Headspace Solvent Microextraction of Trihalomethane Compounds into a Single Drop

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Abstract

Headspace solvent microextraction (HSME) into a single drop is developed for the determination of six trihalomethanes, CH_2Cl_2, $CHCl_3$, C_4H_9Cl, CCl_4, C_2HCl_3, and C_2Cl_4, in aqueous solution. A drop of benzyl alcohol containing bromoform, as an internal standard, is used for extraction. The analytes are extracted by suspending a 3-µL drop directly from the needle of a microsyringe. The needle passes through the septum of a vessel, and the needle tip appears above the surface of the solution. After the prescribed extraction time, the drop is drawn back into the syringe. The syringe is then removed, and its content is injected directly into a gas chromatography column for analysis. The main parameters affecting the HSME process, such as stirring speed, microdrop volume, sample solution temperature, microsyringe needle temperature, sample volume, sampling time, solution pH, extracting solvent, and ionic strength of the solution, are studied. Also, the linear range and precision of the method are examined.

Introduction

Liquid–liquid extraction (LLE) is a widely used and generally accepted sample preparation method for a large variety of applications. Nevertheless, it suffers from several limitations, such as the need for large volumes of expensive, toxic, and high purity organic solvents; labor intensity; tending for emulsion formation; and poor potential for automation. Moreover, it is extremely time consuming. Initial efforts to address the problems of large solvent consumption and poor automation included the development of flow injection extraction. This had the advantages of high speed, low cost, and reduced solvent/sample consumption. In this method, extraction is quantitative, and measuring optical absorption in the organic phase performs analyte determination. Although the method is attractive, solvent consumption is still on the order of several hundred microliters per analysis. More recently, effort has been placed on miniaturizing the extraction process. The primary goal of techniques to miniaturize LLE sample preparation has been to greatly reduce the organic solvent–aqueous phase ratios. Two general methods have evolved. These are single-drop extraction, in which the extraction phase is a discrete drop of immiscible solvent suspended in a sample or extraction into a liquid film in contact with the sample (1). Recently, Jeannot and Cantwell (2) proposed solvent microdrop extraction from water into an 8-µL drop of organic solvent located at the end of a Teflon rod. Later, they simplified the liquid-phase microextraction (LPME) method by suspending a drop directly from the tip of a microsyringe needle immersed in the aqueous phase (3–8). He and Lee (9,10) investigated static and dynamic LPME from water. These methods suffer from two limitations. One is the choice of solvent for microextraction that is immiscible in water, and the other is the impossibility for extraction of analytes from stirred aqueous solutions. For solving these problems, headspace microextraction into a single drop was developed (11–13). In the present work, we have used headspace solvent microextraction (HSME) for the extraction and gas chromatography (GC) analysis of trihalomethanes compounds. Also, factors affecting the HSME process, such as stirring speed, microdrop volume, sample solution temperature, microsyringe needle temperature, sample volume, sampling time, pH and ionic strength of sample solution, and extracting solvent, were examined.

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Experimental

Instrumentation

All of the separations were carried out on a Hewlett-Packard (Avondale, PA) model 5890 series II GC with a flame ionization detector. Separations were performed on a 20-m × 0.53-mm i.d. fused-silica capillary column with 1.5-µm DB-5 coating (J&W Scientific, Folsom, CA). Helium was used as the carrier gas at 3.5 mL/min. The injection port was held at 150°C and used in the split mode with split flow of 3.5 mL/min. Oven temperature programming was used to facilitate separation, with an initial oven temperature of 35°C (held for 8 min), ramping at a rate of 40°C/min to a temperature of 100°C (held for 5 min), and finally up to 150°C (held for 6 min) at a rate of 20°C/min. The detector oven was held at 150°C. A microsyringe with an angled-cut needle tip (Hamilton, Reno, NV) was used for extractions and injections.

The syringe plunger operated in the glass barrel (not in the needle). Two circulating water baths (Braun UM-S, Melsungen, Schwarzenberger, Germany) were used for adjusting the temperatures of the syringe needle and sample solutions with an accuracy of ± 0.01°C. Figure 1 shows the apparatus used for the HSME.

Reagents

Reagent-grade carbon tetrachloride, trichloroethylene, cyclododecane, 1-octanol, octane, vinyl acetate, tetrachloroethylene, 1-chlorobutane, cyclohexanol, ethylene glycol (Merck, Darmstadt, Germany), dodecane (Aldrich, Milwaukee, WI), benzyl alcohol, bromoform, heptanol (Fluka, Buchs, Switzerland), chloroform, toluene, hexane, dichloromethane, and ethanol (Panreac, Barcelona, Spain) were used as received.

Extraction procedure

The extraction procedure was carried out using a 10-µL microsyringe, 8- × 1.5-mm magnetic stirring bar (VWR Scientific Products, West Chester, PA), and a magnetic stirrer. Also, a two-compartment recirculating cell (one for sample temperature control and the other for needle temperature control, constructed in the laboratory) and a 7-mL extraction vial with a poly(tetrafluoroethylene)–silicon septum (Supelco, Bellefonte, PA) were used. The maximum syringe volume and the delivery volume were set to 1.0–4.0 µL via a change adapter.

For an extraction, benzyl alcohol containing bromoform (50 µg/mL) as an internal standard was drawn into the syringe. The needle of the syringe was then inserted into the internal tube of the two-compartment cell above the extraction vial. This was in such a way that the needle passed through the septum of the extraction vial. The needle tip protruded to a depth of approximately 5–15 mm (depending on the sample volume) above the surface of the stirred analyte solution. To form the extraction drop in the headspace of the solution, the plunger was depressed, causing the solvent to be suspended from the needle tip. The drop was exposed to the headspace of the analyte solution for a given extraction time. The drop was then drawn back into the syringe. The needle was consequently removed from the vial, and its contents were injected into the GC for the analysis.

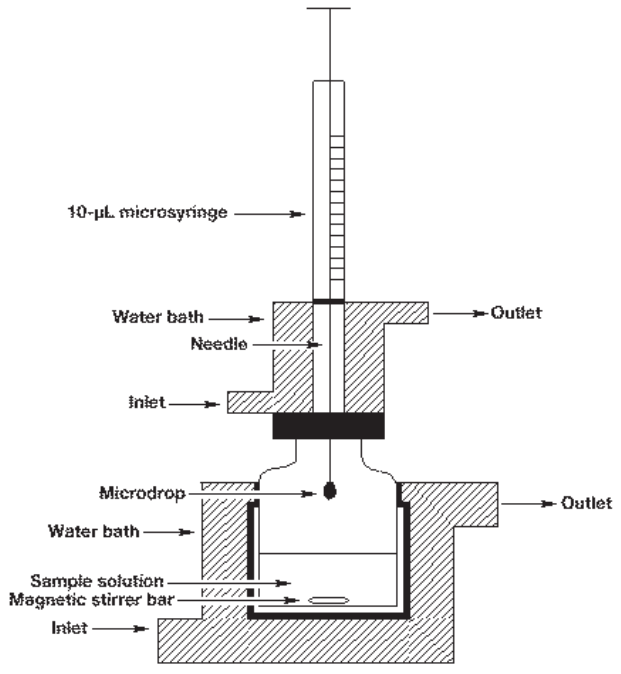

Figure 1. Schematic diagram of the HSME apparatus.

Results and Discussion

Method development

Method development was examined on trihalomethanes extraction from an univariate approach. The parameters influencing the HSME procedure, including the nature of solvent used as extractant, stirring speed, volume of the microdrop, temperature of bulk analyte solution and microdrop, volume of analyte solution, extraction time, and the ionic strength and pH of analyte solution, were optimized independently. All quantitations made in this study were based on the relative total peak area of analytes to the internal standard (bromoform) from the average of three replicate measurements.

Nature of microdrop solvent

In order to find which solvent was more appropriate for the extraction of trihalomethanes, several solvents such as dodecane, toluene, *n*-heptane, ethanol, cyclododecane, 1-heptanol, *n*-hexane, cyclohexanol, ethylene glycol, octane, vinyl acetate, 1-hexanol, 1-octanol, benzene, decane, benzyl alcohol, and water were tested. Among 17 different solvents examined, only the peaks of benzyl alcohol were separated completely from the sample peaks. Thus, benzyl alcohol was chosen as the extraction solvent for the further studies.

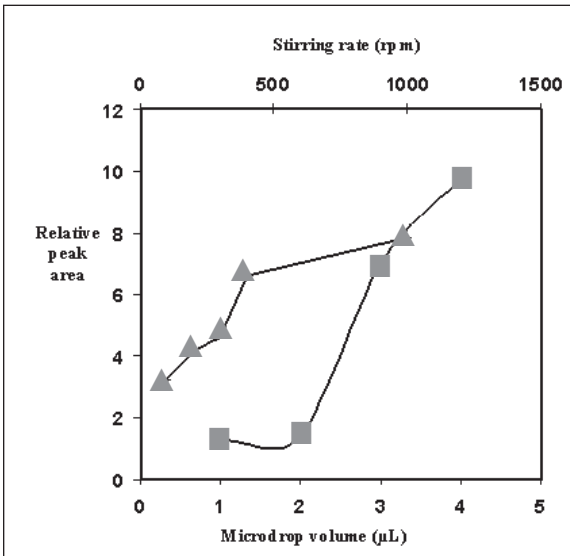

Figure 2. Effects of the stirring speed (▲) and microdrop volume (■) on extraction efficiency. Conditions for stirring speed experiments: standard solution of analytes, 1 µg/mL; sample volume, 2 mL; drop size, 3 µL; microsyringe needle temperature, 3°C; sample temperature, 35°C; and extraction time, 10 min. Conditions for microdrop volume experiments as before, except 400 rpm for stirring rate and varying drop size.

Stirring rates

Sample stirring often reduces the time required to reach the equilibrium. This decreases extraction time by enhancing the diffusion of the analytes toward the microdrop. The extraction efficiency of a 1-µg/mL aqueous solution of compounds as a function of stirring speed is illustrated in Figure 2. One can see that, for a 10-min extraction time, the increase in stirring speeds from 100 to 400 rpm improves significantly the analytical signal. At higher stirring speeds, the amount of analyte extracted continues to increase with a relatively smaller rate. The evaluation of these results should take into account that, for the headspace extraction, two distinct mass-transfer steps are occurring simultaneously, one from the sample solution to the headspace and the other from the headspace to the drop (14). The suitability of the HSME technique for the extraction of compounds in water depends on the transfer of the analyte from the aqueous phase to the gaseous phase. For volatile compounds, the controlling step in the HSME process is the diffusion of the analyte into the drop. On the other hand, for compounds, which are less volatile and have high water solubility, the mass transfer from the water to the gaseous phase may be the rate-controlling step in the HSME process (15). Thus, the increase in the extracted masses will be observed for the less volatile analytes with increasing the speed of stirring. Therefore, in the present work, the extraction equilibrium may be approximately established at a stirring rate of 400 rpm, after 10 min.

Microdrop volume

Figure 2 shows that an increase in the volume of the microdrop (up to 4 µL) results in a sharp enhancement in the extraction efficiency of the system. However, at larger volumes (i.e., > 3 µL), the microdrop reveals a great tendency to fall down from the tip of the microsyringe. Thus, a 3-µL drop size was chosen as the optimized volume.

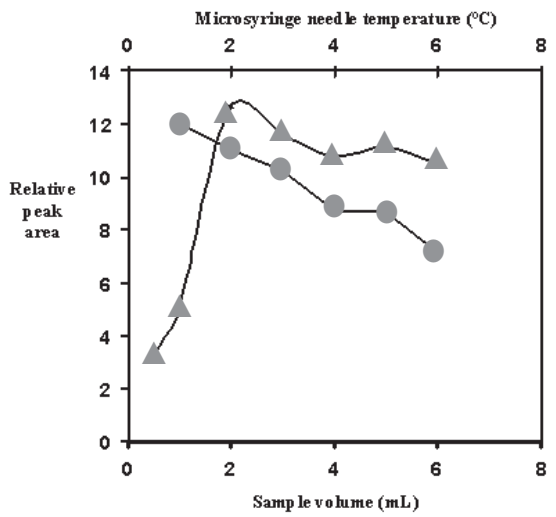

Figure 3. Effects of microsyringe needle temperature (●) and sample volume (▲) on extraction efficiency. Conditions for microsyringe needle temperature experiments: standard solution of analytes, 1 µg/mL; sample volume, 2 mL; stirring rate, 400 rpm; sample temperature, 20°C; drop size, 3 µL; and extraction time, 10 min. Conditions for sample volume experiments as before, except 1°C for microsyringe needle temperature and varying sample volume.

Sample solution and microdrop temperature

The effect of sample solution temperature on the extraction efficiency was investigated by varying the temperature in the range of 10–60ºC. Results showed that the amount of analyte delivered into the microdrop and, consequently, sensitivity of the method were increased with increasing temperature of the stirred sample solution up to 20ºC. At higher temperatures, overpressurization of the sample vial occurs. This may cause the gradual loss of analytes through the sides of the syringe needle. Thus, the sample solution temperature was held at 20ºC for further studies.

HSME is a process that involves the partition of analytes from the aqueous phase to the gas phase and eventually into the microdrop. This occurs according to their partition coefficients, K_d, (16). At high temperatures, the vapor pressure of analytes and, hence, their concentrations in headspace increase.

Variation of extraction efficiency with microsyringe needle temperature in the range of 1–6ºC is shown in Figure 3.

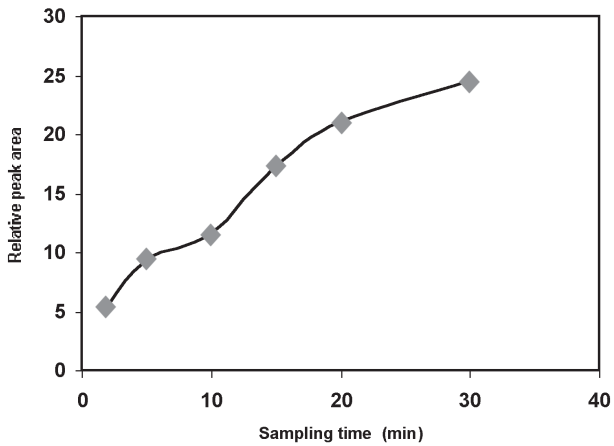

Figure 4. Extraction time profile for determination of optimum sampling time: standard solution of analytes, 1 µg/mL; microsyringe needle temperature, 1°C; stirring rate, 400 rpm; sample temperature, 20°C; drop size, 3 µL; and sample volume, 2 mL.

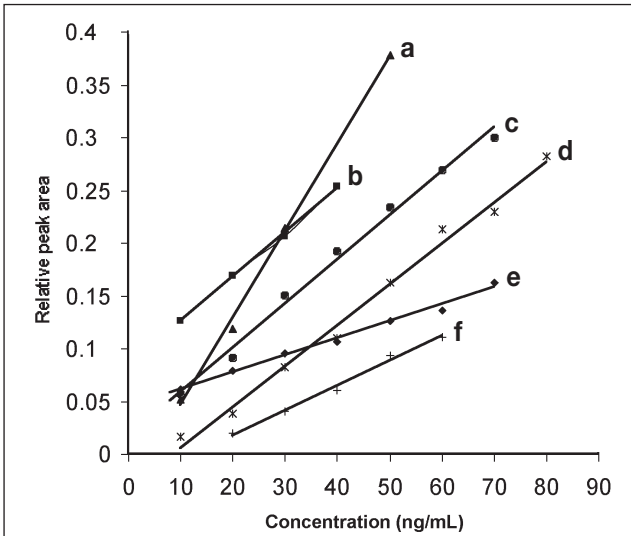

Figure 5. Calibration curves of trihalomethanes in optimal conditions: (a) chlorobuthane, (b) chloroform, (c) tetrachloroethylene, (d) trichloroethylene, (e) dichloromethane, and (f) carbon tetrachloride.

Table I. Regression Equations, Correlation Cofficients, and Dynamic Linear Ranges

	a	b	c	d	e	f
	Choloro-buthane	Chloro-form	Tetrachloro-ethylene	Trichloro-ethylene	Dichloro-methane	Carbon tetrachloride
r^2	0.9974	0.9985	0.9923	0.9908	0.9901	0.9918
LOD (ng/mL)	3.2	2.0	9.1	6.6	7.0	5.4
Slope	0.0083	0.0042	0.0042	0.0039	0.0016	0.0024
Intercept	–0.0371	0.0848	0.0177	–0.0329	0.0458	–0.0298
Dynamic linear range (ng/mL)	10.0–50.0	10.0–40.0	10.0–70.0	10.0–80.0	10.0–70.0	20.0–60.0

Table II. Determination of Trihalometanes in Water Samples

Isfahan samples	Compounds	Concentration (ng/mL) Add	Found	RSD% $n = 3$	Relative error%
1	$CHCl_3$	–	13.8	2.4	–
	$CHCl_3$	20.0	33.1	7.9	–2.1
	CCl_4	–	< 20.0	7.4	–
	CCl_4	20.0	36.2	10.0	–
2	$CHCl_3$	–	13.2	5.8	–
	$CHCl_3$	10.0	21.8	6.2	–6.1
	CCl_4	–	< 20.0	15.2	–
	CCl_4	10.0	24.6	12.1	–
3	$CHCl_3$	–	< 10.0	7.0	–
	$CHCl_3$	20.0	29.1	6.9	–
	CCl_4	–	< 20.0	6.5	–
	CCl_4	20.0	29.4	2.2	–
	C_2Cl_4	50.0	50.2	7.3	0.4
	C_2HCl_3	50.0	52.4	5.5	4.7
	C_4H9Cl	40.0	42.6	2.1	6.5
	CH_2Cl_2	50.0	51.3	17.7	2.6
4	$CHCl_3$	–	< 10.0	17.8	–
	$CHCl_3$		25.6	3.8	–
	CCl_4		39.0	18.0	–2.5
	C_2Cl_4		51.6	8.9	3.2
	C_2HCl_3		49.8	6.2	–0.4
	C_4H_9Cl	40.0	40.8	4.6	2.0

Maximum extraction occurs in low temperatures. Thus, further extraction was performed at 1ºC.

Sample volume

For high sensitivity headspace extraction, the volume of the gaseous phase should be minimized (15). The volume of headspace into which the analytes diffuse affects the extraction of the analytes. Because all previous extractions used 2-mL aqueous samples, herein we investigated the effect of sample volumes on extraction efficiency. The optimum ratio of aqueous volume to headspace volume for headspace analysis in 7-mL vials was determined by varying the sample volume (amounts of 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 mL). The results are shown in Figure 3. The extracted amount of trihalomethanes increased sharply with increasing sample volume, reached a maximum yield at an aqueous volume of 2 mL, and again decreased at a sample volume of 3 mL. Headspace analysis above 2-mL water sample volume was therefore used for further investigation. The ability to work with larger headspace volume without decreasing the response of the method has an important practical advantage; there is a lower tendency for very small water droplets to stick on the microdrop, which results in a cleaner operation. Similar results have been reported for the headspace solid-phase microextraction (SPME) of methyl tert-butyl ether in water samples (17) and headspace SPME of volatile and semivolatile pollutants in soil (18).

Extraction time

In the HSME method, the amount of analyte transferred into the microdrop is expected to increase with increasing its exposure time to the headspace of the stirred sample solution. However, the HSME is not an exhaustive extraction method, and the analyte is partitioned between the bulk aqueous phase, the headspace, and the microdrop. Thus, the amount of analyte transferred into the microdrop reaches its maximum when the equilibrium is established. For analytes with low volatility, low concentrations on the headspace are expected. Hence, longer time periods are needed for reaching the equilibrium. Also, analytes with higher molecular weights are expected to need longer equilibrium times. This is because of their lower diffusion coefficient. The equilibrium time is inversely proportional to the diffusion coefficient (16). Our results in Figure 4 show that the equilibrium for trihalomethanes is not reached even after 30 min. Nevertheless, for quantitative analysis, it is not often necessary for the analytes to reach the equilibrium. In such a case, allowing sufficient mass transfer into the microdrop in an adopted time should give reproducible extraction efficiencies (8). Thus, in the present work, a 10-min extraction time was adopted for further studies.

Evaluation of the method performance

Dynamic linear ranges (calibration curves) were calculated using 10 spiking levels of trihalomethanes in the concentration range of 1–100 ng/mL. For each spiking level, three replicate analyses were performed. The calibration curves are given in Figure 5. The corresponding regression equations, correlation coefficients (r^2), and dynamic linear ranges are shown in Table I. The limits of detection (LODs) of the proposed method for the determination of trihalomethanes were studied under the optimal experimental conditions. LODs obtained from $C_{LOD} = KS_b/m$ (19), where $K = 3$, S_b is the standard deviation of six replicate blank measurements, and m is the slope of the calibration curve. The LODs obtained were in the range of 2.0–9.1 ng/mL (Table I).

In order to assess the applicability of the newly developed extraction system to real samples, four water samples were obtained from four different pools of Isfahan Tap Water Refining Company (Isfahan, Iran) and tested by the recommended procedure (Table II). It can be seen that the results of three analyses of each sample obtained by the proposed method and amounts

added are in satisfactory agreement. On the other hand, the proposed method revealed good reproducibilities with relative standard deviation values in the range of 2.1–18.0%.

Conclusion

A novel HSME using very low organic solvent (1–4 µL) and sample solution volumes (1–4 mL) was described. There is no need for a delicate and expensive apparatus for the proposed method. Although the precision and accuracy are still not ideal, the extreme simplicity and cost effectiveness of HSME make it quite attractive when compared with the SPME and other labor-intensive methods such as LLE or solid-phase extraction. The results presented in this work confirm the applicability of the proposed method for the determination of trihalomethanes in water samples.

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