Analysis of Chemical Warfare Agents in Water Using Single-Drop Microextraction

Yang-Gi Park, Sung Ki Kim, Kihwan Choi,† Byung-Hoon Son, Ju-Sub Park, and Hong-Ku Kang*

Department of Chemistry and Environmental Analysis, Chemical Defense Research Institute, Seoul 137-180, Korea
**E-mail: hongkukang@empal.com*
†Department of Chemistry, Seoul National University, Seoul 151-747, Korea

Received April 6, 2008, Accepted October 7, 2008

Single-drop microextraction (SDME) is an extraction methodology where the drop plays an essential role as extracts. It was evaluated for the GC-MS determination of nerve agents, one class of the chemical warfare agents (CWAs). Since these nerve agents are highly toxic, it is important to detect the nerve agents in the environmental samples. Several affecting factors including extraction solvents, stirring rate, extraction time, and amounts of salt were optimized. The limit of detections (LODs) were 0.1 - 10 ng/mL and the relative standard deviations (RSDs%, n=5) were in the range of 6.3% to 9.0% for four nerve agents. Without pretreatment of the environmental samples, 5-103 fold enrichments and 48-100% recovery were accomplished. These results demonstrated the feasibility of this method for on-site and off-site analysis of water sample collected from suspicious CWAs site.

Key Words: Single-drop microextraction, Chemical warfare agents, Nerve agents

Introduction

The Organization for Prohibition of Chemical Weapons (OPCW) inspects relevant industrial or military sites to implement Chemical Weapons Convention (CWC) which bans the development, production, stockpiling, and transferring of these dreadful weapons in various countries.[1] This inspection includes various verification activities (on-site analysis by OPCW inspectors in alleged point and off-site analysis designated by OPCW laboratories). A key point of verification activities is to analyze chemical warfare agents (CWAs) regardless of sample types. Therefore, a pre-treatment of CWAs from the complex environmental samples is very important for analyzing its trace. The pre-treatment techniques for analyzing CWAs include liquid-liquid extraction,[2] solid-phase extraction,[3] and solid-phase microextraction.[4] These extraction techniques were adopted when preparing samples of different sample matrices. Each extraction technique has their strengths and weaknesses and can be used according to the properties of sample and matrix.

Recently a fast, simple, inexpensive and solvent free sample preparation technique has been used for extracting CWAs from water. This technique, so called liquid-phase microextraction (LPME) can be performed as a pre-treatment which makes extraction and pre-concentration occur simultaneously. This technique involves hollow fiber protected liquid-phase microextraction[5,6] and single-drop microextraction.[7,8] In case of hollow fiber liquid-phase microextraction, a hollow fiber containing organic solvent is attached into the tip of the syringe needle, and the analytes of interesting are extracted into the organic solvent through a thin layer of a hollow fiber and then detected by GC or GC-MS,[9] typically. After the organic solvent is retracted into the syringe, the hollow fiber is discarded. Therefore, a carry-over effect can be removed. The extraction of CWAs and convention related compounds (CRCs) has been carried out using this technique.[5] The CWAs were directly extracted into organic solvent through hollow fiber and the CRCs having low volatility were extracted and derivatized before GC-MS analysis.[10]

Single-drop microextraction (SDME) was also developed as a solvent minimized sample preparation method, and since small amount of solvent is used, exposure to toxic organic phase can be minimized.[11] A drop of the solvent suspended at the tip of syringe needle is exposed to an aqueous phase. After extraction for a prescribed time, the droplet is retracted into the syringe and is injected into the GC or GC-MS for analysis.[7] Since SDME is simpler and faster than other liquid extraction techniques, this microextraction has been very frequently applied to various studies.[12,13]

In the present report, SDME is applied to the extraction of CWAs from water sample.[7] The chemicals chosen for this study were five highly toxic nerve agents, tabun (GA), sarin (GB), soman (GD), and GF. The objectives of the present study are to investigate the effect of several parameters using this extraction method and to study the applicability of SDME to determine nerve agents in water sample.

Experimental

Reagents and Chemicals. The compounds selected for extraction were four nerve agents (> 98%); ethyl *N,N*-dimethylphosphoramidocyanidate (GA), isopropyl methylphosphonofluoridate (GB), pinacolyl methylphosphonofluoridate (GD), and cyclohexyl methylphosphonofluoridate (GF). The nerve agents were all synthesized at CDRI, the Chemical Defense Research Institute (Seoul, Korea). Figure 1 shows the structures of the nerve agents tested. Organic solvents such as toluene, trichloroethylene, carbon tetrachloride, octane and acetonitrile were purchased from Sigma (St. Louse, MO, USA). Decane as internal standard was also purchased from Sigma. Sodium chloride (NaCl) was obtained from Merk (Darmstadt, Germany). Deionized water was prepared from a NANO pure II purification system (Barnstead, Dubuque, IA, USA) for spiking the aqueous samples. The stock solutions were prepared in acetonitrile with concentration levels of 2000 μg/L for each agent and all of working solutions were prepared at

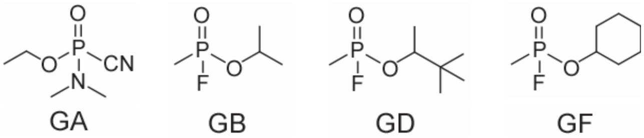

Figure 1. The structure of nerve agents.

10 μg/L concentration by diluting appropriate volume of stock solution with deionized water daily.

The sea water sample was obtained from the sea in front of the Inchen, Korea and the river water sample from the Han River, Seoul, Korea. Finally, field water was collected from Mt. In-rueng, Seoul, Korea. All water samples were collected in polystyrene bottles and filtered through a 0.45 μm microfilters (Millipore, MA, USA).

Safety Considerations. The CWAs are very toxic chemicals. The synthesis and storage should be done in an efficient fume hood with intensified ventilation. Protective mask, glove, and suit should be worn when the agents are handled. A 10% sodium hydroxide solution or other decontaminants should be always prepared and used for decontamination of nerve agents.

GC-MS Analysis. The GC/MS analysis was performed using an Agilent 6890 GC equipped with a mass selective detector (Agilent Technologies, SanJose, CA, USA). Analysis was conducted with a HP-5MS column (30 m, 0.25 mm, 0.25 μm) purchased from J&W Scientific (Folsom, CA, USA). Helium was used as carrier gas and set at 0.7 mL/min. The temperature program used for the analysis was: from 50^{o}C (1 min) to 250^{o}C (21 min) at 10^{o}C/min. The injector was set to 250^{o}C with a splitless mode. The MS system was operated in the electron ionization (EI) mode, and for determination of nerve agents, selected ion monitoring (SIM) mode was performed. A mass scan from *m/z* 50 to 500 was used, and the ions selected for GC-SIM analysis were *m/z* 70 and 133 for GA, *m/z* 99 and 125 for GB, *m/z* 99 and 126 for GD, *m/z* 99 and 137 for GF, and *m/z* 85 and 124 for decane, acting as an internal standard.

Extraction Procedure. A 3-mL volume of aqueous sample was placed in a 4-mL extraction vial containing a PTFF-coated magnetic stirring bar (7 mm×2 mm) and the extraction vial was maintained at 25^{o}C with a circulating water bath. A model #701, 10-μL syringe with a 22° bevel needle tip (Hamilton, NV, U.S.A) was used. Before extraction, the syringe was rinsed with extracting solvent 10 times to avoid bubble formation and contamination. A 1.5 μL of the appropriate organic solvent was drawn into the syringe for the extraction and a 0.5 μL of organic solvent was expelled to remove the air bubbles. After the needle passed through the septum, the needle tip was kept below the surface of the solution and the plunger was depressed until the 1.0 μL droplet of the organic phase was exposed to the sample. Magnetic stirring at 100 - 800 rpm was applied for 10 - 40 min during the extraction. After extraction, the plunger was withdrawn and the drop was retracted carefully into the syringe. The syringe was then transferred to the heated injector of GC/MS system and analysis was carried out. The analytical signal was taken as the area ratio of the analyte peak to the internal standard peak.

Results and Discussion

A SDME is a process of the partition of analyte between the water sample and the extracts. To enhance the extraction efficiency, several parameters such as selection of solvent, stirring rate, extraction time, and addition of salt were investigated. To obtain optimized conditions, the ratio of the peak area of analyte to the peak area of internal standards were used in GC/MS analysis.

Organic Solvent. Partitioning of the analytes between the sample and the extracting solvents depends on the properties of the organic solvents used as extracting solvents. Factors that should be considered in selecting organic solvent are high extraction efficiency, low volatility, low solubility in water, and the compatibility with direct injection into GC system.[13] Several organic solvents such as trichloroethylene, toluene, carbon tetrachloride, and octane were used to investigate the extraction efficiency of nerve agents. The experiments were performed using deionized water solution containing 10 μg/L concentrations of nerve agents for 10 min at stirring of 200 rpm, and three repetitive extractions were successively conducted for each solvent. As illustrated in Fig. 2, trichloroethylene showed higher extraction efficiency. In addition, negligible solubility of trichloroethylene in water (0.1% at 25^{o}C) increased the drop stability. Therefore, trichloroethylene was chosen for subsequent experiments.

Stirring Rate. The effect of stirring rate on the extraction efficiency was investigated between ranges of 0 rpm to the maximum of 800 rpm. As shown in Fig. 3, the extraction efficiency of GD, GF gradually increased up to the 400 rpm, while there was just a little increase after the 400 rpm. These trends were caused by enhancing contacting chance between organic droplet and analytes in an aqueous solution. The extraction efficiency of GA and GB showed only a negligible change with increasing stirring rate. Hence, an optimum allowable stirring rate on the magnetic stirrer was 400 rpm for various analytes. These trends are also consistent with the theory proposed by Nernst and further developed by Lewis and Whitman. The aqueous phase mass transfer coefficient is defined by[12]

$$\beta_{aq} = D_{aq} / \delta_{aq} \tag{1}$$

where δ_{aq} is the thickness of liquid layer, D_{aq} is the diffusion

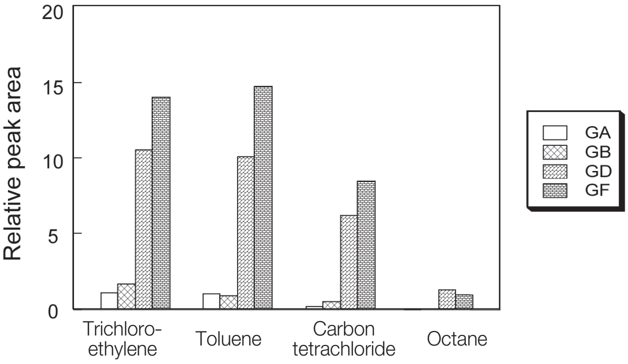

Figure 2. Extraction efficiency of nerve agents with different organic solvents for trichloroethylene, toluene, carbon tetrachloride, and octane. Extraction conditions: concentration, 10 μg/mL; extraction time, 10 min. n=3

coefficient in the aqueous phase. According to “Eq. (1)”, it is concluded that extraction efficiency increases with increasing stirring rate, because faster stirring makes δ_{aq} decrease, so that the value of β_{aq} gets bigger. As a result, we selected the 400 rpm as an optimum stirring rate.

Extraction Time. To investigate the effect of extraction time, extractions were performed at 10, 20, 30, and 40 min. As can be seen in Fig. 4, the amount of extracted GD and GF increased with increasing the extraction time up to 30 min. After 30 min, enrichment of GD, GF slightly increased. For GA and GB, enrichment became flat from 10 min. It seems that equilibrium between two phases was established within 10 min. Since dissolution of microdrop was observed after 40 min, 30 min was selected as the optimum extraction time.

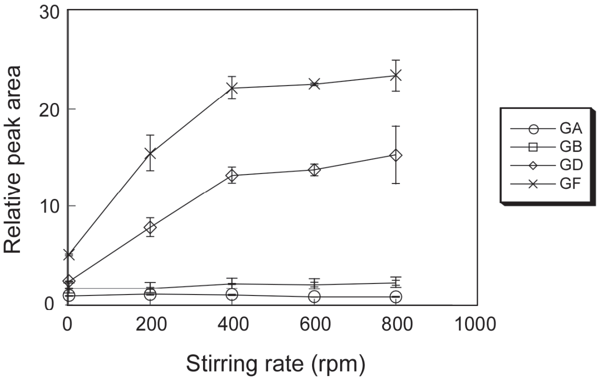

Figure 3. Influence of stirring rate on the extraction efficiency. Extraction conditions: concentration, 10 μg/mL; organic solvent, trichloroethylene; extraction time, 10 min. n=3

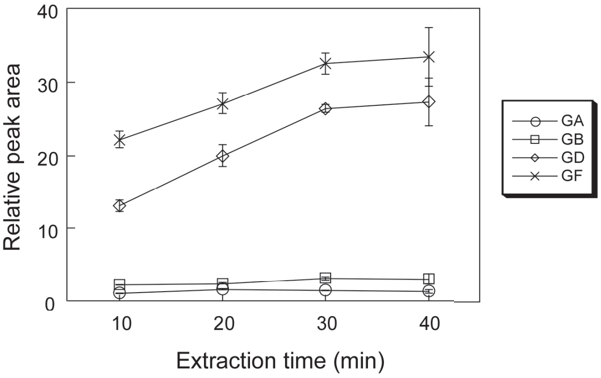

Figure 4. Influence of extraction time on the extraction efficiency. Extraction conditions: concentration, 10 μg/mL; organic solvent, trichloroethylene; stirring rate, 400 rpm. n=3

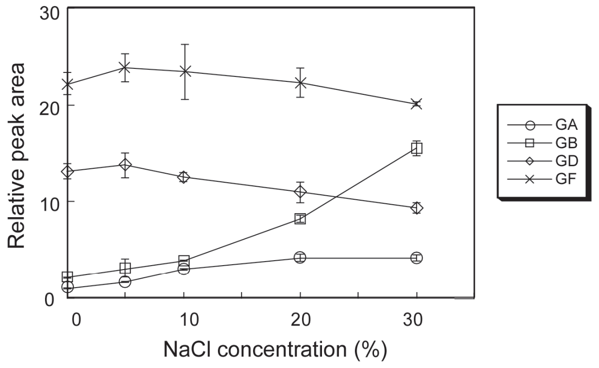

Figure 5. Influence of salt addition on the extraction efficiency. Extraction conditions: concentration, 10 μg/mL; organic solvent, trichloroethylene; stirring rate, 400 rpm; extraction time, 10 min. n=3

Salt Addition. The addition of salt generally increases the extraction efficiency. Since the salt reduces the solubility of analytes in water, it makes the diffusion of analytes to organic phase easy (salting out effect). However, there are cases that the extraction efficiency does not change despite the addition of salt.[9,15] The reason is generally due to the physical properties of the Nernst diffusion film by dissolved salt that reduce the rate of diffusion of the analytes into the drop.[14,15] These two effects by the addition of salt were shown in Fig. 5. With the gradual addition up to 30% of NaCl, the extraction efficiency of GA and GB was increased, while extraction efficiency of GD and GF was slightly decreased. 20% NaCl was selected as optimum condition since extracted amount of GA was not increased and that of GD and GF was decreased with 30% NaCl.

Quantitative Analysis. Under the optimized conditions, repeatability, linearity, and limit of detection (LODs) were determined. The trichloroethylene microdrop contained 80 μ g/ml of decane as internal standard in order to quantify the nerve agents. As shown in Table 1, the linearity was observed in the range of 0.01-1 μg/mL for nerve agents. The correlation coefficient (r^2) ranged from 0.9872 to 0.9953. The LODs were 2, 2, 0.2, and 0.1 ng/mL at a signal-to-noise ratio of 3, and the enrichment factors (EF) 33.7, 8.7, 108.7, and 93.7 were for GA, GB, GD, and GF, respectively. The good LODs satisfy the requirement (1-10 μg/ml) of OPCW for analysis of trace CWAs. The assessment of repeatability was investigated in five replicate experiments. The relative standard deviations (RSDs%) was from 6.3% to 9.0% for nerve agents.

Matrix Effects. To evaluate the feasibility of the SDME method for extraction from water sample, sea, river, and field water samples were applied. The environmental water samples were extracted using the SDME method in optimized

Table 1. Linearity, RSDs% (n=5), limit of detection (LOD), and enrichment factor (EF) for the SDME of nerve agents from deionized water

Compound	Linear range (μg/mL)	R^2	RSDs% (n=5)	LOD (ng/mL)	EF
GA	0.01 - 1	0.9872	9.0	2	33.7
GB	0.01 - 1	0.9909	8.6	2	8.7
GD	0.01 - 1	0.9953	7.3	0.2	108.7
GF	0.01 - 1	0.9970	6.3	0.1	93.8

Table 2. Calculated relative recovery (RR) and enrichment factor (EF) of nerve agents spike to various water samples. n=3

Compound	Sea water		River water		Field water	
	RR (%)	EF	RR (%)	EF	RR (%)	EF
GA	84.4	28.4	74.6	25.1	87.3	29.4
GB	56.6	4.9	93.7	8.1	78.1	6.8
GD	70.1	76.2	95.2	103.4	85.0	92.3
GF	48.0	45.0	99.5	93.3	76.8	72.0

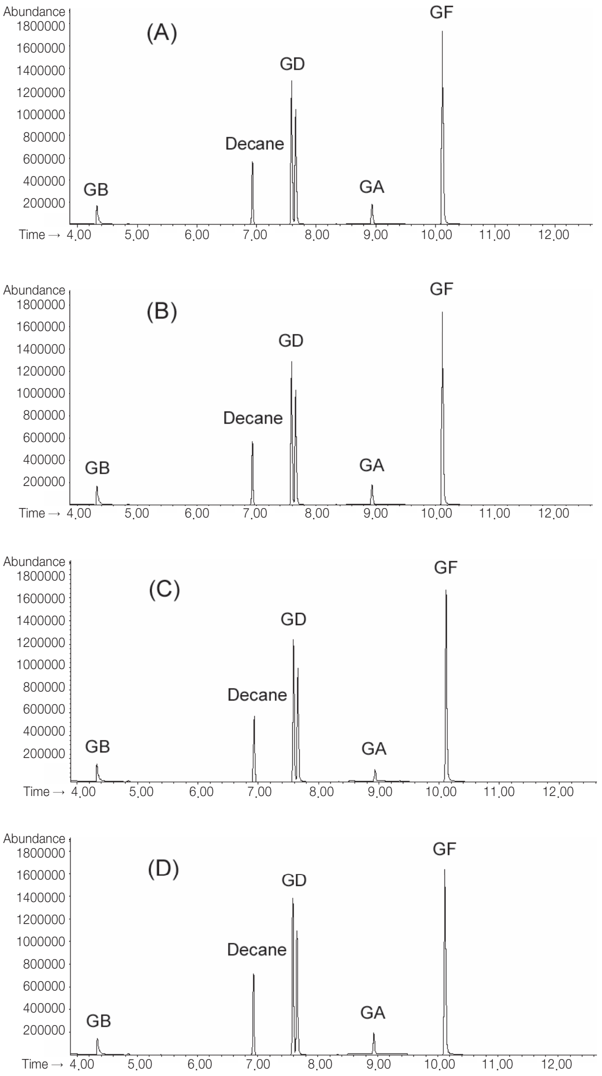

Figure 6. Total ion chromatography of nerve agents in GC-MS analysis obtained by using optimized conditions. (A) Deionized water sample, (B) sea water sample, (C) river water sample, and (D) field water sample. Extraction conditions: concentration, 1 μg/mL; organic solvent, trichloroethylene; stirring rate, 400 rpm; extraction time, 30 min; salt concentration, 20%.

conditions (organic solvent: trichloroethylene, extraction time: 30 min, stirring rate: 400 rpm, salt: 20%) and analyzed by GC-MS. All of the water samples were spiked with four nerve agents at 1 μg/mL concentration level to assess the matrix effects. A typical total chromatogram (TIC) of nerve agents obtained by SDME is shown in Fig. 6. The two peaks of GD of chromatogram are due to its characteristics having two isomers. The resolution and peak shapes after SDME of sea, river, and field water samples are comparable with that of deionized water. The relative recovery is defined as the ratio of the signal intensity of nerve agents spiked in environmental waters to that of the same amount spiked in deonized water.[13] This value is shown in Table 2. As we can see, the relative recovery in seawater for four nerve agents was lower than that of river and field water. That is because the cations presented in seawater catalyze degradation of nerve agents[4] and a basic condition of seawater increase the hydrolysis of nerve agents during the extraction. Also, the enrichment factors (EF, defined as the ration of the peak area obtained after SDME to the peak area obtained with no extraction) were 5(GB) and 76(GD) for sea water, 8(GB) and 103(GD) for river water, 7(GB) and 92(GD) for field warter. (Table 2) A good recovery and enrichment of analytes proved that this method is versatile for the analysis of nerve agents in environmental samples.

Conclusion

In the present work, SDME combined with GC-MS was applied to analyze several kinds of nerve agents (GA, GB, GD, and GF) in water samples. The optimized extraction conditions of solvent selection, extraction time, stirring rate, and salt content were trichloroethylene, 30 min, 400 rpm, and 20%, respectively. Also, low LODs (2 - 0.1 ng/mL) and good repeatability (6.3% - 9.0%) showed the possibility which can be used for the verification of CWAs. And because of not only quick procedure and minimal requirement but also precision and reproducibility, SDME is expected to be useful and suitable method to verify CWAs.

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