Solid-Phase Microextraction and Gas Chromatography–Electron Capture Detection Analysis of Trace Organochlorine Pesticides in Water Using Novel Benzo-15-Crown-5 Sol-Gel Coating

Danhua Wang[1], Jiagang Peng[1], Jun Xing[1], Caiying Wu[1,*], and **Ying Xu[2]**

[1]College of Chemistry and Molecular Science, Wuhan University, Wuhan, 430072, China and [2]Institute of Hydrobiology, Chinese Academy of Science, Wuhan, 430072, China

Abstract

A novel dihydroxy-terminated benzo-15-crown-5 is synthesized and applied to prepare the solid-phase microextraction (SPME) fiber coating with sol-gel technology. Headspace SPME, as a simple, solvent-free method, is applied to the analysis of 16 organochlorine pesticides (OCPs) present at trace levels in a water sample. A homemade crown ether fiber coated with 80-µm thickness was used for extraction. Analyses are performed using gas chromatography–electroncapture detection. The optimization of the extraction process is studied. Compared with commercially available SPME fibers, polydimethylsiloxane, the new phases show better selectivity and sensitivity toward OCPs. The linear concentrations range from 1 to 1000 ng/L, the detection limits are in the range of 0.01–0.5 ng/L, the recoveries are over 85%, and relative standard deviations are below 7.2% for these OCPs.

Introduction

Pesticides have been widely applied in agriculture, either directly to soil or sprayed over crop fields. They can enter ground water as contaminants via filtration through the soil, by deposition, or by surface run-off. Organochlorines characteristically have very low solubilities in water, are fat soluble, and are resistant to metabolism. The combination of their persistence in the environment, toxicity, and ability to bioaccumulate has caused them to be labeled as environmental hazards. Thus, organochlorine pesticides (OCPs) are receiving much attention in the European Union and are included in the lists of the top priority pollutants to maintain water quality (1).

The primary steps in water analysis are the separation and preconcentration of the trace target analytes from the aqueous matrix. The two most popular analytical techniques, liquid–liquid extraction (LLE) and solid-phase extraction (SPE), are traditionally used for this purpose. LLE often employs large quantities of expensive, toxic solvents that can be harmful to the operator and environment. The procedure is time consuming, tedious, and often requires preconcentration of the sample prior to analysis. SPE has increased in popularity as a sample preparation technique because it overcomes a few of the disadvantages encountered with LLE, as it is not as time consuming and requires less solvent. However, disadvantages include plugging of cartridges, significant background interferences, and poor reproducibility (2).

The solid-phase microextraction (SPME) technique was introduced by Berladi and Pawliszyn (3) in 1989, which is a solvent-free analytical technique. The SPME technique has many attractive features compared with the traditional sample preparation methods. It can integrate the extraction, preconcentration, and sample introduction into one simple step. This convenient and solvent-free extraction method is also sensitive, inexpensive, and portable. Most works concerning the determination of OCPs were performed using manual SPME, polydimethylsiloxane (PDMS) fiber, and electron-capture detection (ECD) (4–6) or mass spectrometry (MS) (1,2,7–9) detection. The lowest limit of detection (LOD) has been reported in the range between 0.15 and 0.30 ng/L for 13 OCPs from water (10).

It is well known that crown ethers have wide applications in chemistry, especially in analytical chemistry, resulting from the cavity structure and strong electronegative effect of hetetroatoms on the crown ether ring. They have been widely used as chromatographic stationary phases for their strong directional force and good selectivity (11–13) and SPME coating (14–15). We specially synthesized a new dihydroxy-terminated benzo-15-crown-5 (DOH-B15C5) and preparated benzo-crown ether polysiloxane coating with sol-gel technique. OCPs are nonpolar compounds, and our homemade benzo-15-crown-5 sol-gel coating has the double characteristic for nonpolar and polar compounds because of the main structure of polysiloxane and the functionality benzo-15-crown-5. Therefore, extraction of OCPs using our homemade benzo-15-crown-5 sol-gel coating will be suitable.

* Author to whom correspondence should be addressed: email cywu@whu.edu.cn.

Experimental

Instrumentation and reagents

The SPME holder for manual sampling was obtained from Supelco (Bellefonete, PA). To compare the extraction efficiencies of different extraction phases, commercially available SPME fibers, PDMS (100 µm, Supelco) were used and compared with homemade fiber. The separations were carried out in a 6890N system equipped with a µ-electron capture detection (µ-ECD) system (Agilent, Palo Alto, CA). A 30-m × 0.32-mm i.d., 0.25-µm SPB-5 capillary column was used. High purity nitrogen was used as the carrier gas at a linear velocity of 20 cm/s. The gas chromatography (GC) injector was splitless for 2 min. The temperatures of the injector and detector were maintained in the ranges of 280°C and 290ºC, respectively. To analyze the OCPs, the GC was programmed to hold at 100ºC for 2 min, then heated at 10ºC/min to 190ºC for 2 min, and then 25ºC/min to 280ºC, which was held for 5 min. Ultrapure water from a Milli-Q system (Millipore, Bedford, MA) with electrical resistance 18 MΩ was used in all cases. The fused-silica fiber (140-µm o.d.) with protective polyimide coating was obtained from the Academy of Post and Techcommunication (Wuhan, China).

Hydroxy-terminated silicone oil (OH-TSO, molecular weight average 3500), tetraethoxysilane (TEOS), and ploy(methylhydrosiloxane)(PMHS) were obtained from the Chemical Plant of Wuhan University (Wuhan, China). Trifluoroacetic acid was purchased from Aldrich (Allentown, PA). All solvents used in this study were analytical-reagent grade. The stock solution of OCPs was prepared by dissolving each compound with methanol to 1 µg/mL at room temperature. Stock and working standards were stored at 4ºC in the refrigerator. The aqueous solutions were prepared daily by diluting standard solution with ultrapure water to give the corresponding solution for extraction.

Structure of crown ether and coating

The structure of crown ether and coating are shown in Figures 1 and 2.

Preparation of SPME fiber

Before coating the sol-gel stationary phase, the protective polyimide layer was removed from the fiber by dipping it into acetone for several hours at 25ºC. Then the fiber was dipped in 1M NaOH solutions for 1 h to expose the maximum number of silanol groups on the surface of the fiber, cleaned with water, and then it was placed in 0.1M HCl solutions for 20 min to neutralize the excess NaOH, cleaned again, and dried.

The sol solution was prepared at 25ºC as follows: 20 mg of DOH-B15C5 was dissolved in methylene chloride, and then 90 mg of OH-TSO, 10 mg of PMHS, and 100 µL of TEOS were added and mixed thoroughly. Eighty microliters of TFA containing 5% water was added to the resulting solution with ultrasonic agitation for 3 min. The mixture was centrifuged at 13,000 rpm for 5 min. The little white precipitate at the bottom of the tube was removed, and the clear sol solution was used for fiber coating.

The treated fiber was dipped vertically into the sol solution and held inside the sol solution for 5 min, during which a sol-gel coating was formed on the bare outer surface of the fiber end. For each fiber, this coating process was repeated several times in the same sol solution from 5 to 15 min until the thickness of the coating wanted was obtained. The fiber was removed and placed in a desiccator at room temperature for 24 h; the unattached end of the fused silica was then inserted into the outer stainless steel tube of the assembly with epoxy resin by manual operation and conditioned at 200–350ºC under nitrogen for 3 h in the GC injection port. The thickness of the fiber measured by microscope was semidiameter of coated fiber minus semidiameter of bare fiber. The final thickness of the fiber was 80 µm. The length of coated fiber was 1 cm. The concentration of DOH-B15C5 in the coated fiber was 9.1%.

Headspace extraction procedure

Sodium chloride (5 g) was added to a standard 25-mL headspace (HS) vial containing a magnetic spin bar and 15-mL water sample. To prevent sample evaporation, the HS vial was sealed with a septum. The HS vial was placed in a heating bath for 30 min to reach thermal equilibrium before extraction. Exposing the coated fiber end to the gas phase performed the extraction. The organic analytes were adsorbed from the gas onto the fiber. After the extraction, the fiber was withdrawn into the needle and immediately inserted into the heated GC injector port for desorption. The thermal desorption step was conducted in the splitless mode for 2 min.

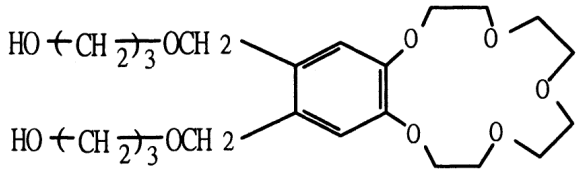

Figure 1. Structure of 4,5-dihydroxy(propoxy)methyl-benzo-15-crown-5.

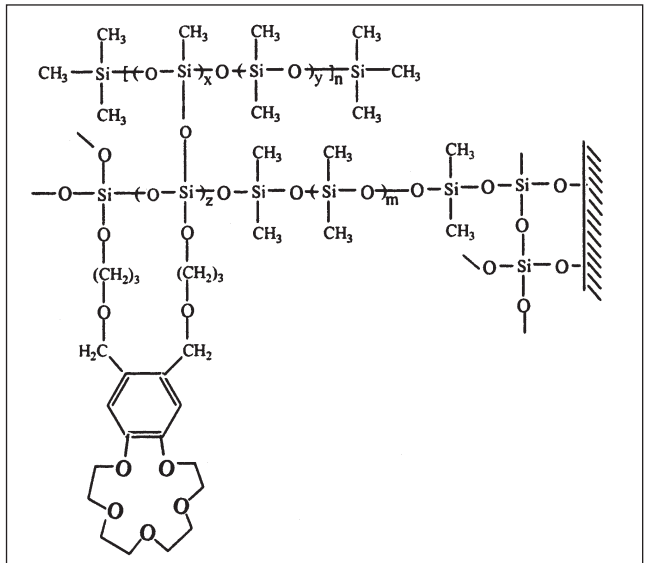

Figure 2. Structure of benzo-15-crown-5 sol-gel coating fiber.

Results and Discussion

Selection of optimum conditions for SPME

The GC equipped with μ-ECD was used to trace the optimum SPME methodology conditions. In general, the extraction efficiency is heavily dependent upon the extraction conditions and the kinds of the fibers. During the HS-SPME procedure, distribution and adsorption equilibrium of the analytes must be established between the aqueous and gaseous phases and between the gaseous and solid phases. The equilibrium is affected by various factors, including the nature of the fiber, the identity of the analytes, the extraction time, and the extraction temperature. The optimum experimental conditions should be investigated for each compound and for the fiber selected. Figure 3 is a typical gas chromatogram of an HS-SPME using the crown ether fiber for 0.1 μg/L OCPs under the optimized conditions.

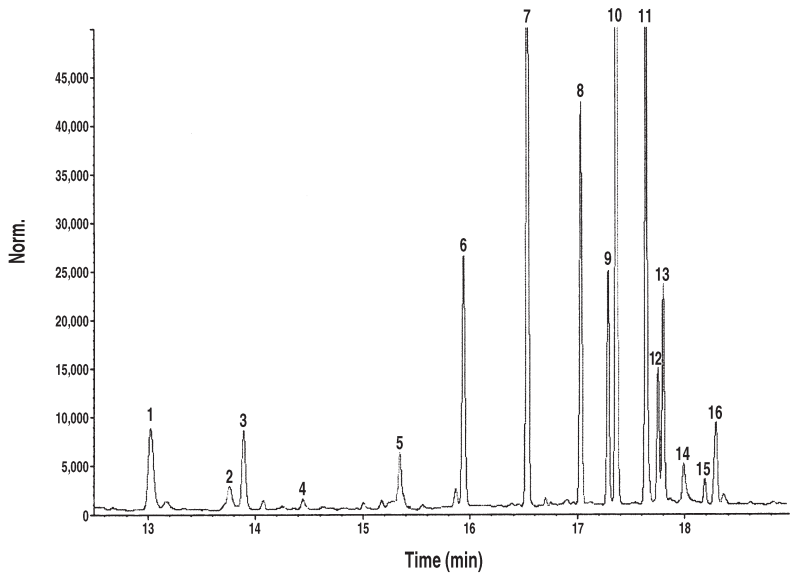

Figure 3. SPME-GC–ECD analysis of 0.1 g/L OCPs using the 80-μm 9.1% crown ether fiber: (1) α-HCH, (2) β-HCH, (3) γ-HCH, (4) δ-HCH, (5) heptachlo, (6) aldrin, (7) heptachlor epoxide, (8) endosulfan I, (9) *p,p*'-DDE, (10) dieldrin, (11) endrin, (12) endosulfan II, (13) *p,p*'-DDD, (14) endrin aldehyde, (15) endosulfan sulfate, and (16) *p,p*'-DDT. The extraction temperature was 90°C, extraction time was 40 min, desorption time 2 min, and they were agitated and saturated with salt.

Equilibrium extraction time and desorption time

Figure 4 shows the extraction time profile of the sol-gel crown ether fiber for 0.1 μg/L OCPs under agitation and saturated with salt sampling conditions at 90ºC. The results indicate that the equilibration time is very short, approximately 40 min for all 16 kinds of OCPs. The porous structure helps faster mass transfer during extraction, thus the equilibration time is shorter. For the same advantage, the desorption time is very short. Analytes that were adsorbed by the porous layer diffuse from fiber into the carrier gas rapidly. For all analytes, the desorption process is very fast and can be completed within 60 s at 280ºC. To eliminate the carryover, 2 min was selected for desorption. Such short extraction and desorption equilibration times arise from the porous structure of the coating and result in a short analysis time.

Extraction temperature

The extraction temperature profile is shown in Figure 5. It can be seen that with the increase of extraction temperature, the extraction quantities of all analytes are different. Most of the analytes are maximum at 90ºC, but α-HCH, β-HCH, and heptachlor are maximum at 70ºC and γ-HCH and endosulfan I are at 80ºC. Therefore, 90ºC was selected as the extraction temperature for OCPs.

Agitation and NaCl addition

The "salting out" effect is widely used to increase the effectiveness of an organic solvent to extract organic compounds dissolved in water. NaCl is often added to the sample in order to

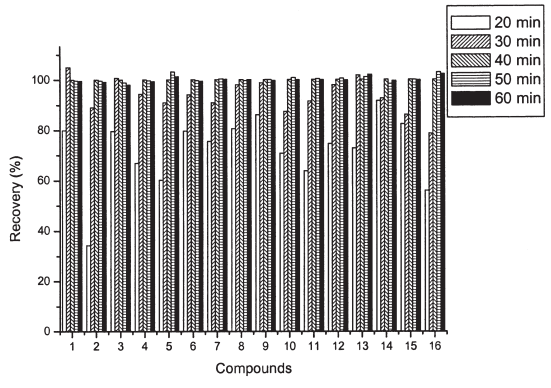

Figure 4. The extraction time profile of the 80-μm 9.1% crown ether fiber for 0.1 μg/L OCPs. The compounds are the same as in Figure 3, the extraction temperature was 90°C, desorption time was 2 min, and they were agitated and saturated with salt.

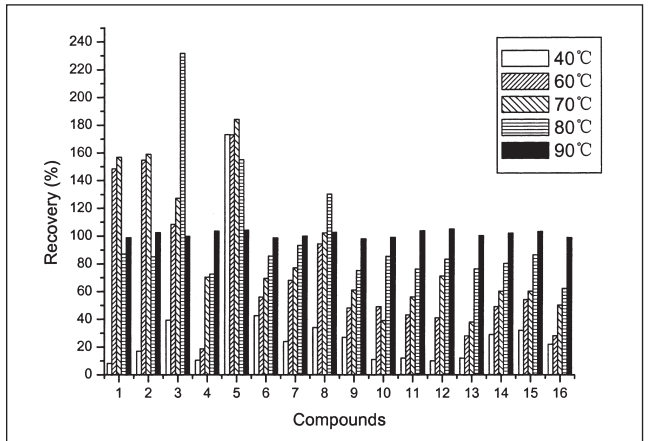

Figure 5. The extraction temperature profile of the 80-μm 9.1% crown either fiber for 0.1 μg/L OCPs. The compounds are the same as in Figure 3, and the extraction time was 40 min.

increase the ionic strength and enhance the amount of analyte extracted by fiber. The results obtained show that the peak areas increase significantly when salt is added compared with agitation. The maximum peak areas of these compounds were obtained when the sample solution was saturated with 5 g NaCl and stirred with a magnetic stirrer bar, thus this condition was selected for subsequent experiments.

Extraction quantity and selectivity

Comparison of extraction quantities with commercial coating

According to the previously stated experiments, the optimum analytical conditions for OCPs with the HS-SPEM–GC method were 40 min of extraction at 90ºC, 2 min desorption at 280ºC, the aqueous phase saturated with 5 g NaCl, and constant stirring at 85% of maximum. Figure 6 is the comparison of different fibers under optimum conditions. From Figure 6, it can clearly be seen that the extraction quantities of 80 µm crown ether coating are much higher than 100 µm PDMS fiber. There are some reasons for this good function: the 3-D network in the coating provides higher surface area and sample capacity, the phenyl in crown ether enhances π–π interaction with OCPs (but PDMS does not), and the high thermal desorption temperature overcomes the sample carry over problem.

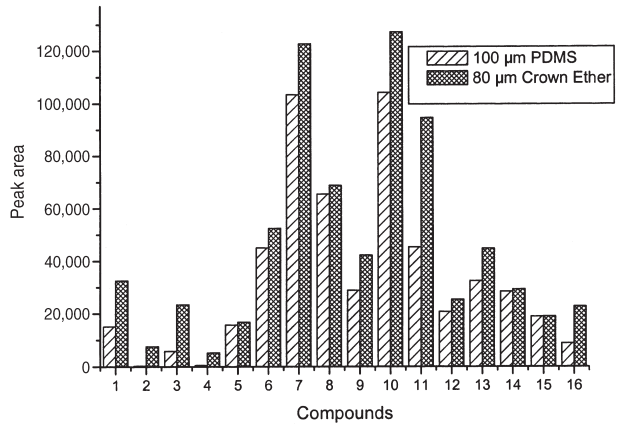

Figure 6. The comparison of extraction quantities using two different fibers for 0.1 µg/L OCPs under optimum conditions. The compounds are the same as in Figure 3.

Linear range, correlation coefficients, sensitivity, and precision

LODs, linearity, and precision were studied (see Table I). For all compounds, the LODs were between 0.01–0.5 ng/L. The linearity of response was studied over a vast concentration range, 1–1000 ng/L. The correlation coefficients were over 0.9975. The precision of the method for replicate analysis of aqueous solutions was also studied under optimum conditions. For the solution containing 0.1 µg/L of each compound, the relative standard deviation (RSD) was below 7.2%, which shows acceptable precision. These results exhibit that the crown ether fiber used in this study is better than literature data (10).

Application to real samples

The optimized SPME methods were applied successfully to the analysis of two polluted lake and a river water samples using the crown ether fiber. Data are collected in Table II. The concentrations of pollutants were very low. The recoveries were calculated from the East Lake (Wuhan, China) sample spiked with 100 ng/L standard. The spiking recovery of the East Lake water sample was between 85.9% and 107.6%, respectively. The found concentration of OCPs was calculated from the linear regression equation of the calibration curve for standards going through the HS-SPME and GC–ECD processes:

Recovery = (net concentration/ addition concentration) × 100 Eq. 1

Net concentration = apparent concentration – native concentration Eq. 2

Table I. LODs, Linear Range, Correlation Coefficients, and Precision for the Analysis of OPCs with HS-SPME–GC Using the Sol-Gel Crown Ether Fiber

Compound	LOD* (ng/L)	Linear Range (ng/L)	Correlation Coefficient	RSD (%, *n* = 6) 100 ng/L
α-HCH	0.10	0.1–10^3	0.9996	2.7
β-HCH	0.42	1–10^3	0.9999	5.4
γ-HCH	0.16	0.1–10^3	0.9990	7.2
δ-HCH	0.50	1–10^3	0.9975	7.0
Heptachlor	0.22	0.1–10^3	0.9998	5.3
Aldrin	0.06	0.1–10^3	0.9996	4.0
Heptachlor epoxide	0.01	0.1–10^3	0.9978	6.3
Endosulfan I	0.04	0.1–10^3	0.9998	3.2
p,p'-DDE	0.07	0.1–10^3	1.0000	3.0
Dieldrin	0.01	0.1–10^3	0.9998	6.2
Endrin	0.02	0.1–10^3	0.9997	3.8
Endosulfan II	0.13	0.5–10^3	0.9993	5.4
p,p'-DDD	0.08	0.1–10^3	0.9995	4.9
Endrin aldehyde	0.20	0.1–10^3	0.9989	4.6
Endosulfan sulfate	0.33	1–10^3	0.9999	3.2
p,p'-DDT	0.16	0.1–10^3	0.9989	5.6

* Signal-to-noise ratio = 3.

Conclusion

This paper describes an HS-SPME method using homemade coating that is applied to the analysis of 16 OCPs and in an environmental water sample. ECD detection combined with SPME is highly selective and sensitive. The novel homemade fiber has high, rich efficiency that is

Table II. Determination of Organochlorine Compounds in Polluted Water Samples

Compound	East Lake (ng/L)	Yaer Lake (ng/L)	Shanhoujing River in Zhejiang (ng/L)	Recovery of East Lake (%, *n* = 3) 100 ng/L
α-HCH	7.1	79.9	4.1	93.9
β-HCH	ND*	ND	ND	93.8
γ-HCH	2.3	ND	ND	92.4
δ-HCH	1.0	20.1	15.6	107.6
Heptachlor	ND	ND	ND	97.4
Aldrin	ND	ND	ND	97.2
Heptachlor epoxide	ND	ND	ND	89.7
Endosulfan I	ND	ND	ND	85.9
p,p'-DDE	4.2	8.7	5.9	96.7
Dieldrin	ND	ND	ND	97.8
Endrin	ND	ND	ND	96.7
Endosulfan II	ND	ND	ND	87.3
p,p'-DDD	ND	ND	ND	107.5
Endrin aldehyde	ND	ND	ND	100.7
Endosulfan sulfate	ND	ND	ND	90.1
p,p'-DDT	3.7	10.2	6.2	98.4

* ND, below LOD.

very suitable to analyze trace pollutants in environmental water. The optimum conditions for extraction were investigated at an equilibrium time of 40 min at 90ºC and desorption in a GC injector at 280ºC for 2 min. This method shows a precision below 7.2% (RSD), depending on the compound. Linearity is verified over a wide range, and the LODs are below the ng/L level.

Acknowledgments

This work was supported by the National Natural Science Foundation of China and the Research Fund for the Doctoral Program of Higher Education of China.

References

1. J.P. Pérez-Trujillo, S. Frías, J.E. Conde, and M.A. Rodríguez-Delgado. Comparison of different coatings in solid-phase microextraction for the determination of organochlorine pesticides in ground water. *J. Chromatogr. A* **963:** 95–105 (2002).
2. S. Magdic and J. Pawliszyn. Analysis of organochlorine pesticides using solid-phase microextraction. *J. Chromatogr. A* **723:** 111–22 (1996).
3. C.L. Arthur and J. Pawliszyn. Solid phase microextraction with fused silica optical fibers. *Anal. Chem.* **62:** 2145–48 (1990).
4. W.H. Ho and S.J. Hsieh. Solid phase microextraction associated with microwave assisted extraction of organochlorine pesticides in medicinal plants. *Anal. Chim. Acta* **428:** 111–20 (2001).
5. F.J. López, E. Pitarch, S. Egea, J. Beltran, and F. Hernandez. Gas chromatographic determination of organochlorine and organophosphorus pesticides in human fluids using solid phase microextraction. *Anal. Chim. Acta* **433:** 217–26 (2001).
6. R. Boussahel, S. Bouland, K.M. Moussaoui, M. Baudu, and A. Montiel. Determination of chlorinated pesticides in water by SPME/GC. *Water Res.* **36:** 1909–11 (2002).
7. B.H. Hwang and M.R. Lee. Solid-phase microextraction for organochlorine pesticide residues analysis in Chinese herbal formulations. *J. Chromatogr. A* **898:** 245–56 (2000).
8. T. Kusakabe, T. Saito, and S. Takeichi. Solid-phase microextraction and gas chromatography-mass spectrometry analysis of p,p´-DDE in biological samples. *J. Chromatogr. B* **761:** 93–98 (2001).
9. Al-Saleh, M. Al-Zahrani, M. Al-Shanshoury, G. Mohammed, N. Shinwari, I. Al-Doush, and P. Basile. Comparative quantitative study of DDT and its metabolites in human milk by gas chromatography with electron capture and mass spectrometric detection. *Chromatographia* **55:** 457–61 (2002).
10. C. Aguilar, A. Peñalver, E. Pocurull, J. Ferré, F. Borrull, and R.M. Marcé. Optimization of solid-phase microextraction conditions using a response surface methodology to determine organochlorine pesticides in water by gas chromatography and electron-capture detection. *J. Chromatogr. A* **844:** 425–32 (1999).
11. C.Y. Wu, C.M. Wang, Z.R. Zeng, and X.R. Lu. Preparation and characteristics of a crown ether polysiloxane stationary phase for capillary gas chromatography. *Anal. Chem.* **62:** 968–71 (1990).
12. C.Y. Wu, X.C. Zhou, Z.R. Zeng, X.R. Lu, and L.F. Zhang. Preparation and study of two benzo-crown ether polysiloxane stationary phases for capillary gas chromatography. *Anal. Chem.* **63:** 1874–79 (1991).
13. C.Y. Wu, L.S. Cai, Y.J. Wang, H.M. Han, Z.R. Zeng, Z.Y. Yu, and H. Yuan. Preparation and gas chromatographic characterization of dibenzyl crown ether stationary phases. *Chromatographia* **37:** 374–80 (1993).
14. Z.R. Zeng, W.L. Qiu, and Z.F. Huang. Solid-phase microextraction using fused-silica fiber coated with sol-gel-derived hydroxyl-crown ether. *Anal. Chem.* **73:** 2429–36 (2001).
15. Z.Y. Wang, C.H. Xiao, C.Y. Wu, and H.M. Han. High-performance polyethylene glycol-coated solid-phase microextraction fibers using sol-gel technology. *J. Chromatogr. A* **893:** 157–68 (2000).

Manuscript accepted September 4, 2003.