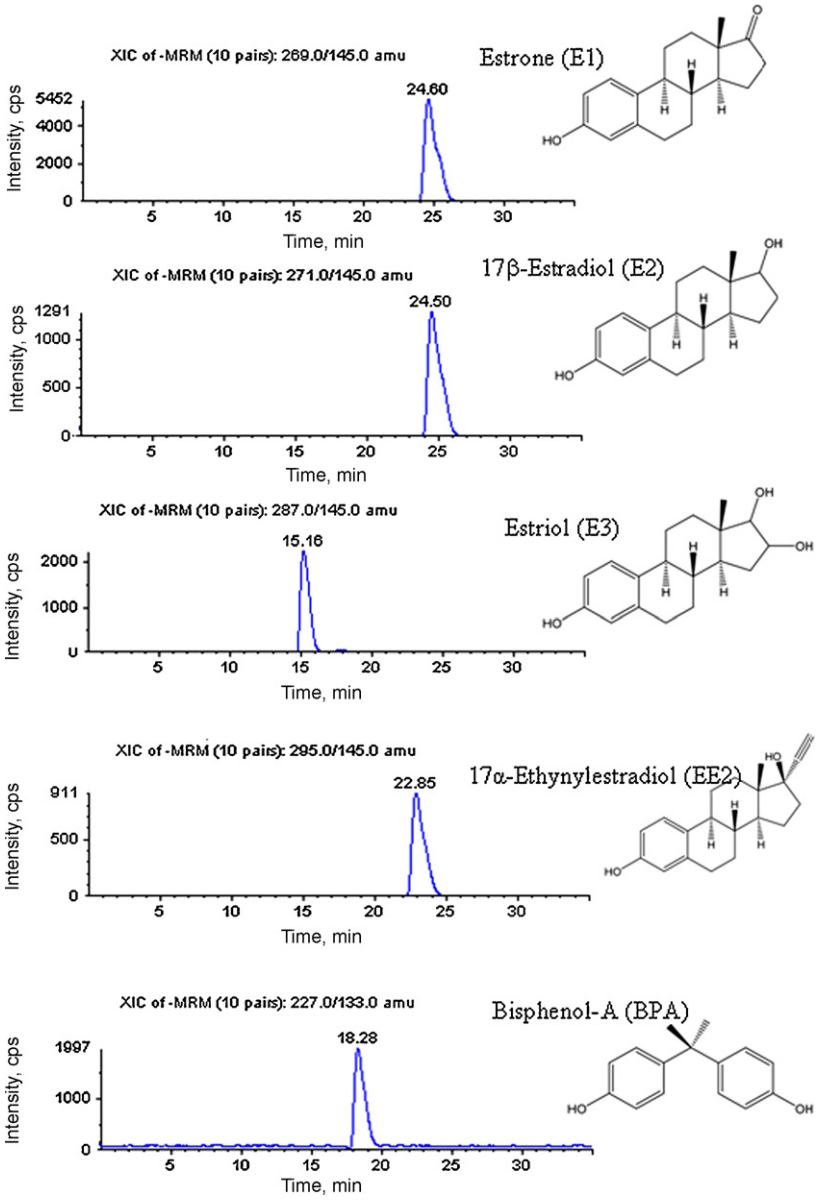


Fig. 3. Typical LC–ESI-MS/MS chromatograms of a standard solution containing 100 ng/ml of E1, E2, E3, EE2 and BPA. Injection volume was 5 μl.

to that of bulk Fe_3O_4 [60]. Particle size distribution obtained from TEM images showed that over 71% of the microspheres were in the diameter range of 300–600 nm. In order to coat the Fe_3O_4 magnetite microspheres with an organic sorbent layer for MM-SPE-MP extraction, we have tried a number of different polymers and polymerization conditions and found that suspension polymerization was able to produce a thin layer of organic polymers on the Fe_3O_4 magnetite microspheres. Aggregation of the coated microspheres was serious with highly hydrophobic polymer coatings, e.g. the styrenic coating produced by the polymerization of divinylbenzene. This is a well-known phenomenon in the preparation of polymer microspheres and nanoparticles [61,62]. In order to obtain core-shell magnetite microspheres that are free from aggregation so that they can be easily dispersed in water samples for extraction application, a more polar monomer, methacrylic acid (MAA), was used together with divinylbenzene to form an amphipolar co-polymeric coating for the magnetite microspheres. The resultant Fe_3O_4/poly(divinylbenzene-*co*-methacrylic acid) (pDVB-MAA) core-shell microspheres were found to be discrete micro-particles with minimal aggregation (Fig. 1e–h). Surface morphology of the Fe_3O_4 microspheres changed from globular to smooth after the polymerization reaction. Particle size distribution of the resultant core-shell particles did not differ significantly with that of the Fe_3O_4 microspheres. From the TEM images of the core-shell microspheres, thickness of the polymer coating was ca. 10 nm. XRD pattern of the core-shell magnetite microspheres was essentially similar to that of bulk Fe_3O_4, indicating that the formation of the polymer coating did not lead to any crystal phase change in the magnetite cores. The presence of the poly(divinylbenzene-*co*-methacrylic acid) coating on the core-shell microspheres was mainly characterized by infrared spectroscopy. Fig. 2 compares the FT-IR spectrum of the Fe_3O_4 microspheres to that of the pDVB-MAA coated core-shell magnetite microspheres. The broad absorption at 3150–3630 cm^{-1} of both materials were due to the υ_{O-H} stretching of the surface Fe–OH groups in the uncoated magnetite microspheres, and the carboxyl acid groups in pDVB-MAA coating of the core-shell microspheres. The absorption peak at 1720 cm^{-1}, typical of $\upsilon_{C=O}$ stretching, also demonstrates the presence of carboxyl functional groups in the core-shell microspheres. Absorption bands at 2831–2916 and 1046–1083 cm^{-1} were attributable to the υ_{C-H}

stretching and δ_{C-H} bending of the polymer matrix of the coating, respectively. Absorption at 1449 cm^{-1} was attributable to the δ_{O-H} bending of the carboxyl functional groups in the polymer coating. These new peaks in the coated magnetite microspheres confirm the establishment of the pDVB-MAA shell on the microspheres.

3.2. Optimization of LC–MS–MS conditions for the determination of estrogenic EDCs

Operation parameters (except the ion spray voltage) of the electrospray ionization (ESI) interface of the triple quadrupole tandem mass spectrometer were optimized in the negative mode by direct syringe infusion of the EDC standards in methanol: 0.15% aqueous ammonia (75:25, v/v) mobile phase at a flow rate of 10 μl min^{-1}. Table 1 tabulates the optimal ionization parameters and the qualitative and quantitative MRM traces for the MRM detection of each EDC analyte. Fig. 3 shows the LC–MS/MS chromatograms of the estrogenic EDC analytes monitored at their specific MRM transitions. Although chromatographic retention times of E1 and E2 were still rather close to each other under optimal isocratic HPLC elution, they were resolved by the use of MRM detection with qualitative identification via observing at different precursor-to-product MRM transitions.

3.3. Sorption characteristics of the Fe_3O_4/pDVB-MAA core-shell magnetite microspheres towards the selected estrogenic EDCs

The pK_a values of the phenolic groups on the selected estrogenic EDCs are in the range of 9–10 [63], while that of the carboxyl functionalities on the pDVB-MAA polymer coating of the core-shell magnetite microspheres is ca. 4–5. Protonation/deprotonation of these functional groups affects the overall charge of the analytes as well as the MM-SPE-MP sorbent and may, in turn, affect its efficiency. Fig. 4a illustrates the effect of media pH of spiked samples

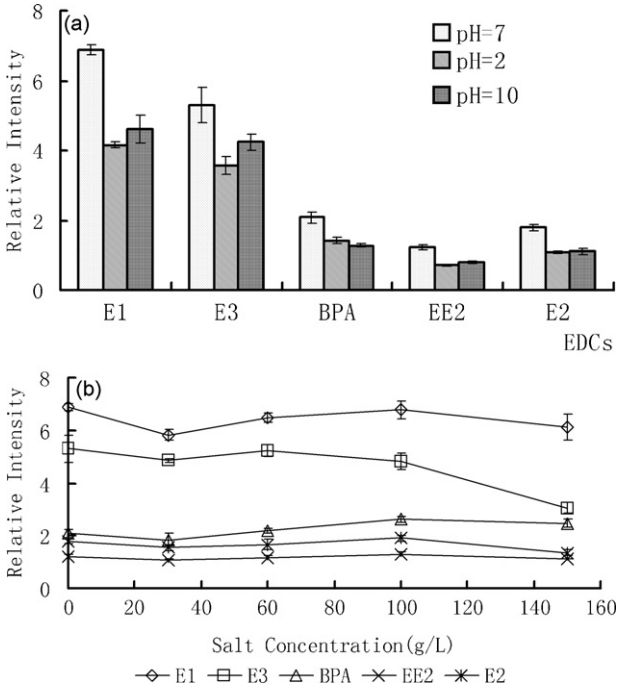


Fig. 4. Effects of media pH (a), and ionic strength (b) on the MM-SPE-MP extraction efficiency of the selected EDCs by the Fe_3O_4/pDVB-MAA core-shell microspheres. Spike level of all the five EDCs was 14.7 ng ml^{-1}. The volume of all the samples involved was 200 ml. Amount of microsphere used was 2 mg and the extraction duration was 15 min. Each data point shows the mean recovery with error bar representing ±1 SD (n = 3).

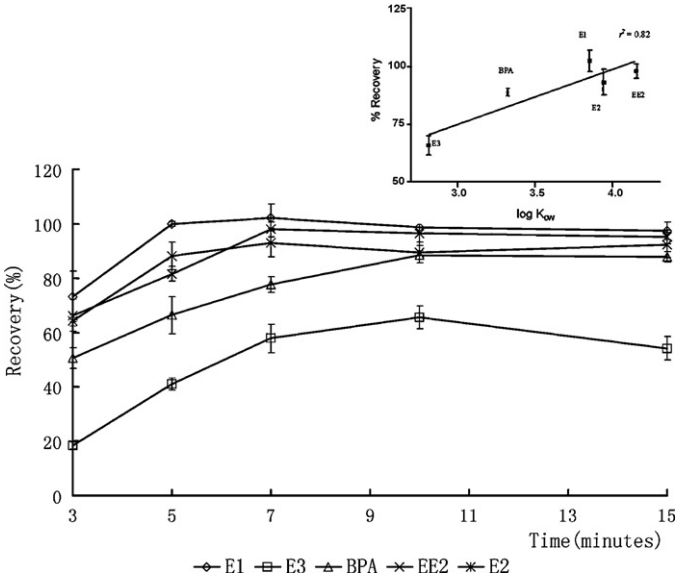


Fig. 5. MM-SPE-MP extraction time profiles of the selected EDCs by the Fe_3O_4/pDVB-MAA core-shell microspheres. Spike level of all the five EDCs was 14.7 ng ml^{-1}. The volume of all the samples involved was 200 ml. Amount of microsphere used was 10 mg. Relationship between log K_{OW} (obtained from Ref. [62–65]) of the EDCs and their recovery is shown in the inset. Each data point shows the mean recovery with error bar representing ±1 SD (n = 3).

on the amount of extracted EDCs (expressed as the corresponding LC–MS–MS responses). Among the three pH values tried (pH 2, 7 and 10), the highest efficiency was achieved at pH 7 for all the selected estrogenic EDCs. At the higher sample media pH (pH 10), significant amount of the estrogenic EDCs and most of the carboxyl functionalities on the pDVB-MAA coating are deprotonated. Coulombic repulsion between the negatively charged phenolate analytes and the carboxylates on the sorbent may hinder the MM-SPE-MP extraction process. On the other hand, at the lower sample media pH (pH 2), protonation of the carboxyl groups in the sorbent may render the pDVB-MAA coating less polar and reduce its affinity for the polar estrogenic EDC analytes. As neutral pH was found to be optimal for the MM-SPE-MP extraction of the estrogenic EDCs, pH of natural water samples in all subsequent analyses in this study was not adjusted unless they have deviated from pH 7 by ±1.

Fig. 4b shows the effects of increasing ionic strength (via the addition of NaCl) of the Milli-Q aqueous matrix on the MM-SPE-MP extraction efficiency. To all the EDCs, addition of NaCl did not improve their efficiency. In fact, a slight reduction of MM-SPE-MP extraction efficiency was observed in E1, E2 and E3 at ionic strength >100 g-NaCl l^{-1}. As a result, no adjustment of media ionic strength was undertaken in subsequent analyses of all the spiked and real natural water samples analyzed in this study.

Time profiles of the MM-SPE-MP processes for the selected estrogenic EDCs are shown in Fig. 5. Partition equilibration for E1, E2 and EE2 were achieved in 7 min. For BPA and E3, partition equilibration were achieved in 10 min. Analyte recoveries were ranged from 66 to 102% in the Milli-Q aqueous matrix, which followed the descending order of E1 > EE2 > E2 > BPA > E3. Analytes with lower log K_{OW} value seemed to require relatively longer equilibration time for partitioning and showed lower analyte recovery (Fig. 5, inset). Amongst the five estrogenic EDCs in this study, water solubility of E3 is considered the highest (its K_{OW} is 11–21-fold lower than the others) and it showed the lowest recovery of ca. 66% at equilibrium. This is followed by BPA (its K_{OW} is 3.4–6.8-fold lower than the other EDCs) which showed a MM-SPE-MP recovery of ca. 89% at equilibrium [64–67]. This trend of analyte recovery is attributable to the amphipolar nature of the pDVB-MAA coating

Table 2
Performance of the MM-SPE-MP coupled LC–MS/MS determination of selected estrogenic EDCs in natural water.[a]

	Tap water				Seawater				Sewage influents			
	Spike level ($ng\,ml^{-1}$)	Detected ($ng\,ml^{-1}$)	% Recovery (% repeatability[c]) ($n=3$)	MDL ($pg\,ml^{-1}$)	Spike level ($ng\,ml^{-1}$)	Detected ($ng\,ml^{-1}$)	% Recovery (% repeatability) ($n=3$)	MDL ($pg\,ml^{-1}$)	Spike level ($ng\,ml^{-1}$)	Detected ($ng\,ml^{-1}$)	% Recovery (% repeatability) ($n=3$)	MDL ($pg\,ml^{-1}$)
E1	–	n.d.[b]	100.3	1.0	–	n.d.	97.9	1.8	–	0.09 ± 0.004	107.8	2.2
	0.50	0.51 ± 0.04	(14.4)		0.50	0.49 ± 0.02	(7.5)		0.52	0.56 ± 0.03	(9.8)	
E2	–	n.d.	96.0	3.0	–	n.d.	101.2	6.2	–	n.d.	110.0	2.3
	0.64	0.61 ± 0.05	(15.0)		0.64	0.65 ± 0.02	(5.6)		0.64	0.71 ± 0.03	(7.7)	
E3	–	n.d.	71.2	3.0	–	n.d.	69.1	10	–	n.d.	55.9	3.0
	0.85	0.60 ± 0.11	(33.7)		0.85	0.58 ± 0.06	(19.0)		0.85	0.48 ± 0.08	(30.6)	
EE2	–	n.d.	108.3	7.0	–	n.d.	110.0	24	–	n.d.	105.0	6.5
	0.75	0.81 ± 0.04	(9.0)		0.75	0.83 ± 0.06	(13.3)		0.77	0.81 ± 0.05	(11.3)	
BPA	–	n.d.	85.8	20	–	n.d.	91.7	36	–	0.28 ± 0.02	105.3	19
	1.11	0.95 ± 0.14	(27.1)		1.11	1.01 ± 0.11	(20.0)		1.16	1.22 ± 0.04	(6.0)	

[a] Amount of Fe_3O_4/pDVB-MAA core-shell magnetite microspheres used was 10 mg; duration of the MM-SPE-MP extraction was 15 min.
[b] Not detected.
[c] Repeatability was determined according to the following equation:

$$\text{Repeatability } (\%) = \frac{t \cdot \sigma}{\bar{x} \cdot \sqrt{n}} \times 100$$

where σ is the standard deviation of the data; $n=3$; $\bar{x}$ is the mean data and t is the compensation factor from the Student's t-table with a confident interval of 95%.

of the microspheres. Although the present analyte recoveries for the selected estrogenic EDCs are considered acceptable, our results suggest that for the efficient MM-SPE-MP extraction of other highly polar analytes, such as pharmaceuticals and polar metabolites of organic contaminants, magnetite microspheres with more polar coatings might be necessary. Nevertheless, partition equilibration of the present MM-SPE-MP extraction process for all the tested estrogenic EDCs is already much faster than those achieved by traditional solid–liquid extraction techniques, such as SPME. In all subsequent analysis of natural water samples spiked with EDCs, a 15 min. MM-SPE-MP extraction duration was adopted.

Extract capacity of the pDVB-MAA coated Fe_3O_4 microspheres for the five EDCs was determined by exposing the micro-particle sorbent to excess EDC (concentration up to 100 μg-EDC ml^{-1}) in Milli-Q water for 15 min. It was revealed that the pDVB-MAA coated Fe_3O_4 micro-particle sorbent was able to extract up to 2.38 ± 0.03 μg-E1 mg^{-1}, 3.16 ± 0.14 μg-E2 mg^{-1}, 0.10 ± 0.01 μg-E3 mg^{-1}, 3.49 ± 0.19 μg-EE2 mg^{-1}, and 1.02 ± 0.07 μg-BPA mg^{-1} ($n=3$) at 25 °C. This extraction capacity was deemed adequate to handle EDCs in real natural water samples. In all subsequent analyses of spiked natural water samples, 10 mg of the pDVB-MAA coated Fe_3O_4 micro-particle sorbent was used.

3.4. MM-SPE-MP-LC–MS/MS determination of estrogenic EDCs in environmental water samples

To further validate this MM-SPE-MP coupled LC–MS/MS method, we tested it on a series of natural water samples, including tap water (pH 7.6; 0.79 mg-TOC l^{-1}), seawater (Ph 7.8; 2.9 mg-TOC l^{-1}) and sewage influents (pH 6.4; 28.3 mg-TOC l^{-1}) (TOC = total organic carbon content), spiked with realistic levels of estrogenic EDCs. Table 2 tabulates the performance of the analytical method on the various sample matrices. Analyte recoveries were estimated by quantification of samples spiked with known amounts of EDC standards. Method detection limits (MDLs) for the five EDCs were taken from the lowest spike levels of the EDCs in the various sample matrices that their corresponding MRM chromatographic peaks still showed a signal-to-noise (S/N) ratio of >5 in a series of five consecutive analyses. For the three sample matrices that have been studied, the MM-SPE-MP coupled LC–MS/MS determination were able to achieve detection limits of <10 pg-EDC ml^{-1} for most of the EDCs, except BPA (MDLs were 20, 36 and 19 pg-BPA ml^{-1} in tap water, seawater and sewage influents respectively) and EE2 in seawater (MDL was 24 pg-EE2 ml^{-1}). These levels of sensitivity are considered adequate for environmental monitoring purposes. In fact, even lower MDL should be achievable by simply using larger sample volume (in the present study, volume of samples was 200 ml). Repeatability of the MM-SPE-MP coupled LC–MS/MS method for most of the EDCs was <20% ($p<0.05$), except for BPA (27.1%) in tap and E3 (19.0–33.7%) in all the three matrices. Similar to their behaviours in the Milli-Q aqueous matrix, BPA and E3 also showed relatively lower recovery. These relatively inferior repeatability and analyte recovery is probably caused by their lower K_{OW} values that rendered them less favourably partitioned onto the amphipolar pDVB-MAA coating of the core-shell magnetite microspheres. No targeted estrogen was found in the tap water and seawater samples, but 0.09 ± 0.004 ng ml^{-1} of E1 and 0.28 ± 0.02 ng ml^{-1} of BPA were found in the sewage influents.

4. Conclusion

A new magnetite Fe_3O_4/poly(divinylbenzene-*co*-methacrylic acid) (pDVB-MAA) core-shell microsphere material has been successfully fabricated and utilized as a sorbent in a magnetic-mediated solid-phase extraction in dispersion mode (MM-SPE-MP)

process for the determination of selected estrogenic endocrine disrupting chemicals (EDCs) in natural waters. The thin pDVB-MAA coating on the Fe_3O_4 magnetite microspheric cores enabled discrete core-shell microspheres to be easily dispersed in the aqueous sample matrix. The high sample–sorbent interfacial area afforded by these microspheres, together with their amphipolar polymer coating, offered adequate extraction capacity and rapid partitioning for trace organics extraction and pre-concentration. Their magnetite cores permitted convenient, rapid and complete retrieval from sample matrices. By coupling this MM-SPE-MP process with LC–MS/MS quantification, parts-per-trillion level of detection sensitivity with good repeatability and analyte recovery was achieved for the determination of E1–E3, EE2 and BPA in natural waters. It is envisioned that with the development of more kinds of polymeric coatings of different polarity and chemical nature, this MM-SPE-MP technique can become a very useful tool for the solid–liquid pre-concentration of trace organics in environmental monitoring as well as other analytical applications, such as food & pharmaceuticals analysis and clinical diagnostics.

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