Solid-phase Microextraction of Phosphate and Methylphosphonate Using Novel Fibers Coated with a Sol-Gel-Derived Silicone-Divinylbenzene Co-Polymer

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Key Words
Gas chromatography
Solid-phase microextraction
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Phosphate and methylphosphonate
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Summary
Co-poly (hydroxy-terminated silicone divinylbenzene) coating was first prepared by sol-gel and cross-linking methods and applied to the solid-phase microextraction of phosphate and methylphosphonate from air and water. The possible mechanism is discussed. The characteristics of the new films were also investigated. Compared with commercial SPME fibers, the novel films showed better selectivity and sensitivity towards phosphate and methylphosphonate. Moreover, the coating proved to be very stable at high temperatures and in different solvents. Some important parameters, such as extraction time, extraction temperature, effect of salt and desorption time were optimized. The limits of detection for dimethyl methylphosphonate (DMMP), trimethyl phosphate (TMP) and tributyl phosphate (TBP) were 1.11, 1.46, and 1.64 \( \mu \text{g L}^{-1} \) in air and 0.34, 2.20, and 0.01 \( \mu \text{g mL}^{-1} \) in water. A relative standard deviation (n = 6) below 7% can be achieved depending on the compounds both in air and in water. Linear ranges were about 1–2 orders of magnitude. The recovery of spiked water samples was from 89.46 to 90.88%.

Introduction
Sample pretreatment and preconcentration is generally required for the determination of trace organic pollutants. Traditional solvent extraction often needs large amount of toxic solvents and time-consuming procedures. Solid-phase microextraction (SPME) was originally developed by Pawliszyn and coworkers in the 1990s [1]. It integrates extraction, concentration and sample introduction into one single step without the use of organic solvents. In recent years, the applications of SPME have been widely extended from volatile organic compounds, and semi-volatile chemicals to non-volatile inorganic pollutants in different fields, including the environment [2], food [3], natural products [4], pharmaceuticals [5], biology [6], toxicology [7], etc. The fibers used in these work were initially poly (dimethylsiloxane) (PDMS) and polyacrylate (PA), and have now been extended to other coatings such as PDMS-divinylbenzene (PDMS/DVB), Carbowax-divinylbenzene (CW/DVB) and Carboxen-PDMS. They are generally prepared by simple physical deposition of the polymer coating on the surface of the silica fiber. The lack of proper chemical binding between the stationary phase and silica fiber surface may be responsible for the low thermal and chemical stability and short lifetime [8–9]. Sol-gel coating technology can reduce these problems by providing efficient incorporation of organic components into the polymeric structures in solution under mild thermal conditions [9–10]. Furthermore, the porous structure of sol-gel coating provides higher surface area, enhances sample extraction capacity and accelerates the speed mass transfer [10–11]. Selectivity can also be improved by changing coating materials and their proportion.

The nerve agents are among the most toxic compounds known. With the Chemical Weapons Convention which came into force on 29, April 1997, chemical warfare agents have been of concern to governments, military leaders, and scientists. The nerve agents sarin, soman, tabun, and O-ethyl-S-[2-(diisopropylamino)ethyl]methylphosphonothiolate (VX) are representatives of methylphosphonate nerve agents. The pretreatment and determination of these compounds in aqueous or environmental matrixes is challenging because they are highly polar compounds and easily to hydrolyzed, and
have no chromophore for UV or fluorescence detection [12]. These difficulties can be overcome by SPME coupled with gas chromatography (GC) or gas chromatography-mass spectrometry (GC-MS) [13].

Alkylphosphonic acids and their monoesters are important hydrolysis products of nerve agents and related species. Dimethyl methylphophonate (DMMP) is structurally similar to methylphosphonate nerve agent, while possessing much less toxicity and has been commonly used as simulant for nerve agents, by many researchers [14–16]. Trimethyl phosphate (TMP) has been used as an internal standard for Sarin and tributyrylphosphate (TBP) has been used as an internal standard for VX.

In this work, co-poly(hydroxy-terminated silicone oil/divinylbenzene) (OH-TSO/DVB) fibers were first prepared by sol-gel and cross-linking technology. DMMP, TMP and TBP were employed as model compounds to assess the extraction procedure and the characteristics of the novel fibers, and simulate the detection of methylphosphonate nerve agents in air and water.

Experimental

Apparatus

The experiments were carried out on an SP-6800A capillary GC system (Shandong, China) equipped with a capillary split/splitless injector system and flame ionization detector. Online data collection and processing was done on Chromatopac model SISC-SPS (Beijing, China).

To mix various solution ingredients thoroughly, an Ultrasonator model SY-1200 (Shengyuan, China) was used. A centrifuge model TGL-16C (Shanghai Anting Instrument Factory, Shanghai, China) was used to separate the solution from the precipitate. The silica fiber (140 lm, o.d.) with protective polyimide coating was provided by the Academy of Post and Telecommunication, Wuhan, China.

A magnetic stirrer DF-101B (Leqing, China) was employed for stirring the sample during extraction. A homemade SPME syringe was used to transfer the extracted sample to the GC injector for analysis. The commercially available polydimethylsiloxane (PDMS, 100 mm), polyacrylate (PA, 85 mm), and co-poly (dimethylsiloxane/divinylbenzene) (PDMS/DVB, 65 mm) coated fibers for comparison were purchased from Supelco (Bellefonte, PA, USA).

Reagents

Hydroxy-terminated silicone oil (OH-TSO) was purchased from Chengdu Center for Applied Research of Silicone (Chengdu, China). Tetraethoxysilane
(TEOS), poly (methylhydrosiloxane) (PMHS), and vinyltriethoxysilane (VTEOS) were obtained from the chemical plant of Wuhan University, China. Trifluoroacetic acid (TFA) and divinylbenzene (DVB) were purchased from Merck, Germany. DMMP, TMP and TBP were obtained from a military source. All solvents used in this study were analytical-reagent grade.

**Fiber Preparation**

Prior to sol-gel coating, the 6 cm long fused-silica fiber was dipped in acetone for 3 h to remove the protective polyimide layer, in a 1 M NaOH solution for 1 h to expose the maximum number of silanol groups on the surface, cleaned with water, and dipped in 0.1 M HCl solution for 30 min to neutralize the excess NaOH, cleaned again with water and air-dried at room temperature.

The sol solution was prepared as follows: 180 µL of DVB, 60 mg of OH-TSO, 100 µL of TEOS, 50 µL of VTEOS, 10 mg of PMHS and 8 mg of benzophenone (BP) were dissolved in 100 µL of methylene chloride and mixed thoroughly by ultrasonic agitation in a plastic tube. A 70-µL volume of TFA containing 5% water was sequentially added to the resulting solution with ultrasonic agitation for another 5 min. The mixture was centrifuged at 12000 rpm for 8 min. The top clear sol solution was collected for fiber coating. A sol-gel coating was formed on the outer surface of the fiber end (about 1 cm), after the fibers were dipped vertically into the sol-gel solution for 30 min. For each fiber, this coating process was repeated several times until the desired coating thickness was obtained. After that the fibers were irradiated under ultraviolet light for 30 min, then placed in a desiccator for 12 h at room temperature and conditioned at 250–380 °C under nitrogen protection for 3 h in the GC injection port to give the final fiber ready for use.

The other fibers were coated with an identical procedure except that the proportions of OH-TSO and DVB were different: 180 mg OH-TSO/0 µL DVB and 180 mg OH-TSO/180 µL DVB.

**GC Conditions**

Separation was carried out on a capillary column (25 m × 0.32 mm i.d.) coated

![Figure 1. Comparison of extraction efficiency of the three kinds of sol-gel-coated OH-TSO/DVB fibers. Extraction temperature, 32 °C; extraction time, 15 min; saturated out with NaCl; constant stirring; desorption time, 5 min; splitless injection.](image)

![Figure 2. Comparison of extraction efficiency of the three kinds of sol-gel-coated OH-TSO/DVB fibers. Extraction temperature, 32 °C; extraction time, 15 min; saturated out with NaCl; constant stirring; desorption time, 5 min; splitless injection.](image)
with SE-54. Nitrogen was used as the carrier gas at a linear velocity of 30 cm s\(^{-1}\). Splitless injections were performed. Temperature was maintained at 250 °C for the injection port, 280 °C for the detector. The temperature program for the analysis was: 50 °C, hold for 2 min, then ramp at 10 °C min\(^{-1}\) to 120 °C, finally ramp at 20 °C min\(^{-1}\) to 250 °C, hold for another 2 min.

### Preparation of Standard Solution

For air sample, stock solution of dimethyl methylphosphonate (DMMP), trimethyl phosphate (TMP) and tributyl phosphate (TBP) were prepared by dissolving 10 mg of each compound in acetone in a 10 mL volumetric flask. For water sample, DMMP, TMP and TBP were dissolved in acetone to make stock solution at a concentration of 10 mg mL\(^{-1}\), 10 mg mL\(^{-1}\), and 1 mg mL\(^{-1}\), respectively.

### Solid-phase Microextraction Procedure

Air sampling was simulated by exposing the SPME fiber to vapor in the headspace of a vial with a septum cap. The needle of the homemade SPME syringe was first passed through the septum of a 12 mL glass vial, and then the fiber was pushed out of the needle into the headspace of the vial containing a 10 μL of the 1 mg mL\(^{-1}\) standard solution, for an appropriate time. After that, the fiber was directly exposed to the injection port of the GC for thermal desorption.

For water samples, a 50 μL standard solution with 10 mg mL\(^{-1}\) DMMP, 10 mg mL\(^{-1}\) TMP and 1 mg mL\(^{-1}\) TBP, 5 mL of deionized water and 1 g NaCl were mixed in a 12 mL vial with a magnetic stirring bar. The extraction and desorption procedures for water samples were identical with that for the air samples. To prevent the analytes from being absorbed onto the glass wall, the vials were acid washed and silanized prior to the experiments. To avoid sample evaporation, the vials were sealed with butyl rubber stoppers wrapped with PTFE sealing tape, and then sealed with aluminum caps.

Commercial fibers were conditioned according to the instructions provided by the supplier.
Results and Discussion

Characteristics of the Sol-gel-derived OH-TSO/DVB Fibers

In sol-gel chemistry, a gel can be formed by the simultaneous hydrolysis and polycondensation of the organo-precursor and other sol-gel active ingredients followed by aging and drying under ambient atmospheres. Unlike the commonly used sol-gel process, in which only one mental alkoxide is used as the precursor to produce the coated silica fiber, our process involves two different coated silica monomers as co-precursors. A commonly used precursor for a glass matrix, TEOS, was hydrolyzed in conjunction with a second monomeric unit containing a vinyl substituent. In this experiment we selected VTEOS as the co-precursor which reacted with DVB by radical cross-linking under UV to produce chemical bonding of DVB to other coating ingredient. Thus, a surface-bonded polymeric coating (OH-TSO/DVB) is formed by sol-gel and cross-linking technology.

The mechanism of the coating process can be represented by the steps outlined in following:

1. TEOS, which used as a precursor, can hydrolyze by mixing it with water.
2. At the same time, VTEOS can also be hydrolyzed under the same condition.
3. A 3-D SiO₂ network is formed by polycondensation reaction of the hydrolyzed products of TEOS.
4. Being hydroxy-terminated, the OH-TSO is incorporated with the silica structures by chemical binding.
5. Hydrolyzed products of VTEOS connect with product from step 4.
6. The silanol groups exposed on the fiber surface can also chemically bind with the polymeric network to create a surface-bonded polymeric coating.
7. A dried gel still contains a very large quantitative of chemisorbed hydroxyls on the surface of the pores, which may create difficulty in desorption of polar analytes extracted and influence the reproducibility of the fiber. In general, some PMHS is added to the solution to partially deactivate the fiber due to its small effect on the SPME characteristics of the coating.
8. When exposed to ultraviolet light, cross-links form between VTEOS and DVB with benzophenone as an immobility catalyst.

Extraction efficiency is different for each coating according to the concentration of coating materials. Figure 2 demonstrates the comparison of three OH-TSO/DVB fibers with different concentration of OH-TSO and DVB: 180 mg OH-TSO/0 μL DVB, 180 mg OH-TSO/180 μL DVB, and 60 mg OH-TSO/180 μL DVB. From the figure, we can see that the concentration of DVB plays a key role in improving the selectivity of the OH-TSO/DVB fibers towards phosphate and methylphosphonate. The 60 mg OH-TSO/180 μL DVB fibers possess the highest extraction efficiency.

The sol-gel coating possess a 3-D porous network structure. It can increase the available surface area on the fiber, thereby, enhancing extraction efficiency. The extraction ability of the novel OH-TSO/DVB coated fiber in extracting phosphate and methylphosphonate was compared with the commercially available PDMS, PA, and PDMS/DVB fibers. The results are represented in Figures 3, 4. It can be seen that the OH-TSO/DVB fiber gives the best extraction efficiency compared with the three commercial
fibers, especially for extracting DMMP, which used as the simulant of Sarin. Therefore, this novel fiber may be a good choice for SPME of the methylphosphonate nerve agent.

Being chemically bonded to the substrate, the sol-gel fiber shows high thermal and chemical stability and the average lifetime of the sol-gel fiber was longer than commercial SPME fibers.

Figure 5 demonstrates the thermal stability of the OH-TSO/DVB fiber. As can be seen from the figure, the response did not significantly decrease after it was conditioned for 2 h at 250 °C, 1 h at 300 °C, 320 °C, 350 °C, 380 °C, successively, which is due to the strong chemical binding provided by sol-gel coating technology. Thus, it can provide higher desorption temperature for analytes with high boiling points, and eliminate the sample carryover problem.

Figure 6 illustrates the solvent stability of the OH-TSO/DVB fiber. The extraction efficiency did not decrease or even had a small increase after the fiber was dipped in water for 11 h, and in methylene dichloride for 2.5 h, 11 h, respectively, owing to the chemical binding between the coating and the surface of the fiber. Therefore, it’s possible for the direct SPME of a liquid matrix.

**Determination of Phosphate and Methylphosphonate**

The headspace SPME technique is based on the distribution of the analytes in a three-phase system: a coating, a headspace above solution, and an aqueous solution. The different parameters (extraction time, extraction temperature, ionic strength) that influence the partition of analytes in the three-phase system were optimized.

It is generally accepted that reduction of the diffusion layer is necessary to reach equilibrium faster, and this can be easily achieved by sample agitation. Therefore, magnetic stirring was applied during the extraction step. Figure 7 shows the extraction time profiles for phosphate and methylphosphonate in air. All results were done in duplicate to ensure reproducibility. From the figure we can see that the signal reached equilibrium at 3 min for DMMP and TMP, and 5 min for TBP. The optimized time is 5 min for an air sample. Figure 8 shows the extraction time profiles for the three analytes in water. The figure demonstrates that 5 min was required for DMMP and TMP to reach equilibrium, while for TBP 40 min was required to reach equilibrium because of its higher molecular mass and boiling point. The concentration of DMMP and TMP is 10 times of that of TBP, while their peak area is much lower than that of TBP. Therefore, when selecting extraction conditions, DMMP and TMP are of first consideration. To meet the need of fast determination, 15 min was hence selected as optimal, which is sufficient to achieve the required sensitivity for TBP while does not suffer extraction losses for DMMP and TMP.

Temperature has a double impact on SPME: at a higher temperature, diffusion coefficients are higher and therefore a faster distribution equilibrium is achieved, but partition coefficients are lower and unavoidably result in a poorer extraction efficiency. The influence of temperature depends on the result of these two effects. Figure 9 represents the extraction temperature profiles for phosphate and methylphosphonate in air.
DMMP and TMP reached equilibrium at 32 °C, and TBP, at 60 °C. Therefore, the optimum temperature was 60 °C for air sampling. Figure 10 demonstrates the extraction temperature profiles for the three compounds in water. DMMP and TMP gave the largest response at 25 °C, and then decreased with increasing temperature, but TBP did not achieve the highest signal intensity until the temperature reached 50 °C. Selecting 32 °C as the optimum extraction temperature gave satisfactory sensitivity for all analytes.

Previous studies have shown that ionic strength can improve extraction efficiency, particularly for those compounds with a lower solubility. The ionic strength was modified by adding various amount of NaCl in 5 mL deionized water. Figure 11 illustrated that the extraction for all analytes increased with the increase of NaCl content, then approached a plateau or even decreased. Therefore, 1 g NaCl was added to 5 mL deionized water in the subsequent experiments.

To avoid carryover effects that may occur among subsequent SPME analyses, the time needed for complete desorption of analytes from the OH-TSO/DVB fiber has to be carefully determined. The carryover was measured with a series of blank analyses following the initial desorption. Total desorption of the three analytes was obtained in 5 min.

Tables I, II, summarize the detection limit (LODs), relative standard deviations (RSDs) and linear ranges for the extraction of phosphate and methylphosphonate from air and aqueous solution using the OH-TSO/DVB fiber. The LODs were based on the lowest detectable peak that had signal three times of the background noise (signal/noise = 3). Six consecutive extractions of air samples

![Figure 10](image1.png) Influence of extraction temperature on the amounts of phosphate and methylphosphonate extracted from water. Extraction time, 15 min; saturated with NaCl; constant stirring; desorption time, 5 min.

![Figure 11](image2.png) Effect of NaCl content on the extraction efficiency from water. Extraction time, 15 min; extraction temperature, 40 °C; constant stirring; desorption time, 5 min.

### Table I. Limits of detection linear range and precision for phosphate and methylphosphonate in air.

<table>
<thead>
<tr>
<th>Compound</th>
<th>LOD (µg L⁻¹)</th>
<th>RSD (%)</th>
<th>Linear range (µg mL⁻¹)</th>
<th>Regression equation (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMMP</td>
<td>1.11</td>
<td>4.29</td>
<td>0.0980–4.90</td>
<td>Y = 1.20911E6 + 2.18671E7*X (0.9997)</td>
</tr>
<tr>
<td>TMP</td>
<td>1.46</td>
<td>4.79</td>
<td>0.0972–4.86</td>
<td>Y = 1.28077E6 + 2.47079E7*X (0.9997)</td>
</tr>
<tr>
<td>TBP</td>
<td>1.64</td>
<td>5.88</td>
<td>0.0165–0.827</td>
<td>Y = -689217.49 + 9.20245E7*X (0.9994)</td>
</tr>
</tbody>
</table>

### Table II. Limits of detection linear range and precision for phosphate and methylphosphonate in water.

<table>
<thead>
<tr>
<th>Compound</th>
<th>LOD (µg mL⁻¹)</th>
<th>RSD (%)</th>
<th>Linear range (µg mL⁻¹)</th>
<th>Regression equation (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMMP</td>
<td>0.34</td>
<td>5.04</td>
<td>4.508–450.8</td>
<td>Y = 95725.21 + 2.2018.31*X (0.9999)</td>
</tr>
<tr>
<td>TMP</td>
<td>2.20</td>
<td>3.67</td>
<td>7.512–375.6</td>
<td>Y = 166230.19 + 2.1220.38*X (0.9986)</td>
</tr>
<tr>
<td>TBP</td>
<td>0.01</td>
<td>6.44</td>
<td>0.2462–19.67</td>
<td>Y = 92216.62 + 1.03634E6*X (0.9990)</td>
</tr>
</tbody>
</table>
and spiked water samples were performed to investigate the precision of SPME. LODs were 1.11, 1.46 and 1.64 \( \text{ng} \text{L}^{-1} \) for the three analytes in air, but they were poorer in water, with 0.34 \( \text{ng} \text{L}^{-1} \) for DMMP, 2.20 \( \text{ng} \text{L}^{-1} \) for TMP, and 0.01 \( \text{ng} \text{L}^{-1} \) for TBP, respectively. Linear ranges were about 1–2 orders of magnitude for both air and aqueous sample. Based on six injections of the mixture using the SPME procedure, a relative standard deviation below 7% was achieved, depending on the compound.

The established SPME method was successfully applied to analyze a tap water sample; none of the analytes were detected. Therefore, 50 \( \mu \text{L} \) of standard solution was spiked into 5 mL of the tap water to calculate the recovery of the method. The areas of the peaks obtained, when these spiked tap water samples were analyzed by the same procedure as the standard solution, were compared with the corresponding peak areas obtained with the standard. Table III represents the recovery of phosphate and methylphosphonate in tap water. The recovery of two of the compounds is about 90%. The relative standard deviation \((n=4)\) was between 4.90–8.45%.

Figures 12 and 13 are chromatograms of air and tap water samples spiked with phosphate and methylphosphonate extracted by the OH-TSO/DVB fiber, and then analyzed on an SE-54 column under the same extraction conditions.

### Conclusion

A novel SPME fiber OH-TSO/DVB was first prepared by sol-gel and cross-linking technology and the characteristics of the new fiber were also evaluated. It possessed high thermal (to 380 °C) and solvent stability and long lifetime. Compared with commercial SPME fibers, the sol-gel-coated OH-TSO/DVB fiber showed better selectivity and sensitivity towards phosphate and methylphosphonate, especially for DMMP, acting as a simulant of methaphosphonate nerve agent. Therefore, the novel sol-gel OH-TSO/DVB fiber may be a good choice for analyzing warfare agents with SPME. Some important parameters using the new fiber were investigated. The optimum conditions are: extraction time, 5 min; extraction temperature, 60 °C for air sample, and 15 min, 32 °C, 1 g NaCl, constant stirring for water sample.

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References


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