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Determination of Compound-Specific Carbon Isotope Ratios of Chlorinated Methanes, Ethanes, and Ethenes in Aqueous Samples

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Compound-specific carbon isotope ratio analysis is a promising tool to assess the origin and fate of organic contaminants in groundwater. The aim of this study was to develop and evaluate a reliable, fast method to determine carbon isotope ratios of chlorinated methanes, ethanes, and ethenes in aqueous samples. Direct solid-phase microextraction (dSPME) and headspace solid-phase microextraction (hSPME) were selected as extraction method and compared to headspace equilibration. For dSPME and hSPME, deviations between carbon isotope ratios in the aqueous phase and on the SPME fiber were $\leq$ 0.40‰. For headspace equilibration, molecules in the gas phase were enriched in ^{13}C compared to molecules in the aqueous phase by up to 1.46‰, in particular for chlorinated methanes. The absence of significant carbon isotope fractionation during dSPME and hSPME could be explained by the fact that both the aqueous phase and the SPME fiber coating discriminate against molecules with ^{13}C to a similar degree, and thus no net carbon isotope fractionation occurs. If aqueous phase/gas-phase carbon isotope fractionation during headspace equilibration is taken into account, all methods, dSPME, hSPME, and headspace equilibration, provide accurate $\delta^{13}C$ values with a similar precision. Direct SPME was the most sensitive method with detection limits as low as 130 ppb.

Introduction

Contamination of groundwater with chlorinated solvents is a common environmental problem due to the toxicity and persistence of these compounds (*1*). Recently, the use of compound-specific stable isotope ratios was proposed as a tool to investigate the behavior and fate of chlorinated solvents in the subsurface (*2*). Isotope ratios may be used to demonstrate intrinsic biodegradation of chlorinated solvents (*3*−*7*) or abiotic transformation of chlorinated solvents by reactive barriers (*8*). These applications are based on the fact that a kinetic isotope effect may occur during biotic or abiotic transformation of organic compounds. As a result, the remaining fraction of the compound becomes enriched in heavy isotopes, while the product is depleted in heavy isotopes. In the absence of biodegradation, isotope ratios analyses may help to distinguish between different events or sources of contamination (*2*). The aim of this study was to develop and evaluate a method to determine compound-specific carbon isotope ratios of chlorinated solvents and dechlorination products in aqueous samples.

A variety of techniques to extract organic compounds from the aqueous phase have been developed, among them headspace equilibration, solid-phase microextraction (SPME), solid-phase extraction, liquid−liquid extraction, and purge-and-trap. In this study, SPME was chosen as extraction method, since SPME is a solvent free, fast, and sensitive method. The SPME method involves exposing a fused silica fiber coated with a polymeric phase to the aqueous sample (*9*, *10*). The fiber can either be directly immersed into the aqueous phase of the sample (direct SPME) or placed in the headspace above the sample (headspace SPME). In this study, direct SPME is abbreviated as dSPME and headspace SPME as hSPME, while SPME refers to the SPME method in general. Compared to dSPME, the hSPME method has the advantage that extraction of nonvolatile compounds, which may disturb the chromatographic resolution of target compounds, can be minimized. The hSPME method has a similar sensitivity for concentration analyses of volatile hydrocarbons as purge-and-trap methods (*11*). Therefore, SPME was expected to be a very sensitive method for isotope analyses of chlorinated solvents in aqueous samples. The SPME method was compared to headspace equilibration (*12*), a method that was previously evaluated by Slater et al. (*13*) for carbon isotope analysis of trichloroethene (TCE). In contrast to techniques that involve solvents, SPME and headspace techniques can be used to analyze isotope ratios of compounds with low boiling points such as dichloromethane.

The extraction methods investigated in this study, dSPME, hSPME, and headspace equilibration, rely on phase transfer processes which may lead to differences in the isotope ratios of a compound in different phases (isotope fractionation). To be able to determine carbon isotope ratios accurately, the degree of isotope fractionation during partitioning of a compound between the involved phases has to be known. Dias and Freeman (*14*) investigated carbon isotope fractionation during aqueous phase/SPME fiber partitioning of various non-chlorinated hydrocarbons. They found that hydrophobic compounds extracted by a nonpolar fiber were slightly enriched in ^{13}C ($\leq$0.5‰), while organic acids extracted by a polar fiber were depleted in ^{13}C ($\leq$1.5‰). Isotope fractionation was attributed to mass-dependent energy shifts during partitioning of the organic compound between the aqueous phase and the SPME fiber coating. Harris et al. (*15*) used a hSPME method for carbon isotope analysis of gasoline range hydrocarbons in oils. They found a nonsystematic ^{13}C depletion or enrichment in the extracted compounds compared to results from a purge-and-trap method. Carbon isotope fractionation during headspace equilibration of toluene and trichloroethene (TCE) was investigated by Slater et al. (*13*), and differences in carbon isotope ratios between molecules in the aqueous phase and the headspace were found to be $\leq$0.5‰.

In this study, the precision, accuracy, and sensitivity of compound-specific carbon isotope analysis was investigated for nine chlorinated methanes, ethanes, and ethenes. The selected compounds are among the most frequently detected organic contaminants in groundwater (*16*). The compounds were extracted by dSPME, hSPME, and headspace equilibration from aqueous solutions and measured using a gas chromatography combustion isotope-ratio mass spectrometry (GC-C-IRMS) system. To determine the accuracy of the methods, carbon isotope fractionation during dSPME, hSPME, and headspace equilibration was evaluated. The occurrence of carbon isotope fractionation during dSPME and hSPME

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TABLE 1. List of Compounds

compound	abbreviation	mixture	purity (%)	manufacturer
vinyl chloride	VC	A	99.5[a]	Fluka, Buchs, Switzerland
trans-1,2-dichloroethene	tDCE	A	98[a]	Aldrich, Milwaukee, U.S.A.
cis-1,2-dichloroethene	cDCE	A	97[a]	Aldrich, Milwaukee, U.S.A.
trichloroethene	TCE	A	99.7[b]	Dow, Midland, U.S.A.
tetrachloroethene	PCE	A	99.8[b]	Dow, Midland, U.S.A.
dichloromethane	DCM	B	99.9[a]	EM Science, Gibbstown, U.S.A.
chloroform	CF	B	99.8[a]	Aldrich, Milwaukee, U.S.A.
carbon tetrachloride	CT	A	99.9[a]	Fisher, Fair Lawn, U.S.A.
1,2-dichloroethane	1,2-DCA	B	99.8[a]	Aldrich, Milwaukee, U.S.A.

[a] Specified by manufacturer. [b] Determined by GC-FID.

depends on the magnitude of mass dependent effects in the aqueous phase and the SPME fiber coating. The headspace studies provide information on isotope effects associated with the aqueous phase. To evaluate isotope effects during interaction of the compounds with the SPME fiber coating, gas phase/SPME fiber partitioning experiments were performed. Furthermore, aqueous phase/SPME fiber partition coefficients were determined, and the effect of salt addition on the SPME and headspace extraction efficiency was investigated to allow for comparison of the sensitivities of the SPME and headspace methods.

Material and Methods

Analytical System. Compound-specific carbon isotope ratios were determined in the Environmental Isotope Laboratory of the University of Waterloo using a GC-C-IRMS system. The GC-C-IRMS system consisted of a Agilent 6890 GC (Agilent, Palo Alto, U.S.A.) with a split/splitless injector, a Micromass combustion interface operated at 850 °C, a cold trap cooled to −100 °C using liquid nitrogen, and a Micromass Isochrom isotope-ratio mass spectrometer (Micromass, Manchester, U.K.). The GC was equipped with a DB-VRX column (60 m × 0.25 mm, 1 μm stationary phase, J&W Scientific, Folsom, U.S.A.). The following oven temperature program was used: 50 °C for 2 min, 12 °C/min to 100 °C, 20 °C/min to 220 °C, 220 °C for 2 min, and the injector was set at 270 °C. The isotope-ratio mass spectrometer was operated at a trap current of 400 μM to optimize for linearity. A higher trap current would lead to an increase in sensitivity of the instrument, however, at the expense of stability and precision. For all experiments, injection sizes were selected such as to obtain peak heights between 1.0 and 5.0 V. Within this peak size range, the linearity of the system was better than 0.14‰. The lower limit of peak sizes corresponded to a minimal amount of 1.5 nmol carbon (C) on the GC column.

For SPME injections, a narrow bore SPME sleeve was installed (Supelco, Bellefonte, PA). During SPME injections, the split vent was closed for 1 min. All SPME extractions were performed using 100 μM poly(dimethylsiloxane) (PDMS) fibers (Supelco). For gas injections, the injector was equipped with a split/splitless sleeve (Restec, Bellefonte, U.S.A.). A split ratio of 10:1 was chosen to maximize the sensitivity of the headspace method. All $^{13}C/^{12}C$ ratios are reported in the usual delta notation ($\delta^{13}C$) referenced to the VPDB (Vienna Peedee Belemnite) standard (*17*). The $\delta^{13}C$ value is defined as $\delta^{13}C = (R_s/R_r - 1) \times 1000$, where R_s and R_r are the $^{13}C/^{12}C$ ratios of the sample and the international standard, respectively.

Determination of Carbon Isotope Ratios of Pure Phase Chlorinated Methanes, Ethanes, and Ethenes. The $\delta^{13}C$ of the pure phase compounds used in this study (Table 1) were determined using (i) a CE Instruments elemental analyzer (CE Instruments, Rodano, Italy) coupled to a Micromass Isochrom isotope-ratio mass spectrometer (EA-IRMS) and (ii) the GC-C-IRMS instrument described above. For both systems, carbon isotope ratios of target compounds were determined relative to external CO_2 reference gas (*18*). In addition, various amounts of reference CH_4 with a known $\delta^{13}C$ (−43.04‰) was injected similarly as the target compounds. In case of EA-IRMS, 3−5 μL of pure phase compounds or 0.5 mL of vinyl chloride (VC) was injected onto the combustion tube of the elemental analyzer (EA) using a syringe injection port attached to the EA. The values of target compounds were normalized and linearity corrected against the reference CH_4, which led to adjustments of the $\delta^{13}C$ values between 0.10 and 0.23‰.

When using the second system (GC-C-IRMS), two mixtures of chlorinated methanes, ethanes, and ethenes (A and B) were prepared that contained the compounds at equimolar ratios with respect to carbon (Table 1). Aliquots of the mixtures were injected into 500 mL glass bottles that had been flushed with helium for 5 min and closed with an open screw cap containing a Teflon lined silica septum. Vinyl chloride gas was added using a Hamilton gastight locking syringe (Hamilton, Reno, U.S.A.). The final concentrations of each compound in the glass bottles were 15 and 60 μM C per compound, respectively. After allowing at least 30 min for evaporation of the compounds added as liquid phase, gas samples of 1 mL volume from the glass bottles were injected into the split/splitless injection port of the GC using a gastight locking syringe. Since the value of reference CH_4 determined by GC-C-IRMS (−43.08 ± 0.07‰, n = 9) corresponded well to its calibrated value (−43.04‰) and the instrument exhibited good linearity, the $\delta^{13}C$ value obtained relative to external CO_2 were not corrected. The mean $\delta^{13}C$ values are reported with the corresponding 95% confidence intervals, which were obtained by multiplying the standard uncertainties with a coverage factor based on the t-distribution (*19*).

Determination of Partition Coefficients. In contrast to aqueous phase/gas phase partition coefficients (Table 2), the aqueous phase/SPME fiber partition coefficients are not documented for all of the compounds used in this study. Since the detection limits of the SPME method depends on the aqueous phase/SPME fiber partition coefficients, they were determined. Partition coefficients are defined by

$$K_{yx} = \frac{C_y}{C_x} \quad (1)$$

where C_x and C_y are the concentration of the compound in phase x and y, respectively (w = aqueous phase, g = gas phase, f = SPME fiber coating). To determine aqueous phase/SPME fiber partition coefficients (K_{fw}), aqueous solutions were prepared by injecting aliquots of pure phase mixtures A and B, respectively, through a Teflon lined septum into bottles containing organic free distilled water and a magnetic stirrer. The solutions contained 10, 50, and 150 μM C of each compound. After at least 3 h of mixing, the solutions were transferred into 17 mL glass vials with open screw caps and Teflon lined septa. Prior to dSPME extraction, 0.8 mL of

TABLE 2. Dimensionless Partition Coefficients at 25 °C for Aqueous Phase/Gas Phase Partitioning (K_{gw}), Aqueous Phase/SPME Fiber Partitioning (K_{fw}), and Octanol/Aqueous Phase Partitioning (K_{ow})[e]

compd	aqueous/gas K_{gw}	aqueous/SPME fiber K_{fw}	aqueous/octanol K_{ow}	aqueous/gas K_{gw}^{NaCl}/K_{gw}	aqueous/SPME fiber K_{fw}^{NaCl}/K_{fw}
VC	1.080[a]	50	4[b]	4.6	nd[d]
tDCE	0.377[a]	95	123[c]	5.1	5.1
cDCE	0.154[a]	72		5.1	5.5
TCE	0.386[a]	380	263[b]	5.7	2.1
PCE	0.716[a]	1630	759[b]	4.1	1.0
DCM	0.087[a]	23	14[b]	4.8	3.8
CF	0.147[a]	84	85[b]	5.9	5.3
CT	1.230[a]	760	537[b]	4.5	1.4
1,2-DCA	0.044[b]	36	30[b]	4.6	4.9

[a] Reference *34*. [b] Reference *35*. [c] Reference *36*. [d] nd, not determined. [e] Increase of partition coefficients by adding 5 M NaCl to aqueous phase. Aqueous phase/SPME fiber partition coefficients were determined using 100 μm poly(dimethylsiloxane) SPME fibers.

solution was removed to avoid contact of the needle holding the SPME fiber with the aqueous phase and a 14 mm magnetic stir bar was added. Three standards of each concentration level were extracted for 20 min by immersing the fiber into the aqueous phase. The peaks were recorded with a FID detector and peak areas were calculated. Using the peak areas and response factors, the amount of each compound on the fiber was calculated. Based on the amount of each compound on the SPME fiber and the concentration in the aqueous phase, K_{fw} values were obtained (*10*). The standard uncertainty of K_{fw} was calculated based on standard uncertainties of peak areas and response factors (*19*). The average relative standard uncertainty was 14%.

Evaluation of Carbon Isotope Fractionation during SPME and Headspace Equilibration. ***Analysis of Aqueous Standards by dSPME and hSPME.*** Aqueous standards were prepared using two mixtures of chlorinated methanes, ethanes, and ethenes (Table 1). The concentrations in the aqueous standards were chosen such that 1.5 or 6 nmol C of each compound was on the fiber after extraction. The required concentrations in the aqueous phase were calculated based on partition coefficients, volume of standards, and volume of the fiber coating (*10*). To prepare aqueous standards, aliquots of the pure phase mixtures were injected into 125 mL glass bottles containing organic free distilled water. The solutions were stirred for at least 3 h. For dSPME studies, glass vials were completely filled with the prepared solutions. The volume of the vials (17 mL) was chosen such that only a small amount of each compound (≤6%) was extracted, which maximizes the sensitivity of the method (*20*). Before analysis, 0.8 mL of solution was removed, and a 14 mm long Teflon-coated magnetic stir bar was added. For hSPME studies, 30 mL of solution was transferred into a 42 mL vial containing a 21 mm Teflon-coated magnetic stir bar. Vinyl chloride was added as gas phase to the headspace. The vials were vigorously shaken by hand for 2 min and then placed on a lateral shaker (120 rpm) for at least 2 h.

To determine whether the duration of extraction influences $\delta^{13}C$ values, tests were conducted with extraction times of 10, 20, and 30 min. Peak areas and $\delta^{13}C$ values for different extraction times were similar within the range of analytical uncertainty. For all further experiments, an extraction time of 20 min was used. The required extraction time may vary depending on the stirring rate, and in the case of hSPME, it also depends on the headspace volume and the shape of the vial (*20*). Three measurements were made for standards of each concentration level using separate vials. Additional standards were prepared for dSPME in a 5 M NaCl solution using similar amounts of the compounds as for the lower concentration level of the standards described above. The effect of NaCl on the partition coefficients was quantified based on peak areas of standards with and without NaCl.

Analysis of Aqueous Standards by Headspace Equilibration. For headspace equilibration studies, aqueous standards were prepared similarly as for SPME studies. Aqueous phase concentrations were chosen such that similar equilibrium concentrations of each compound in the headspace (15 or 60 μM C) were obtained. The concentrations corresponded to approximately 1.5 or 6 nmol C per compound on the GC column when 1 mL was injected at a split ratio of 10:1. To prepare aqueous standards, aliquots of the pure phase mixtures were injected into bottles containing organic free distilled water. The pure phase mixtures contained the same compounds as for the SPME experiments but at different ratios taking into account differences in partition coefficients. After 3 h of stirring, a headspace was introduced into bottles by replacing 20 mL of the aqueous phase by 99.995% helium. The bottles were shaken vigorously by hand for 2 min and then placed on a lateral shaker (120rpm) for at least 2 h in a water bath set to 25 °C. Headspace gas samples of 1 mL were injected into the GC using a locking gastight syringe. Three separate bottles were analyzed for each concentration level. Additional standards were prepared for headspace equilibration in a 5 M NaCl solution using similar amounts of the compounds as for the lower concentration level of the standards described above. The effect of NaCl on the partition coefficients was quantified based on peak areas of standards with and without NaCl.

Evaluation of Carbon Isotope Fractionation during SPME of Gaseous Standards. To investigate carbon isotope fractionation during partitioning of the compounds into the fiber coating, gas-phase standards were prepared using pure phase mixtures. The pure phase mixtures contained the compounds in ratios such that a similar amount of each compound was extracted. The compound ratios in the pure phase mixtures were calculated based on the gas phase/SPME fiber partition coefficients, which were estimated using the following equation (*10*)

$$K_{fg} = \frac{K_{fw}}{K_{gw}} \quad (2)$$

where K_{fw} is the aqueous phase/SPME fiber partition coefficient and K_{gw} is the aqueous phase/gas phase partition coefficient. Gas-phase standards were prepared by injecting aliquots of the pure phase mixtures and gaseous VC into 500 mL glass bottles, which had been flushed with helium for 5 min and closed with an open screw cap containing a Teflon-lined septum. After allowing at least 30 min for evaporation of the compounds added as liquid phase, the SPME fiber was exposed to the gas phase for 20 min.

Calculation of Fractionation Factors. Isotope fractionation during phase transfer processes can be expressed using the enrichment factor ϵ, which corresponds approximately to the difference between the isotope ratios of a compound in phase y and x (*21*)

$$\epsilon_{yx} \approx \Delta\delta^{13}C_{yx} = \delta^{13}C_y - \delta^{13}C_x \quad (3)$$

where $\delta^{13}C_y$ and $\delta^{13}C_x$ are the equilibrium isotope ratios of a compound in phase y and x, respectively.

For two-phase systems, the following equation is obtained that relates ϵ_{yx} to the initial $\delta^{13}C$ value of the added compound and the measured $\delta^{13}C$ of compound in phase y

$$\epsilon_{yx} \approx \delta^{13}C_y - \delta^{13}C_x = \frac{\delta^{13}C_y - \delta^{13}C_o}{r_x} = \frac{\Delta\delta^{13}C_{yo}}{r_x} \quad (4)$$

where $\delta^{13}C_o$ is the initial isotope ratio of the added compound and r_x is the fraction of compound in phase x.

For hSPME (w = aqueous phase, f = SPME fiber coating, g = gas phase), the enrichment factor can be calculated using the following equation

$$\epsilon^*_{fw} \approx \delta^{13}C_f - \delta^{13}C_w = \frac{\delta^{13}C_f - \delta^{13}C_o}{r_w} + \frac{r_g(\delta^{13}C_g - \delta^{13}C_f)}{r_w} = \frac{\Delta\delta^{13}C_{fo}}{r_w} + \frac{r_g\epsilon_{gf}}{r_w} \quad (5)$$

where r_g and r_w are the fractions of compound in the gas phase and aqueous phase, respectively.

The enrichment factors were calculated based on the GC-C-IRMS $\delta^{13}C$ values of the compounds, which were considered to represent $\delta^{13}C_o$, and the average $\delta^{13}C$ of all measurements in each experiment. All ϵ_{yx} and ϵ^*_{fw} values are reported with the corresponding 95% confidence intervals, which were obtained by multiplying the standard uncertainties with a coverage factor based on the t-distribution (*19*). The standard uncertainties were calculated based on the standard uncertainties of the measured $\delta^{13}C$ values using the law of propagation of uncertainty. In this study, vials with larger volumes than required for practical applications were chosen to maximize r_x. Larger r_x lead to larger $\Delta\delta^{13}C_{yo}$ (eq 4) and thus make it possible to detect enrichment factors ϵ_{yx} with smaller values for a given standard uncertainty of $\delta^{13}C_o$ and $\delta^{13}C_y$.

If the isotopic enrichment factors are known, the original isotope ratio of compounds in samples that have been analyzed using dSPME and headspace equilibration can be calculated with the following equation

$$\delta^{13}C_o = \delta^{13}C_y - \epsilon_{yx}r_x \quad (6)$$

In case of hSPME, the following equation is valid

$$\delta^{13}C_o = \delta^{13}C_f - r_w\epsilon^*_{fw} + r_g\epsilon_{gf} \quad (7)$$

Results

SPME Partition Coefficients and Effect of Salt. The calculated K_{fw} values are listed in Table 2. For chlorinated ethenes and methanes, the values are higher the more chlorinated the compounds are. Chlorinated ethenes have higher K_{fw} values than chlorinated methanes with a corresponding number of chlorine atoms. Compounds with high octanol/water partition coefficients (K_{ow}) have high K_{fw} values (Table 2) as previously observed (*20*).

For compounds having a SPME partition coefficient (K_{fw}) below 300, addition of 5 M NaCl leads to an increase of the partition coefficient by a factor of 3.8−5.5, while for compounds having a K_{fw} larger than 300 little or no increase is observed (Table 2). For headspace equilibration, the increase of the partition coefficient is in the same range (4.1−5.9) for all compounds.

Stable Carbon Isotope Ratios of Pure Phase Chlorinated Methanes, Ethanes, and Ethenes. The $\delta^{13}C$ values determined by EA-IRMS and GC-C-IRMS are similar within the range of analytical uncertainty (Table 3). This indicates that split injection into the GC, which is used in the headspace experiments, does not cause significant carbon isotope fractionation. The standard uncertainties are slightly smaller for EA-IRMS than for GC-C-IRMS measurements, which reflects the larger number of handling steps and higher complexity of the GC-C-IRMS system.

Carbon Isotope Fractionation during SPME. Enrichment factors between compounds in the aqueous phase and on the SPME fiber were calculated for dSPME (ϵ_{fw}) and hSPME (ϵ^*_{fw}), respectively, using eqs 4 and 5, respectively. The enrichment factors are not significantly different from zero and within the range of analytical uncertainty, for all compounds except PCE and CT (Table 4). Extraction of PCE and CT by hSPME is accompanied by a detectable but small carbon isotope fractionation. Addition of 5 M NaCl does not lead to a significant change of carbon isotope fractionation during dSPME (Table 4). Enrichment factors ϵ_{gf} for extraction of gas standards by SPME were quantified using eq 4. Molecules in the gas phase are enriched in ^{13}C compared to molecules on the SPME fiber. The largest carbon isotope fractionation occurs for chlorinated methanes.

TABLE 3. Stable Carbon Isotope Ratios of Chlorinated Methanes, Ethanes, and Ethenes Determined Using an Elemental Analyzer Coupled to an Isotope-Ratio Mass Spectrometer (EA-IRMS) and a Gas Chromatograph Coupled to an Isotope-Ratio Mass Spectrometer (GC-C-IRMS)

compound	EA-IRMS $\delta^{13}C$ (‰ VPDB) $n = 4$	GC-C-IRMS $\delta^{13}C$ (‰ VPDB) $n = 6$
VC	−28.62 ± 0.14	−28.62 ± 0.31
tDCE	−22.23 ± 0.16	−22.44 ± 0.18
cDCE	−23.23 ± 0.11	−23.33 ± 0.07
TCE	−29.48 ± 0.08	−29.39 ± 0.20
PCE	−27.27 ± 0.08	−27.20 ± 0.24
DCM	−53.59 ± 0.10	−53.53 ± 0.14
CF	−63.62 ± 0.14	−63.43 ± 0.22
CT	−32.48 ± 0.22	−32.34 ± 0.15
1,2-DCA	−30.73 ± 0.02	−30.63 ± 0.25

Carbon Isotope Fractionation during Headspace Equilibration. Enrichment factors for aqueous phase/gas-phase partitioning (ϵ_{gw}) were calculated using eq 4. For all compounds, molecules in the gas phase are enriched in ^{13}C compared to molecules in the aqueous phase (Table 4). The magnitude of isotope fractionation is larger for chlorinated methanes than for chlorinated ethanes and ethenes. It does not significantly depend on the degree of chlorination within a compound class. In standards with NaCl, ϵ_{gw} is slightly smaller for most of the compounds than in standards without salt (Table 4); however, the differences are within the range of uncertainty for most compounds.

Detection Limit. The minimum detection limit of the SPME method is lower than that of the headspace method for all compounds except VC (Table 5). Addition of salt (salting out) leads to lower detection limits. For dSPME, salting out has a particularly large effect on compounds with a low K_{fw} and thus a high detection limit. By adding 5 M NaCl, a dSPME detection limit <1 ppm can be reached for all compounds except DCM. The reported detection limits are specific to the GC-C-IRMS system used in this study which requires a minimum of 1.5 nmol C on the GC column. For other GC-C-IRMS systems, the detection limits would differ depending upon the amount of carbon required.

Discussion

Our findings demonstrate that extraction of chlorinated methanes, ethanes, and ethenes by dSPME and hSPME is generally not accompanied by significant carbon isotope fractionation. In contrast, detectable isotope fractionation was observed during headspace equilibration, in particular for chlorinated methanes. This indicates that carbon isotope effects occur during interaction of the compounds with water molecules. The absence of significant isotope fractionation during extraction of aqueous standards by dSPME and hSPME suggests that in case of SPME, isotope effects in the aqueous phase are compensated with isotope effects in the fiber coating. This conclusion is consistent with the observation that enrichment factors for gas phase/aqueous phase and gas phase/SPME fiber partitioning have a similar magnitude

TABLE 4. Carbon Isotope Fractionation during Analysis of Aqueous Standards by Direct SPME (dSPME), Headspace SPME (hSPME), and Headspace Equilibration[a]

	dSPME ϵ_{fw}		hSPME ϵ^*_{fw}	headspace equilibration ϵ_{gw}		SPME of gas standards ϵ_{gf}
	dist water	5 M NaCl	dist water	dist water	5 M NaCl	
VC	nd[b]	nd[b]	−0.01 ± 0.14	0.33 ± 0.20	0.21 ± 0.16	0.39 ± 0.17
tDCE	−0.17 ± 0.33	−0.37 ± 0.27	−0.15 ± 0.16	0.38 ± 0.23	0.28 ± 0.19	0.80 ± 0.16
cDCE	−0.04 ± 0.35	−0.21 ± 0.23	0.05 ± 0.15	0.61 ± 0.26	0.69 ± 0.15	0.60 ± 0.14
TCE	−0.12 ± 0.25	0.06 ± 0.26	0.03 ± 0.24	0.59 ± 0.35	0.37 ± 0.24	0.68 ± 0.31
PCE	−0.25 ± 0.26	−0.29 ± 0.33	−0.37 ± 0.18	0.53 ± 0.37	0.17 ± 0.17	0.69 ± 0.26
DCM	0.28 ± 0.34	0.40 ± 0.22	0.04 ± 0.16	1.30 ± 0.28	1.27 ± 0.17	0.98 ± 0.50
CF	0.38 ± 0.44	0.40 ± 0.22	−0.18 ± 0.21	1.46 ± 0.34	1.03 ± 0.22	0.97 ± 0.24
CT	−0.29 ± 0.30	−0.09 ± 0.43	−0.26 ± 0.17	1.38 ± 0.42	1.29 ± 0.16	1.39 ± 0.38
12DCA	0.10 ± 0.21	−0.10 ± 0.19	0.01 ± 0.19	0.69 ± 0.26	0.06 ± 0.20	0.74 ± 0.33

[a] Carbon isotope fractionation during analysis of gas standards by SPME. Isotope fractionation is expressed as enrichment factors in ‰ (eqs 4 and 5) with the corresponding 95% confidence intervals ($n = 6$). [b] nd, not determined.

TABLE 5. Detection Limit for Carbon Isotope Analysis Using Direct SPME (100 μm Polydimethylsiloxane Fiber) and Headspace Equilibration, Respectively[a]

	direct SPME (ppm)		headspace equilibration (ppm)	
	no salt	5 M NaCl	no salt	5 M NaCl
VC	1.5	nd[b]	0.51	0.17
tDCE	1.2	0.24	2.1	0.49
cDCE	1.6	0.29	4.9	1.0
TCE	0.40	0.19	2.7	0.61
PCE	0.13	0.13	2.0	0.62
DCM	8.4	2.2	15.0	3.3
CF	3.3	0.63	13.0	2.8
CT	0.51	0.36	2.2	0.80
1,2-DCA	3.1	0.64	18.0	4.1

[a] The detection limit corresponds to the initial concentration required in the aqueous phase to obtain 1.5 nmol C on the GC column. [b] nd, not determined.

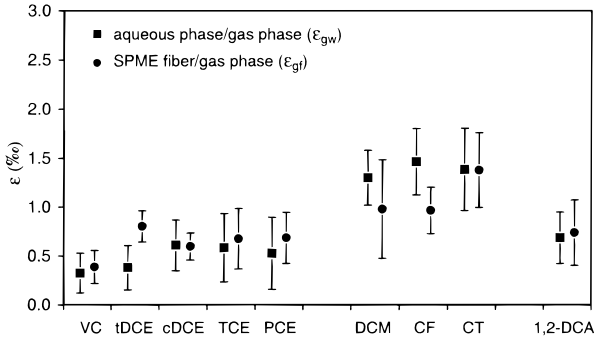

FIGURE 1. Carbon isotope fractionation during aqueous phase/gas phase and SPME fiber/gas phase partitioning. Positive enrichment factors ϵ signify an enrichment of ^{13}C in the gas phase. The error bars represent the 95% confidence intervals ($n = 6$).

(Figure 1). In both cases, an inverse isotope effect is observed whereby the molecules in the gas phase are enriched in ^{13}C compared to molecules in the aqueous and in the SPME fiber coating, respectively.

A similar inverse isotope effect is frequently observed during gas phase/liquid-phase partitioning in GC columns (*22*−*25*), which results in a slightly faster elution of molecules with heavy isotopes than molecules with light isotopes during GC separation. The SPME fiber has a similar chemical composition as nonpolar GC columns (dimethylpolysiloxane). Therefore, similar isotope effects during gas phase/liquid-phase partitioning are expected for SPME fiber and GC column. In this study, an inverse isotope effect during GC separation was observed for all compounds which is consistent with the observation that during SPME partitioning, molecules in the gas phase are enriched in ^{13}C compared to molecules on the fiber. An inverse isotope effect with respect to carbon has also been observed during evaporation of pure phase solvents such as CF, CT, and TCE (*26*−*30*). For example, Huang et al. (*29*) found that under equilibrium conditions, TCE in the gas phase was enriched in ^{13}C by 0.9‰ (25 °C) compared to pure phase TCE. The inverse isotope effect during evaporation and GC separation of organic compounds has been rationalized in terms of a mass-dependent effect of the van de Waals interactions on the vibrational energy of molecules in the liquid phase (*31*, *32*). Due to this effect, molecules with heavy isotopes have slightly higher energies in the liquid phase than molecules with light isotopes, which explains their higher volatility. A similar effect probably occurs during partitioning of chlorinated methanes, ethanes, and ethenes into the fiber coating.

When using dSPME and hSPME to analyze aqueous samples, the measured $\delta^{13}C$ can generally be considered to represent the $\delta^{13}C$ of the compounds originally dissolved in the aqueous phase, and no correction is required. A similar conclusion has been drawn by Dayan et al. (*8*) for carbon isotope analysis of PCE, TCE, cDCE, and tDCE by hSPME. However, when using headspace equilibration, isotope fractionation during gas phase/aqueous phase partitioning has to be taken into account, particularly for chlorinated methanes, and especially if values obtained by different analytical methods are compared (e.g. EA-IRMS for compounds present as nonaqueous phase liquid; GC-C-IRMS and headspace equilibration for dissolved compounds; GC-C-IRMS and gas injection for compounds in unsaturated zone). If carbon isotope fractionation during aqueous phase/gas phase partitioning is taken into account, dSPME, hSPME, and headspace equilibration techniques provide $\delta^{13}C$ values with a similar accuracy and precision. The 95% confidence interval of $\delta^{13}C_o$ (eqs 6 and 7), which can be considered as a measure of the overall analytical uncertainty of the method, ranges from 0.24 and 0.47 ‰ for dSPME, from 0.15 to 0.24 ‰ for hSPME, and from 0.29 to 0.57 ‰ for headspace equilibration. The good agreement between $\delta^{13}C$ values measured in standards without and with added NaCl indicates that for both SPME and headspace equilibration, the results are not affected by variations in ionic strength in aqueous samples.

The dSPME method has the advantage of having a lower detection limit and requires less sample volume than the headspace method. For hSPME, the detection limit is up to 30% higher than for dSPME, depending on K_{gw} of the compound (*20*), but is still lower than for headspace equilibration. By choosing other fibers than the one used in this study, lower detection limits than those given in Table 5 can be obtained for some compounds. For example, a detection limit of 150 ppb was reached for 1,2-DCA by using a PDMS-DVB fiber. For hSPME, a further decrease of the

detection limit could be reached by simultaneously heating the sample and cooling the fiber (*20*), for headspace equilibration by heating the sample (*13*). The headspace equilibration method has the advantage that in addition to obtaining isotope ratios of chlorinated solvents and degradation products, those of gaseous end products (ethene, CO_2, and CH_4) can be determined as well, using the same method or even during the same GC run (*5*).

Application in Groundwater Studies. The analytical techniques tested in this study can be easily adapted for analyzing chlorinated methanes, ethanes, and ethenes in groundwater samples. The results of this study make it possible to combine headspace equilibration, dSPME and hSPME methods according to their merits. For example, chlorinated compounds present in high concentration and gaseous end products can be analyzed by headspace equilibration and compounds at low concentration by dSPME. For such a combined analysis, no more than the content of a standard 40 mL VOC vial is required if the water that is displaced to create a headspace is used for dSPME. This approach was used to analyze groundwater samples from field sites, and a similar precision as reported in this study was achieved (*33*). Given the high precision of the measurements and given that shifts of $\delta^{13}C$ values during abiotic and biotic degradation of chlorinated solvents are in the range of tens of ‰ (*5*−*8*), the methods investigated in this study are very sensitive to trace abiotic and biotic transformation of these compounds in groundwater.

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