

In situ polar organic chemical integrative sampling (POCIS) of steroidal estrogens in sewage treatment works discharge and river water

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A passive sampler (the polar organic chemical integrative sampler; POCIS) was assessed for its ability to sample natural estrogens (17 β -estradiol, E2; estrone, E1 and estriol, E3) and the synthetic estrogen (17 α -ethynylestradiol, EE2) in the outlet of a sewage treatment works over several weeks. The performance of the POCIS was investigated and optimised in the laboratory before field deployment with high recoveries (66–99%) were achieved for all estrogens. Moreover, it was shown that POCIS does not exhibit any preferential selectivity towards any of the target compounds. The sampling rates of E1, E2 and E3 were 0.018 ± 0.009 , 0.025 ± 0.014 and 0.033 ± 0.019 L d⁻¹, respectively. Following field deployments of 28 days in the discharge of a sewage works, POCIS was shown to enhance the sensitivity of estrogen detection, especially for E3, and provide time-weighted average (TWA) concentrations of E1, E2 and E3, ranging from undetectable to 12 ng L⁻¹ upstream of the outflow of a sewage treatment works, 13 to 91 ng L⁻¹ at the outflow and 8 to 39 ng L⁻¹ downstream of the outflow. This revealed that E1, E2 and E3 are not completely removed during sewage treatment, with concentrations most likely being maintained by contributions from conjugated estrogen analogues. Grab water samples showed considerable variation in the concentrations of estrogens over a longer period (6 months). The results confirm that POCIS is an effective and non-discriminatory method for the detection of low concentrations of estrogens in the aquatic environment.

Introduction

Most monitoring of organic pollutants in the aquatic environment depends on active sampling performed by the collection of discrete grab, spot or bottle samples of water at a specific time.^{1–10} When organic pollutants are present at low concentration this necessitates the collection of large volumes of water and many steps are generally required for sample preparation, espe-

cially filtration and pre-concentration. This results in analytical determinations that are more time-consuming and have a higher analytical sensitivity. Moreover, the subsequent determination provides information only at the specific time of sampling resulting, potentially, in episodic pollution events being missed and no account of variations in target compound concentrations over time being taken. Such problems may be mitigated by increasing the frequency of sampling or by installing automatic samplers, however, this can be both costly and time-consuming.

In order to overcome these problems, passive sampling approaches involving continuous collection of analytes *in situ* and the determination of time weighted average (TWA) concentrations of target analytes have been developed.^{11,12} Passive sampling is insensitive to variations in the concentration

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Environmental impact

Estrogenic endocrine disrupting compounds (EDCs) are present in UK watercourses and, even at very low concentrations (>parts per trillion), are capable of eliciting an estrogenic response in aquatic organisms. A typical example is the feminisation of male wild fish that have been exposed to sewage effluent. The passive organic chemical integrative sampler (POCIS) offers a time-weighted average, rather than instantaneous (*i.e.* grab sampling), determination of EDC concentrations thereby enabling more accurate assessment of risk. This work validates the use of POCIS by showing the absence of any preferential uptake of four EDCs (17 β -estradiol, estrone, estriol and 17 α -ethynylestradiol) and, additionally, provides sampling rates for three of these target compounds. Field results from POCIS and previous grab sampling studies are compared.

of organic compounds over short periods of time and has the potential to provide a useful means for the long-term monitoring of pollutants (ranging from weeks to months) in the environment^{11,12} which has contributed to its uptake as a popular sampling method.^{13–16} In this work, the polar organic chemical integrative sampler (POCIS), a passive sampler originally introduced by the U.S. Geological Survey, Biological Resources Division,^{12,17} has been used to determine low concentrations of natural and synthetic estrogens in the aqueous environment. Advantages conferred by this method include the capacity to adjust the selectivity and capacity of the POCIS, by judicious selection of an appropriate adsorbent, and a low membrane susceptibility to bio-fouling. Other potential benefits of using POCIS include sampling cost reductions, enhancement of analytical sensitivity and, most importantly, the capacity to use the sampler in locations where temporally variable concentrations of contaminants are of concern.¹⁸ However, the POCIS may be damaged easily when deployed in highly turbulent waters and is also a target for vandalism.

Natural estrogens (17 β -estradiol, E2; estrone, E1 and estriol, E3) and the synthetic estrogen (17 α -ethynylestradiol, EE2) were chosen as targets since these compounds are the primary estrogens suspected of causing estrogenic effects in fish due to their high potencies.^{19–22} For example, the production of the female yolk protein precursor, vitellogenin, which is synthesized by the liver exclusively in response to estrogenic compounds, was observed in male wild fish such as the roach and rainbow trout when exposed to sewage effluent in the UK.²¹ Estrogens have been detected in the environment in sewage discharge and surface waters in the nanogram per litre range.^{1–10} E1 is always detected as the dominant estrogen, whereas E2 and EE2 appear more sporadically.^{1–6,8,10} The concentrations of estrogens in sewage effluent are variable, with E1 ranging between <0.1 and 82.1 ng L⁻¹, while E2 ranges from <0.4 to 48.0 ng L⁻¹. EE2 has been detected in the <0.2 to 7.5 ng L⁻¹ range, while E3 is below the limit of detection with the exception of a high concentration of 28 ng L⁻¹ observed in an Italian sewage treatment works.⁴ If POCIS is to be used with confidence in field studies then its ability to trap target compounds, with no preferential selectivity, must be demonstrated. In addition, uptake rates for the target compounds must be determined and the durability of the sampling system in the field assessed. Therefore, the objectives of this paper were to: (i) assess the effectiveness of POCIS in the laboratory for the trapping of estrogens spiked into water, (ii) determine the concentrations of estrogens at the outflow of a sewage treatment works and in the river upstream and downstream of the outflow using POCIS, and (iii) compare the results obtained by POCIS with those obtained by previous grab sampling studies. If shown to be an effective means of sampling trace organic pollutants then POCIS will provide a more cost-effective and realistic means of determining organic pollutants in the aquatic environment.

Experimental

POCIS configuration

Commercially available POCIS discs (EST, St Joseph, MO, USA; type “generic”) were used for all analyses. They comprise

an adsorbent layer contained between two polyethersulfone membrane disks sandwiched between two stainless steel support rings.¹² The membrane contains 0.1 μm sized pores and has a 7 cm diameter. The adsorbent layer contains 200 mg of a triphasic adsorbent mixture comprising: isolate ENV+ (a hydroxylated polystyrene–divinylbenzene resin) and Amborsorb 572 (a carbonaceous adsorbent) dispersed on S-X3 Bio-beads (a styrene–divinylbenzene copolymer; 20 : 80, w/w; see ref. 12 for a detailed description of adsorbents). In this paper, each POCIS disc was attached to a stainless steel support rod for use in selectivity and field investigations.

POCIS extraction efficiency

The efficiency of POCIS extraction for the four target estrogens (E1, E2, E3 and EE2) was investigated in the laboratory before field deployment. Three nanograms of the authentic estrogen mixture (E1, E2, E3 and EE2) were added to 50 mL of double distilled water and sonicated for 10 min. After sonication, the water was passed through a glass column packed with adsorbent retained by solvent-extracted glass wool. The column was pre-rinsed with methanol and the eluate discarded. After passing the estrogen mixture through the column the water was discarded and the column was left for 5 min. It was then eluted with methanol that was collected in a round-bottom flask (fraction 1). After adding 3 ng of E2-*d*₃ (internal standard, synthesised as described by Samuels *et al.*) onto the adsorbent the column was left for a further 5 min and was then eluted with 50 mL of methanol/toluene/dichloromethane (1 : 1 : 8, v/v/v; fraction 2).²³ This fraction was then mixed with an additional 20 mL of methanol. Five millilitres of dichloromethane was then passed through the column (fraction 3). All three fraction solutions were reduced in volume using a rotary evaporator to *ca.* 1 mL, and fractions 1 and 2 dried over anhydrous sodium sulfate (Fisher Scientific, Leicestershire, UK) packed in a pasture pipette to remove any residual water before removing the last of the solvent under a gentle stream of nitrogen. All fractions were dissolved in 500 μL of dichloromethane/methanol (1 : 1, v/v) and a 100 μL aliquot derivatised with *tert*-butyldimethylsilyl chloride (TBDMSCl; see Derivatisation method below) prior to analysis by GC/MS-SIM. The GC/MS results of the three fractions were combined and then compared to those obtained for the authentic estrogen mixture that was only derivatised. The recoveries were determined by comparing the ratio of peak areas of the authentic estrogen mixture of three fractions recovered from POCIS to the peak area of E2-*d*₃ with the ratio of peak areas of the same authentic mixture which was only derivatised, *i.e.* not subjected to the extraction protocol, to the peak area of E2-*d*₃.

Selectivity and sampling rate

One and a half litres of double distilled water was added to each of nine containers and the first set of three containers was spiked with a mixture of authentic estrogens (E1, E2, E3 and EE2), giving an overall concentration of 100 ng L⁻¹ for each estrogen per container, and left for 30 min. A POCIS disc and support rod were suspended in each of the first set for 7 days (room temperature in the dark with continuous stirring). The second and third sets of replicates were treated in the same way as the

first set except that the POCIS discs were immersed for 14 and 28 days, respectively. After immersion for the various periods all of the POCIS discs were removed and extracted as above. Extracts were dissolved in 500 μL of dichloromethane/methanol (1 : 1, v/v) and a 100 μL aliquot was derivatised with TBDMSCl prior to analysis by GC/MS-SIM. The control sample comprised an aliquot of the authentic standard mixture, derivatised and analysed as above.

The POCIS sampling rate (L d^{-1}) was calculated from the concentration of the estrogen remaining in the water (determined as for grab samples, see below) following each period of exposure (7, 14 and 28 days). Using the following equation:

$$R_s = \frac{C_i - C_t}{C_i} \times \frac{V}{t}$$

where R_s is the sampling rate, C_i is the initial concentration of the estrogen, C_t is the concentration of the estrogen at time t , V is the volume of water and t is the number of exposure days.

Field deployment of POCIS

The sampling points chosen for the field deployment of the POCIS were at the outflow of the Wootton Bassett Sewage Treatment Works, Wiltshire, UK and upstream and downstream of the outflow. The Wootton Bassett sewage treatment works serves a population of *ca.* 13 000. The works uses trickling filters as the secondary treatment and aerated denitrifying sand filters as the tertiary treatment. The final treated sewage effluent is discharged into Hancock's river. Additional POCIS sampling apparatus was constructed to avoid the turbulent flow of the river damaging the samplers. The surface of each individual POCIS was covered by a mesh screen to avoid physical damage to the POCIS surface. Further protection to the POCIS was afforded by a cage that helped to maintain the four POCISs in position during the period of deployment. Three cages were placed on the Hancock's river bed in August 2006 for 28 days at each of the three sampling points indicated above. The temperature of the water was 19 °C on the day of deployment and 18 °C on the day of retrieval. The cages containing the POCISs were retrieved in September 2006. They were transferred to the laboratory, where the POCISs were removed from each cage. Each POCIS was removed from the mesh screen and rinsed with water, wrapped with methanol-rinsed aluminium foil and stored at -20 °C. Extraction of field POCISs was modified in light of the results obtained from the POCIS extraction efficiency experiment. Prior to processing, the POCISs were removed from the freezer and allowed to warm to room temperature. Each POCIS was then rinsed under running water to remove any further materials attached to the surface of the sampler. The membrane and support rings of the POCIS were separated carefully and adsorbent transferred with methanol into a glass column plugged with extracted glass wool and pre-rinsed with methanol. Methanol used for transferring was collected in a round-bottom flask (fraction 1). The membrane was also rinsed with methanol and the washings were combined with fraction 1. Three hundred nanograms of E2-*d*₃ was added to the column which was then eluted with 50 mL of methanol/toluene/dichloromethane (1 : 1 : 8, v/v/v) and 5 mL of dichloromethane, respectively, into the flask containing fraction 1. Twenty millilitres of methanol

were then added to the same flask and all solvent was removed subsequently by rotary evaporation. The dried extract was dissolved in 2.5 mL of methanol before being passed through anhydrous sodium sulfate packed into a pasture pipette. Solvent was removed under a gentle stream of nitrogen at 40 °C. Estrogen extracts were dissolved in 300 μL of dichloromethane/methanol (1 : 1, v/v) and a 100 μL aliquot derivatised with TBDMSCl before analysis by GC/MS-SIM.

In addition to passive sampling, 500 mL grab samples of surface river water were collected at the three sampling points during four sampling days in 2006 (day 1, 14th March; day 2, 11th April; day 3, 18th August; day 4, 15th September). All grab samples were collected in pre-cleaned glass bottles and the contents preserved by adding 1% formaldehyde immediately to prevent microbial degradation.³ Samples were kept at 4 °C in the dark and vacuum filtered through glass fibre filters (1.2 μm , Grade GF/C Whatman, Maidstone, UK) within 1–2 days of collections. The filters containing suspended particulate matter were cut into small pieces with stainless steel scissors and stored under nitrogen at -20 °C until required for extraction. Filtrate dissolved samples (referred to as the dissolved fractions) and the particulate matter trapped on the filter (referred to as the particulate fractions) were extracted as described below.

After the addition of 150 ng of E2-*d*₃, the filtrates from the water obtained by grab sampling were extracted 3 times with 25 mL aliquots of dichloromethane. Each of the dichloromethane extracts was then combined to produce the total estrogen extract and evaporated by rotary evaporation. After the addition of 150 ng of E2-*d*₃, the particulates trapped on the filter were extracted 3 times with 10 mL aliquots of dichloromethane, ultrasonicated for 15 min and centrifuged for 5 min in each case. Solvent was removed from the combined particulate extracts by rotary evaporation. Both river water and water particulate extracts were dissolved in 100 μL of dichloromethane and 2.5 mL of a 0.2 M acetate buffer (pH 4.8) and then hydrolysed with 25 μL of the enzyme β -glucuronidase/arylsulfatase (Merck, Darmstadt, Germany) at 37 °C for 15 h. After cooling to room temperature, the hydrolysed samples were extracted 3 times with 10 mL aliquots of dichloromethane. These extracts were combined and reduced to a small volume by rotary evaporation, and then dried over anhydrous sodium sulfate. Estrogen extracts were dissolved in 250 μL of dichloromethane/methanol (1 : 1, v/v) and a 100 μL aliquot derivatised with TBDMSCl before analysis by GC/MS-SIM.^{24,25}

Derivatisation

Estrogens were analysed as their corresponding *tert*-butyldimethylsilyl (TBDMS) ethers as preliminary work (not shown) revealed that this method gave a high derivative yield, were stable and gave good GC/MS responses for all four targets, yielding abundant [M-57]⁺ ions that were particularly suitable for a GC/MS-SIM acquisition. The analytes were treated with 50 μL of 1 M imidazole in dimethylformamide and 50 μL of 1 M TBDMSCl in dimethylformamide and heated for 30 min at 60 °C.²⁴ After cooling, the mixture was diluted with 100 μL of ethyl acetate and 1 mL of hexane and passed through a short column of Sephadex LH-20 (3 cm; GE Healthcare BioSciences AB, Uppsala, Sweden) in order to remove excess derivatising

reagent.²⁶ The silylated estrogens were eluted with 2 mL of ethyl acetate/hexane (1 : 1, v/v) and evaporated under a gentle stream of nitrogen at 40 °C. The derivatives were dissolved in 100 µL of ethyl acetate by vortex-mixing prior to analysis by GC/MS-SIM.

Instrumental analysis

The GC/MS analyses were performed on a ThermoFinnigan Trace GC-MS fitted with a PTV injector. Injections were made using splitless mode (0.5 min splitless time) onto a CPSil-5CB column (50 m × 0.32 mm i.d. × 0.12 µm film thickness, 100% dimethylpolysiloxane phase; Varian Chrompack). The GC oven temperature was maintained at 40 °C for 1 min and then programmed to 200 °C at 10 °C min⁻¹, then to 300 °C at 3 °C min⁻¹ and maintained at 300 °C for 20 min. Helium was used as the carrier gas at a flow rate of 2 mL min⁻¹. The interface and source temperatures were maintained at 300 °C and 200 °C, respectively, with an emission current set to 150 µA. The sample injection volume was 1 µL. The data acquisition and processing were performed using the ThermoFinnigan Xcalibur software suite. MS was performed using electron ionisation (70 eV) and operating in full-scan mode from *m/z* 50–650 with a cycle time of 0.7 s for the qualitative analysis and SIM mode for the quantitative analysis. SIM analyses monitored the following ions: *m/z* 327 and 384 for E1, *m/z* 353 and 410 for EE2, *m/z* 367 and 443 for E2, *m/z* 369 and 446 for E2-*d*₃ and *m/z* 245 and 367 for E3. Peak identifications were based on GC/MS-SIM retention times by comparison with the retention times of an authentic estrogen mixture (E1, E2, E3 and EE2) that was analysed under the same GC/MS-SIM conditions. Co-injections were performed with the authentic estrogens after each sample run. Quantification was carried out by comparing the peak area of analytes with the peak area of the internal standard (E2-*d*₃) and by using the internal standard calibration curves that were established in the standard matrices using authentic estrogen mixtures at concentrations of 10, 50, 100, 300 and 500 pg µL⁻¹, each including a constant concentration of 300 pg µL⁻¹ of E2-*d*₃. The calibration curves were all linear, exhibiting *r*² values of >0.99. Detection limits of estrogens as their TBDMS derivatives were 0.5 pg for E1, E2 and E3, and 10 pg for EE2, based on 1 µL injected on column and the signal-to-noise ratio (*S/N*) = 3. Quantification was effected for estrogens that gave responses with a *S/N* > 10.

Results and discussion

POCIS extraction efficiency

As a result of initial investigations²⁶ it was determined that the extraction procedure recommended by the POCIS supplier²⁷ gave low percentage recoveries; *i.e.* 16% to 24% for all 4 estrogens, it should be noted that this is the same extraction procedure adopted by other POCIS studies of this type.^{13,15,16} Therefore, an improved extraction method was adopted employing methanol, methanol/toluene/dichloromethane and dichloromethane as eluents to recover estrogens from POCIS. Significantly, all estrogens were recovered at higher efficiencies, namely: 66 ± 8% for E1, 99 ± 13% for EE2, 96 ± 2% for E2 and 73 ± 1% for E3. This optimised method was used for all subsequent extractions of POCISs used in the laboratory and field experiments.

POCIS selectivity and sampling rate

To assess whether the relative concentration of different estrogens adsorbed by the POCIS is different from that adsorbed without the POCIS, the adsorption selectivity of POCIS was investigated. Selectivity was determined from the relative abundance of adsorbed estrogens after 7, 14 and 28 days and that of estrogens in the authentic control not adsorbed by the POCIS. The relative abundances were calculated by normalising the concentrations of EE2, E2, and E3 with the concentration of E1 (Fig. 1). The results reveal that the relative abundance of EE2, E2 and E3 adsorbed by the POCIS over the 28 days does not differ significantly when compared with the control (ANOVA test, *P* = 0.01). For example, the relative abundance of E2 ranged from 126–140% over the 28 days, while the control value was 132%. Thus, the POCIS does not selectively trap different estrogens and provides an extract that reflects the true distribution of estrogens when sampling the aquatic environment.

Fig. 2 shows the laboratory uptake of estrogens adsorbed by the POCIS over 28 days. The analytes continue to be adsorbed for at least 28 days for E1, E2 and E3 and 14 days for EE2, the first three being consistent with the behaviour of a range of pharmaceuticals reported previously in a stirred system.¹⁸ On the basis of these findings an optimal sampling period of 28 days was chosen for the field deployment of the POCIS since the main estrogens detected in a pilot study of grab water samples at the sewage works outflow showed E1, E2 and E3 to be the major estrogens present. Study of the estrogen uptake over 28 days provides an estimate of the sampling rate. E1, E2 and E3 have sampling rates of 0.018 ± 0.009, 0.025 ± 0.014 and 0.033 ± 0.019 L d⁻¹, respectively, *i.e.* 18 ± 9, 25 ± 14 and 33 ± 19 mL of the 1000 mL of water in one day is cleared of E1, E2 and E3, respectively. As far as we are aware no estimates exist of the sampling rate of E3 by POCIS. Interestingly, these clearance figures are somewhat lower than those reported for a range of organic chemicals, including diuron, isoproturon and azithromycin, under stirred conditions (0.03–0.12 L d⁻¹).¹⁸ However, Matthiessen *et al.* reported a sampling rate of 0.09 to 0.129 L d⁻¹ for E2 in a similar laboratory experiment whilst in

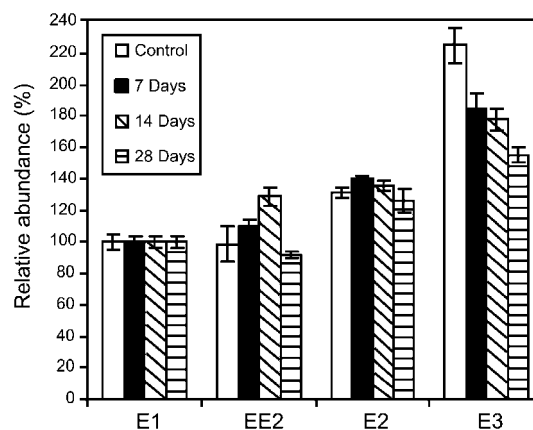


Fig. 1 Comparison of the selectivity of POCIS in trapping the different estrogens compared to the control (mixture of authentic estrogen standards: E1, EE2, E2 and E3) and after 7, 14 and 28 days of exposure. Values are means and errors are SD (*n* = 3).

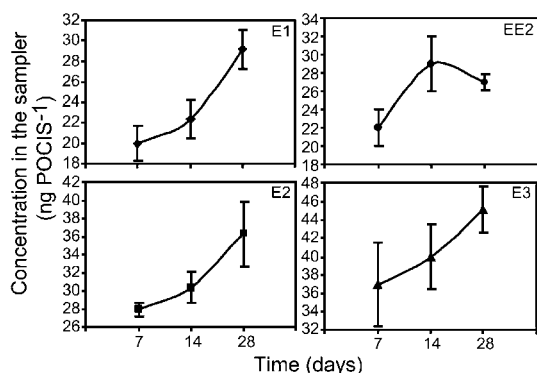


Fig. 2 Uptake of E1, EE2, E2 and E3 adsorbed by the POCIS under stirred conditions in 1.5 L of water for 7, 14 and 28 days. Values are means and errors are SD ($n = 3$).

another study Zhang *et al.* reported sampling rates between 0.036 L d⁻¹ for bisphenol A and 0.069 L d⁻¹ for E1.^{14,28} These latter values from more recent work are comparable with our own determinations. The laboratory-determined sampling rates of estrogens provided the basis of all subsequent determinations of the concentrations of estrogens from field POCIS sampling.

Estrogens detected by POCIS and grab sampling

The dissolved estrogens obtained from POCIS, and the dissolved and particulate associated estrogens obtained from grab sampling were converted to their TBDMS derivatives. Examples of estrogen analyses of POCIS samples together with co-injections are shown in Fig. 3. Fig. 4 and 5 depict the partial GC/MS-SIM chromatograms of TBDMS derivatives of estrogens and mean concentrations of estrogens detected by POCIS, respectively. These concentrations of estrogens were calculated using the sampling rates obtained above to extrapolate to the TWA concentrations of estrogens in the water (ng L⁻¹) using the relationship established by Alvarez.¹² Only the dissolved estrogens are reported since only dissolved estrogens are retained by the POCIS adsorbent. GC/MS analyses of POCIS extracts not only revealed the presence of estrogens but also other components at higher concentrations. Peak identifications of these

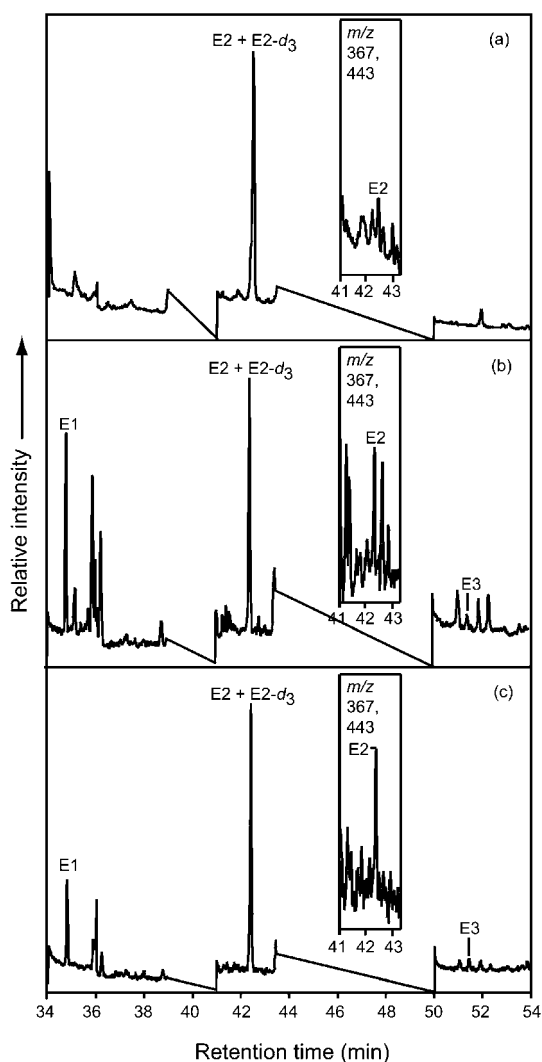


Fig. 4 Partial GC/MS-SIM profiles of the TBDMS derivatives of estrogens collected by POCIS upstream of the outflow (a), at the outflow (b) and downstream of the outflow (c). The inset blocks represent partial SIM mass chromatograms of the TBDMS derivatives of E2 (m/z 367 and 443). The ions monitored were m/z 327 and 384 for E1, m/z 353 and 410 for EE2, m/z 367 and 443 for E2, m/z 369 and 446 for E2- d_3 , and m/z 245 and 367 for E3. For full experimental details, see text.

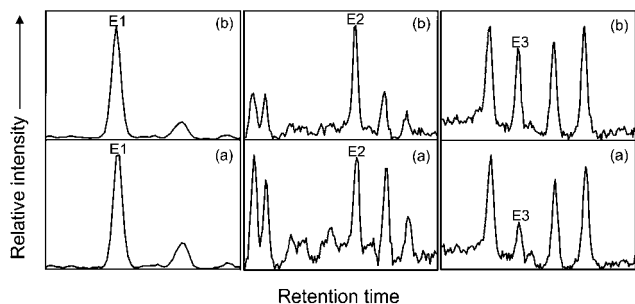


Fig. 3 Partial GC/MS-SIM profiles of the TBDMS derivatives of estrogens collected by POCIS at the outflow, together with the distributions of unknown components (a) compared to those of co-injected authentic estrogens (b). The ions monitored were m/z 327 and 384 for E1, m/z 367 and 443 for E2 and m/z 245 and 367 for E3. For full experimental details, see text.

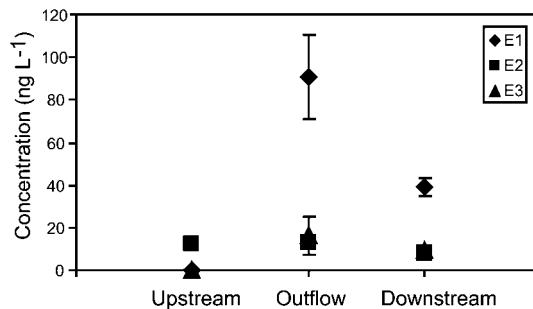


Fig. 5 Plot of the POCIS concentrations (ng L⁻¹) of E1, E2 and E3 upstream of the outflow, at the outflow and downstream of the outflow. Values are means and errors are SD ($n = 4$ for upstream of the outflow, and $n = 3$ for the outflow and downstream of the outflow).

components were made by comparison with their known EI mass spectra of the trimethylsilyl (TMS) derivatives.²⁶ Other components adsorbed by the POCIS are sterols (*i.e.* coprostanol, cholesterol, 5 α -cholestanol, sitosterol, 5 α -stigmastanol), sugars (*i.e.* glucopyranose, disaccharides) and fatty acids (*i.e.* palmitic acid, stearic acid). Furthermore, POCIS extracts exhibit a very high concentration of bis(2-ethylhexyl) phthalate compared with estrogens and other components at all sampling points. This component likely derives from the adsorbent as it was also detected in the POCIS blank.

For grab samples considerable variation is observed in the concentrations of estrogens over long time scales (6 months). The mean concentrations of dissolved and particulate associated estrogens collected by grab sampling upstream of the outflow, at the outflow and downstream of the outflow during four sampling days are shown in Fig. 6. E1 and E2 were detected in higher concentration than E3 at all sampling points, while EE2 was not detected at any of the sampling points.

E2 is the dominant estrogen observed in both the dissolved and particulate fractions at all sampling points compared with E1 and E3. The mean concentration of E1 is higher upstream of the outflow compared to the outflow probably due to the oxidation of E2 as this compound was also detected in high concentration upstream of the outflow by POCIS. Compared to E1 and E2, E3 is present at lowest concentration in the soluble and particulate fractions at all points. These results provided a basis for interpretation of the trends observed with integrative sampling using the POCIS.

The POCIS yields different relative concentrations of estrogens compared to those detected by grab sampling (see Fig. 5 and 6). For example, the concentrations of soluble E1 and E2 detected by POCIS in the outflow were 91 and 13 ng L⁻¹, respectively, while those detected by grab sampling were 21 and 36 ng L⁻¹. These differences arise from the fact that the concentrations of estrogens detected by POCIS are the TWA

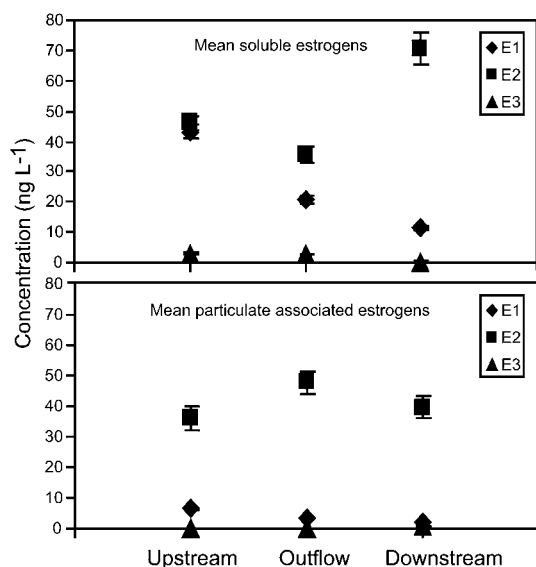


Fig. 6 The mean concentrations (ng L⁻¹) of E1, E2 and E3 in water collected by grab sampling upstream of the outflow, at the outflow and downstream of the outflow over four sampling days. Values are means and errors are SD ($n = 8$).

concentrations of free estrogens over the exposure periods, whereas those detected by grab sampling reflect the concentrations at the specific sampling time, the latter also reflecting the variations of total (free and conjugated) estrogen concentrations. Interestingly, the concentration of free E1 determined using POCIS is over four times that of total E1 determined by grab sampling although a direct comparison with single grab samples is not particularly secure. The sensitivity of detection of E3 is improved by POCIS since it involves the pre-concentration of a target compound retained by the POCIS adsorbent during the long exposure time.

On the first day of deployment, rain was heavy in the early morning but stopped before deployment. Upstream of the outflow receives drainage from a disused canal which possibly receives discharge from another river near a park and farm. Estrogens in the agriculture run-off from this area, if it were raining during the POCIS period, could be responsible for the high concentration of E2.

The concentrations of soluble estrogens detected by POCIS at the outflow were higher than at the other sampling points, with E1 present at the highest concentration. This indicates that estrogens survive the Wootton Bassett treatment process, most likely explained by a further release from conjugated estrogens. Certain estrogens such as estrone-3-sulfate could pass through the treatments and latterly be deconjugated into E1 which is detected at the outflow.²⁹ Some E1 at the outflow could also derive from the oxidation of E2 during treatment which has also been observed previously in an aerobic degradation study²⁶ and elsewhere.^{30,31}

The concentrations of estrogens detected by POCIS were lower downstream of the outflow, with E1 present in highest concentration followed by E3 and E2. For example, the mean concentration of E1 was detected at 91 ng L⁻¹ at the outflow and decreased to 39 ng L⁻¹ downstream of the outflow. The reductions in the concentrations of E1, E2 and E3 downstream of the outflow were determined to be 57%, 38% and 44%, respectively, compared to those at the outflow. These reductions in concentrations of estrogens are possibly due to the dilution by the flow of the river or degradation by bacteria in the river. Binding to the particulates may also cause a reduction in the concentrations of estrogens since particulate associated estrogens were detected in the downstream grab samples. EE2 was undetectable by POCIS and grab sampling from all sampling points. This is not surprising since its concentration is low in effluent, being possibly near its limit-of-detection, as seen in other grab sampling studies.^{1,4,6,27,32} An important consideration is the concentrations of estrogens observed in effluents in terms of their estrogenic potencies.^{19–22} The concentrations of E1 and E2 observed at all sampling points by POCIS and grab samples are within the range likely to cause adverse effects on the fish in the river close to the outflow of the Wootton Bassett sewage treatment works.

Comparison of estrogen determinations by POCIS and literature grab sampling in the discharges

The concentrations of soluble estrogens detected by POCIS and reported values for literature grab samples at the outflow of sewage treatment works are compared in Fig. 7.^{1,3,4,6,28,33,34} The main estrogen detected by POCIS at the sewage treatment works

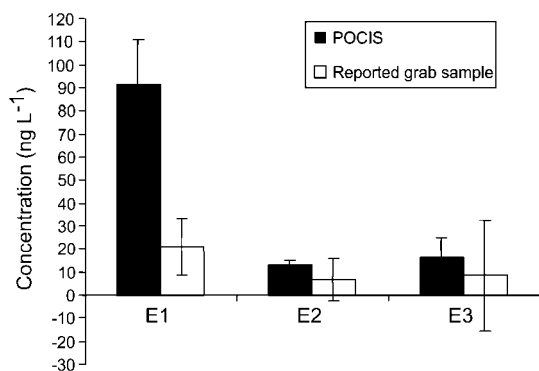


Fig. 7 Comparisons of the concentrations (ng L⁻¹) of soluble estrogens detected by POCIS and reported values for literature grab samples at outflows. Values are means and errors are SD (POCIS: $n = 3$; grab sampling: $n = 12$ from ref. 1,3,4,6,23,26 and 27).

outflow was E1 which is similar to that reported by literature grab sampling although not by grab sampling in this study. This may be due to the grab samples in this study being taken when the concentration of E2 was abnormally high, whilst POCIS provides an average concentration of E2 during the deployment period. As seen in Fig. 7, estrogens adsorbed by the POCIS were detected in higher concentrations than values reported for literature grab samples. For example, the mean concentration of E1 detected by POCIS was 91 ng L⁻¹, while the literature value for grab samples is 21 ng L⁻¹. These differences arise from the fact that the detection of estrogens is improved with POCIS due to the increased signal-to-noise ratio resulting from the pre-concentration of estrogens and the combination of optimised extraction and POCIS. POCIS pre-concentrates estrogens during exposure time with the adsorbent acting as the sink for estrogens in the aquatic environment. This makes estrogens, especially E3, more easily detectable with POCIS as seen the higher concentrations of estrogens present in the POCIS extracts (Fig. 3 and 4).

Conclusions

This paper assesses the usefulness of POCIS for the determination of four target estrogens (E1, E2, E3 and EE2) in the aquatic environment. The performance of POCIS in the field is confirmed by both laboratory experiments with an authentic estrogen mixture and field experiments involving comparisons of POCIS with literature grab samples. The results show unequivocally that POCIS provides a more reliable assessment of the TWA concentrations of estrogens in a riverine system compared to the large variations of mean concentrations of estrogens observed in grab samples from seven previous studies. Therefore, POCIS is an effective method to use for the detection of estrogens in aquatic environments.

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