Application of solid-phase microextraction and gas chromatography with electron-capture and mass spectrometric detection for the determination of hexachlorocyclohexanes in soil solutions

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Abstract

Solid-phase microextraction (SPME) is a method for the extraction of organic compounds from aqueous samples. The analytes are extracted into a stationary phase placed on a fused-silica fibre and are thermally desorbed in the injector of a gas chromatograph. The connection of GC with electron-capture (ECD) and mass spectrometric (MS) detection with the SPME method makes it possible to determine low concentrations of organochlorine compounds in aqueous solutions. With hexachlorocyclohexanes (HCHs) detection limits between 5 ng/l (for α- and γ-HCH with the combination of SPME and GC–ECD) and 80 ng/l (for β-HCH with the combination of SPME and GC–MS) were calculated. The SPME–GC method was used to investigate the mobility of HCHs in wetland soils near Bitterfeld. The results of this study show the high mobility of β-HCH despite the low water solubility and the long persistence of β-HCH in soils. The proportion of β-HCH in the total HCH concentration is higher in soil solutions (80–90%) than in soils.

1. Introduction

Hexachlorocyclohexanes (HCHs), mainly β-HCH, are widespread in the riverine area of the river Mulde near Bitterfeld, so it is necessary to investigate the mobility of HCHs in the wetland soils to assess the danger for ground water, plants, animals and humans. The large number of samples and the urgency of this problem require a rapid and inexpensive method for the extraction of HCHs from aqueous samples, in particular from soil solutions. Such a method was found using solid-phase microextraction (SPME). First described by Pawliszyn and co-workers [1–6], SPME is a solvent-free, rapid and inexpensive method for the extraction of organic compounds from aqueous samples. The SPME technique is based on chemically modified fused-silica fibres fixed inside a syringe. The fibre with the immobilized organic film is exposed to aqueous samples and organic compounds are extracted from the water into the silicon phase by

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diffusion processes. After this procedure the microextractor is directly inserted into the split–splitless or on-column injector of a gas chromatograph.

First applications of this method to the determination of contaminants in water have shown that SPME is a practical alternative to other commonly used extraction techniques (headspace, purge-and-trap, liquid–liquid extraction).

The main objective of this work was the optimization of the SPME procedure for the measurement of HCHs and the application of the optimized procedure coupled with GC–ECD and GC–MS for the determination of these compounds in environmental samples, especially for the determination of the concentration distributions of α-, β- and γ-HCH in soil solutions from different sites.

2. Theoretical

The amount of an analyte absorbed by the fibre at equilibrium (infinite volume assumed) is linearly dependent on the concentration of the analyte in the aqueous phase according to

$$n_s = KV_sC_{aq}$$

were n_s is the number of moles of the analyte absorbed by the stationary phase, K is the distribution constant of a compound between the stationary and the aqueous phase, V_s is the volume of the stationary phase and C_{aq} is the initial concentration of the analyte in the aqueous phase.

Louch et al. [4] showed that in a finite volume V_{aq} the amount of the analyte in the stationary phase is given by

$$n_s = \frac{KV_sV_{aq}C_{aq}}{KV_s + V_{aq}}$$

This means that a linear relationship between the concentration of analytes in aqueous samples and the response of a GC detector is to be expected if the absorption conditions in the sample vial and the desorption conditions in the injection port of the GC are reproducible. Louch et al. [4] also developed a mathematical model for the dynamics of the absorption process. Assuming that the dynamics of extraction is a diffusion-determined process and solving Fick's second law of diffusion, they calculated time profiles for perfectly stirred samples of infinite volume.

The diffusion of analytes into the fibre coating from an unstirred solution of finite volume was also calculated. It was shown that the time necessary to reach equilibrium for a perfectly agitated sample is relatively short. Without mixing, the equilibrium time increases considerably.

3. Experimental

3.1. Site description and extraction of soil solution

The sites Bobbau, Keller and Spittel are located in the floodplain of the stream Spittelgraben. This stream flows into the river Mulde and was used for several decades as a waste water channel of the chemical industry. During flood events the Spittelgraben covers the wetlands with its highly contaminated water. The sites Spittel and Keller are situated only a few metres from the river bank. They have often been flooded by the Spittelgraben and the sandy soils of this riverine area are very polluted with heavy metals and organic contaminants [7,8]. The site Bobbau is further away from the edges of the Spittelgraben and the sandy soil shows low contamination. The loamy soil in Greppin is located in the floodplain of the river Mulde and is highly polluted with heavy metals and organic contaminants [7,8].

Four small lysimeters (five lysimeters for the Spittel site) with a surface soil area of 400 cm^2 and a depth of 25 cm were used for the extraction of soil solution. The lysimeters were obtained as undisturbed monoliths. Some soil properties are presented in Table 1. The experiments, including the simulation of several rainfall events, were carried out under greenhouse conditions. Irrigation of the lysimeters was ef-

Table 1
Soil properties

Site	pH	Clay + fine silt (%)	Carbon (%)	CEC (cmol$_c$ kg^{-1})^a
Bobbau	6.1	6	2.7	10.1
Keller	4.4	13	24	32.6
Spittel	3.3	8	7.6	33.1
Greppin	6.4	22	10.5	25.3

^{a} Cation exchange capacity, measured in cmol$_{charge}$ per kg of soil.

fected with distilled water. After completed percolation through the soil monoliths the percolates were collected in glass bottles, filtered through 0.45-μm filters and stored at $-18°C$ until analysed. Altogether 24 percolates per site (34 for the Spittel site) were analysed for HCHs.

3.2. SPME procedure and chromatographic conditions

The studies were carried out with an SPME device from Supelco (100-μm polydimethylsiloxane solid-phase microextraction fibre assembly).

The SPME procedure is very simple. First the fibre is withdrawn into the needle of the syringe and the syringe is used to penetrate the septum of the sample vial. Then the fibre is inserted in the aqueous phase. When equilibrium is reached, the fibre is again withdrawn into the needle and the syringe needle is removed from the vial. The last step is the thermal desorption of the analytes in the injector of the gas chromatograph.

The gas chromatograph used was a Chrompack CP 9000 device with ECD. For the experiments a 25 m × 0.32 mm I.D. Ultra 1 capillary column (Hewlett-Packard) with a 0.52-μm film thickness was used. The carrier gas and the make-up gas were nitrogen. A split–splitless injector was used in the splitless mode and maintained at 200°C. after the optimization experiments a 2.0-min desorption time was chosen. The column temperature programme was as follows: initial temperature 60°C (held for 3 min), increased at 10°C/min to 250°C and held at the final temperature for 20 min. The detector temperature was 250°C.

For the identification of the HCHs some GC–MS measurements were performed. A combination of an HP 5890II gas chromatograph and a mass spectrometer (Hewlett-Packard) was used. A capillary column of 0.25 mm I.D. and a 0.25-μm film thickness were employed. The carrier gas was helium 6.0 (99.9999% pure). A split–splitless injector in the splitless mode was used and the temperature programme was the same as described for the GC–ECD measurements. The transfer line and mass spectrometer were held at 280°C. The mass spectrometer worked in the single-ion monitoring (SIM) mode.

For calibration and optimization of the SPME, doubly distilled water was spiked with a mixture of α-, β-, γ- and δ-HCH dissolved in methanol.

4. Results and discussion

4.1. Optimization of SPME

The theoretical considerations show the necessity to optimize carefully the mixing process, the exposure time of the fibre in an aqueous sample and the desorption time (exposure time of the fibre in the GC injection port). Optimization also includes the determination of the linear dynamic range of the SPME procedure in connection with the chromatographic detector used.

The first step is to examine the time required for the HCHs to reach equilibrium depending on the turning speed of the stirrer. Fig. 1 shows the peak area of the ECD signal versus the exposure time for lindane. Without stirring (A) the equilibrium is reached after a time $\gg$ 60 min. Using a magnetic stirrer at 250 turns/min (B) the equilibrium time is reached within 40–60 min. An increase in the stirring speed up to 1000 turns/min (C) ensures that the aqueous sample is
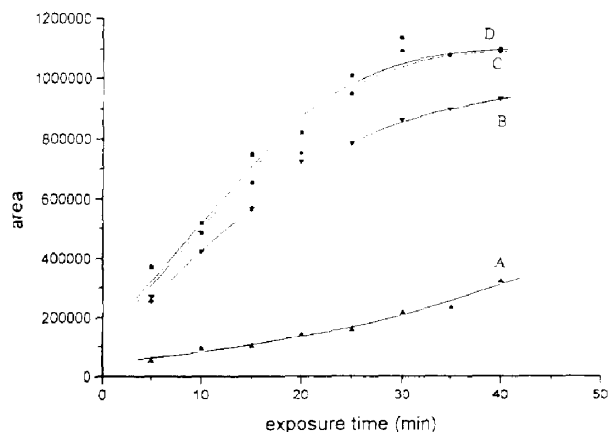
Fig. 1. Exposure time profiles for γ-HCH as a function of the stirring conditions. Concentration of γ-HCH, 0.6 ng/ml water. (A) Without stirring; (B) stirring speed 250 turns/min; (C) stirring speed 1000 turns/min, central position; (D) stirring speed 1000 turns/min, non-central position.
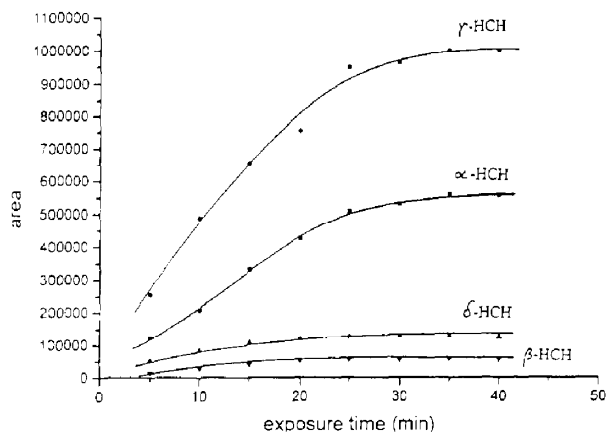

Fig. 2. Exposure time profiles for α-, β-, γ- and δ-HCH under optimum stirring conditions. Concentration of α-, β- and δ-HCH, 0.3 ng/ml water; concentration of γ-HCH, 0.6 ng/ml water.

nearly perfectly agitated and equilibrium is reached within 20–30 min. In these cases the fibre was placed in the middle of the 4-ml vial used and therefore also in the middle of the rotation paraboloid formed by magnetic stirring. In a further case (D) the fibre was exposed at a distance 6 mm from the centre. That means the fibre was placed in a region of violent agitation of the sample. The equilibrium time was also 20–30 min and the results in experiments C and D were nearly identical. Because of the better reproducibility, the central position (C) was chosen.

Fig. 2 shows the equilibrium time profiles for α-, β-, γ- and δ-HCH under optimum conditions (stirring speed 1000 turns/min). Because the exposure time profiles under constant conditions (speed of the stirrer) are well reproducible, it is possible to choose exposure times lower than the equilibrium time. The only disadvantage is the decrease in sensitivity. For the measurement of a large number of soil solutions with relatively high HCH concentrations an exposure time of 10 min and a optimum stirring speed of 1000 turns/min were chosen.

The next step is to ensure that the exposure time of the fibre within the GC injector is long enough to desorb the compounds completely from the silicon phase. Fig. 3 shows desorption

time profiles of the HCHs for an injector temperature of 200°C. The desorption is nearly completely after 0.5 min and a desorption time of 2 min was chosen for the soil solution measurements.

Depending on the partition coefficient of an analyte, the sample can be significantly depleted in a single extraction. Fig. 4 shows the decrease in the concentration of analytes in a 4-ml vial as a function of the number of extractions. Because of this decrease in concentration, for each 4-ml sample only one extraction was performed.
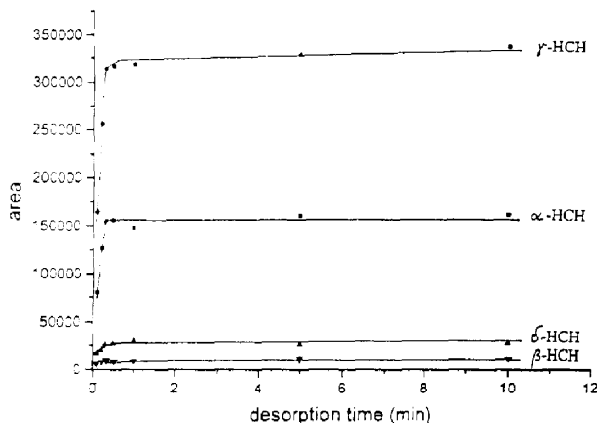

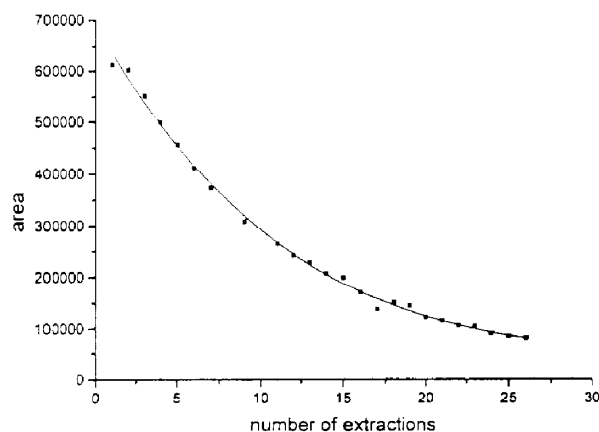Fig. 3. Desorption time profiles for α-, β-, γ- and δ-HCH. Concentration of α-, β- and δ-HCH, 0.3 ng/ml water; concentration of γ-HCH, 0.6 ng/ml water.

Fig. 4. Dependence of the ECD signal for γ-HCH on the number of extractions. Initial γ-HCH concentration, 0.6 ng/ml water.

Fig. 6. Linearity of the SPME–MS method for α-, β-, γ- and δ-HCH.

After establishing the exposure time and the desorption time, the linear dynamic range of the detectors coupled with the SPME procedure was investigated. In Fig. 5 the ECD signal versus the concentration of the HCHs in doubly distilled water is shown. For α-, γ- and δ-HCH the linear dynamic range exceeds more than three orders of magnitude, and for β-HCH the linear range extends to more than 300.

MS was applied in the SIM mode with the characteristic ions at m/z 109, 183, and 219. The linearity of the SPME–GC–MS procedure (Fig. 6) is between two and three orders of magnitude.
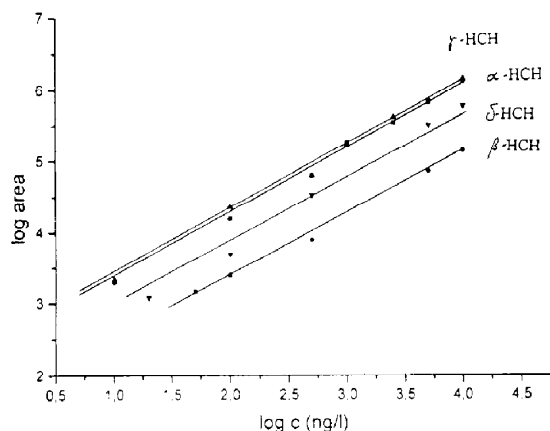
A peak with a signal-to-noise ratio of 3 was defined as the detection limit. Under these conditions the detection limits in Table 2 were calculated.

4.2. Analysis of soil solutions

After optimization, the SPME–GC–ECD procedure was used for the determination of α-, β- and γ-HCH in soil solution. GC–MS combination was used when the identification of the HCHs was difficult. Fig. 7 shows the ECD results for a Spittel site sample with a dominant β-HCH peak (4.28 μg/l), an α-HCH concentration of 0.12 μg/l and a γ-HCH concentration of 0.03 μg/l. The peaks following that of γ-HCH are caused by other chlorinated compounds which are superimposed on the δ-HCH peak, but the chromatograms obtained using MS in the SIM mode showed that the δ-HCH concentrations in the samples are neglectible. Fig. 8 shows

Fig. 5. Linearity of the SPME–ECD method for α-, β-, γ- and δ-HCH.

Table 2
Detection limits with the SPME–GC combinations

Compound	SPME–GC–ECD (ng/l)	SPME–GC–MS (ng/l)
α-HCH	5	12
β-HCH	32	80
γ-HCH	5	13
δ-HCH	12	40

Fig. 7. Chromatogram of a soil solution obtained using ECD. Numbers at peaks are retention times in min.

that none of the three peaks with retention times of 18.62, 18.77 and 18.89 min corresponds to δ-HCH, because neither the retention time (18.84 min) nor the ion intensity ratio relate to this compound.

The concentration distributions of α-, β- and γ-HCH in Bobbau, Keller, Spittel and Greppin samples (see Fig. 9) were determined on 106 samples of the soil solutions. To ensure that the external calibration (addition of HCHs to doubly distilled water) is transferable to the soil solutions, some of the contaminated samples were

Fig. 8. Chromatogram of a soil solution obtained using MS. Time in min.
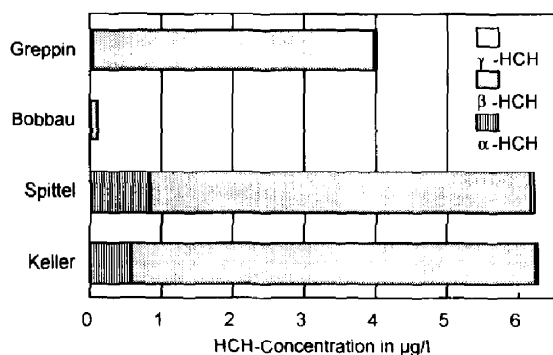
Fig. 9. Concentration of HCHs in soil solutions from different sites.

spiked with known α-, β- and γ-HCH concentrations.

The increase in the peak areas was comparable to the peak areas obtained after adding the HCHs to doubly distilled water. This experiment demonstrated that we could assume that the soil solution matrix in this case would not affect the calibration and that we could transfer the calibration graphs obtained for clean water.

Fig. 9 demonstrates the dependence of the HCH concentration on the distance of the site from the river bank and consequently on the frequency of flood events in the riverine area of the Spittelgraben with high HCH concentrations in the Keller and Spittel and low concentrations in the Bobbau sites. Nevertheless, the β-HCH concentration in the probably low-contaminated soil from the Bobbau site exceeds the drinking water limit for German drinking water supplies (0.1 μg/l) for chlorinated pesticides. At the Greppin site, which is located in the floodplain of the river Mulde, the HCH concentrations in the percolates are less than those at the Keller and Spittel sites. The dominance of β-HCH within the HCHs and its high concentration and also the low concentrations of γ-HCH found in the percolates cannot be explained by the solubility of the HCHs. γ-HCH is the most soluble isomer and β-HCH is only slightly soluble in water [9]. However, the degradation of α- and γ-HCH is faster than the degradation of β-HCH [10,11]. Probably relative enrichment of β-HCH took place in the soils in these riverine areas.

Additionally, an influence of the dissolved organic matter in the soil solution on the β-HCH concentration is assumed. The dissolved organic matter is able to increase the solubility of hydrophobic compounds and therefore its concentration in the soil solution [12,13]. Further, α- and β-HCH are by-products of lindane production, so substantial soil contamination with γ-HCH cannot be expected. The differences in the β-HCH concentration in the percolates between the Spittel and Keller sites on the one hand and the Greppin site on the other may also result from the influence of soil properties on the mobility of β-HCH. At the Greppin site the soil texture is heavier and the pH higher than at the Spittel and Keller sites, but the Spittel and Keller soils contain more organic matter than the Greppin soil. The high mobility and long persistence of β-HCH become clear on comparing the proportions of β-HCH in the total HCH concentration between the soil and the soil solution. Borsdorf et al. [14] determined for some soils in the floodplain of the river Mulde a proportion of β-HCH in the total HCH concentration varying between 52% and 72%. This proportion as determined for the soil solutions of the four sites discussed in this paper varies between 80% and 99%. This is an indication of the highest mobility and the longest persistence of β-HCH among the other HCHs, although β-HCH shows the lowest water solubility. Additional experiments are necessary to elucidate the influence of the soil and the components of the soil solution on the mobility and persistence of β-HCH.

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