〈特別研究課題〉　機能性高分子・繊維充填型ニードルを応用した
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機能性高分子·繊維充填型ニードルを応用した精密室内環境測定

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Novel Needle-Type Sample Preparation Device Packed with Polymeric and Fibrous Materials for the Analysis of Various Volatile Compounds in In-Room Environments

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Chapter 1

Analysis of Volatile Organic Solvents by Polymer-Packed Sample Preparation Device

1.1 Abstract

　A novel in-needle extraction device has been developed for the preconcentration of gaseous organic compounds prior to the determination in gas chromatography (GC). As the extraction medium, a copolymer of methacrylic acid and ethylene glycol dimethacrylate was synthesized. Based on the results in the preliminary experiments, the desorption conditions such as desorption temperature and time have been optimized along with the evaluation of the extraction efficiency. The storage performance of the needle extraction device was also studied. The results clearly demonstrated the excellent extraction performance for typical organic solvents and also suggested the future possibility such as in the applications for the analysis of working environments.

1.2 Introduction

Miniaturization of sample preparation process has been recently focused, especially in separation science and the related research fields, because the down-sizing could be one of the most promising solution to realize the recent requirements such as improved selectivity and sensitivity, and also low-cost and environmentally-friendly features [1-4]. On-line coupling of the novel miniaturized sample preparation methods to the corresponding microscale separation system was also studied in order to ensure the effective hyphenation between these techniques [3]. As a miniaturized sample preparation method in liquid chromatography (LC), in-tube solid-phase microextraction (in-tube SPME) was developed, where a section of open-tubular column in gas chromatography (GC) was introduced as the extraction medium [5-10]. Several other miniaturized sample preparation processes for the subsequent LC separation have been proposed such as wire-in-tube SPME [11], fiber-in-tube solid-phase extraction (fiber-in-tube SPE) [12-16] and liquid-phase microextraction (LPME) [17-19], and the successful on-line coupling of these sample preparation and separation techniques has been demonstrated as summarized in some recent review articles [3,20,21].

For the GC analysis of volatile organic compounds, solvent-less microscale sample preparation methods have been developed [22-25]. SPME is one of the most successful examples having attractive features over traditional sample preparation methods with a large amount of organic solvents [22,23], however, it requires a careful handling during the extraction and desorption processes, where the exposed fused-silica rod coated with polymeric materials is fragile. Needle-type microextraction device is alternative sample preparation device [26-34]. Recent studies showed that the needle-type microscale sample preparation possesses the most of the advantageous features of the SPME, but without the above disadvantage, for the preconcentration of volatile organic compounds prior to the GC separation. Compared to the SPME, a wide variety of the extraction media could be used and easy to handle during the extraction and desorption processes that will be an attractive feature for automation and on-line coupling to typical GC instruments, although a continuous flow of the gaseous sample through the extraction needle should be supplied during the sampling.

In this work, a novel in-needle sample preparation device [34] was introduced for the GC analysis of several organic solvents commonly used in typical chemical laboratory. Because of a wider selectivity for both polar and non-polar analytes, a copolymer of methacrylic acid and ethylene glycol dimethacrylate, typically employed as the material for molecularly imprinted polymers (MIPs) [35,36], was synthesized to prepare the extraction medium instead of other polymeric materials such as polystyrene-divinylbenzene (PS-DVB) and polydimethylsiloxane (PDMS). A relatively easier synthetic procedure is another advantageous feature of the present method. The polymeric beads were packed into a section of a specially prepared needle. During the sampling of gaseous sample mixtures a vacuum sampling device was attached to the needle extractor, while a desired volume of the samples could be passed through the needle. The extraction needle was then transferred to the heated GC injection port for the thermal desorption, and the simultaneous injection to the GC column was made. The extraction performance of the polymer-packed needle extractor has been evaluated. Taking into

account the applications including the working environment analysis, the storage power of the needle device for the extracted analytes under the room temperature was also studied along with the consideration to the repeatability for run-to-run and the reproducibility for needle-to-needle.

1.3 Experimental

Reagents

All reagents, solvents and sample solutes were of analytical grade and purchased either from Kishida Chemical, Osaka, Japan or Tokyo Kasei Kogyo, Tokyo, Japan unless otherwise specified. Water was purified by a Milli-Q Water Purification System (Millipore, Tokyo, Japan).

Preparation of Standard Samples

Standard gas samples were prepared with two-step or three-step dilution of each organic solvent. First an appropriate amount of the organic solvent was injected into a vacuum glass vessel of 1.0 L volume to prepare the corresponding gas sample of 10 μg/mL, where N_2 gas was used for the dilution after vaporizing the organic solvent in the vessel. Next, 10 mL of the above gas sample was injected to a gas sampling bag (Tedlar Bag; GL Sciences, Tokyo Japan) of 1.0 L volume followed by the dilution process with N_2 (990 mL) therein, resulting the gas sample of 100 ng/mL. In the case of the standard sample having lower concentration, a similar process was carried out once more. The standard samples containing two or more organic solvents were also prepared in a similar manner.

Synthesis of Polymer-Based Extraction Medium

First, 15 g of poly(vinyl alcohol) having a degree of polymerization of 500 were dissolved into 500 mL of water at 50℃, and then sodium chloride (15 g) was also dissolved. To the solution, the mixture of methacrylic acid (2.58 g; 0.03 mol), ethylene glycol dimethacrylate (29.7 g; 0.15 mol), di-*n*-butyl phthalate (27.44 g) and azobisisobutyronitrile (0.52 g) was added drop wise over about 3 min, while the solution was kept at 50℃. Next, the solution was subject to the temperature-programmed heating at the rate of 1.0℃/min to 85℃. The polymerization reaction was initiated at about 80℃. In order to ensure the successful polymerization, the temperature of the reaction mixture was maintained at 85℃ for 1 h with vigorous stirring. After the polymerization reaction was completed, the resulting beads of the copolymer was sequentially washed with hot water, acetone, water and acetone (for five times each), and let dried at the room temperature.

The polymer-beads were sieved to obtain the spherical beads having the diameter between 150 and 180 μm. The specific surface area (SSA) measured by the nitrogen adsorption method was 390 m^2/g. The beads were packed into the specially designed needle (85 mm x 0.5 mm I.D., 0.7 mm O.D.) as illustrated in Figure 1, where a section of about 30 mm length was packed with the copolymer beads. To place the beads in the appropriate position in the needle, a small amount of a heat-resistant polymer fiber [37,38] was also packed at the both end of the packed section. The typical amount of beads packed in the needle is about 2 mg.
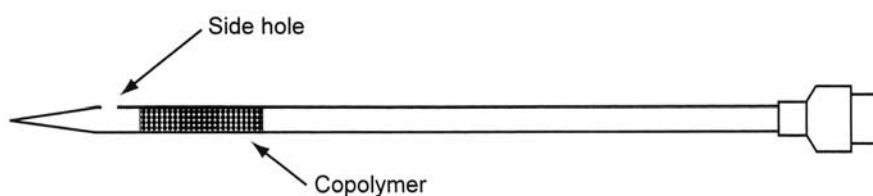
Figure 1. Illustration of the in-needle sample preparation device

Extraction Procedure

The needle was attached to a commercially-available vacuum sampling device (Komyo Rikagaku Kogyo, Tokyo, Japan). For the extraction process the needle was inserted into the gas sampling bag containing the standard sample, while the sample gas was introduced to the vacuum sampling device via the extraction needle. Typical sampling volume was 50 mL, and it takes about 6 min to complete the sampling with the vacuum sampling device. After the extraction, the needle was attached to a injection syringe (1.0 mL) containing N_2 gas, and inserted to the injection port of the GC. The desorption and injection were made simultaneously by injecting the N_2 through the needle at the heated injection port, where the needle was heated for several seconds before the N_2 injection. The desorption temperature, the desorption time and the volume of the N_2 has been optimized as described below.

GC Measurements

An Agilent 6890N gas chromatograph (Yokogawa Analytical Systems, Musashino, Japan) with a split/splitless injection port and a flame ionization detector (FID) was used for all the GC measurements. All the injections were made by split mode with the ratio of 25:1. As the carrier gas, N_2 was used, and the carrier gas and air were supplied from the respective gas cylinders through the cartridge packed with molecular sieve. For the GC separation, a fused-silica column having a poly(ethylene glycol) coating, HR-20M (30 m x 0.25 mm I.D., d_f= 0.5 μm; Shinwa Chemical, Kyoto, Japan) was employed with an appropriate preconditioning before the use. Typical injection and detection temperatures were 200 and 220℃, respectively. The other separation conditions such as carrier gas flowrate, column head pressure, and temperature programs were determined by the results of preliminary experiments for each samples. All GC measurements were done at least three times, and the relative standard deviations (RSDs) for the retention times were less than 1.0%. The data collection was made with Borwin Chromatography Data Handling Software (Jasco, Tokyo, Japan) running on a personal computer.

1.4 Results and Discussion

Optimization of Desorption Conditions

For the optimization of desorption conditions, D (%) has been defined as the following equation:

$$D\ (\%)\ =\ \frac{\text{Peak area in the 1st desorption}}{\text{Total peak area in the 1st and 2nd desorption}}\times 100$$

where the D (%) was calculated based on the ratio of the peak area obtained in the 1st desorption to the total peak area obtained as the sum of the peak area in the 1st and 2nd desorption. The definition of the above equation was derived from the results in the preliminary experiments, in which the desorption of more than 99.99% of the analytes was made in the first two desorption for the sequential desorption process for more than three times. That means, the quantitative desorption could be evaluated based on the above equation.

The effect of desorption temperature was studied by setting the temperature of the GC injection port between 150 and 230℃. For the optimization of desorption conditions, the extraction conditions were tentatively determined based on the preliminary experiments, however, the validity of those conditions have been confirmed as described below. As found in Figure 2, the desorption for these two typical organic compounds was almost quantitatively made at about 180℃, however, at the same time, a partial decomposition of the copolymer at the temperature of higher than 220℃ was also found, although more systematic investigations should be needed to precisely evaluate the thermal stability of the copolymer by the thermogravimetric analysis (TGA). In addition, the extraction power was maintained in the repeated use, typically more than 100 times with the desorption temperature at lower than 210℃. This is quite consistent with the above observation for the partial decomposition of the copolymer started at about 220℃. Therefore, the desorption temperature of 200℃ was used in the following experiments.
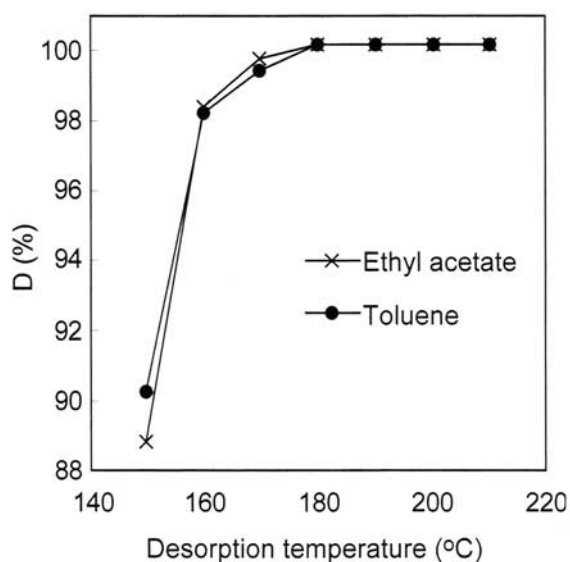

Figure 2. Optimization of the desorption temperature. Extraction conditions: desorption time, 10 sec; injection volume of N_2, 0.5 mL. Chromatographic conditions: column head pressure, 50 kPa; column temperature 150℃ (isothermal). Other conditions are in the text.

The optimization of the desorption time, that is the preheating time of the needle at the injection port before injecting the desorption gas, was carried out as shown in Figure 3. The results showed that nearly perfect desorption was obtained with the desorption time of 7 s. However, a slight peak broadening, especially for the analytes eluted earlier, was found in the preliminary experiments if the desorption time was more than 10 s. A longer preheating time is also not desirable in terms of the analysis time, therefore, the desorption time of 10 s was chosen as the optimum value and used in all the experiments below.

Similar optimization for the volume of the desorption gas was conducted. The results summarized in Figure 4, indicating that more than 0.4 mL of the N2 gas is necessary for the complete desorption of the analytes. Even a larger volume could be injected to conventional packed column, the volume of 0.5 mL was determined as the optimum volume for split injection to the typical open-tubular capillary column used in this work.
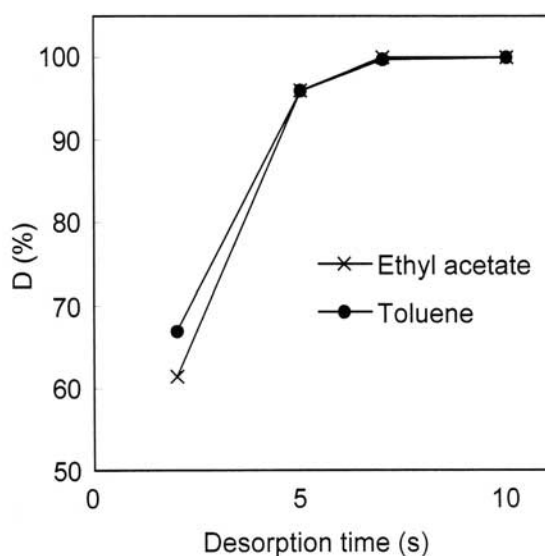

Figure 3. Effect of the desorption time. Conditions: desorption temperature, 200℃; injection volume of N_2, 0.5 mL. Other conditions are the same as in Figure 2.
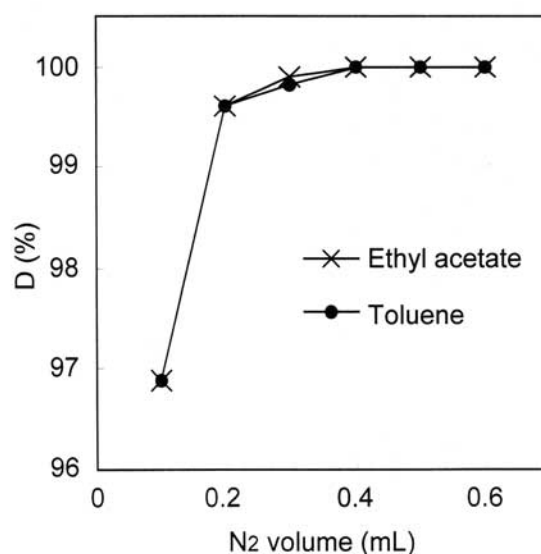
Figure 4. Evaluation of the optimum N_2 volume for the desorption. Desorption temperature and time, 200℃ and 10 sec, respectively. Other conditions are the same as in Figure 2.

Evaluation of Preconcentration Performance

Taking into account the concentration of the standard gas sample of 100 ng/mL each and the sampling volume of 50 mL, the total amount of these organic compounds should be 5 μg each, if assuming the complete extraction. Table 1 summarize the linear calibration ranges and the corresponding correlation factors for each sample probes. The data clearly demonstrated the practical extraction power for the quantification of typical gaseous organic compounds in the laboratory, although the extraction for more complex sample mixtures should be further studied.

On successful optimization of the desorption conditions, the extraction efficiency was evaluated. A standard gas sample containing 5 ng/mL each of four typical organic solvents, hexane, acetone, ethyl acetate and toluene was prepared, and 50 mL of the sample was extracted with the needle extraction

Table 1. Linear range of the calibration curve and the correlation coefficients.

	hexane	acetone	ethyl acetate	toluene
Linear range (ng/mL)	1-500	1-400	1-600	1-1000
r	0.997	0.999	0.999	0.999

N_2 volume: 0.5 mL.   Other conditions are the same as in Fig. 4.

device, while another sample containing 500 ng/mL each was also prepared for the direct injection of 0.5 mL volume. Figure 5 shows the comparison of the needle extraction with the direct injection, and the obtained peak area data were summarized in Table 2. Compared to the direct injection of 0.5 mL sample, the extracted sample generates relatively sharp peak shape, suggesting the extraction was made at short section of the extraction medium and desorbed quickly during the desorption gas injection of even the same volume. The quantitative extraction by the needle device could be confirmed in Table 2, because the total amount of each analyte is the same in these two standard samples.

Reproducible and repeatable extraction could be one of the most important requirements as an extraction technique, especially in the case of multiple and multipoint sampling for environmental analysis. In Table 3, the RSDs for run-to-run (n=5) and needle-to-needle (n=5) measurements were tabulated. Although the RSDs for needle-to-needle were somewhat larger than that of run-to-run, as expected, these data demonstrated that the needle extraction will offer a good repeatability and reproducibility.
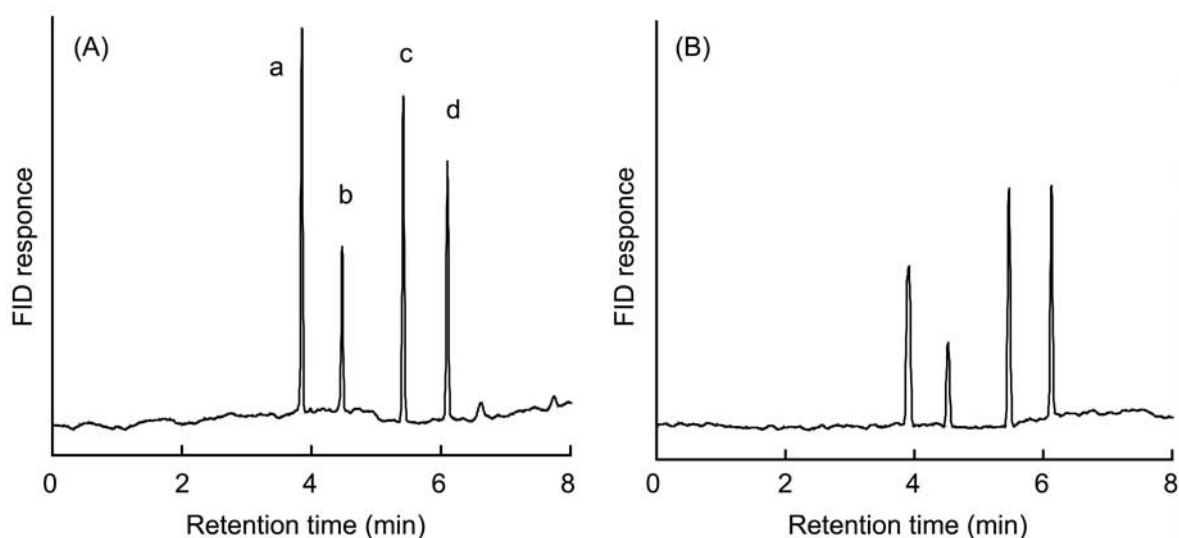

Figure 5. Chromatograms with (A) and without (B) the sample preconcentration by the needle extraction device. Column temperature was programmed from 80℃ to 160℃ at the rate of 10℃/min. Other conditions are the same as in Table 1. Peaks: (a) hexane; (b) acetone; (c) ethyl acetate; and (d) toluene.

Table 2. Comparison of the peak area obtained with the needle extraction and the direct injection.

	hexane	acetone	ethyl acetate	toluene
Direct injection *	3059	1275	3039	2522
Needle extraction **	3063	1388	3139	2588
Recovery (%)	100.1	108.9	103.3	102.5

*Direct injection: sample concentration, 500 ng/mL; injection volume, 0.5 mL.
**Needle extraction: sample concentration, 5 ng/mL; sampling volume, 50 mL.

Table 3. Relative standard deviations (RSDs) of the peak area for the run-to-run and needle-to-needle measurements.

	hexane	acetone	ethyl acetate	toluene
run-to-run	3.11	4.26	1.78	2.80
needle-to-needle	8.52	5.38	7.91	8.44

Sample concentration: 100 ng/mL each.   Other conditions are the same as in Fig. 5.

Table 4. Storage performance of the needle extraction device for various organic solvents over 7 days at room temperature.

		hexane		acetone		ethyl acetate		toluene	
		%Rec.*	%RSD	%Rec.*	%RSD	%Rec.*	%RSD	%Rec.*	%RSD
3 days	1 ng/mL	106.5	4.6	101.0	4.7	95.76	4.3	96.33	4.0
	10 ng/mL	98.67	2.7	102.8	3.0	104.1	3.7	93.88	2.6
	100 ng/mL	101.1	5.8	101.2	6.3	106.8	7.3	97.77	6.4
7 days	1 ng/mL	91.11	3.3	96.88	5.7	92.32	4.1	92.38	2.8
	10 ng/mL	105.8	6.1	101.2	5.1	99.65	4.6	90.77	3.3
	100 ng/mL	103.3	0.8	98.87	1.1	95.79	2.5	82.12	1.4

* %Rec.: Recovery (%).   See the text for the definition.   Conditions are the same as in Fig. 5.

Storage Performance of the Needle Extractor

As a gas sampling device, the storage performance will be an advantageous feature, especially for the multipoint sampling at the same time. To evaluate the storage performance of the needle extraction device, the needle was stored up to 7 days at the room temperature, with the PTFE caps at both ends of the needle, after the sampling. The results are summarized in Table 4, where the recovery (%) was calculated on the basis of the immediate quantification by the needle extraction. The excellent storage power of the needle extraction device was shown. The stability of aromatic solutes for the long storage time, more than 3 days, might be improved further by changing the chemical structure of the copolymer.

1.5 Conclusions

Miniaturized sample preparation with in-needle extraction device has been successfully developed for the sample preparation of volatile organic solvents. The specially designed needle was packed with porous beads of the polymeric material that showed the excellent performance for the extraction and a suitable thermal stability for typical analysis in GC. The extracted analytes were quantitatively desorbed by simply passing N_2 at the heated injection port in GC. The extracted samples could be determined even after the storage at the room temperature for several days.

More extensive work is currently underway in our laboratory including, the coupling of the needle extraction device to the polymer-coated fiber-packed capillary columns with a higher sample loading capacity, the design and synthesis of the novel polymeric extraction medium, and also the applications of the in-needle extraction technique to the in-room environmental analysis.

1.6 References

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Chapter 2

Analysis of Volatile Aldehydes with Simultaneous Derivatization/Preconcentration
by Fiber-Packed Sample Preparation Device

2.1 Abstract

   Novel in-needle sample preparation device has been developed for the determination of volatile aldehydes in gaseous samples. The needle device is designed for the gas chromatographic (GC) analysis of aldehydes and ketones commonly found in typical in-house environments. In order to prepare the extraction device, a bundle of the polymer-coated filaments was longitudinally packed into a specially-designed needle. Introducing the derivatization reaction with 2,4-dinitrophenylhydrazine (NDPH) in the needle, the aldehydes and ketones were derivatized to be the corresponding hydrazones and simultaneously extracted in the extraction needle. The reproducible preparation of the extraction needle was established along with the repeatable derivatization/extraction that ensure the successful determination of aldehydes. The storage performance of the extraction needle was also evaluated at the room temperature for three days. The results demonstrated the successful

applications of the fiber-packed extraction device for the sample preparation of gaseous sample of aldehydes, and the future possibility of the extraction device for the analysis of the in-house environments.

2.2 Introduction

Miniaturized sample preparation techniques have been increasingly studied in the research field of separation science due to the promising possibility to meet the recent requirements such as enhanced sensitivity and selectivity, and more economical and ecological features [1-3]. The effective hyphenation of the miniaturized sample preparation to microscale separation methods was also investigated, allowing the successful on-line coupling of these techniques without disadvantages that typically found in an off-line multistep process for the analysis of complex sample mixtures [3]. For the liquid-phase separation methods such as liquid chromatography (LC) and capillary electrophoresis (CE), various types of novel sample preparation techniques have been reported, where most of the publications were mainly focused on the increased sensitivity and selectivity of the target analytes with a specially-designed concentration process [4-10]. Downsizing the sample preparation it could make possible to obtain the enhanced mass sensitivity and reduce the sample size, although the suitable optimization of the hyphenated system should be considered.

As the sample preparation designed for the analysis of volatile compounds in gas chromatography (GC), solid-phase microextraction (SPME) [11,12] is one of the successful approaches with several attractive features over traditional sample preparation techniques that require a significant amount of organic solvent in the procedure. A wide variety of the SPME applications have been developed, however, conventional SPME requires a careful handling during the extraction and desorption processes, because the exposed fused-silica rod with polymeric coating is somewhat fragile. Alternative sample preparation techniques were developed with a needle-shaped extraction device [13-19]. It has been shown from the recent studies that the needle-type microscale sample preparation has the most of the advantages of the conventional SPME, but without the above disadvantage for the preconcentration of volatile organic compounds prior to the analysis in GC. For the needle-type extraction, a continuous flow of the gaseous sample through the extraction needle should be supplied during the sampling process, however, a wide variety of the extraction media can be employed and easy to handle during the extraction and desorption processes, which must be additional attractive features for the automation and on-line coupling to typical GC instruments.

Introducing the synthetic fibers as extraction medium, novel miniaturized sample preparation has been developed, where several hundreds of the polymeric filaments were longitudinally packed into a short capillary to prepare the extraction cartridge [20-26]. The method, so-called fiber-in-tube solid-phase extraction (FIT-SPE), was successfully applied to the sample preparation of complex sample mixtures, especially in LC [20-25]. Using the FIT-SPE technique, a trace amount of analytes could be extracted onto the surface of the fine filaments in the extraction capillary by simply passing the aqueous sample solution into the extraction tube. The extracted analytes are desorbed by pumping a small amount of

solvents in a similar manner and are simultaneously transferred to the injection loop of LC system for the subsequent chromatographic separation. Several advantageous features can be found with the FIT format such as: an increased surface area of the extraction medium and a reduced pressure drop through the extraction capillary.

On the basis of the above successful applications of synthetic fine fibrous materials as the medium for the sample preparation in liquid phase separations, a novel in-needle sample preparation device was developed in this work, which is designed for the GC analysis of aldehydes and ketones commonly found in typical in-house environments. Simultaneous derivatization with 2,4-dinitrophenylhydrazine (DNPH) was made during the sampling process of the gaseous sample, and the derivatized analytes were desorbed by passing a small amount of organic solvent through the extraction needle in the heated GC injection port. The basic extraction performance of the fiber-packed needle extractor has been evaluated along with the optimization of several experimental parameters on the derivatization, extraction and desorption. Taking into account the applications including the in-house environment analysis, the storage power of the needle device for the extracted analytes under the room temperature was also studied.

2.3 Experimental

Reagents

All reagents, solvents and sample solutes were of analytical grade and purchased either from Wako Pure Chemical Industries, Osaka, Japan or Tokyo Kasei Kogyo, Tokyo, Japan unless otherwise specified. Water was purified by a Milli-Q Water Purification System (Millipore, Tokyo, Japan).

Preparation of Standard Samples

Standard gas samples were typically prepared with two-step or three-step dilution of each sample solute. First an appropriate amount of the solute was injected into a vacuum glass vessel of 1.0 L volume to prepare the corresponding gas sample of about 2.0-3.0 mg/L, where N_2 gas was used for the dilution after vaporizing the organic solvent in the vessel. Next, 10 mL of the above gas sample was injected to a gas sampling bag (Tedlar Bag; GL Sciences) of 1.0 L volume followed by the dilution process with N_2 (0.99 L) therein, resulting the gas sample concentration of about 20-30 μg/L.

In the case of the standard sample having lower concentration, a similar process was carried out once more. The standard samples containing two or more samples were also prepared in a similar manner. For the preparation of formaldehyde gas sample, a home-made vaporizer was used, in which solid paraformaldehyde was vaporized to generate the gaseous standard sample followed by a similar multi-step dilution process as described above.

Polymer Coating onto the Filaments

Prior to the packing process into the needle-shaped device, the polymer-coated filaments have been prepared as the same procedure as previously described [27-32], where the coating treatment was made in

a conventional fused-silica capillary of 1 m length packed with a bundle of the filaments. A bundle of filaments (166±2 filaments) was longitudinally packed into the fused-silica capillary of 0.32 mm i.d. To ensure the reproducible preparation of fiber-packed capillary, the total number of packed-filaments was precisely counted before and after the packing process. As the coating material for the preparation of polymer-coated filaments, HR-1, 100%-methyl-polysiloxane (Shinwa Chemical Industries, Kyoto, Japan) was employed.

First, a fiber-packed capillary was connected to the pressure-proof vessel containing 10 mL of acetone and washed with the solvent pumped by N_2 gas at the pressure of 500 kPa. After the same volumes of the following solvents; water, acetone and chloroform, were pumped in the similar manner, the capillary was allowed to dry at room temperature for about 2 h using N_2 gas flow. Second, the capillary was subject to heating in a GC oven with the flow of N_2 gas. The temperature was programmed from room temperature to 300℃ at 2℃/min and then held for about 10 h. Next, the solution of the coating material (typically 3%) in n-hexane containing a cross-linking reagent (0.8% to the polymer), benzoyl peroxide, was pumped through the packed capillary. After the total volume of the polymer solution (0.5 mL) was pumped, the N_2 flow was maintained for more than 5 h. Then, the column was installed in the GC oven again and the programmed heating was carried out as follows: from 40℃ to 300℃ at 0.5℃/min and then hold for more than 48 h to ensure the complete reaction.

The successful polymer-coating treatments were confirmed by the separation of a standard sample containing three n-alkanes, n-dodecane, n-tetradecane and n-hexadecane on the polymer-coated fiber-packed capillary as a GC column [28], and the relative standard deviations (RSDs) for the retention factors were less than 2.0% on three capillaries separately prepared with the same coating, where the average values of the retention factors for three consecutive runs on each capillary were used for the calculation. The RSDs for multiple injection onto the same capillary were less than 1.5% (*n*=3) for all the capillaries studied.
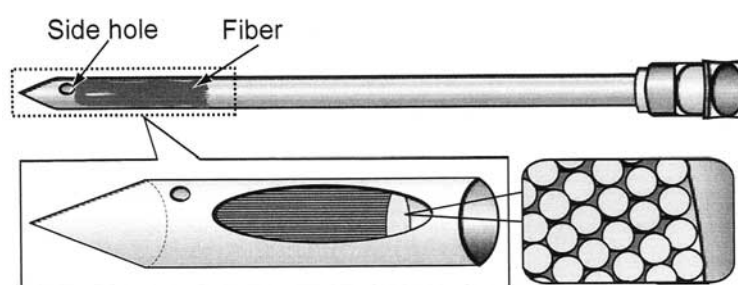

Figure 1. Miniaturized sample preparation device prepared with polymer-coated fibrous material.

Preparation of the Extraction Needle

For the preparation of the extraction needle, a bundle of the polymer-coated filaments was taken out from the capillary, and packed into the specially-designed needle [19] (85 mm x 0.5 mm i.d., 0.7 mm o.d.) as illustrated in Figure 1, where a section of about 20 mm length was packed with the filaments, and one end of the packed section was positioned just before the side hole of the needle. The number of

filaments packed was 830, and the corresponding RSD (*n*=5) was less than 1.5%.

In order to ensure the parallel alignment of all the polymer-coated filaments in the needle, the packing process (Figure 2) was carried out [24,32]: (1) insert an appropriate length of poly(vinylidene fluoride) (PVF) fishing line (25 μm o.d.) as the first guide fiber (line (a) in Figure 2) into the needle, while the guide fiber should have an extra length to form a loop at the outside of the needle; and (2) then the second PVF guide fiber (line (b) in Figure 2) is inserted into the loop of the first guide fiber; (3-4) pull the first guide fiber from the other end of the needle, resulting another loop of the second guide fiber; (5) the bundle of the filaments (40 mm length for 20 mm packed section) to be packed is inserted into the loop of the second guide fiber, where the front-end of the bundle should be appropriately bended to make sure the smooth introduction; (6) pull the second guide fiber from the side hole of the needle with careful attention to produce the uniform introduction of the bundle; and finally (7) carefully pull out the second guide fiber, followed by the attachment of the syringe connector to the resulting polymer-coated fiber-packed needle as depicted in Figure 1.
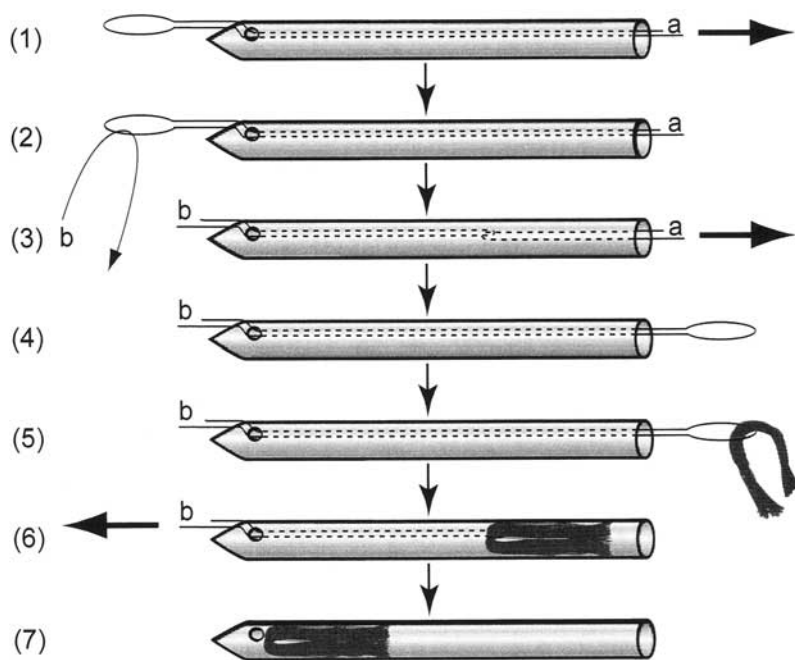

Figure 2. Packing procedure of the polymer-coated filaments into the needle-type extraction device. Two lines indicated as (a) and (b) are the guide fibers for the packing process.

GC-MS Measurements

An Agilent 6890N gas chromatograph (Yokogawa Analytical Systems, Musashino, Japan) with a split/splitless injection port and a HP 5972A mass selective detector (MSD) was used for all the GC measurements. All the injections were made by split mode with the typical ratio of 5:1. As the carrier gas, helium was used. For the GC separation, a fused-silica column having a PDMS coating, DB-1 (15 m x 0.25 mm i.d., d_f= 0.25 μm; J&W Scientific, Folsom, CA, USA) was employed with an appropriate preconditioning before the use. The mass spectrometer was operated either total ion monitoring mode

(TIM) or selected ion monitoring mode (SIM) with electron impact ionization. The ionization voltage was 70eV. The other separation conditions such as carrier gas flowrate, column head pressure, and temperature programs were determined by the results of preliminary experiments for each samples.

All GC measurements were done at least three times, and the RSDs for the retention times were less than 1.0%. The data collection was made with Borwin Chromatography Data Handling Software (Jasco, Tokyo, Japan) running on a personal computer.

2.4 Results and Discussion

Simultaneous Derivatization and Extraction of Aldehydes

For the derivatization of aldehydes, the most well-known specific reaction of carbonyl compounds with DNPH resulting the corresponding hydrazones [33-41] was used. Figure 3 shows the reaction scheme of the DNPH derivatization, where aldehydes and ketones quantitatively react with DNPH to form the derivatives. Except for formaldehydes, it has been demonstrated that the formation of *E*- and *Z*-stereoisomers could be expected for the aldehydes as well as asymmetric ketones, although the isomerization was also reported in several specific conditions [36-41].
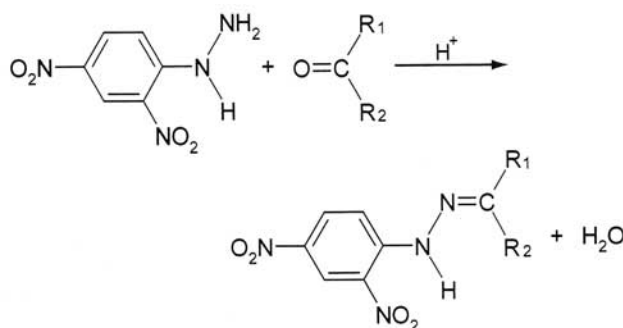

Figure 3. Scheme of the derivatization reaction for aldehydes and ketones with DNPH.

On the basis of the preliminary experiments for the derivatization in the needle extraction device, an acetonitrile solution (0.1 mg/mL) of DNPH hydrochloric acid salt was employed as the derivatization reagent as found in Figure 4. Prior to the sample gas introduction, the DNPH solution was pumped through the polymer-coated fiber-packed needle at about 16 μL/min for 4 min, and then the remaining solution was vented by passing N_2 of 1 mL. The needle was attached to a commercially-available vacuum sampling device [19] (Komyo Rikagaku Kogyo, Tokyo, Japan). For the extraction process, the needle was inserted into the gas sampling bag containing the standard sample, while the sample gas was introduced to the vacuum sampling device via the extraction needle with the derivatization reagent therein. Typical sampling volume was 50 mL, and it takes about 8 min to complete the sampling with the vacuum sampling device, where the RSD (*n*=5) for the sampling time was less than 1.0% on the same needle, although the RSD for needle-to-needle was somewhat larger, about 3.3% regarding five needles separately prepared. The completion of the gas sampling for each run was confirmed by the indicator equipped in the vacuum sampling device.
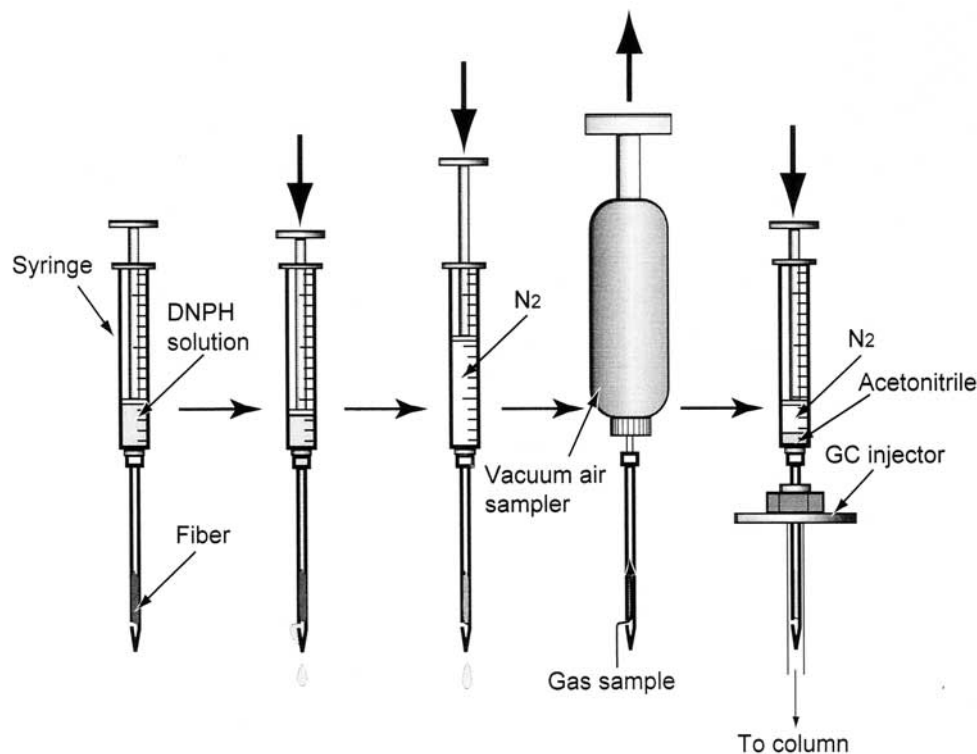
Figure 4. Preconditioning of the extraction device, and subsequent sampling of gaseous samples and the desorption/injection in the GC injector.

Optimization of the Basic Desorption Conditions

After the derivatization/extraction process, the needle was attached to a injection syringe containing pure acetonitrile and N_2 gas, and inserted to the injection port of the GC as illustrated in Figure 4. The desorption and injection were made simultaneously by injecting acetonitrile and N_2 through the needle at the heated injection port. The injection was made immediately after the insertion of the needle to the injector, since it has been confirmed that no preheating time was necessary for the effective desorption under the optimized conditions for the desorption temperature and the volume of the solvent injected as described below.

Taking into account the solubility of both DNPH hydrochloric acid salt and the resulting hydrazones formed by the derivatization reaction with aldehydes, and also the required chromatographic separation from the derivatives and unreacted DNPH, acetonitrile was chosen as the desorption solvent, although a more suitable solvent could be found as an alternative. In the preliminary experiments, it was found that the desorption was significantly insufficient without the desorption solvent, that means the desorption could be made with a flow of heated solvent, but not successfully completed only with N_2 gas flow through the heated needle.

The optimum volume of the desorption solvent was studied with the DNPH derivative of propionaldehyde (PA) as a representative of the volatile aldehydes, where 50 mL of the standard gas sample containing 23.7 μg/L (corresponds 10 v/v ppm at 25℃) was extracted with the needle extraction device. Changing the volume of the desorption solvent, acetonitrile, the resulting

chromatograms were monitored with the SIM mode set at *m/z* 238. The peak area for the PA-DNPH derivative was calculated as the sum of the peak areas for *E*- and *Z*-isomers. As shown in Figure 5, an optimum volume (about 30 μL) of the desorption solvent could be found. The peak area was decreased with insufficient volume of the solvent. At the same time, however, the desorption efficiency was also decreased with the solvent volume of more than 40 μL, possibly resulted by an instantaneous temperature drop of the injector due to the vaporization of unusually large volume of acetonitrile in the injector. Similar trend was observed for acetone, an representative of volatile ketones, as plotted in Figure 5. The above interpretation was also supported by the fact that a certain amount of the carry-over was found with the needle desorbed under those undesirable desorption conditions.
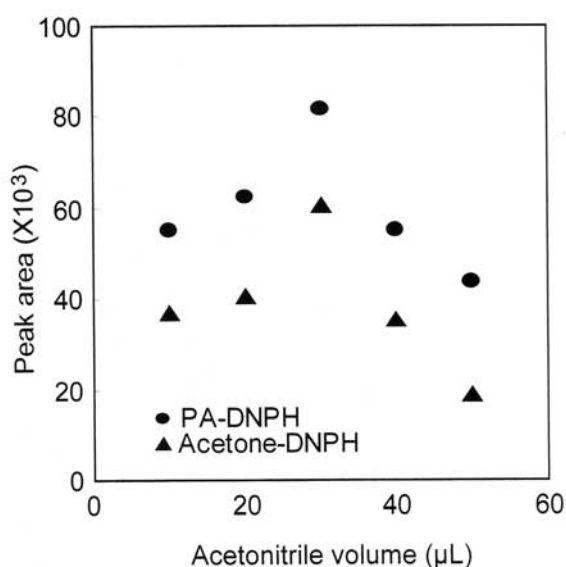

Figure 5. Optimization of the volume of desorption solvent. Extraction conditions: desorption temperature, 170℃; sample gas concentration, 23.7 μg/L for both PA and acetone (corresponding about 10 v/v ppm). GC conditions: temperature program, 150℃ (1.0 min) to 220℃ at 10℃/min; column head pressure, 80 kPa. Other conditions are in the text.
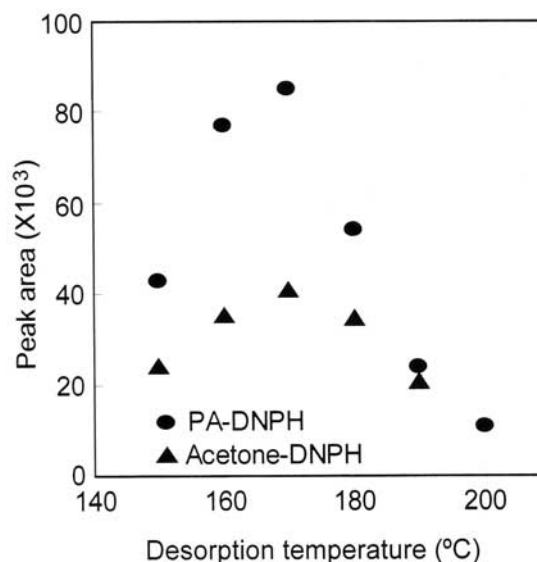
Figure 6. Effect of the desorption temperature. Conditions: desorption solvent and the volume, acetonitrile (30 μL). Other conditions are the same as in Figure 5.

The desorption temperature in the GC injector was also optimized. The plots in Figure 6 clearly suggest the existence of the optimum desorption temperature about 170℃ for the desorption with 30 μL of acetonitrile as the solvent. The insufficient desorption was observed both at lower and higher desorption temperatures. The decreased desorption efficiency was attributed to the unsatisfactory heating, because the carry-over was found at the lower desorption temperature. The drop of the peak areas at the higher temperature could be interpreted as the partial decomposition of the DNPH derivatives under these excessively-heated conditions, although a more extensive studies should be scheduled for the thermal stability of these derivatized species.

Performance of the Needle Extraction Device

On the basis of the above successful optimization on the volume of desorption solvent and the

temperature, resulting the practically complete desorption (more than 99.99%) at the first desorption, the extraction performance was evaluated. The linear calibration ranges and the corresponding correlation factors for the determination of several compounds are summarized in Table 1, in which the DNPH derivatives of formaldehyde (FA), acetaldehyde (AA), and acetone was also tabulated.

The quantification was carried out with the SIM mode for the corresponding target ions: FA-DNPH (m/z: 210), AA-DNPH (m/z: 224), PA-DNPH (m/z: 238) and acetone-DNPH (m/z: 238). All the concentrations were calculated as that of underivatized analytes in Table 1. Considering the sampled gas volume of 50 mL and the concentration range found in typical in-house measurements, the data clearly demonstrate the practical quantification range for these compounds with excellent correlation coefficients. As an extraction technique, the repeatable and reproducible extraction could be one of the requirements to ensure the determination, especially for the multiple and/or multipoint sampling in environmental analysis. The RSDs for run-to-run (n=5) and needle-to-needle (n=5) measurements are shown in Table 2. The RSDs for needle-to-needle were slightly larger than that of run-to-run, however, these data indicate a good repeatability and reproducibility for the needle preparation including the derivatization reaction in the needle.

Table 1. Linear range of the calibration curve and the correlation coefficients.

	linear range (ng/L)	r
FA	10 - 20000	0.996
AA	20 - 40000	0.999
PA	20 - 40000	0.998
acetone	60 - 60000	0.999

Table 2. Relative standard deviations (%) of the peak area for the run-to-run (n=5) and needle-to-needle (n=5) measurements.

	run-to-run	needle-to-needle
FA-DNPH	0.45	5.25
AA-DNPH	3.51	5.72
PA-DNPH	4.20	4.45

Conditions: desorption temperature, 170°C. Other conditions are the same as in Figure 6.

Figure 7 shows a typical separation of DNPH derivatives of three aldehydes that have been derivatized and simultaneously extracted with the needle extraction device. The top chromatogram was obtained by the TIM mode with the m/z range of between 200 and 250, while the other chromatograms were extracted from the above TIM data set at the corresponding m/z number. Introducing the time-programmed SIM mode as depicted in Figure 8A, the lowest quantification level could be improved more. The estimated lowest quantification level for FA (50 mL sampling) was improved to be less than few ng/L, because the determined concentration of FA in Figure 8A was 25

ng/L (corresponds about 20 v/v ppb). Similar improvement was also confirmed for the determination of acetone and methyl ethyl ketone (MEK) as found in Figure 8B. In addition, the linearity of the calibration curve (Table 1) could be also extended with an increased sampling volume, it can be said that the present method will be successfully applied to the FA determination in typical air samples.
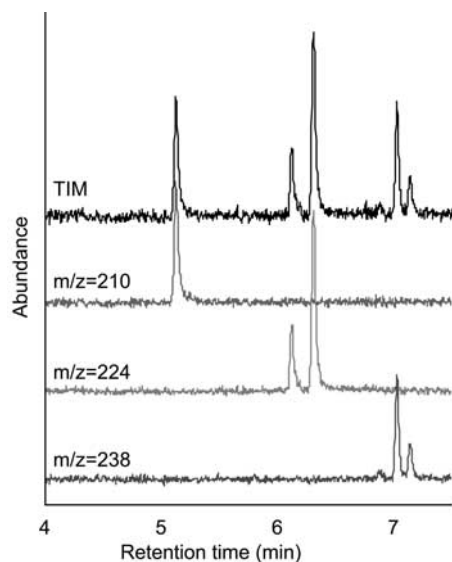

Figure 7. Typical GC-MS separation of the aldehydes derivatives. Extraction conditions: desorption temperature, 170℃; desorption solvent and the volume, acetonitrile (30 μL). Sample gas concentration: 0.6 μg/L for FA, 3.6 μg/L for AA and 4.7 μg/L for PA, corresponding 0.5, 2.0 and 2.0 v/v ppm, respectively. GC conditions are the same as in Figure 5. The peaks for FA-DNPH, AA-DNPH and PA-DNHP in TIM trace were assigned by the corresponding extracted chromatograms at *m/z*: 210, 224 and 238, respectively.
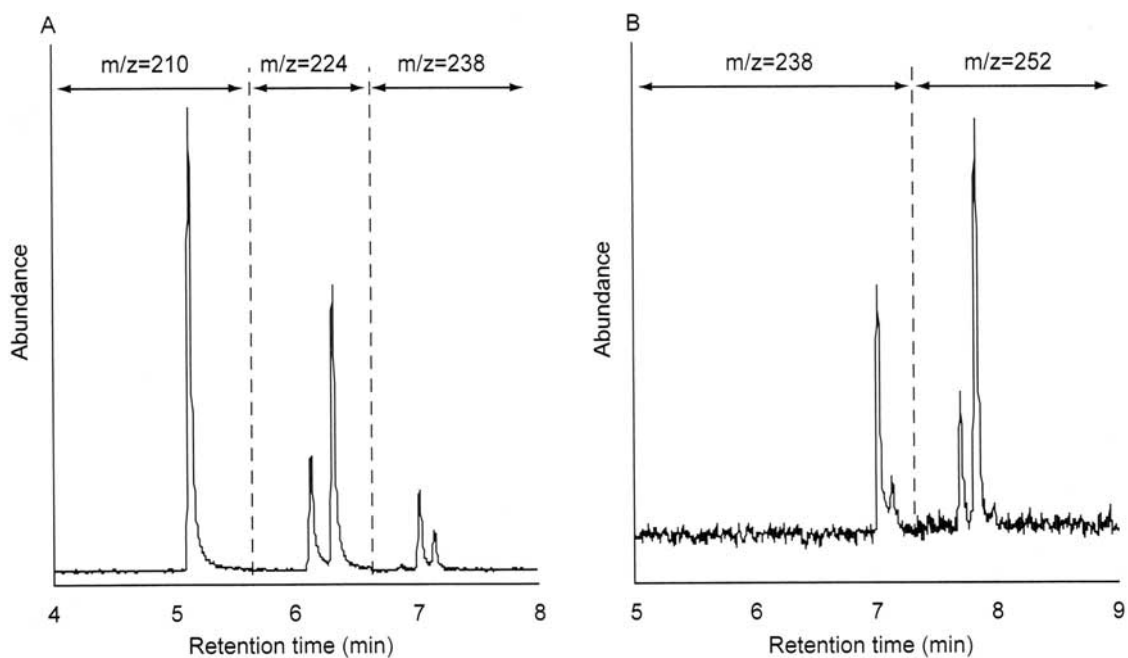

Figure 8. Chromatograms for the separation of the DNHP derivatives of (A) aldehydes and (B) ketones with programmed SIM mode. Peaks: (A) FA-DNPH (*m/z*: 210; 4.0-5.6 min; 25 ng/L as FA), AA-DNPH (m/z: 224; 5.6-6.6 min; 300 ng/L as AA) and PA-DNPH (*m/z*: 238; 6.6-8.0 min; 400 ng/L as PA), and (B) acetone-DNPH (*m/z*: 238; 5.0-7.4 min; 500 ng/L as acetone) and MEK-DNPH (m/z: 252; 7.4-9.0; 600 ng/L as MEK). The extraction and GC conditions are the same as in Figure 5, except for the final temperature of 230℃ regarding the chromatogram in Figure 8B. Other conditions are described in the text.

In Table 3, the storage performance of the needle extraction device was summarized, where the recovery (%) was calculated based on the immediate quantification. Up to three days after the sampling, the needles were stored at the room temperature (about 24℃). During this storage period, all the needles were sealed with the plugs manufactured by a small piece of polytetrafluoroethylene (PTFE). The excellent storage performance of the needle could be confirmed in Table 3, suggesting the advantageous feature for the large number of sampling at the same time. The limit of detection (LOD) and the limit of quantification (LOQ) are summarized in Table 4. Although further optimization should be carried out to improve the performance of the present approach, especially on the injection conditions, these data clearly demonstrated the practical employment of the present method to the aldehydes determination in typical in-house environments.

Table 3. Storage performance of the needle extraction device for aldehydes at room temperature.

	24 h	72 h
FA-DNPH	98.4	97.6
AA-DNPH	96.2	92.7
PA-DNPH	99.9	99.2

The values are calculated as the recovery (%) based on the immediate analysis. Conditions: desorption temperature, 170°C. Other conditions are the same as in Figure 6.

Table 4. Limits of detection (LOD) and quantification (LOQ).

	LOD (ng/L)	LOQ (ng/L)
FA	1.2	3.6
AA	3.6	10.8
PA	4.7	14.1
acetone	11.7	35.1

The extraction and GC conditions are the same as in Table 2.

2.5 Conclusions

Simultaneous derivatization/extraction for the volatile aldehydes was studied with the specially-designed needle extraction device packed with a bundle of polymer-coated filaments. Although some additional studies regarding a more systematic optimization for extraction and determination procedures might be necessary along with the additional investigations for the derivatization reaction and the isomerization, the above results suggest the practical applicability of the method to the analysis of volatile aldehydes in typical in-house air. On the basis of the excellent storage power of the needle extraction device, the extracted samples could be determined even after the storage at the room temperature for at least three days.

With the miniaturized needle-type extraction device, more extensive work is currently underway in our laboratory including, the determination of volatile ketones in air samples, the coupling of the needle extraction device to the polymer-coated fiber-packed capillary columns [27-32] with a higher sample loading capacity, and the design and synthesis of the novel polymeric extraction medium based on the surface derivatization reaction of the filaments [42] to be packed into the needle.

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