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Semi-automated hollow-fibre membrane extraction, a novel enrichment technique for the determination of biologically active compounds in water samples

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Abstract

An automated hollow fibre membrane extraction technique was developed for the GC–MS determination of pharmaceutical and endocrine disrupting compounds in water samples. Enrichment was carried out inside a porous polypropylene hollow fibre membrane, which separated the aqueous and organic phases and regulated the transfer of analytes. *n*-Octanol placed inside the hollow fibre was used as the acceptor solution. A water–solvent ratio of about 300:1 was used to concentrate the analytes. After 1 hour's extraction of the water sample under magnetic stirring, 1 μl of the *n*-octanol phase was automatically injected from the hollow fibre into the GC–MS. Development work included examining the influence of different sample matrices, volumes, extraction times and extraction solvents. The detection limits, linearity and standard deviations of the method were determined using drugs such as ibuprofen, phenazone and carbamazepine as well as the endocrine disrupting compounds, technical nonylphenols, bisphenol A, 17α-ethinylestradiol and tonalide by way of example.

Keywords: Hollow fibre membranes; Extraction methods; Water analysis; Endocrine disruptors; Drugs

1. Introduction

Monitoring the widespread presence of biologically active substances in aquatic systems is becoming increasingly important. They include pharmaceutical products and their metabolites as well as endocrine disrupting compounds. These compounds mainly enter the environment via municipal sewage plants. Water solubility and persistence prevent their complete degradation, allowing them to enter surface and ground water [1–3].

The polarity and low concentration levels of these compounds in the environment make them a serious challenge for analytical methods. Another major problem is their structural diversity. Therefore, universal methods are required which allow as many analytes as possible to be determined simultaneously.

The most common methods for determining biologically active compounds are liquid–liquid and solid-phase extraction (SPE) [4–11]. These methods are relatively robust and enable large sample amounts to be handled, resulting in detection limits in the lower ng/l range. However, they are rather time-consuming and often involve several clean-up and preconcentration steps. Solvent-free solid-phase microextraction (SPME) has also been used for

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endocrine disrupting compounds and drugs [12–14]. Although faster and simpler, SPME is not as sensitive as SPE. SPME is therefore more suitable for screening analysis at higher concentration ranges.

One promising way of determining organic compounds in water samples is to enrich them with hollow fibre membranes. Rasmussen et al. reported the application of a polypropylene hollow fibre for the liquid-phase microextraction (LPME) of several drugs and pharmaceutical compounds in urine and human plasma [15–18]. The authors obtained detection limits down to 2 ng/ml. For analyte enrichment they used a hollow fibre filled with a suitable solvent, usually *n*-octanol, and exposed it to the sample. After extracting the analytes under vibration, the acceptor solution was collected in inserts by using a small head pressure and analysed using capillary gas chromatography (GC), capillary electrophoresis (CE) or high-performance liquid chromatography (HPLC).

The high preconcentration factors obviously make hollow fibre extraction especially attractive for water analysis. It has already been used to extract nitrophenols with subsequent capillary liquid chromatography [19]. Enrichment is based on the pH gradient between the sample and the aqueous acceptor phase (HCl and NaOH, respectively) within the hollow fibre. Efficient analyte preconcentration is achieved by immobilizing *n*-octanol inside the pores of the hollow fibre, resulting in enrichment factors of up to 380.

The objective of our studies was to develop a hollow fibre extraction device which allows the enrichment of biologically active compounds from water samples and their subsequent automatic GC–MS determination. We also investigated how various process parameters such as enrichment time and sample matrix affect extraction efficiency.

2. Experimental

2.1. Chemicals

Tonalide, ibuprofen, phenazone and carbamazepine were supplied by Promochem (Wesel, Germany). Bisphenol A, technical (t) nonylphenols and 17α-ethinylestradiol were purchased from Sigma. Estradiol diacetate used as internal standard was obtained from ICN Biomedicals (Aurora, OH, USA) and 4-*n*-nonylphenol was supplied by Dr. Ehrenstorfer (Augsburg, Germany). All solvents for standard solutions as well as *n*-octanol (99%), sodium chloride, sodium hydroxide and hydrochloric acid were obtained from Merck (Darmstadt, Germany) and were used without further purification.

2.2. Hollow fibre extraction

The principle of hollow fibre extraction is shown in Fig. 1. The Q3/2 Accurel microporous hollow fibre membrane consisted of polypropylene (Membrana, Wuppertal, Germany). The fibre was cut into pieces 6 cm long with an internal diameter of 600 μm and a wall thickness of 200 μm. The pore size was 0.2 μm. Before being used, the fibres were shaken in acetone for 2 h to remove polypropylene oligomers and other potential contaminants and air-dried. Afterwards, the hollow fibre was attached with one end to a funnel-shaped injection guide consisting

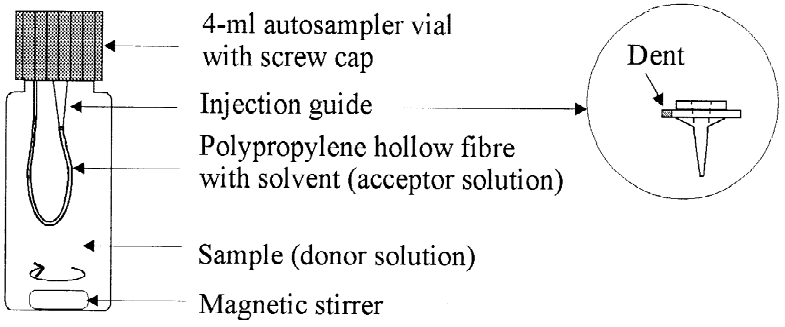

Fig. 1. Schematic set up of the hollow-fibre extraction.

of stainless steel. Additionally, in the injection guide a small dent is placed that holds the other end of the fibre unsealed. Thus, during injection the extraction solvent can move within the fibre and will not be partially pushed out by the intruding syringe needle. Furthermore, the advantage of such a hollow-fibre ''loop'' compared with a straight fibre is, that there are no problems with air bubbles when filling the autosampler syringe.

Before extraction the hollow fibre was filled with 40 μl of *n*-octanol using a microliter syringe. Subsequently, the solvent-filled fibre was immersed into a 4-ml autosampler vial with screw cap (Agilent Technologies, Palo Alto, CA, USA). The vial contained a 5-ml water sample, so that the fibre is completely immersed in the sample. The enrichment was performed under magnetic stirring (1000 rev./min).

2.3. Instrumental parameters

After extraction the sample vial was placed into a GC autosampler (7683 series, Hewlett-Packard, San José, CA, USA). One μl of the *n*-octanol phase was automatically taken from the hollow fibre and injected into the GC–MS system. GC was performed with a 6890 A series gas chromatograph equipped with a mass-selective detector 5973 (Agilent Technologies). Injections were carried out in pulsed splitless mode at a temperature of 280 °C. The injector was coupled to a retention gap (2.5 m×0.32 mm I.D.) and a 30 m×0.25 mm I.D., 0.25 μm HP-5MS capillary column (J&W). Helium was used as carrier gas at a column flow of 1 ml/min. The GC oven temperature programme started at 120 °C. This temperature was maintained for 3 min and then increased at a rate of 10 °C/min to 300 °C.

The MS parameters were as follows: interface temperature 280 °C, source temperature 230 °C, electron impact (EI) ionization mode, 70 eV. Analysis was performed in the selected ion monitoring (SIM) mode using the characteristic ions given in Table 1.

3. Results and discussion

3.1. General considerations

Some of the compounds most frequently detected in aquatic systems were chosen as target analytes for these studies. They belong to different classes of pharmaceutical products and xenoestrogens, and are listed together with their range of application in Table 1.

Introducing an enrichment factor (EF) is useful in order to compare the experiments. It can be defined as a quotient between the final concentration of the analyte in the extraction solvent within the hollow fibre c_e and the initial concentration of the analyte within the aqueous sample c_s:

$$EF = c_e / c_s \quad (1)$$

The final concentration c_e was determined by calibration with standard solutions in *n*-octanol containing the corresponding analytes at concentrations up to 100 times higher than in the sample. Furthermore, extraction recoveries (*R*) can be calcu-

Table 1
Investigated biologically active compounds with performance parameters of the hollow fibre extraction (sample: 5 ml distilled water, pH 7 for t-nonylphenols, bisphenol A, tonalide, 17α-ethinylestradiol; 5 ml saturated NaCl solution pH 7 for ibuprofen, phenazone, carbamazepine)

Analyte	Range of application/effective as	Water solubility (μg/l)	log K_{ow}	SIM target ions	LOD (μg/l)	Correlation coefficient (c=0.1–100 μg/l)	RSD (n=5) (%)	Recovery, R (%)
t-Nonylphenols	Industrial detergent/xenoestrogen	7	5.76	107, 135, 220	0.1	0.9989	11.0	20.1
Bisphenol A	Plasticizer/xenoestrogen	120	3.32	213, 228	0.3	0.9986	21.1	23.6
Tonalide	Musk/xenoestrogen	1.25	5.7	243, 258	0.02	0.9985	13.1	35.2
17α-Ethinylestradiol	Synthetic estrogen	11.3	3.67	160, 213, 296	0.02	0.9952	5.3	33.8
Ibuprofen	Analgetic, antirheumatic	21	3.97	161, 163	0.02	0.9996	11.1	48.7
Phenazone	Analgetic	51,900	0.38	173, 188	0.04	0.9995	9.3	1.8
Carbamazepine	Analgetic, antiepileptic	17.7	2.45	193, 236	0.02	0.9993	12.4	15.5

LOD, limit of detection.

lated as a percentage of the total analyte amount originally contained in the sample n_s and the amount enriched in the extract n_e:

$$R\,(\%) = (n_e/n_s) = (V_e/V_s) \cdot \mathrm{EF} \quad (2)$$

where V_e is the volume of the extraction solvent and V_s the volume of the sample. Exactly observing the extraction time guarantees a reproducible loss of extraction solvent by diffusion through the membrane into water. An extraction volume of 13 μl in mean (±1 μl) was defined. This volume of solvent remaining was determined by withdrawing the extract in a microlitre syringe. It was used to calculate the recoveries *R* in Table 1.

3.2. Selection of a suitable solvent (acceptor solution)

When selecting a suitable solvent, two factors need to be taken into account. First of all, for enrichment to be effective, the analytes should be well soluble in the solvent used. Another factor is the appropriate viscosity of the solvent applied to avoid diffusion through the porous hollow fibre membrane into the aqueous phase. Therefore *n*-octanol [15] and *n*-octanol mixed with butyl acetate or dihexyl ether [16] were used as acceptor solution. The application of octanol for the extraction of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid [20] and chloroform for the extraction of cocaine and its metabolites [21] has also been described, although in these cases enrichment times of only 10 and 3 min, respectively, were used because of the leakage of the solvent. The sensitivity of this method was reported to be in the μg/l range.

To determine the bioactive analytes in this study, several organic solvents such as *n*-octanol, butylacetate, dihexyl ether, *n*-alkanes (C_8–C_{11}), xylene, mesithylene and *tert*.-butylbenzene were tested for usage with the hollow fibre technique. These experiments demonstrated that *n*-octanol yielded a reproducible enrichment associated with a reasonable amount diffused through the membrane into the aqueous phase. Dihexyl ether proved unsuitable owing to interference with some important target ions in the SIM mode. All the other solvents displayed greater leakage, and so only shorter extraction times could be applied. Due to the time dependence of the extraction yields in this case, lower enrichment factors have to be expected. For example, the calculation of enrichment factors for extraction with *n*-octan and an enrichment time of 10 min resulted in EF values below 10 for the relatively polar compounds bisphenol A and 17α-ethinylestradiol. In order to attain optimal detection limits on the basis of these results, *n*-octanol was chosen for all additional investigations.

Indeed, the usage of *n*-octanol with a boiling point of 178.5 °C has consequences for the subsequent GC–MS analysis. To accomplish tailing-free peaks, an initial column temperature of at least 120 °C is necessary. Hence only compounds with boiling points above 250 °C can be determined reproducibly in this way. Further investigations regarding the modification of the hollow fibres used are necessary for the utilization of GC common solvents.

3.3. Influence of the enrichment time

To determine the influence of the enrichment time, aqueous standard solutions were extracted within different times of between 5 and 90 min. Fig. 2 shows the extraction time profiles of all the compounds investigated at a concentration of 10 μg/l in distilled water.

Despite the analytes belonging to different substance classes, the course of the curves for all analytes is comparable. Optimum extraction yields are obtained after an enrichment time of 60 min. Therefore, this time was selected for the subsequent enrichment experiments. The uniform behaviour of all the analytes allows their simultaneous determination within one analysis run.

3.4. Influence of the donor solution

The enrichment of organic compounds from water samples is significantly influenced by the properties of the sample solution. Depending on the substance class of the analyte, recovery can for example be increased by optimally adjusting the pH or adding sodium chloride.

In order to optimize the extraction yield for the individual compounds, the influence of various sample parameters was investigated. Table 2 shows the

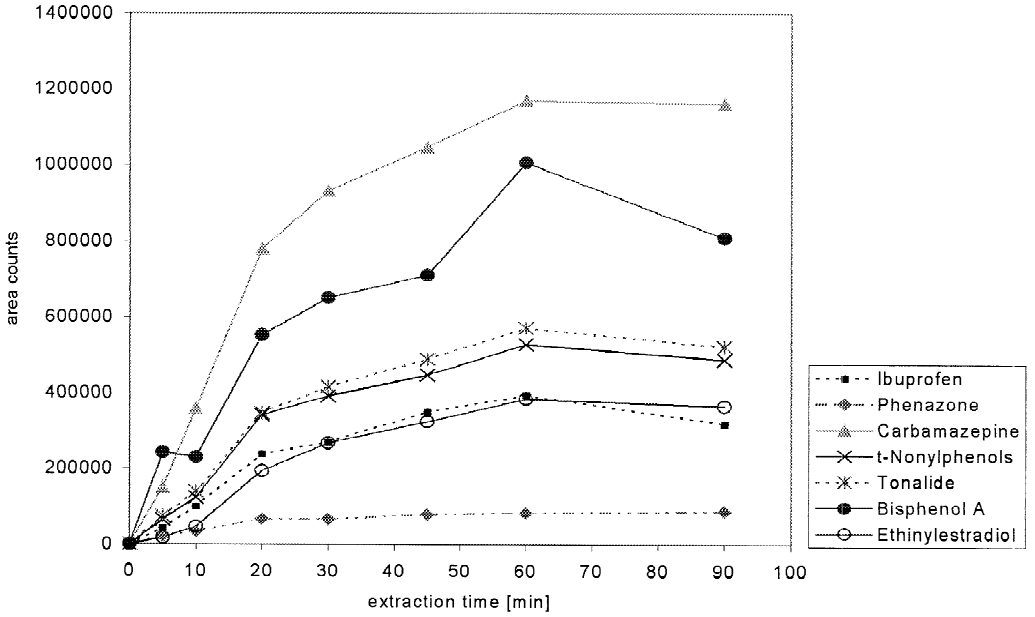

Fig. 2. Enrichment time profiles for the hollow-fibre extraction of the biologically active compounds at a concentration of 10 μg/l in distilled water.

correlation between sample matrix and enrichment factor.

The enrichment of the analytes within the fibre is significantly influenced by their hydrophobicity and their solubility in the extraction solvent. With *n*-octanol as the extraction solvent, the enrichment of the analytes according to their octanol–water partition coefficient (K_{ow}) was to be expected. However, as can be seen from Table 2, the highest enrichment factors were achieved for ibuprofen, bisphenol A and 17α-ethinylestradiol with log K_{ow} values between 3.32 and 3.97. For example, ibuprofen in distilled water with pH 7 provided an enrichment factor of 187. t-Nonylphenols and tonalide possess higher log K_{ow} values of 5.7. Yet in both cases only enrichment factors below 100 were yielded. This indicates that not only the distribution equilibrium of the analytes between water and octanol but also the enrichment of the compounds within the microporous hollow fibre membrane has to be taken into account.

In order to assess adsorptive effects of the polypropylene material, empty hollow fibre membranes were shaken in aqueous standard solutions containing known amounts of all analytes. After drying,

Table 2
Influence of various sample matrices on the enrichment factor of the bioactive compounds ($c = 10$ μg/l)

Analyte	Enrichment factor			
	Distilled water pH 7	Distilled water pH 2	Distilled water pH 10	Saturated sodium chloride solution
t-Nonylphenols	77	82	114	29
Tonalide	91	111	118	35
Bisphenol A	135	151	9	189
17α-Ethinylestradiol	130	144	101	125
Ibuprofen	187	254	0.3	415
Phenazone	7	2	1.8	13
Carbamazepine	60	39	30	166

these membranes were extracted with acetone. Analysing the obtained extract by GC–MS showed that nearly 75% of the t-nonylphenols and 90% of the tonalide was to be found in the extract whereas all the other compounds could not be detected. This means that in the case of analytes with a very high K_{ow} value and extremely low water solubility, adsorption within the hydrophobic polypropylene membrane plays an important role. More detailed investigations on these processes were not carried out because the main issue of this study was the liquid–liquid partition of the target analytes.

Lowering the pH to 2 only for ibuprofen led to a significant improvement in the extraction yield because of its carboxyl group. The influence on all the other compounds including bisphenol A and 17α-ethinylestradiol, both containing two OH groups, was rather small. Using a pH of 10 did not significantly improve enrichment efficiency. However, the enrichment factors for bisphenol A and ibuprofen dropped markedly due to their OH and carboxylic groups, respectively.

The addition of sodium chloride increased extraction efficiency, especially for ibuprofen and carbamazepine. In both cases the EFs were more than doubled to values of 415 and 166, respectively. Interestingly, the extraction yields of t-nonylphenols and tonalide in the presence of salt markedly declined. The reduction of the water solubility due to the sodium chloride content probably led to the greater adsorption of these two compounds in the hollow fibre membrane.

Phenazone with the lowest log K_{ow} value (0.38) and the largest water solubility (51.9 g/l) of all the compounds investigated delivered an enrichment factor of just 13 for sodium chloride saturated samples. Nevertheless, because of its favourable signal-to-noise ratio for the target ions in GC–MS analysis, satisfactory detection limits were achieved (Table 1).

3.5. Evaluation of the hollow fibre technique

The performance parameters of the hollow fibre technique such as detection limits, correlation coefficients, relative standard deviations and recoveries are listed in Table 1. The specified values for the endocrine disrupting compounds, t-nonylphenols, bisphenol A, tonalide and 17α-ethinylestradiol were determined in distilled water with pH 7, while those of ibuprofen, phenazone and carbamazepine were achieved using sodium chloride-saturated sample solutions. In all cases a sample volume of 5 ml was used.

In particular the detection limits within the ng/l range demonstrate the capability of the method for determining organic compounds in water samples. If necessary the sensitivity could be further enhanced using larger sample volumes. However, longer extraction times must be applied to reach the distribution equilibrium. For example, by using sample volumes of 20 ml and extraction times of 120 min, the detection limits for the pharmaceutical compounds were lowered to values between 15 ng/l for phenazone and 7 ng/l for carbamazepine. The relatively high detection limits for t-nonylphenols and bisphenol A are due to blind values. In the case of t-nonylphenols they come from the hollow fibre. This was proved by heating up some fibres in the tube of a thermodesorber. In the GC–MS chromatogram obtained, t-nonylphenols were evidenced.

The standard deviations achieved were in the range of 5.3% for 17α-ethinylestradiol and 21.1% for bisphenol A. Regarding the relatively polar nature of the compounds investigated, these values are acceptable. After all they are analyzed without derivatization, which requires an inert GC–MS system.

Hollow-fibre extraction had already been tested for the determination of bioactive compounds in real samples. Fig. 3 shows the GC–MS chromatogram of the influent of the tern-sewage plant in Grosslehna near Leipzig. 4-*n*-Nonylphenol, [$^2H_{14}$]bisphenol A and estradiol diacetate were used as internal standards. The endocrine disrupting compounds, t-nonylphenols, bisphenol A, tonalide and in some cases 17α-ethinylestradiol were thus detected and determined in concentrations between 0.03 and about 4 μg/l. Hence, hollow-fibre extraction is also a useful tool for analysing very contaminated samples. Using the established methods of liquid–liquid or solid-phase extraction, the analysis of such polluted samples normally would take several clean-up steps.

One essential advantage of the hollow fibre technique is the relatively high enrichment factors, which can even be achieved with small sample and solvent volumes. This is attributable to the favourable

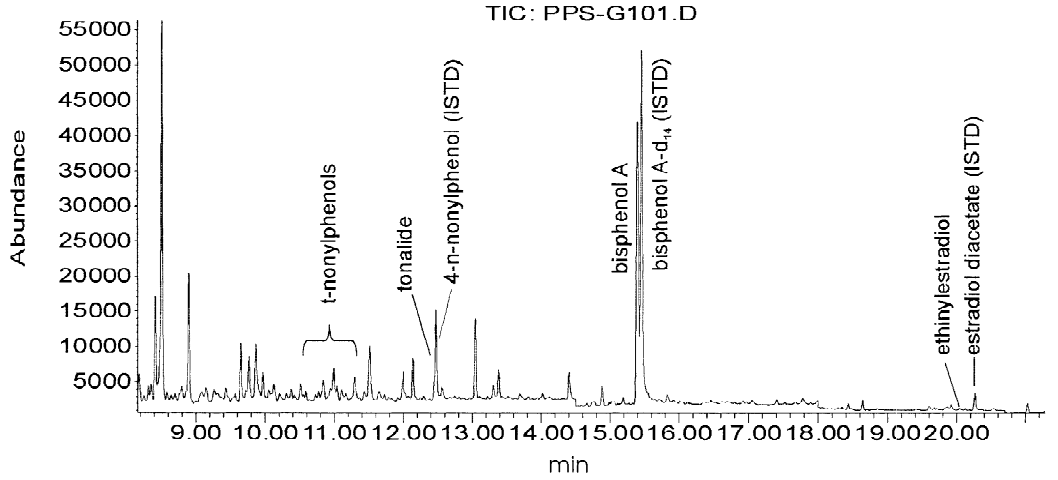

Fig. 3. GC–MS chromatogram of the hollow fibre extract for an influent of the tern-sewage plant in Grosslehna. The peaks correspond to 5 μg/l for the internal standards 4-*n*-nonylphenol, [$^{2}H_{14}$]bisphenol A and estradiol diacetate. Analyte concentrations determined were 3.70 μg/l t-nonylphenols, 0.77 μg/l tonalide, 2.05 μg/l bisphenol A and 0.035 μg/l ethinylestradiol.

geometric dimensions of the hollow fibre. Assuming a hollow fibre with a length of 6 cm and an internal diameter of 600 μm, the resulting inner volume is ~17 μl. Hence applying just a 5-ml sample volume would result in a water/solvent ratio of about 300:1, while larger sample volumes increase this ratio. Due to the high enrichment factor the injection of 1 μl of the extract is usually sufficient to secure the required sensitivity. Large volume injections [22,23] often entailing the pollution of the liner and capillary column as well as additional optimization efforts are not required.

Furthermore, the automation facility should be underlined. After extraction, the sample vial can be placed directly into a GC autosampler. Using appropriate injection guides, the technique can be used with all commercial autosampler systems.

The polypropylene hollow fibres used are low-cost articles, thus allowing a new piece of hollow fibre to be used for each individual analysis to avoid carry-over effects of the analytes.

4. Conclusion

This article introduces a new analytical method for biologically active compounds in water samples based on enrichment with polypropylene hollow fibres. Advantages of the new method over established enrichment techniques such as liquid–liquid and solid-phase extraction are the small amount of solvent required and the low sample consumption. Although hollow fibre extraction cannot match either of these methods in terms of detection limit, limits in the ng/l range can be achieved if the right analysis conditions are chosen. Furthermore, no preconcentration or clean-up steps are necessary.

Other benefits of the hollow fibre technique are its low cost, the possibility of automation, and its simple handling, which requires only a negligible amount of material.

The investigations reveal the potential of hollow fibre extraction as a simple but nevertheless effective tool in water analysis. Its advantages make automatic hollow fibre enrichment especially suitable for screening analysis. Moreover, given appropriate sample volumes the technique is also a useful tool for trace analyses.

Further investigations are required for the use of solvents other than octanol. One way of keeping more suitable solvents in the fibre is probably to immobilize stationary phases within the pores of the hollow fibre. This could also improve selectivity for several classes of substances.

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