ELSEVIER

Journal of Chromatography A, 740 (1996) 139–145

JOURNAL OF CHROMATOGRAPHY A

Short communication

Optimizing split/splitless injection port parameters for solid-phase microextraction

John J. Langenfeld, Steven B. Hawthorne*, David J. Miller

University of North Dakota, Energy and Environmental Research Center, P.O. Box 9018, Grand Forks, ND 58202, USA

Received 30 August 1995; revised 9 February 1996; accepted 9 February 1996

Abstract

Desorption profiles of analytes using a conventional split/splitless injection port after solid-phase microextraction were determined in real-time by direct coupling of the injection port to a mass spectrometer. Desorption times for aromatic hydrocarbons increased with the analyte's boiling point, and decreased with injection port temperature. For example, naphthalene (b.p. 218°C) was efficiently desorbed in <12 s at 200°C and higher temperatures, while benz[*a*]anthracene required >60 s at 200°C, but only 15 s at 300°C. As expected, desorption profiles from a 7-μm poly(dimethylsiloxane) fiber were initially narrower than the 100-μm fiber; however, the profiles from the 7-μm fiber showed significantly more tailing indicating the presence of more available active sites (possibly on the silica core) for thinner fiber coatings. All desorption profiles were wider than could be accepted for capillary GC, but good chromatographic peak shapes were obtained by cryogenic focusing in the GC column, increasing the GC column film thickness, and reducing the injection port liner volume from 1 ml to 0.25 ml.

Keywords: Desorption; Solid-phase microextraction; Extraction methods; Injectors; Optimization; Hydrocarbons, aromatic; Polynuclear aromatic hydrocarbons

1. Introduction

Solid-phase microextraction (SPME) is a relatively new extraction technique that is gaining acceptance for solventless extraction of water samples. Unlike conventional extraction methods that aim for quantitative analyte removal from a sample (i.e., purge and trap, solid-phase extraction and liquid–liquid extraction), SPME is an equilibrium method that relies on analyte partitioning between a water phase or headspace above water [1–3], solid sample [4–7], or an air sample [8,9], and a fused-silica fiber coated with a liquid-polymeric phase [e.g., poly(dimethylsiloxane)].

SPME is mechanically simple to perform compared to conventional extraction techniques. The sorbent-coated fiber is exposed to a sample, and after a specified time the fiber is removed from the sample and inserted into the heated injection port of a gas chromatograph. Thermal desorption inside the injection port transfers the analytes from the fiber into the GC column, and chromatographic separation is performed in a normal manner. As well as being a new extraction technique, SPME can also be considered a new solvent-free GC injection method [10]. To date, most of the developmental work on SPME has been performed using gas chromatographs with

*Corresponding author.

0021-9673/96/$15.00
PII S0021-9673(96)00140-9

septum-equipped programmable injectors (SPIs) produced by Varian [1–9]. The SPI has properties that make it ideally suited for solvent-free applications such as SPME including a small internal volume, the ability to be heated independently of the GC oven, and direct injection capabilities that resemble on-column injection [11]. However, SPI are not as common as the split/splitless injection port, which is one of the oldest and most popular injection ports available. Split/splitless injection ports also have potential to be used successfully with SPME (most notably in the splitless mode where maximum sensitivity would be achieved), but only limited work has been done coupling these systems [12,13].

The optimization of SPME injections requires an understanding of the interactions between the desorption rate of absorbed analytes (and the effect of the sorbent characteristics such as film thickness) with injection port parameters (e.g., temperature and liner volume) as well as chromatographic conditions. To better understand the interactions of these parameters, the effects of injection port temperature on the desorption process and the subsequent effects of chromatographic conditions were studied independently. To study thermal desorption profiles, the split/splitless injection port was directly coupled to a mass spectrometer with a short length of fused-silica tubing (i.e., no chromatographic column). This allowed real-time desorption profiles to be determined at various injection port temperatures with both 7-μm and 100-μm fibers. Once the desorption profiles were established, injection port and chromatographic parameters including split/splitless liner volume, cryogenic trapping temperature, and column film thickness were examined.

2. Experimental

2.1. Standards

Individual aromatic hydrocarbons (benzene, naphthalene, phenanthrene, fluoranthene, benz[*a*]anthracene, and benzo[*a*]pyrene) were obtained from Aldrich (Milwaukee, WI, USA) and were of 99+% purity. A single 12–250 μg/ml standard of the six aromatic hydrocarbons was prepared in Fisher Optima Grade acetone (Fisher Scientific, Pittsburgh, PA, USA). The standard was prepared so that approximately the same mass of each component was extracted by the fiber by taking into account the values of K determined for the test compounds from a 100-μm poly(dimethylsiloxane) fiber [14]. A 20-μl aliquot of this standard was spiked into a 40-ml vial (Supelco, Bellefonte, PA, USA) containing 38 ml of Fisher HPLC-grade water. Prior to sealing the vial, a magnetic stir bar (2.54 cm long×0.79 cm diameter) was added to the water so that the solution could be agitated during SPME (note that there was no headspace above the water once the magnetic stir bar was added).

2.2. SPME procedure

SPME was performed by inserting a commercially-available 100-μm or 7-μm poly(dimethylsiloxane) fiber (Supelco) through the teflon-lined septum on the vial, and depressing the plunger of the fiber holder so that the fiber was directly exposed to the water standard until absorption equilibrium was achieved for all of the hydrocarbons (previously determined to be 5 h for benzo[*a*]pyrene from a 100-μm poly(dimethylsiloxane) fiber [14]). Once the extraction was completed, the fiber was retracted back inside its protective needle and removed from the water standard.

2.3. Real-time fiber desorption profile monitoring

Fiber desorption profiles were examined by directly coupling the split/splitless injection port of a Hewlett-Packard 5890 Series II GC to a Hewlett-Packard 5972 mass spectrometer. A 70 cm×0.1 mm I.D.×0.16 mm O.D. piece of fused-silica tubing (Polymicro Technologies, Phoenix, AZ, USA) was installed in the split/splitless injection port in the same manner as a GC column, while the other end of the tubing was fed through the transfer line of the mass spectrometer until it was about 1 mm from the repeller. The GC oven, GC-MS transfer line, and the detector were maintained at 320, 320 and 220°C, respectively. The head pressure of helium was set at 91 kPa. A 7.9 cm×2-mm I.D. (250 μl internal volume) glass insert was used in the injection port,

which was maintained at either 200, 250, 300 or 350°C. The GC system was operated in the split mode so the dead volume of the injection port would not affect the desorption measurements [11]. Using this setup, the hold-up time of the system was measured to be approximately 0.5 s, resulting in real-time measurements of the desorption profile of each analyte.

Prior to determining the desorption profiles, the mass spectrometer was turned on for approximately 1 min so the baseline could stabilize. Once a flat baseline was established, the SPME fiber (with absorbed hydrocarbons) was introduced into the split/splitless injection port. Desorption profiles were recorded with the mass spectrometer operating in the selected ion monitoring (SIM) mode for the molecular ion of each aromatic (e.g., m/z 78 for benzene, m/z 128 for naphthalene, etc.), and the electron multiplier voltage was held at 2556 V.

2.4. Gas chromatographic analysis

Analysis by GC was performed by directly inserting the SPME fiber into the split/splitless injection port of a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detection (FID) system. The desorptions were performed by piercing the injection-port septum with the SPME needle and depressing the plunger to expose the coated fiber to the heated injection port. The columns used were either a 60 m×0.25 mm I.D. (0.25 μm film thickness) DB-5 column or a 60 m×0.25 mm I.D. (1 μm film thickness) DB-5 column (J&W Scientific, Folsom, CA, USA). Hydrogen was used as the carrier gas at a linear velocity of 50 cm/s. The initial column temperature was maintained at either −30, 0, 30, or 60°C for 3 min in the splitless mode during the desorption (the GC oven was cryogenically cooled with liquid nitrogen to achieve oven temperatures below 30°C), and after the 3-min holding time the GC oven was ramped at 20°C/min to 320°C, and held at 320°C for 5 min. Either a 7.9 cm×4 mm I.D. or 7.9 cm×2 mm I.D. (1 ml and 250 μl internal volumes, respectively) glass liner was used in the injection port (maintained at 300°C throughout the entire separation), and the FID temperature was maintained at 320°C.

3. Results and discussion

3.1. Effects of temperature and fiber-coating thickness on desorption profiles

In SPME, analytes in water are absorbed by a fused-silica fiber coated with a liquid-polymeric extraction phase. The SPME device not only acts as an extraction device, but also as a sample-introduction device for gas chromatography. For efficient and quantitative transfer of analytes from the fiber coating to the GC column, the following two steps must occur: (1) reasonably rapid and quantitative desorption of the target hydrocarbons from the fiber sorbent coating and (2) quantitative transfer of the aromatics from the injection port to the GC column followed by efficient focusing of the analytes at the head of the column to minimize band broadening effects due to the finite desorption times and the dead volume of the injection port. Although the considerations of the second step are fairly well understood because they are important for analogous solventless injection systems (e.g., thermal desorption from a resin trap), the SPME desorption step is difficult to understand and evaluate independently. Understanding the desorption rate is important to optimize qualitative (chromatographic peak shape) results and avoid quantitative errors, especially since Potter and Pawliszyn previously reported up to 15% carryover of anthracene, benz[a]anthracene, and benzo[a]pyrene after a 1-min fiber desorption at 300°C with a 15-μm poly(dimethylsiloxane) fiber [15]. Since desorption rates have not been accurately measured for SPME, real-time monitoring by coupling the split/splitless injection port directly into the ion source of a mass spectrometer was used to determine the rate of the desorption process.

Fig. 1 compares the desorption rates of naphthalene (b.p. 218°C) and benz[a]anthracene (b.p. 400°C) using a 100-μm or 7-μm fiber at either 200, 250 or 300°C. Each peak represents the intensity of the MS response (in the selected ion monitoring mode) as a function of time, and the peak heights for each aromatic are normalized to the ion abundance for a 300°C desorption. At 200°C, the desorption of naphthalene (b.p. 80°C) was quite rapid and was complete in only ~12 s (as determined by the width of the desorption peak at the baseline). Less volatile

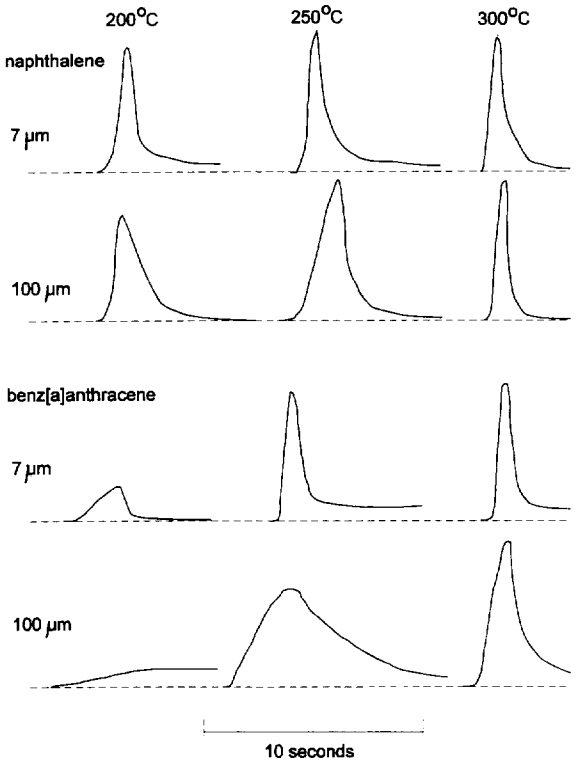

Fig. 1. Comparison of real-time desorption profiles of naphthalene and benz[*a*]anthracene using 7-μm and 100-μm fibers at 200, 250 and 300°C. The peak heights of each analyte are normalized to the abundance desorbed from each fiber at 300°C.

aromatic hydrocarbons such as benz[*a*]anthracene (b.p. 400°C) were desorbed at a much slower rate at 200°C, as evident by the lower peak heights (relative to the peak heights obtained at 300°C for each compound) and the tailing effects that often extended for several minutes. Note that because of tailing effects the peak heights shown in Fig. 1 do not reflect the area under each peak. Instead, it was found that the peak areas after a 5-min desorption were nearly identical regardless of the desorption temperature.

Elevated desorption temperatures generally had negligible effects on the desorption rate of volatile aromatic hydrocarbons such as naphthalene, and desorption of naphthalene was complete in ~12 s whether the desorption temperature was 200 or 300°C. However, significantly faster desorption rates were obtained when the desorption temperature was increased from 200 to either 250 or 300°C for the less volatile high-molecular-mass aromatics. For example, the desorption rate of benz[*a*]anthracene increased at 250°C, as evident by the sharper peak height and reduced tailing effects compared to desorption at 200°C. Further increasing the desorption temperature to 300°C resulted in significantly reduced tailing and faster desorption estimated to be about 16 s. Although the desorption rate of naphthalene was not significantly affected by elevated desorption temperatures, desorption at 300°C was the only condition that rapidly desorbed all of the aromatics from the 100-μm fiber.

Fig. 1 also shows the effect of the fiber coating thickness on the desorption profiles of naphthalene and benz[*a*]anthracene. Minimal differences were obtained between the desorption profiles of naphthalene from the 100-μm and 7-μm fiber. However, less volatile aromatics such as benz[*a*]anthracene were desorbed more rapidly from the 7-μm fiber than from the 100-μm fiber, especially at 250°C and 300°C. Surprisingly, tailing effects were more prevalent for all of the PAHs when they were desorbed from the 7-μm fiber (compared to the 100-μm fiber). Although the reasons are unclear, tailing peaks usually signify heterogeneity in a system (i.e., active sites). Previous reports have suspected heterogeneity in the 7-μm poly(dimethylsiloxane) fibers from Supelco when measured equilibrium distribution constants (K) were found to be higher with a 7-μm fiber than a 100-μm fiber [14]. These results suggested that aromatics may be more exposed to active sites on the fused-silica core with the thinner 7-μm coating (in addition to absorption in the fiber coating) due to the higher core-surface area/coating volume ratio, as well as the relatively higher fraction of analytes exposed to active sites (as a result of the lower capacity of the 7-μm coating compared to the 100-μm coating).

To further examine the presence of active sites in the coated fibers, a separate standard including phenol (aromatic acid) and pyridine (aromatic base) was prepared in water, extracted with a 100-μm or 7-μm fiber and desorbed at 250°C. The real-time desorption profiles are shown in Fig. 2. Desorption of pyridine and phenol was fairly rapid and only slight tailing effects were observed with the 100-μm fiber. Although the desorption rate of naphthalene

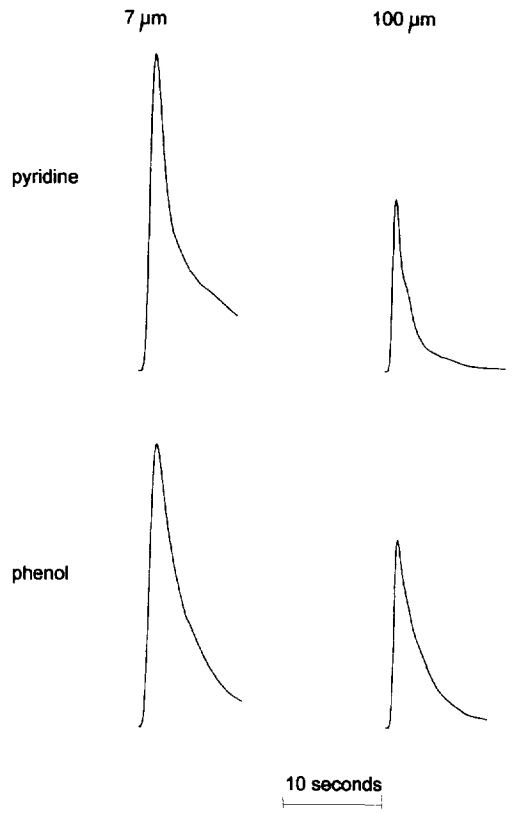

Fig. 2. Comparison of real-time desorption profiles of pyridine and phenol using 7-μm and 100-μm fibers at 250°C. The peak heights of each analyte are normalized to the abundance desorbed from each fiber at 300°C.

was fairly independent of the desorption temperature (see Fig. 1), elevated temperatures slightly increased the desorption rate and reduced tailing effects of phenol (b.p. 182°C) and pyridine (b.p. 115°C) desorbed from a 100-μm fiber. On the other hand, desorption of pyridine and phenol from the 7-μm fiber showed tailing effects that were much worse than with the 100-μm fiber, as demonstrated by the continued detection of pyridine and phenol from the 7-μm fiber even after 1 or 2 min desorption. Again, these results demonstrate that experimental desorption times are slower than theoretical predictions, and tailing effects are prevalent for both non-polar and polar analytes as shown in Fig. 1 and Fig. 2. None of these desorption phenomena are accounted for in previous SPME theoretical studies.

3.2. Effects of liner volume, trapping temperature and column film thickness

Once the analytes are desorbed from the SPME fiber, they must be transported from the split/splitless injection port to the GC column in an efficient manner. Neither SPME desorption or transport from the injection port liner to the column are instantaneous, so the initial peak width of the hydrocarbons (especially the more volatile species) will be larger than the acceptable high-resolution capillary chromatography peak width unless measures are taken to refocus the compounds in narrow injection bands using cryogenic trapping. Fig. 3 shows the effect of cryotrapping temperature on the peak shape and width of the most volatile hydrocarbon tested (benzene) using a wide-bore glass insert (1-ml volume) and a narrow-bore insert (250-μl volume). All desorptions shown in Fig. 3 were performed with a 100-μm fiber and an injection port temperature of 300°C. A 60-m DB-5 column (0.25-mm I.D.) with a 0.25-μm film thickness was used for the GC-FID separation. Each peak represents the FID response as a function of time (all peaks shown are at the same FID attenuation).

Peak widths were unacceptably high for benzene (18 s wide at one-half of the peak height) when the trapping temperature was maintained at either 30°C or 60°C with the wide-bore insert, and severe tailing effects were observed which reflect the 1-ml dead volume of the glass-liner insert. Decreasing the trapping temperature to 0°C narrowed the peak width of benzene to 6 s, but only cryogenic trapping at −30°C efficiently focused benzene so the peak had acceptable symmetry and peak width expected from high-resolution capillary chromatography.

Although the trapping temperature affected the symmetry and peak width of benzene with the wide-bore insert, it was much less important when using a narrow-bore insert (250 μl) because benzene could be transported more rapidly from the injection port liner to the GC column. Trapping temperatures up to 60°C could even be used with the DB-5 column with only a slight gain in the peak width (i.e., 3.6 s). These peak widths and shapes are similar to those obtained by conventional solvent injections in the splitless mode. Although large volume glass liners are often required for solvent injections in the

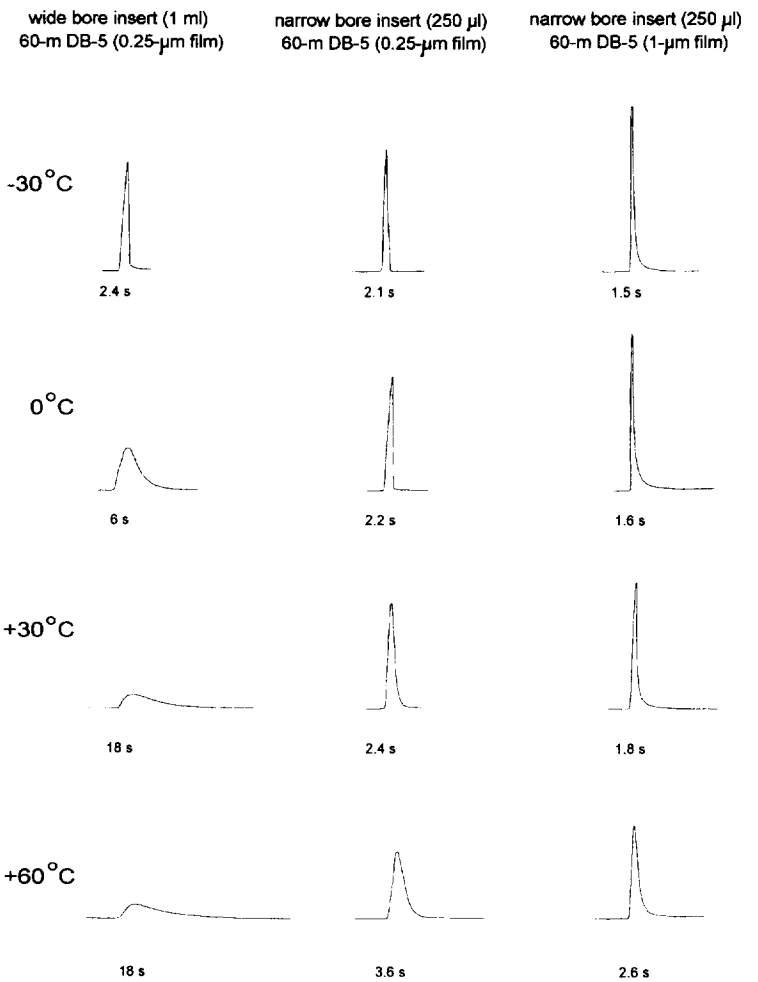

Fig. 3. Effect of glass-liner volume, cryogenic trapping temperature and column-film thickness on the chromatographic peak symmetry and width of benzene desorbed from a 100-μm poly(dimethylsiloxane) fiber at 300°C. The FID peak attentuation is the same for all of the conditions, and the GC conditions are given in Section 2.

splitless mode to accommodate the expanded solvent vapors, SPME is solvent-free and small volume liners can be used without backflushing contaminants into the carrier gas lines of the GC system. Furthermore, these results demonstrate that a smaller liner volume puts less demands on cryogenic focusing to obtain narrow chromatographic peaks.

Although cryogenic trapping is an effective method for focusing broad injection bands after splitless injection by SPME, utilizing a GC column with a thicker film may also aid in the refocussing of broad injection bands and lessen the demand for cryogenics. Fig. 3 also shows the effect of the GC column film thickness on the peak shape of benzene using a 60 m×0.25 mm I.D. DB-5 column with a thick film (1-μm film thickness) and a narrow-bore glass insert (250 μl). The peak widths of benzene focused onto a 1-μm film DB-5 column were only slightly narrower than with the 0.25-μm film DB-5 column at trapping temperatures up to 30°C. For example, when the trapping temperature was maintained at 30°C, the peak width of benzene with the 0.25-μm film DB-5 was 2.4 s, while the peak width with the 1-μm film DB-5 was 1.8 s. However, when the 1-μm film DB-5 column was used, trapping temperatures up to 60°C could be used to refocus

benzene so that it had acceptable symmetry and peak width, even though benzene has a boiling point of 80°C. These results demonstrate that when the dead-volume of the injector is minimized by using a narrow-bore glass liner, good chromatographic peak shape and widths can be obtained as long as the GC oven temperature is held at least 50°C below the elution temperature of the most volatile target hydrocarbon when using conventional capillary GC columns (i.e., 0.25 μm column film thickness). Alternatively, a thicker film GC column (e.g., 1-μm film) allows trapping temperatures as high as 20°C from the boiling point of the most volatile target hydrocarbon, thus reducing the need for cryogenic cooling for most target analytes.

4. Conclusions

Efficient desorption of non-volatile analytes from SPME fibers with 7-μm and 100-μm coatings can be achieved using a conventional split/splitless injection port at 300°C. However, desorption times are often longer than previously predicted by theoretical studies. The efficiency of desorption from 7-μm fibers can be poorer than from 100-μm fibers, especially for more polar analytes, because of increased interactions with the silica core. For SPME desorptions with a conventional split/splitless injection port (splitless mode), narrow-bore injection port inserts (250-μl volume) give much narrower chromatographic peaks than splitless (wide-bore) inserts with a 1-ml volume. With a narrow bore insert and a 1-μm film thickness column, efficient focusing of compounds as volatile as benzene can be achieved without cryogenic cooling (GC oven temperatures as high as 60°C).

Acknowledgments

The financial support of the US Environmental Protection Agency, Office of Exploratory Research is gratefully acknowledged.

References

[1] C.L. Arthur, K. Pratt, S. Motlagh, J. Pawliszyn and R.P. Belardi, J. High Resolut. Chromatogr., 15 (1992) 741.
[2] D.W. Potter and J. Pawliszyn, J. Chromatogr., 625 (1992) 247.
[3] C.L. Arthur, L.M. Killam, S. Motlagh, M. Lim, D.W. Potter and J. Pawliszyn, Environ. Sci. Technol., 26 (1992) 979.
[4] Z. Zhang and J. Pawliszyn, Anal. Chem., 65 (1993) 1843.
[5] B. MacGillivray, J. Pawliszyn, P. Fowlie and C. Sagara, J. Chromatogr. Sci., 32 (1994) 317.
[6] Z. Zhang and J. Pawliszyn, Anal. Chem., 67 (1995) 34.
[7] Z. Zhang and J. Pawliszyn, J. High Resolut. Chromatogr., 16 (1993) 689.
[8] M. Chai and J. Pawliszyn, Environ. Sci. Technol., 29 (1995) 693.
[9] M. Chai, C.L. Arthur, J. Pawliszyn, R.P. Belardi and K.F. Pratt, Analyst, 118 (1993) 1501.
[10] C.L. Arthur, M. Chai and J. Pawliszyn, J. Microcol. Sep., 5 (1993) 51.
[11] K. Grob, Anal. Chem., 66 (1994) 1009A.
[12] R. Eisert, K. Levsen and G. Wunsch, J. Chromatogr. A, 683 (1994) 175.
[13] L.P. Sarna, G.R.B. Webster, M.R. Friesen-Fischer and R. Sri Ranjan, J. Chromatogr. A, 677 (1994) 201.
[14] J.J. Langenfeld, S.B. Hawthorne and D.J. Miller, Anal. Chem., 68 (1996) 144–155.
[15] D.W. Potter and J. Pawliszyn, Environ. Sci. Technol., 28 (1994) 298.