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Polymer-coated hollow-fiber microextraction of estrogens in water samples with analysis by gas chromatography–mass spectrometry

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Abstract

A novel sorbent, dihydroxylated polymethylmethacrylate (DHPMM), coated on hollow-fiber membrane, is used for the polymer-coated hollow-fiber microextraction of trace amounts of natural and synthetic estrogens, such as diethylstilbestrol, estrone, 17β-estradiol and 17α-ethynylestradiol, in aqueous samples. In this procedure, estrogens were extracted using the functionalized polar DHPMM polymer with derivatization using *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide followed by gas chromatography–mass spectrometric analysis. The detection limits for estrogens in aqueous sample were between 0.03 and 0.8 ng l^{-1} and the calibration curves were linear over the concentration range 0.05–10 μg l^{-1} and had correlation coefficients of >0.994. The relative standard deviations (RSDs) were <15% ($n = 3$). This simple, accurate, sensitive and selective analytical method is applicable to the determination of trace amounts of estrogens in reservoir and potable water samples.

Keywords: Endocrine disruptors; Polymer-coated hollow-fiber microextraction; Environmental analysis; Extraction methods; Solid-phase microextraction

1. Introduction

Endocrine disrupting compounds (EDCs) are widespread environmental contaminants that affect the endocrine systems of wildlife and human beings [1]. Decreasing sperm count in human males, increasing breast cancer in women and reproductive abnormalities in human beings represent some evidence of the effects of natural and synthetic EDCs found in the environment [2,3]. Among the wide range of EDCs, estrogens are of particular interest due to their high estrogenic potency [2]. These estrogens are classified as natural hormones which include 17β-estradiol, its main metabolites estriol and estrone and synthetic contraceptive additives, such as 17α-ethynylestradiol and diethylstilbestrol (DES) [4,5] (Fig. 1). Previously, DES was commonly used in the USA for the treatment of high-risk pregnancies, and was prescribed to more than five million pregnant women in the 1940s [6]. In the 1970s and 1980s, possible association between treatment of DES during pregnancy and its endocrine disruption effects were documented by several epidemiologists [7,8]. Estradiol has also been used in the management of the menopausal syndrome and in diverse cancers, mainly prostate and breast cancer [9].

Invariably, estrogens used by the general population and the manner described above, are entering the aqueous environment by various ways including excretion, and incomplete removal in wastewater treatment plants. Municipal sewage effluents are a major source of estrogens in the aquatic media [10,11]. Estrogens have been shown to provoke endocrine disruption in certain fish at ultratrace levels [10,12]. Their determination therefore requires high-sensitivity analytical methods. There are limited analytical techniques reported in the literature for estrogen analysis, normally after liquid–liquid extraction (LLE) [13], solid-phase extraction (SPE) [14–18] and solid-phase microextraction (SPME) [19] of aqueous samples. In SPME, sorbent coatings for polar compounds, such as estrogens are usually limited to only polyacrylate (PA) coating although more coatings for more polar compounds are being introduced. Some of the commercial SPME coatings are not stable with the coated phase being detached from the silica core [19] after two or three estrogen extractions. Accordingly, there are only few reports on the SPME of estrogens, combined with gas chromatography–mass

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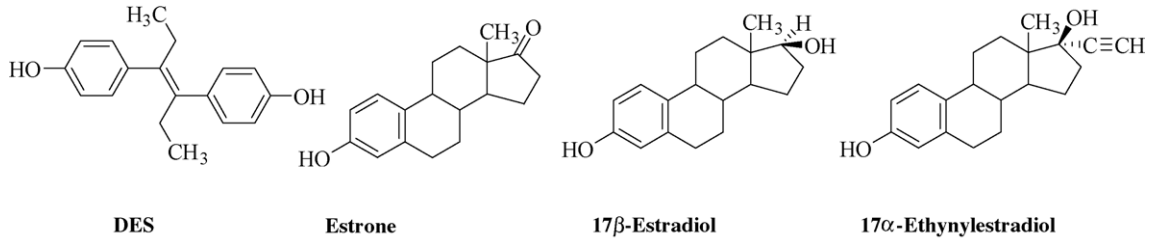

Fig. 1. Chemical structures of estrogens studied.

spectrometry (GC–MS) [20] and high performance liquid chromatography [21]. In these reports, relatively high quantification limits were obtained. With the alternative stir bar sorptive extraction (SBSE) technique, only polydimethylsiloxane (PDMS)-coated stir bars are commercially available. Strictly speaking, the non-polar PDMS is not suitable for the extraction of polar analytes [22]. Recently, to improve the extraction of the estrogens using SBSE, multiple PDMS-coated stirrer bars were used [23].

For ultratrace estrogen analysis, GC–MS has been commonly used with derivatization [11,24]. Different derivatization reagents have been used for estrogens in order to improve sensitivity and selectivity. These include pentafluorobenzyl [25], *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) [26], *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) [27] *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) [28] and direct aqueous derivatization using acetic anhydride [29]. Among these methods, BSTFA, MTBSTFA and MSTFA are the most popular derivatization approaches for estrogens [30].

In this work, an efficient extraction procedure, in which a novel functional polymer coated on hollow-fiber membrane was used as sorbent material followed by MSTFA derivatization of estrogens, in combination with GC–MS detection, is reported for the analysis of these compounds in water samples.

Compared with SPME sorbent materials, the novel polymer described has a high number of functional groups (–OH) that makes it more amenable for the extraction of polar compounds. Results from this procedure (polymer-coated hollow-fiber microextraction (PC-HFME)) [31,32] are compared with those from SPME. Finally, the developed method was applied to the determination of estrogens in reservoir and potable water samples.

2. Experimental

2.1. Chemicals and reagents

Pure estrogen standards were obtained from Aldrich (Milwaukee, WI, USA). All HPLC-grade organic solvents, hydrochloric acid, sodium hydroxide and sodium chloride were purchased from Merck (Darmstadt, Germany). Ultrapure water was prepared on a Milli-Q (Milford, MA, USA) system. Diluted standards and mixtures of them were prepared in methanol. The derivatization reagent MSTFA was purchased from Aldrich.

2.2. Materials

Accurel Q3/2 polypropylene hollow-fiber (600 μm inner diameter, 200 μm wall thickness; 0.2 μm wall pore size) was purchased from Membrana (Wuppertal, Germany). The SPME fiber holder and fibers (PDMS, 100 μm; PDMS-divinylbenzene (DVB), 65 μm; and PA, 85 μm) and extraction vials, septa and aluminium caps were purchased from Supelco (Bellefonte, PA, USA) and used without modification. Before extraction the fibers were conditioned in the GC injection port based on the manufacturer's recommended procedure. The ultrasonicator was purchased from Midmark (Versailles, OH, USA) and the magnetic stirrer/hot plate was obtained from Heidolph (Kelheim, Germany).

2.3. Synthesis of hydrogel and coating on HFM

The synthesis of dihydroxylated polymethylmethacrylate (DHPMM) has been reported previously [33]. Briefly, the alcoholic –OH of solketal (2,2-dimethyl-1,3-dioxolan-4-ylmethanol) 1 was protected using tosyl chloride to give the tosylated derivative 2. Reaction of 2 with 3-hydroxy benzyl alcohol gave intermediate 3 which on reaction with methacryloyl chloride in the presence of triethylamine as base yielded monomer 4. Free radical polymerization of 4 was carried out in toluene using azobisisobutyronitrile as the initiator. Deprotection of the precursor polymer 5 under acidic conditions gave the hydroxylated polymethacrylate, DHPMM in 70% yield. The obtained polymer was found to be soluble in polar solvents like alcohols and acetone but insoluble in hexane, dichloromethane (DCM), toluene, *iso*-octane and *n*-nonane.

To coat the porous HFM, the latter was cut into 1.2-cm lengths and immersed in a 0.5-g ml^{-1} solution of DHPMM in methanol for 24 h. The functional polymer formed a thin layer on the HFM. Physical characterization of the polymer-coated HFM was carried out; scanning electron micrographs, and attenuated total reflection fourier transform infrared spectra indicate the presence of hydroxylated groups on the fiber surface.

2.4. PC-HFME procedure

Twenty millilitres of ultrapure water in a 25-ml screw-cap vial was spiked with 2.5 $\mu g\,l^{-1}$ of each estrogen. The sample pH was adjusted to 4 and sodium chloride concentration to 30% (w/v). A polymer-coated HFM was placed in the sample solution, and extraction was performed for 30 min. The solution was stirred

at 105 rad s^{-1} (1000 rpm; 1 rpm = 0.1047 rad s^{-1}). After equilibrium was established, the fiber was removed with a pair of tweezers and dabbed dry in a lint-free tissue. The analyte containing HFM was desorbed ultrasonically in a 150-μl auosampler-crimper vial with 100 μl of DCM for 20 min. After complete desorption of analytes, the polymer-coated HFM was removed from the vial and then 50 μl of MSTFA was added. The mixture was kept in a hot water bath for 30 min at 60 °C. To increase the sensitivity of the quantitative analysis, the extract volume was reduced to dryness using nitrogen gas and made up to 20 μl with DCM. Finally, 5 μl was injected into the GC–MS. This accounts for the high sensitivity of the method. Derivatized extracts were analyzed immediately, and their temporal stability was not systematically studied. However, the silylated estrogens were stable for at least 2 days, during which no degradation was observed.

2.5. SPME procedure

An estrogens-spiked (5 μg l^{-1} of each analyte) 10-ml sample solution (pH and sodium chloride concentration were adjusted to 2 and 30% (w/v), respectively) was extracted by direct immersion SPME with stirring (at 105 rad s^{-1}). Equilibrium was established after 160 min. After extraction, and prior to derivatization, the fiber was exposed for 2 min to ultrapure water in order to remove excess sodium chloride from its surface. For on-fiber derivatization, the SPME fiber was placed in the headspace of a 3-ml GC autosampler vial containing 100 μl of MSTFA at 60 °C, for 30 min. All these extraction and derivatization conditions were optimized in the present work, based on previous results [19]. Finally, the fiber was desorbed in the injection-port of the GC for 5 min at 280 °C. Possible carryover was minimized by keeping the fiber in the injector for an additional 5 min. Blanks were run periodically to confirm the absence of contaminants.

Fig. 2 shows chromatograms of extracts after PC-HFME and SPME (with PA fiber), with samples spiked with estrogens at the same concentration. Significant differences between PC-HFME and SPME extracts are clearly shown. Extraction was faster in PC-HFME (30 min) than for SPME (160 min).

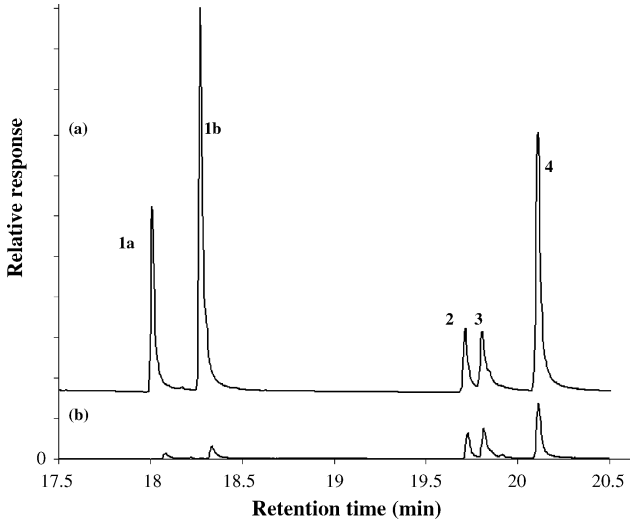

Fig. 2. Total ion chromatogram of MSTFA-derivatized estrogens. Reservoir water spiked at the (5 μg l^{-1}) of each estrogen (a) after PC-HFME and (b) SPME (PA fiber). Peak identification [1a and 1b] DES, [2] Estrone, [3] 17β-estradiol and [4] 17α-ethynylestradiol. Extraction conditions are given in the text.

2.6. Derivatization procedure

Reagents used for the silylation of estrogens are destroyed in the presence of water. Therefore, microextraction and derivatization steps cannot be performed simultaneously. Recently, Cela and co-workers [18] evaluated the performance and selectivity of MSTFA, BSTFA and MTBSTFA at different derivatization conditions. Shareef et al. reported that BSTFA and MTBSTFA derivatization leads to formation of degradation products of trimethylsilyl and *t*-butyldimethylsilyl derivatives [34]. Based on these results, it was decided to use MSTFA as derivatization agent with similar reported conditions in the present work [18,19]. Different amounts (50–100 μl) of MSTFA, the influence of derivatization time (from 10 to 60 min) and temperature (from 40 to 80 °C) were evaluated. The amount of MSTFA did not have any impact on derivatization. During long derivatization times at a high temperature (>60 °C) a significant decrease in the peak areas of the silylated compounds was observed. Probably, under these conditions analytes were partially desorbed from the fibers. Short derivatization times at low temperatures led to the incomplete silylation of analytes. At a derivatization time of 30 min and at 60 °C with 50 μl of MSTFA, complete derivatization was observed; additionally, two DES peaks with the same mass spectra, corresponding to the *cis* and *trans* isomers, were obtained [18,19,35]. The combined peak areas were used for quantification.

2.7. GC–MS analysis

Analysis was carried out using a Shimadzu (Tokyo, Japan) QP2010 GC–MS system equipped with a Shimadzu AOC-20i autosampler and a DB-5 fused silica capillary column (30 m × 0.32 mm I.D., film thickness 0.25 μm, from J & W Scientific, Folsom, CA, USA). Helium was used as the carrier gas at a flow rate of 2.1 ml min^{-1}. Five microlitres of sample was injected into the GC–MS with a splitless injection-port under splitless mode after a sampling time (holding time) of 2 min. The injection temperature was set at 300 °C, and the interface temperature at 270 °C. The GC temperature programme was as follows: 50 °C (2 min); 20 °C min^{-1} to 100 °C; 10 °C min^{-1} to 200 °C; 20 °C min^{-1} to 300 °C (2 min). All samples were analyzed in selective ion monitoring (SIM) mode with a detector voltage of 1.5 kV.

3. Results and discussion

3.1. Optimization of PC-HFME

Initially, 10 ml samples (spiked with individual analytes at 2.5 ng ml^{-1}) were extracted with a single polymer-coated HFM. To improve analyte enrichment, multi-fibers were tested for extraction. Trials on two, three and four pieces of polymer-coated HFM fibers were used to check the efficiency

of PC-HFME. Desorption in a 150-μl autosampler-crimper vial only allowed two pieces of HFM. To desorb four pieces we had to use a larger capacity crimper vial. The latter however decreased the sensitivity as the volume of solvent used was necessarily increased to accommodate the additional fibers. On comparison, extraction with two fibers gave ∼40% higher peak area response. Thus, for subsequent experiments two polymer-coated HFM were used. In PC-HFME, the extraction efficiency and selectivity of the coatings to the analytes depend on the interactions between the analytes and the DHPMM-coated HFM, which most probably include hydrogen bonding, π–π, dipole–dipole, dipole-induced-dipole, and dispersion (hydrophobic interaction) forces. The PC-HFME and SPME parameters that were optimized include extraction time, sample ionic strength, pH, desorption solvent, desorption time, and various aspects of the derivatization procedure.

The effect of extraction time on estrogens extraction was investigated by monitoring the peak area response with exposure time over 5, 10, 15, 20, 25, 30, and 40 min. The amount of analyte that can be extracted depends on the partition coefficient of the analyte between the aqueous sample and the polymer coating on the fiber. The extraction profile showed an initial rapid partitioning between these two phases, followed by a slower uptake profile. Fig. 3 shows that the peak areas increase with sampling time in the range of 5–30 min, and decreases after 30 min. At strong sample agitation with longer extraction time, analytes may slightly desorb from the fiber. Similar behavior has been observed in many microextraction procedures. Therefore, 30 min was deemed sufficient for PC-HFME. As long as conditions were carefully maintained from experiment to experiment, quantitative rigor was not compromised.

It is well-known that increasing the ionic strength of the analyte solution can favor partitioning of organic compounds from an aqueous phase on to a polymer absorbent. The effect of salt on extraction efficiency was determined by adding sodium chloride to 10 ml water samples at 5, 10, 15, 20 and 30% (w/v). Fig. 4 shows the extraction efficiency of PC-HFME on addition of sodium chloride. Salt addition, possibly contributes to the ionization of polar functional groups on the estrogens and DHPMM and also decrease the solubility of the estrogens which

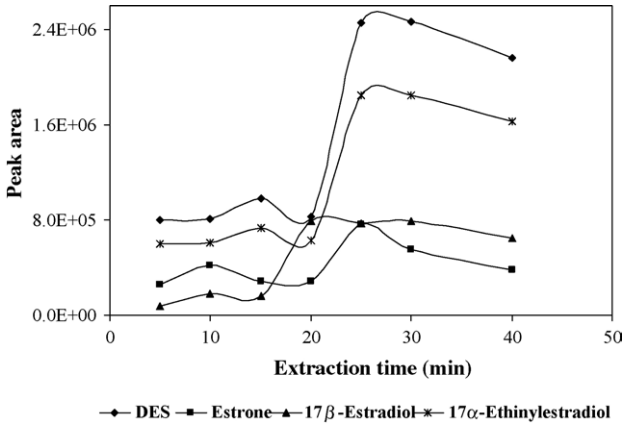

Fig. 3. Effect of extraction time on PC-HFME. Stirring speed was 105 rad s^{-1}. Sample pH and ionic strength was not adjusted.

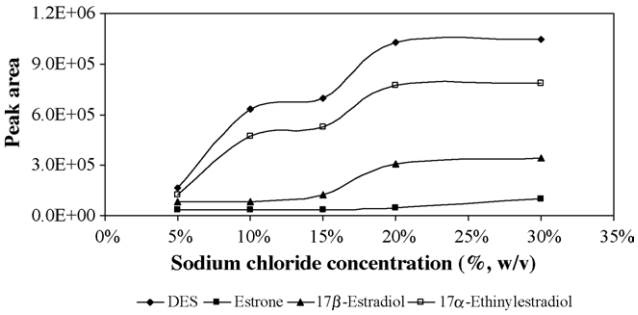

Fig. 4. Effect of sodium chloride on PC-HFME ($n = 3$).

results in better extraction efficiency. Since the extraction efficiency for most of the compounds increased up to 30% (w/v), all subsequent experiments were conducted at this concentration.

Since estrogens are ionizable compounds (pK_a values around 9–10), their extraction behavior at various pH (2–12) was studied; the results are shown in Fig. 5. At low pH (<4) extraction was not found to be better than that obtained at pH 4, whereas pH higher than 8 led to significant losses of DES. At pH 12, the analytes were fully ionized and extraction recovery was minimal [36]. pH 4 was deemed to be suitable for extraction.

As in SBSE [37] and SPE [14–18], in PC-HFME, the analytes were desorbed using a suitable solvent after extraction. Selection of a suitable solvent is one of the prerequisites of PC-HFME. The polypropylene HFM is not soluble in most organic solvents. The DHPMM coating is soluble in methanol and acetone, and insoluble in DCM, hexane, isooctane, toluene and *n*-nonane. The latter solvents were evaluated and the results are shown in Fig. 6. DCM gave the best results and was used as the eluting solvent of choice.

Next, desorption time over the range of 2–25 min was investigated. Analyte desorption peak areas were not significantly increased after 10 min (although there was a slight increase in desorption of 17α-ethinylestradiol after 10 min; data not shown). An optimized desorption time of 20 min was selected for subsequent experiments. After the first desorption, fibers were further desorbed to test carryover effects. No carryover was observed, indicating that analytes were completely desorbed from the fiber during DCM desorption. This means that fibers could be re-used without compromising extraction efficiency for up to 20 analyses.

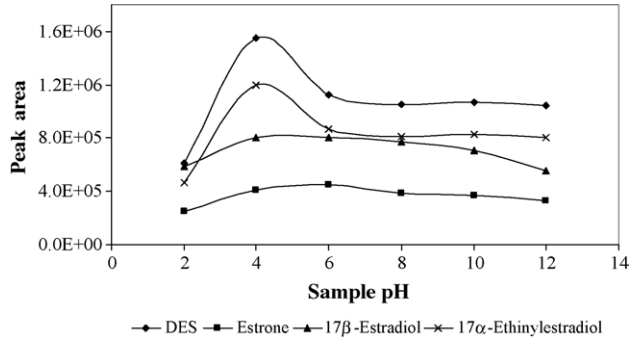

Fig. 5. Effect of sample pH on PC-HFME ($n = 3$).

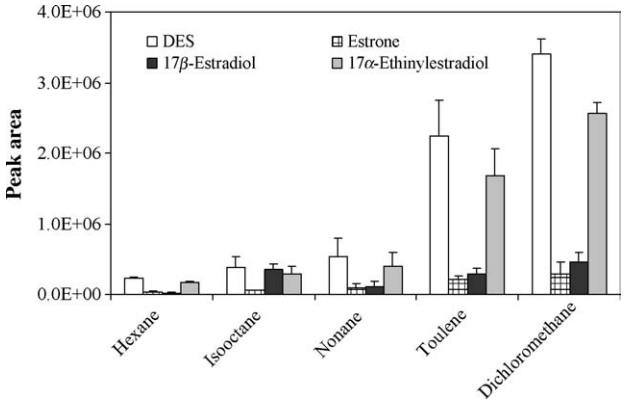

Fig. 6. Desorption profile of estrogens using different solvents ($n = 3$).

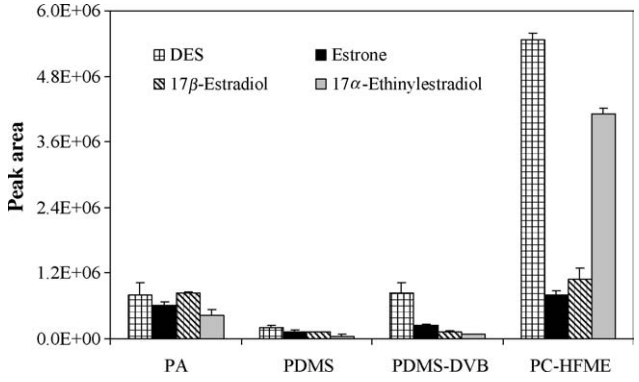

Fig. 8. Comparison of PC-HFME with SPME with different SPME fiber coatings Concentration of each analyte, 5 $\mu g\,l^{-1}$ ($n = 3$).

The sample volume size on PC-HFME extraction efficiency was evaluated between 5 and 50 ml. The efficiency increased from 5 to 25 ml and then decreased (Fig. 7). Although the extraction concepts are similar, substantial differences between both methods were observed. For example, the coated HFMs have higher affinity towards target analytes, with two fibers giving higher active surface area than the single SPME fiber; also the former fibers tumbled freely in the sample which increases extraction capacity. PC-HFME enrichment (based on GC–MS peak area) was optimum when extracting from 25 ml of sample. Analyte enrichment decreased considerably for sample volumes higher than 25 ml. Therefore, a sample size of 25 ml was selected for further experiments.

3.2. Comparison of PC-HFME with SPME

Fig. 8 shows the comparison of PC-HFME with SPME using PA, PDMS and PDMS-DVB fibers, for solutions spiked at identical concentrations. Among the SPME fibers, PA fiber gave higher responses than PDMS-DVB and PDMS. When comparing with the PC-HFME data, only 15% of DES, 10% of 17α-ethynylestradiol, and ∼75% of 17β-estradiol and estrone were extracted using the PA fiber. It clearly shows the selectivity of the DHPMM and benefits of the higher extraction capacity of PC-HFME (since two fibers were used). The PC-HFME is faster (30 min extraction time versus 160 min) and gave better enrichment than SPME which allowed us to determine sub-ppb level concentrations of estrogens in aqueous samples.

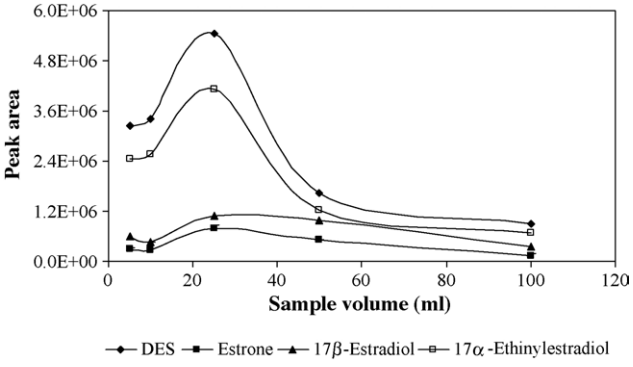

Fig. 7. Influence of sample volume on PC-HFME ($n = 3$).

3.3. Quantitative results of PC-HFME and SPME

To assess the applicability of PC-HFME, linearity, repeatability and limits of detection (LODs) were investigated using the previously optimized extraction conditions. Since the estrogens are present in real world samples at the $ng\,l^{-1}$ range, linearity was tested with estrogen-spiked sample concentrations of 0.025, 0.05, 0.1, 1, 2.5, 5 and 10 $\mu g\,l^{-1}$. The calibration plots were linear over this range of concentrations with correlation coefficient (r) between 0.994 and 0.997. The limits of detection for all target analytes were determined by progressively decreasing the concentrations of analytes until signals were just detected at a signal-to-noise ratio of 3 (S/N = 3) after PC-HFME. The LODs ranged from 0.03 to 0.8 $ng\,l^{-1}$. While determining the LODs, blanks were carried out to confirm that no sample carryover occurred. Three replicates were used to calculate LODs. Limits of quantification (LOQs) at S/N = 10 were calculated and are listed in Table 1. The LOQs of the PC-HFME method were comparable with those previously reported for SPME–GC–MS-MS [19]. The relative standard deviation (RSD) was performed by extracting ultrapure water spiked at 5 $\mu g\,l^{-1}$ of each compound (three replicates). PC-HFME analyte enrichment was higher than those obtained by SPME. This could be due to the DHPMM having functional groups which have higher electrostatic interaction with estrogens and the higher surface active area of polymer-coated HFMs. SPME quantitative information, such as linear range, precision and LODs were also evaluated. The correlation coefficients were from 0.990 to 0.999 for linear range between 0.5 and 20 $ng\,ml^{-1}$. The LOQs obtained in this study were lower than previously reported for extraction with a PA fiber [19] (Table 1).

3.4. Application of PC-HFME to real samples and recovery test

We tested the performance of PC-HFME with reservoir water and tap water samples. First, blank extraction of reservoir and tap water samples using the present method was carried out. No

Table 1
Quantitative data: linearity, precision, LODs (S/N = 3) and LODs of PC-HFME and SPME

Analytes	PC-HFME[a]				SPME (PA fiber)[b]				SPME–GC–MS-MS[c]
	Correlation coefficient	%RSD ($n = 3$)	LODs ($ng\,l^{-1}$)	LOQs (S/N = 10)	Correlation coefficient	%RSD ($n = 3$)	LODs ($ng\,l^{-1}$)	LOQs (S/N = 10)	LOQs (S/N = 10)
DES	0.997	3	0.03	0.1	0.990	7	5.5	18.3	0.2
Estrone	0.995	4	0.7	2.3	0.999	8	2.4	8.0	1.0
17β-Estradiol	0.994	6	0.8	2.7	0.996	9	2.1	7.0	0.7
17α-Ethinylestradiol	0.996	7	0.1	0.3	0.997	6	3.3	11.0	3.0

[a] Linear range for PC-HFME, 0.025–10 $\mu g\,l^{-1}$.
[b] Linear range for SPME, 0.5–20 $\mu g\,l^{-1}$.
[c] Ref. [19].

Table 2
Recoveries (%) of estrogens from reservoir and tap water by PC-HPME ($n = 3$)

Analytes	Spiked reservoir water		Spiked tap water	
	0.5 $\mu g\,l^{-1}$	5 $\mu g\,l^{-1}$	0.5 $\mu g\,l^{-1}$	5 $\mu g\,l^{-1}$
DES	89 ± 11	86 ± 5	90 ± 15	99 ± 2
Estrone	98 ± 13	102 ± 4	100 ± 2	102 ± 3
17β-Estradiol	92 ± 3	110 ± 12	108 ± 10	100 ± 2
17α-Ethynylestradiol	100 ± 7	108 ± 5	87 ± 11	104 ± 5

estrogens were detected in both tap and reservoir water samples. Singapore has limited water resources; it purchases some of its raw water supplies from neighboring countries. Another major source is from rainwater catchments. Both type of water are stored in reservoirs before treatment. Domestic wastewater is also reclaimed by membrane microfiltration and reverse osmosis, which is then blended with the reservoir water. The blended water then undergoes the normal treatment process. Data from the Singapore Ministry of Environment and Water Resources regular monitoring program were compared. The targeted estrogens were not detected in tap water samples. To assess the matrix effects on PC-HFME, two different concentrations (0.5 and 5 $mg\,l^{-1}$) of individual estrogens were spiked in reservoir and tap water samples. Extraction recoveries and reproducibility of PC-HFME were determined. The recoveries of the method were tested by triplicate analysis ($n = 3$) of the spiked sample and the results are listed in Table 2. Recoveries for reservoir water sample were between 86 and 110% with RSD values between 3 and 13%. For tap water samples, between 87 and 108% with better RSD values (between 3 and 12%) were obtained. These results clearly demonstrate that real sample matrices had little effect on the efficiency of PC-HFME, which is therefore suitable for analysis of trace levels of estrogens from real water samples.

4. Conclusions

A novel sorbent has been synthesized and used for the extraction of estrogens using PC-HFME from environmental water samples. The method has been shown to be linear, precise, and accurate, but its most outstanding analytical feature is its high extraction capacity, allowing good sensitivity for real sample analysis. PC-HFME is simple and the sensitivity can be increased by using more than one fiber for each extraction. The LODs when two fibers were used were $<1\,ng\,l^{-1}$. The relative recoveries for tap water were between 87 and 108% with good precision between 2 and 15%, and for reservoir water, between 86 and 110% with precision between 3 and 13% for spiked samples at concentrations of 0.5 and 5 $\mu g\,l^{-1}$. This simple, accurate and highly sensitive method is potentially useful for the analysis of estrogens in environmental waters.

Acknowledgements

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