In-Tube Solid-Phase Microextraction and On-Line Coupling with High-Resolution GC

The guest authors validated an in-tube solid-phase microextraction (SPME) device, which was designed for on-line coupling with a capillary gas chromatography (GC) system, for the trace analysis of organic contaminants in water. They used a 5 m × 0.53 mm, 1.2-µm d_f poly(dimethylsiloxane) phase capillary column in place of a fiber. Conceptually, in-tube SPME is similar to fiber SPME, but the extraction device has a piece of fused-silica GC capillary column in place of a fiber. Consequently, in-tube SPME should preserve the advantages of SPME and could offer improved enrichment efficiency, quantitation, and automation through the on-line coupling with a chromatograph. By using a piece of bonded-phase capillary GC column for sorption, a larger amount of stationary phase and a more robust film are obtained, relative to outside-coated films of conventional SPME fibers. These differences result in higher enrichment factors and longer extractor life.

Because many capillary GC stationary phases are commercially available, in-tube SPME enables easy changing of the extraction-phase polarity, which extends the application range of the method.

The analysis of aqueous samples using in-tube SPME–GC has been reported in the literature (9–11). For the extraction step, the sample solution was pushed or pulled through the capillary extractor at a reasonable flow rate. The adsorbed analytes next were desorbed with a minimum amount of stripping solvent for off-line collection before chromatographic analysis (9). In addition, the capillary extractor could be assembled manually in front of the GC analytical column with a press-fit connector and a piece of precolumn. A temperature-programmed GC run completed the procedure of both thermal desorption and analysis (10,11). Using those approaches, researchers obtained promising results and avoided the problems that are observed with fiber SPME such as the bleed from the ultrathick film and the appearance of ghost peaks.

On-line extraction–capillary GC is an attractive method for the analysis of aqueous samples. Several methods for on-line extraction GC have been reported in the literature, including membrane extraction (12), liquid–liquid extraction (13), and solid-phase extraction with small packed cartridges (14). In contrast to these conventional methods, the complete removal of water can be achieved easily by using an open-tubular capillary. Mol and co-workers (15) developed a method using open-tubular trapping columns for on-line extraction–capillary GC in the analysis of aqueous samples. In that study, they used two switching valves and organic solvent for desorption.

In this installment of “Sample Prep Perspectives,” we will present a novel device.

Guest Authors
Hanwen Wang, Wenmin Liu, and Yafeng Guan

Ronald E. Majors
Sample Prep Perspectives Editor
for coupling on-line in-tube SPME with capillary GC. We will demonstrate this method’s application for the analysis of contaminants in water.

Quantitation

As shown in equation 1, it is common for analysts to calculate the theoretical recovery or to evaluate quantitation based upon equilibrium theory (16):

\[
 n = C_0 \frac{K_0 V_s}{1 + \left( K_0 V_s/V_0 \right)} \quad [1]
\]

where \( n \) is the amount of extracted analyte, \( C_0 \) the initial concentration of the analyte in the matrix, \( K_0 \) the distribution constant of the analyte, \( V_s \) the volume of the stationary phase, and \( V_0 \) the volume of sample.

A concept of negligible depletion extraction recently was proposed for easier quantitation and higher enrichment factors (17). For in-tube SPME, the extraction process will not influence the free concentration of the analyte in the matrix when a sufficient amount of sample solution (quasi-infinite relative to extracting phase volume) passes through the extractor. In this case, \( K_0 V_s \ll V_0 \), and the absolute amount of extracted analyte can be obtained easily from equation 2:

\[
 n = K_0 V_s C_0 \quad [2]
\]

Experimental

On-line in-tube SPME instrument setup and procedure: Figure 1 is a schematic of the on-line in-tube SPME device developed in our study. The system consists of a six-port valve and three gas flow controllers (both from Fuli Corp., Wenling, China), a homemade stainless steel micro tee piece, a homemade 5 m \( \times \) 0.53 mm, 1.2-\( \mu \)m \( d_i \) conventional cross-linked OV-1 (poly(dimethylsiloxane)) capillary column used as extractor, and a mini water-circulating pump (Tengda Corp., Tianjing, China). A homemade oven capable of heating at a rate of 290 °C/min to temperatures greater than 320 °C provided fast and uniform heating for the capillary extractor. A deactivated 1 m \( \times \) 100 \( \mu \)m fused-silica capillary (Ruifeng, Yongnian, China) in close contact with a piece of heating resistor wire was used as the analyte transfer line from the in-tube SPME system to the GC system. An adiabatic sleeve covered the transfer line to maintain heat.

During the extraction period (the solid-line position of the valve in Figure 1), the sample solution was forced through the capillary extractor by the push of auxiliary gas and the suction force of the mini water-circulating pump. A negative pressure at the N point of the tee piece was generated because of the suction of the mini water-circulating pump. The head pressure of the GC column forced the carrier gas through the transfer line to the tee piece (as shown on the Figure 1). Because the pressure at point P always is higher than that at point N, we avoided the problem of direct influx of the aqueous solution into the GC system during the extraction process.

After the aqueous sample was drained completely from the extractor, the six-port valve was switched to the dotted-line position for desorption. To achieve lower detection limits in high-resolution GC and to obtain a sharp desorption band, the extractor should heat up as fast as possible (at a rate as high as 290 °C/min). The desorption of analytes from the capillary extractor occurs very fast because they are purged by the auxiliary gas through the capillary. The thermal desorption time is approximately 2–6 min for the 5 m \( \times \) 0.53 mm capillary column under a 6-mL/min purge-gas flow. Another path of auxiliary gas at a flow rate of approximately 1–2 mL/min is used as makeup gas through the tee piece to prevent any back diffusion of analytes. It must be noted that the desorbed analytes are introduced directly to the high-resolution GC system after the switching valve, instead of through the valve, to eliminate the possibility of any carryover or dead volume along the sample transfer line. On the other hand, using the high-temperature switching valve is unnecessary in this system design. The more expensive valve is not required for this system design, which makes the device less expensive.

Finally, the desorbed analytes were transferred to the homemade cold retention gap in the model 6890N gas chromatograph (Agilent Technologies, Beijing, China), with an initial oven temperature of 30 °C, through the hot capillary transfer line and were refocused on the head of the analytical column by the retention gap (18). We used a sequential temperature-programmed high-resolution GC run to accomplish the separation and detection of analytes of interest. Thus, the total process of analyzing organic compounds in aqueous samples, including the on-line extraction, thermal desorption, and sampling to high-resolution GC, was automated using the above-mentioned in-tube SPME device.

Results and Discussions

Evaluation of on-line in-tube SPME coupled with high-resolution GC: We used aqueous samples containing a series of alkanes (C10–C19) at the microgram-per-liter level to evaluate the performance of our apparatus. As Table I shows, the in-tube SPME capillary extractor has an increased amount of solid stationary phase and a much larger exchanging surface, when compared with the fiber SPME system, which result in drastic increases of the extraction efficiency and enrichment factor.
In the experiments, a 5 m × 0.53 mm, 1.2-µm d_i poly(dimethylsiloxane) phase capillary extractor provides approximately 10 µL of solid phase for extraction, which is roughly 10-fold more solid phase than that of an SPME fiber (~1 µL). Figures 2a and 2b show that the extraction of the same 15-mL aqueous sample with a 10-µg/L concentration of each alkane to obtain an approximately 30-fold concentration of the analytes of interest requires 2.5 min for in-tube SPME versus 35 min for fiber SPME. When the extraction time was limited to 5 min, we were able to extract only few analytes of interest by fiber SPME (see Figure 2d). On the other hand, with a 40-min extraction time, the peaks obtained by in-tube SPME are roughly 50-fold higher than that of fiber SPME (extraction for 35 min), even for components with concentrations as low as 2 µg/L (Figure 2c). In addition, the baselines of chromatograms from in-tube SPME are very smooth because the capillary extractors have thinner and stronger film of bonded phase, and ghost peaks normally appear with fiber SPME.

We performed six replicate experiments of on-line extraction, desorption, and detection for each concentration of samples to examine the method’s reproducibility. The precision of quantitation, obtained using a 5 m × 0.53 mm, 1.2-µm d_f poly(dimethylsiloxane) capillary, varies from 5% to 15% relative standard deviation (RSD) (for n = 6), depending upon the alkanes and concentration of samples studied. Table II shows the precision and linearity for all compounds investigated under different concentration conditions. The average RSD was 8.0% for the 0.5-µg/L concentration level and 5.3% for the 20-µg/L concentration level.

We determined linearity by extracting spiked aqueous samples with concentrations ranging from 0.1 µg/L to 100 µg/L. The method was linear throughout at least three orders of magnitude. The coefficient

Table I: Comparison of a typical apolar capillary extractor (5 m × 0.53 mm, 1.2-µm d_i) and a 100-µm apolar SPME fiber

<table>
<thead>
<tr>
<th>Extractor Type</th>
<th>Character of Extractants</th>
<th>Phase Volume (µL)</th>
<th>Exchanging Surface of Extraction (mm²)</th>
<th>Type of Mixing During Extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary extractor</td>
<td>Bonded on the inner wall</td>
<td>9.9852*</td>
<td>8321†</td>
<td>Turbulence of flow convection</td>
</tr>
<tr>
<td>100-µm apolar SPME fiber</td>
<td>Coated outside the fiber</td>
<td>0.9734‡</td>
<td>9.7§</td>
<td>Agitation by stirrer</td>
</tr>
</tbody>
</table>

* V = πD_iD_oL, where D_i is the inner diameter of capillary tube, d_f is the film thickness of solid phase, and L is the length of capillary column.
† A = πD_iL.
‡ V = πD_oD_iL, where D_o is the outer diameter of fiber core (~110 µm), d_f is the coating film thickness of solid phase, and L is the length of fiber (normally 10 mm).
§ A = πD_oL.

Figure 2: Comparison of chromatograms of spiked aqueous samples by in-tube SPME and fiber SPME: (a) extraction of 15-mL samples within 2.5 min with alkanes of 10-µg/L level by on-line in-tube SPME; (b) extraction of 15-mL samples within 35 min with alkanes of 10-µg/L level by fiber SPME; (c) extraction of 300-mL samples within 40 min with alkanes of 2-µg/L level by on-line in-tube SPME; (d) extraction of 15-mL samples within 3 min with alkanes of 10-µg/L level by fiber SPME. Column: 30 m × 0.53 mm, 0.6 µm d_i MXT-1 (Restek Corp., Bellefonte, Pennsylvania) with a 5-m retention gap; carrier gas: hydrogen at 8 mL/min; oven program: 30 °C for 0.5 min, 30–110 °C at 40 °C/min, 110 °C for 1 min, 110–250 °C at 10 °C/min, 250 °C for 10 min; fiber desorption time in (b): 4 min. Peaks: 1 = n-C_{12}, 2 = n-C_{13}, 3 = n-C_{14}, 4 = n-C_{15}, 5 = n-C_{16}, 6 = n-C_{17}, 7 = n-C_{18}, 8 = n-C_{19}.

www.chromatographyonline.com
of correlation achieved was better than 0.99 (Table II). In addition, we found no carryover or memory effects with this on-line coupled in-tube SPME–high-resolution GC system.

**Applications of On-Line In-Tube SPME Coupled with High-Resolution GC**

**Determination of PAHs with on-line in-tube SPME, high-resolution GC, and flame ionization detection:** Polycyclic aromatic hydrocarbons (PAHs) are an important class of environmental pollutants that represent a risk for living organisms and human health. Among various techniques of PAH determination, SPME (19) and stir-bar sorptive extraction (20) recently have gained wide acceptance. For sub-parts-per-billion level measurement, the SPME device has a limited enrichment factor; however, the stir-bar sorptive extraction device, which has a two-orders-higher enrichment factor than the SPME system, needs several hours to reach extraction equilibrium, especially for four-ring and larger polynuclear compounds. In addition, a number of manual steps are necessary to successfully use the stir-bar sorptive extraction technique.

In our experiment, we examined 16 PAH standards spiked in water at microgram-per-liter or sub-microgram-per-liter concentrations. We applied two types of clean water as the sample matrices: Wahaha purified drinking water (Wahaha Corp., Hangzhou, China) (Figure 3a) and tap water from the laboratory faucet (Figure 3b). The resulting chromatogram (Figure 3) shows that the 16 PAHs were well separated without any tailing peaks. The lowest detection limit for most PAHs was estimated to be less than 0.01 µg/L, much lower than that estimated by the fiber SPME technique. In addition, the overall extraction time was within 40 min for sample volume of 400 mL, quite short compared with stir-bar sorptive extraction or fiber SPME. It was interesting to note that trace amounts of plasticizers were found in the purified water, but no peak of butylbenzyl phthalate was found in the tap water (Figure 3). However, many more volatile compounds were found in the tap water, which we suspected were halogenated hydrocarbons that resulted from the chlorination process.

**Determination of chlorinated pesticides with on-line in-tube SPME, high-resolution GC, and electron-capture detection:** To determine chlorinated pesticides in aqueous samples, we coupled our in-tube SPME device on-line with a model 3800 capillary gas chromatograph (Varian Inc., Palo Alto, California), which was equipped with an electron-capture detector. We divided standard mixtures of 26 total pesticides into three groups, and each pesticide compound was spiked directly.
into a silex vial at the 0.25-μg/L level using a microsyringe. Figure 4 depicts the resulting chromatogram. It is noteworthy to point out that the detectability of the peaks of pp′-dichlorodiphenyltrichloroethane (pp′-DDT) and iprodione, which are barely detectable using GC systems that display poor inertness, was rather good. The lowest detection limits of some components such as benzene hexachloride (BHC) were estimated at the sub-nanogram-per-liter level, which was obtained in only 5 min with extraction of a 30-mL aqueous sample. Actually, lower
Determination of phosphorus-containing pesticides with on-line in-tube SPME, high-resolution GC, and pulsed-flame photometric detection:

We coupled the same apparatus on-line with the capillary gas chromatograph, this time equipped with a pulsed-flame photometric detector (operated in the phosphorus mode) to determine the phosphorus-containing pesticides. We spiked 16 standard pesticides at 0.5 μg/L each in a 30-mL aqueous sample before extraction, and the dilution of compounds was performed in a silex vial using a microsyringe. The chromatogram obtained is illustrated in Figure 5. The lowest detection limit observed was 0.05 μg/L for most of the analytes with an extraction time of only 5 min.

Conclusion

We performed in-tube SPME on-line coupling with high-resolution GC by using a simple device for the trace analysis of organic compounds in aqueous samples. The in-tube SPME–high-resolution GC methods demonstrated in our study successfully performed the on-line extraction, desorption, and sampling of various contaminants in water, followed by analysis of high-resolution GC with different detectors. The novel in-tube SPME–high-resolution GC device presents the following advantages over manual fiber SPME–high-resolution GC:

- It allows much higher enrichment factors than those of fiber SPME because of its 10-fold greater volume of extracting phase compared with fiber SPME. In our experiments, the enrichment factors by means of in-tube SPME were at least 50-fold that of fiber SPME, if both experiments were performed under optimum conditions, even though the ratio of extracting phase volume was approximately 10.`
- It provides faster extractions than those of fiber SPME because of the drastically larger exchanging surface.
- It is performed as a fully on-line operation; therefore, it provides high-precision reproducibility.
- The technique’s cost is relatively low because it uses conventional capillary columns and an ordinary switching valve.

References


Hanwen Wang is an assistant professor at the Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, 116012, China, and a technical support engineer for Agilent Technologies (Shanghai), e-mail han-wen_wang@agilent.com. Wenmin Liu is a Ph.D. student at the Dalian Institute of Chemical Physics, Chinese Academy of Sciences. Yafeng Guan is a professor at the Dalian Institute of Chemical Physics, Chinese Academy of Science; the director of the department of analytical chemistry and micro-instrumentation at the Dalian Institute of Chemical Physics; a member of the Scientific Committee on Chemistry of the Chinese Academy of Sciences; vice-chairman of the Chinese Association of Analytical Instrumentation; and a standing member of the Chinese Society on Chromatography.

Ronald E. Majors
“Sample Prep Perspectives” editor Ronald E. Majors is business development manager, consumables and accessories business unit, Agilent Technologies, Wilmington, Delaware, and is a member of LCGC’s editorial advisory board. Direct correspondence about this column to “Sample Prep Perspectives,” LCGC, Woodbridge Corporate Plaza, 485 Route 1 South, Building F, First Floor, Iselin, NJ 08830, e-mail lgcedit@lcgcmag.com.