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New method to determination of naphthalene in ambient air using cold fiber-solid phase microextraction and gas chromatography–mass spectrometry

Helvécio C. Menezes, Breno P. Paulo, Nathália T. Costa, Zenilda L. Cardeal *

Departamento de Química, ICEx, Universidade Federal de Minas Gerais, Avenida Antônio Carlos, 6627 — 31270901 Belo Horizonte, MG, Brazil

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ABSTRACT

Naphthalene, a highly volatile polycyclic aromatic hydrocarbon (PAH), is classified as possibly carcinogenic to humans and can be found in various environments. This paper describes a cold fiber (CF) solid phase microextraction (SPME) sampling method coupled with gas chromatography/mass spectrometry (GC/MS) for determining naphthalene in ambient air. The method uses a 100 μm polydimethylsiloxane (PDMS) fiber to generate gaseous standards using a permeation tube. The method shows good results for many validation parameters. The intra-assay precision shows a relative standard deviation (RSD) ranging from 1.04 to 8.11%; the limit of detection (LOD) is 0.33 ± 0.01 μg/m^3, and the quantification limit (LOQ) is 0.55 ± 0.01 μg/m^3. The method was applied to the determination of naphthalene from real samples collected from indoor and outdoor air. The results have shown the ability of the method to measure trace levels of naphthalene in the air in different environments.

1. Introduction

Naphthalene, the simplest polycyclic aromatic hydrocarbon (PAH), is one of the more volatile of the class of pollutants classified as semivolatile organic compounds (SVOC) by the U.S. Environmental Protection Agency [1]. The International Agency for Research on Cancer (IARC) has classified naphthalene as possibly carcinogenic to humans [2]. This probable human carcinogenicity is based on an increased risk of rare nasal tumors in male rats [3]. The World Health Organization (WHO) is considering the development of an indoor air guideline for naphthalene [4]. Naphthalene can be found in various environments. Because of its volatility, the atmosphere is the main repository [5]. In the air, naphthalene is distributed between the gaseous and particulate phases, depending on the temperature, precipitation and environmental factors [6]. The half-life of naphthalene in the atmosphere is 8 h because of photodegradation by hydroxyl radicals [7]. The largest exposures to this compound occur near sources emitting naphthalene [8]. Chemical industries, burning of biomass, insect repellents, gasoline and oil burning are the main anthropogenic sources of naphthalene [9]. In urban areas, vehicle emissions represent the most important source [10,11]. Occupational exposure occurs from creosote impregnation, the manufacture of mothballs, oil refineries, the manufacture of phthalic anhydride, cooking plants, foundries, and the production of surfactants and pesticides [12,13]. Burning cigarettes contribute significantly to the increase of exposure to naphthalene in micro-environments [14]. Occupational exposure guidelines formulated for naphthalene include a reference exposure limit (REL) of 75 μg/m^3 for a short-term exposure of 15 min and a threshold limit value (TLV) of 50 μg/m^3 (a time-weighted average (TWA) measured over an 8 h period) [15]. Several techniques can be applied to sample volatile and semivolatile organic compounds in the air, including the use of metal containers, bags, and sorbent enrichment [16–18]. These techniques are efficient, but they have many steps. One technique that has proven to be very efficient for sampling volatile compounds in air is solid phase microextraction (SPME). SPME combines sampling and preconcentration in a single step, with subsequent desorption directly into the analytical instrument [19,20]. The present study used a system constructed [21] to generate naphthalene standards by permeation, with a new alternative device for cold fiber (CF) SPME sampling in ambient air. After direct desorption into the injector of the GC, determinations by gas chromatography coupled to mass spectrometry (GC/MS) were performed. The optimized method is simple and sufficiently sensitive for the analysis of naphthalene in environmental air samples.

2. Materials and methods

2.1. Generator of naphthalene standard

The system for the generation of gaseous standards, developed in a previous study [22], is presented in Fig. 1. The air was conducted to a UHP-10ZA Brand Domnick Hunter scrubber after compression to 345 kPa and then passed through a spiral copper pipe for preheating. After the dilution control valve, the air entered the permeation chamber and passed through a permeation tube of polymer material containing

* Corresponding author. Tel.: +55 3134095725; fax: 55 3134095700.
E-mail address: zenilda@ufmg.br (Z.L. Cardeal).

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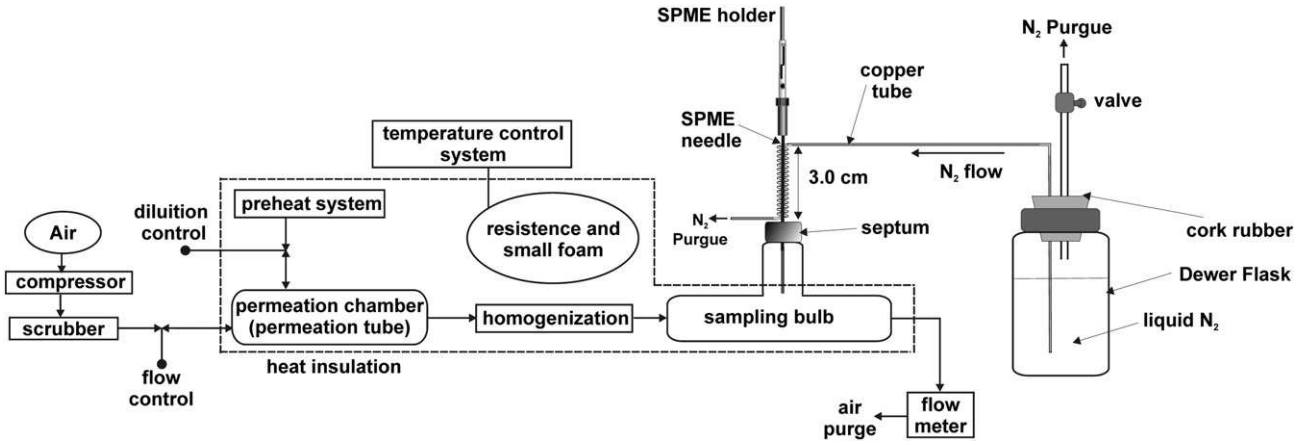

Fig. 1. System for the generation of gaseous standards.

naphthalene. The tube, manufactured by VICI Metronics, Inc., and certified for traceability by NIST (National Institute of Standards and Technology) was 3.5 cm in length, and the rate of permeation was 16.62 ± 0.79 ng/min[1] at 40.0 °C. The tube was maintained at a constant flow with the temperature of 40.0 ± 0.1 °C controlled by a thermostat. The temperature control system was connected to a resistor and a small fan for even distribution of heat throughout all the components inserted into the space bounded by heat insulation (dotted rectangle of Fig. 1). The mixture of air with naphthalene was homogenized in a spiral glass tube before reaching the sampling bulb where the CF-SPME fiber was then exposed. The flow was measured with a Supelco Optiflow 650 digital flowmeter. The system pressure was maintained at 99 ± 1 kPa by a flow control valve in all the experiments. The dilutions required for the construction of the naphthalene calibration curve in ambient air were obtained through the control of the air flow in the permeation chamber. The concentrations of naphthalene were calculated from the expression:

$$C = 10^3 \times Q/F \quad (1)$$

where C (μg/m^3) is the concentration of naphthalene at 101.3 kPa and 298 K, Q (ng/min) is the permeation rate, and F is the corrected flow (mL/min) at 101.3 kPa and 298 K. For each concentration level of the analytical curves, the flow was altered, and after the 150 min equilibrium time, the flow was measured in replicate ($n = 7$). The readings of the blank were obtained after withdrawing the permeation tube and allowing diluent air to flow for 24 h before performing the extraction.

2.2. Cold fiber-SPME device

The device for the extraction using the CF-SPME fiber [23] is connected to the sampling bulb through a silicone septum, as shown in Fig. 1. A copper tube was used to transfer liquid nitrogen from a Dewar flask to the SPME device. One end of the tube was inserted into the Dewar flask through a rubber stopper, and the other end, composed of a 3 cm spiral, held the needle of a manual SPME holder containing a 100 μm polydimethylsiloxane (PDMS) fiber. The SPME device and fiber were obtained from Supelco (Bellefonte, PA, USA). By closing the valve, the liquid nitrogen evaporated slowly and passed through the spiral at a constant rate, absorbing heat from the manual SPME holder and the fiber (Fig. 2). For the extraction, the cooled fiber was immersed in the sample bulb. The air sampling extraction time was 15 min.

2.3. GC/MS analysis

The analysis was performed with an Finnigan Trace DSQ GC/MS equipped with an ion trap mass spectrometer from Thermo Scientific (West Palm Beach, FL, USA), and a capillary column (30 m × 0.25 mm × 0.25 μm) containing 5% diphenyl and 95% dimethylpolysiloxane HP-5MS from Agilent Technology, Inc. (Santa Clara, CA, USA) was used. The oven temperature program began at 35 °C, was held for 1 min, ramped at 15 °C/min to 200 °C, and held for 2 min. The carrier gas was helium at a flow rate of 4.0 mL/min. The injector was operated at 250 °C in splitless mode for 1 min, followed by a 1:20 split ratio (RD). The mass spectrometer was operated in electron ionization mode (EI) with an energy of 70 eV. The ion source temperature was 200 °C, and the GC/MS interface temperature was 280 °C. The analysis was performed in full scan mode (mass range: 50–300 m/z), with a scan time of 3 scans/s. The quantification was achieved by selected ion monitoring (SIM) using the ion fragment m/z 128. The collection of raw data was performed using an X-Calibur 1.4 software system from Thermo Scientific (West Palm Beach, FL, USA).

2.4. Sample collection

The urban air samples were obtained during the period from February to March, 2011, with an average temperature of 24 ± 2 °C, in Belo Horizonte, a city located in southeastern Brazil (19° 55′ S, 46° 56′ W). Belo Horizonte has 2.4 million inhabitants and 1.1 million vehicles in circulation. Eighteen sampling points distributed outdoors (such as avenues and parks) and indoors (such as laboratories, fuel resale stations, parking garages, bathrooms, and car interiors) were selected. A high traffic density (>10,000 vehicles per day) was considered in the selection of the avenues. All the parks selected were within the urban perimeter of the city.

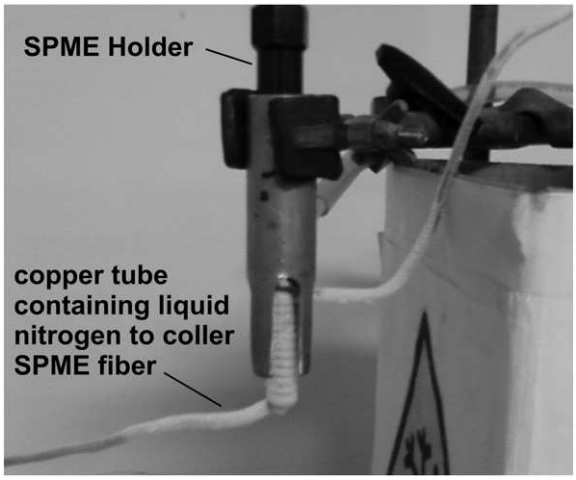

Fig. 2. Cold fiber SPME device.

For each sample collection, the cold fiber was exposed for 15 min in the respiration zone (1.70 ± 0.20 m above ground level). After the collection, the fiber was withdrawn and wrapped in aluminum foil at −15 ± 5 °C until the time of analysis. The maximum period between collection and analysis did not exceed 2 h, so no volatilization of the cold analyte occurred. The analytical protocol for the determination of naphthalene in air samples is schematically depicted in Fig. 3.

2.5. Statistics

Because of the heteroscedasticity of the instrumental responses, the linear model for the calibration curve was constructed by the least squares method weighted by the experimental variance. Basic descriptive analysis was performed using the Origin 8.0 software (OriginLab Corp); p-values below 0.05 were considered significant.

3. Results and discussion

3.1. Analytical characteristics and method validation

A comparative study was performed with the CF-SPME device to evaluate the effect on the extraction yield of cooling the fiber. The experiments were conducted with three extraction replicates for 15 min in the gaseous standard generation system with the permeation tube of naphthalene. The areas obtained for naphthalene at three concentration levels are shown in Fig. 4. In all the evaluated levels, the results showed that the CF-SPME extraction is more efficient than the extraction performed without cooling the fiber. The sorption process that occurred between the analytes and the fiber is exothermic, so the CF-SPME device removes the excess of thermal energy, thereby favoring the mass transfer to the fiber, as was demonstrated by Ghiasvanda and Pawliszyn [24]. To increase the sensitivity, the extractions were performed in CF-SPME mode. The calibration curve was constructed with seven concentration levels, with three steps for each level. The curve obtained ($y = 5261.47x + 10.82$) showed good linearity in the range of 0.55 ± 0.01 to 148.51 ± 0.02 μg/m^3 ($R^2 = 0.998$; p-value < 0.0001). The limits of detection (LOD) and quantification (LOQ) were calculated according to the recommendations of the Eurachem Guide [25] using ten consecutive measurements of the blank. The LOD was 0.33 ± 0.01 μg/m^3, and the LOQ was 0.55 ± 0.01 μg/m^3. In assessing the intra-assay precision (repeatability), ten replicates at the concentration levels of 14.31, 77.42 and 120.97 μg/m^3 were analyzed on the same day. The coefficient of variation (RSD) showed a range of 1.04 to 8.11%, with a mean of 4.75%. These values were lower than the results obtained in other studies for the assessment of naphthalene in ambient air [26,27]. Five replicates at three concentration levels were analyzed on three consecutive days to assess the intermediate precision. The values of RSD obtained in this study varied from 1.04 to 10.03%, with a mean of 5.60%.

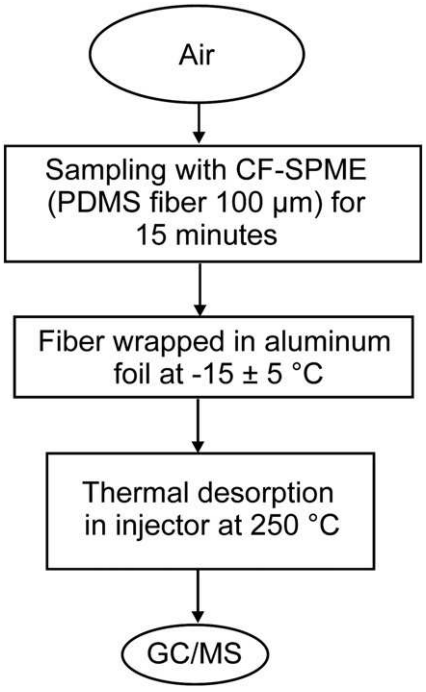

Fig. 3. Analytical protocol for the determination of naphthalene in air samples using CF-SPME-GC/MS.

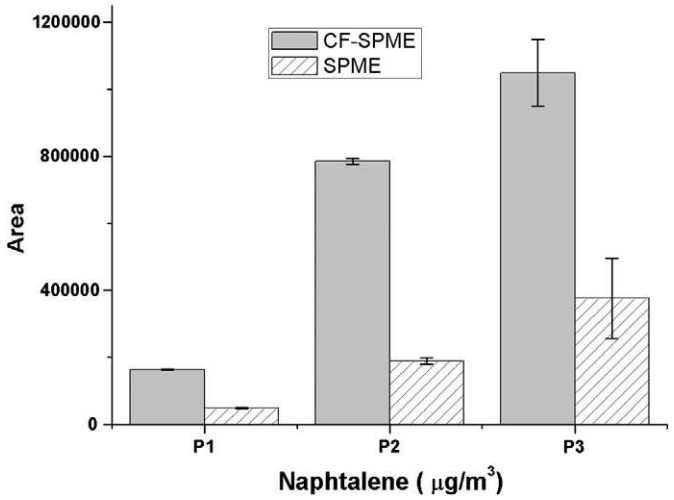

Fig. 4. Naphthalene peak areas at three concentration levels (P1 = 14.30; P2 = 78.50; P3 = 120.90 μg/m^3) to extraction (n = 3) in the system for the generation of gaseous standards with cold fiber-SPME (gray) and SPME (lines).

3.2. Analysis of real samples

The validated method for the determination of naphthalene in the ambient air has been applied to real samples collected in indoor and outdoor environments. Fig. 5 shows the distribution of the results. The average concentrations of naphthalene in indoor and outdoor environments were, respectively, 10.17 ± 14.39 μg/m^3 and 5.47 ± 4.94 μg/m^3. The different types of environments selected for sample collection and the wide variety of emission sources were the probable causes of the large variability observed for naphthalene concentrations in outdoor and indoor ambient air. The median concentration for the indoor air samples in this study was 5.27 μg/m^3. Median concentrations in indoor air reported in the different studies vary considerably, from approximately 0.17 to 4.59 μg/m^3 [28–30]. Indoor concentrations of naphthalene arise from tobacco smoking, use of moth repellents, presence and use of an attached garage, building characteristics, ventilation conditions and emissions from furniture [31–33]. The median concentration determined in outdoor ambient air was 3.81 μg/m^3. Other studies performed in ambient air at urban sites showed a concentration range of 0.01 to 4.15 μg/m^3 [34–36]. In urban areas, concentrations of naphthalene show diurnal and seasonal variations, reflecting variability in emission sources and meteorological influences [37,38].

All samples showed concentrations above the limit of quantification, except for samples 2 and 3 of the outdoor air. Samples 1 to 3 were collected at ecological park sites and are thus close to the background concentrations of naphthalene for urban and suburban centers, which can vary from 0.001 to 1 μg/m^3 [11,29]. The highest concentrations recorded for the outdoor air samples (samples 4 and 5) correspond to

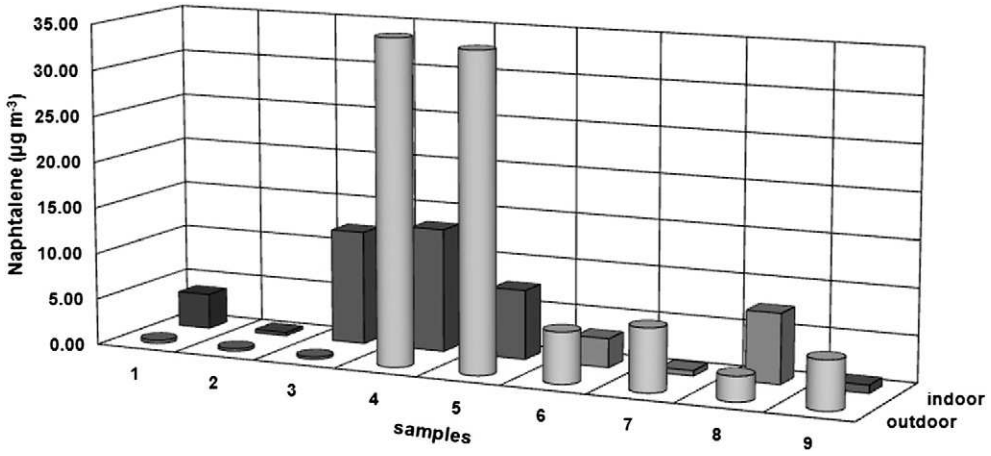

Fig. 5. Concentrations (µg/m^3) of naphthalene in outdoor (cylinders) and indoor (columns) air.

samples taken at fuel resale stations. These values were relatively high because of emissions and fuel evaporation during filling [10]. Samples 6 to 9 were taken on the avenues with heavy traffic, with an average of 12,000 vehicles per day. In indoor environments, the highest concentrations of naphthalene were found in garages at shopping centers (samples 3 and 4) because of the absence of adequate ventilation in these places, resulting in the accumulation of vehicle emissions. Samples 6 to 8 were collected inside parked vehicles. These micro-environments may have elevated levels of naphthalene due to the contribution of the exhaust from other vehicles. The high concentration of naphthalene in sample 8 can be explained by the burning of cigarettes in the vehicle prior to sampling. This residue from smoking is common in environments frequented by smokers and contributes significantly to an increase in the exposure to naphthalene after the act of smoking itself [39]. Residual tobacco smoke pollutants that remain on surfaces after tobacco has been smoked are re-emitted back into the gas phase [40]. Sample 9 represents a sample taken in a public restroom. Occupational environments are represented by samples 1, 2 and 5, which were collected in a research laboratory, in a room with a Xerox copier, and in a garage repair shop, respectively. The presence of naphthalene in indoor and occupational environments constitutes the main contribution to the general population's exposure to this pollutant.

4. Conclusion

This study showed the development of an alternative method for the analysis of naphthalene in ambient air by passive sampling with cooled fiber solid phase microextraction (CF-SPME). The method is simple, fast, and inexpensive. SPME dispensing pumps ensure portability for sampling and pre-concentration. The use of a pattern generation system for gas permeation produced the most appropriate conditions for reproducibility in the atmosphere. This procedure was validated and showed good precision, linearity and sensitivity for the range of environmental interest. The results obtained from the analysis of air samples collected in external and internal environments have demonstrated the ability of the method to measure trace levels of naphthalene in the air in different environments.

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References

[1] USEPA, Compendium of Methods for Determination of Toxic Organic Compounds in Ambient Air, Environmental Protection Agency USA, Center for Environmental Research Information, Cincinnati, 1999.
[2] IARC — International Agency for Research on Cancer, Monographs on the Evaluation of Carcinogenic Risks to Humans — Naphthalene, International Agency for Research on Cancer, France, 2002.
[3] NTP — National Toxicology Program, Toxicology and Carcinogenesis Studies of Naphthalene (CAS No. 91-20-3) in F344/N Rats (Inhalation Studies), National Toxicology Program, Research Triangle Park, USA, 2000.
[4] WHO — World Health Organization, Development of WHO Guidelines for Indoor Air Quality, World Health Organization, Switzerland, 2006.
[5] R. Preuss, J. Angerer, H. Drexler, Naphthalene — an environmental and occupational toxicant, Int. Arch. Occ. Env. Health 76 (2003) 556–576.
[6] M.F. Simcik, T.P. Franz, H.X. Zhang, S.J. Eisenreich, Gas-particle partitioning of PCBs and PAHs in the Chicago urban and adjacent coastal atmosphere: states of equilibrium, Environ. Sci. Technol. 32 (1998) 251–257.
[7] M.J. Bernhard, S.L. Simonich, Use of a bench-top photochemical reactor and solid-phase microextraction to measure semivolatile organic compound-hydroxyl radical rate constants, Environ. Toxicol. Chem. 19 (2000) 1705–1710.
[8] P.S. Price, M.A. Jayjock, Available data on naphthalene exposures: strengths and limitations, Regul. Toxicol. Pharm. 51 (2008) S15–S21.
[9] C.R. Jia, S. Batterman, A critical review of naphthalene sources and exposures relevant to indoor and outdoor air, Int. J. Environ. Res. Public Health 7 (2010) 2903–2939.
[10] J.J. Schauer, M.J. Kleeman, G.R. Cass, B.R.T. Simoneit, Measurement of emissions from air pollution sources. 5. C-1-C-32 organic compounds from gasoline-powered motor vehicles, Environ. Sci. Technol. 36 (2002) 1169–1180.
[11] S.D. Shah, T.A. Ogunyoku, J.W. Miller, D.R. Cocker, On-road emission rates of PAH and n-alkane compounds from heavy-duty diesel vehicles, Environ. Sci. Technol. 39 (2005) 5276–5284.
[12] R. Preuss, H. Drexler, M. Bottcher, M. Wilhelm, T. Bruning, J. Angerer, Current external and internal exposure to naphthalene of workers occupationally exposed to polycyclic aromatic hydrocarbons in different industries, Int. Arch. Occ. Env. Health 78 (2005) 355–362.
[13] S.M. Rappaport, S. Waidyanatha, B. Serdar, Naphthalene and its biomarkers as measures of occupational exposure to polycyclic aromatic hydrocarbons, J. Environ. Monitor. 6 (2004) 413–416.
[14] S.M. Charles, C. Jia, S.A. Batterman, C. Godwin, VOC and particulate emissions from commercial cigarettes: analysis of 2,5-DMF as an ETS tracer, Environ. Sci. Technol. 42 (2008) 1324–1331.
[15] NIOSH, Pocket Guide to Chemical Hazards — Naphthalene, Publication Number National Institute for Occupational Safety and Health, USA, 2005.
[16] K. Demeestere, J. Dewulf, B. De Witte, H. Van Langenhove, Sample preparation for the analysis of volatile organic compounds in air and water matrices, J. Chromatogr. A 1153 (2007) 130–144.
[17] M.R. Ras, F. Borrull, R.M. Marce, Sampling and preconcentration techniques for determination of volatile organic compounds in air samples, Trend. Anal. Chem. 28 (2009) 347–361.
[18] E. Woolfenden, Sorbent-based sampling methods for volatile and semi-volatile organic compounds in air. Part 2. Sorbent selection and other aspects of optimizing air monitoring methods, J. Chromatogr. A 1217 (2010) 2685–2694.
[19] C.L. Arthur, J. Pawliszyn, Solid-phase microextraction with thermal-desoption using fused sílica optical fibers, Anal. Chim. Acta 62 (1990) 2145–2148.
[20] G. Ouyang, J. Pawliszyn, A critical review in calibration methods for solid-phase microextraction, Anal. Chim. Acta 627 (2008) 184–197.
[21] L.C.A. Amorim, J.P. Carneiro, Z.L. Cardeal, An optimized method for determination of benzene in exhaled air by gas chromatography–mass spectrometry using solid phase microextraction as a sampling technique, J. Chromatogr. B 865 (2008) 141–146.

[22] H.C. Menezes, L.C.A. Amorim, Z.L. Cardeal, Sampling of benzene in environmental and exhaled air by solid-phase microextraction and analysis by gas chromatography–mass spectrometry, Anal. Bioanal. Chem. 395 (2009) 2583–2589.
[23] H.C. Menezes, Z. de Lourdes Cardeal, Determination of polycyclic aromatic hydrocarbons from ambient air particulate matter using a cold fiber solid phase microextraction gas chromatography–mass spectrometry method, J. Chromatogr. A 1218 (2011) 3300–3305.
[24] A.R. Ghiasvand, S. Hosseinzadeh, J. Pawliszyn, New cold-fiber headspace solid-phase microextraction device for quantitative extraction of polycyclic aromatic hydrocarbons in sediment, J. Chromatogr. A 1124 (2006) 35–42.
[25] EURACHEM, Guide to Quality in Analytical Chemistry na AID to Accreditation, CITAC/EURACHEM, 2002.
[26] R.A. Rudel, J.G. Brody, J.C. Spengler, J. Vallarino, P.W. Geno, G. Sun, A. Yau, Identification of selected hormonally active agents and animal mammary carcinogens in commercial and residential air and dust samples, J. Air Waste Manage. 51 (2001) 499–513.
[27] R. Barro, J. Regueiro, M. Llompart, C. Garcia-Jares, Analysis of industrial contaminants in indoor air: part 1. Volatile organic compounds, carbonyl compounds, polycyclic aromatic hydrocarbons and polychlorinated biphenyls, J. Chromatogr. A 1216 (2009) 540–566.
[28] E.G. Sanderson, J.P. Farant, Indoor and outdoor polycyclic aromatic hydrocarbons in residences surrounding a Soderberg aluminum smelter in Canada, Environ. Sci. Technol. (2004) 5350–5356.
[29] C. Jia, S. Batterman, C. Godwin, VOCs in industrial, urban and suburban neighborhoods, part 1: indoor and outdoor concentrations, variation, and risk drivers, Atmos. Environ. 42 (2008) 2083–2100.
[30] T.J. Ward, H. Underberg, D. Jones, R.F. Hamilton, E. Adams, Indoor/ambient residential air toxics results in rural western Montana, Environ. Monit. Assess. 153 (2009) 119–126.
[31] S. Batterman, C.R. Jia, G. Hatzivasilis, Migration of volatile organic compounds from attached garages to residences: a major exposure source, Environ. Res. 104 (2007) 224–240.
[32] M.E. Heroux, D. Gauvin, N.L. Gilbert, M. Guay, G. Dupuis, M. Legris, B. Levesque, Housing characteristics and indoor concentrations of selected volatile organic compounds (VOCs) in Quebec City, Canada, Indoor Built Environ. 17 (2008) 128–137.
[33] B. Magee, J. Samuelian, K. Haines, M. Chappel, I. Penn, D. Chin, D. Anders, J. Hinz, Screening-level population risk assessment of nasal tumors in the US due to naphthalene exposure, Regul. Toxicol. Pharm. 57 (2010) 168–180.
[34] Y.J. Liu, L.Z. Zhu, X.Y. Shen, Polycyclic aromatic hydrocarbons (PAHs) in indoor and outdoor air of Hangzhou, China, Environ. Sci. Technol. 35 (2001) 840–844.
[35] S.S. Park, Y.J. Kim, C.H. Kang, Atmospheric polycyclic aromatic hydrocarbons in Seoul, Korea, Atmos. Environ. 36 (2002) 2917–2924.
[36] L. Miller, X.H. Xu, I. Luginaah, Spatial variability of volatile organic compound concentrations in Sarnia, Ontario, Canada, J. Toxicol. Environ. Health A 72 (2009) 610–624.
[37] M.F. Mohamed, D.W. Kang, V.P. Aneja, Volatile organic compounds in some urban locations in United States, Chemosphere 47 (2002) 863–882.
[38] K. Prevedouros, E. Brorstrom-Lunden, C.J. Halsall, K.C. Jones, R.G.M. Lee, A.J. Sweetman, Seasonal and long-term trends in atmospheric PAH concentrations: evidence and implications, Environ. Pollut. 128 (2004) 17–27.
[39] M.H. Becquemin, J.F. Bertholon, M. Bentayeb, M. Attoui, D. Ledur, F. Roy, M. Roy, I. Annesi-Maesano, B. Dautzenberg, Third-hand smoking: indoor measurements of concentration and sizes of cigarette smoke particles after resuspension, Tob. Control. 19 (2010) 347–348.
[40] A. Burton, Does the smoke ever really clear? Thirdhand smoke exposure raises new concerns, Environ. Health Persp. 119 (2011) A71–A74.

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Determination of polycyclic aromatic hydrocarbons from ambient air particulate matter using a cold fiber solid phase microextraction gas chromatography–mass spectrometry method

Helvécio Costa Menezes, Zenilda de Lourdes Cardeal *

Departamento de Química, Universidade Federal de Minas Gerais, Avenida Antonio Carlos, 6627, Pampulha – Belo Horizonte/MG 31270901, Brazil

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAH) from ambient air particulate matter (PM) were analyzed by a new method that utilized direct immersion (DI) and cold fiber (CF) SPME-GC/MS. Experimental design was used to optimize the conditions of extraction by DI-CF-SPME with a 100 μm polydimethylsiloxane (PDMS) fiber. The optimal conditions included a 5 min equilibration at 70 °C time in an ultrasonic bath with an extraction time of 60 min. The optimized method was validated by the analysis of a NIST standard reference material (SRM), 1649b urban dust. The results obtained were in good agreement with certified values. PAH recoveries for reference materials were between 88 and 98%, with a relative standard deviation ranging from 5 to 17%. Detection limits (LOD) varied from 0.02 to 1.16 ng and the quantification limits (LOQ) varied from 0.05 to 3.86 ng. The optimized and validated method was applied to the determination of PAH from real particulate matter (PM10) and total suspended particulate (TPS) samples collected on quartz fiber filters with high volume samplers.

1. Introduction

Polycyclic aromatic hydrocarbons (PAH) are compounds that have proven to be carcinogenic and mutagenic [1]. Their ubiquitous nature is evident in the fact that 16 PAH are considered priority pollutants by the U.S. Environmental Protection Agency (EPA) [2]. Among these priority PAH, benzo[*a*]pyrene (BaP) is used as a marker for carcinogenic risk in Environmental Studies. Recently, the European Union, seeking to avoid adverse effects of PAH to health and the environment, set the target value of 1 ng/m^3 for BaP in air [3]. The main anthropogenic sources of PAH are the incomplete combustion of petrol and diesel in vehicles, incineration of organic waste, burning of wood and coal for household heating, and industrial processes involving coal tars and crude oil products such as creosote and asphalt. PAH can occur as gases, adsorbed/absorbed on particulate matter (PM), or distributed between the gas-particle phases as is the case of semi-volatile materials. The gas-particle partitioning depends mainly on the molar mass of PAH. Those PAH made up of two or three chains are concentrated mainly in the gas phase, whereas those containing up to four chains are adsorbed on the particulate phase [4]. This association facilitates the transport of PAH of larger mass over long distances and contributes significantly to the increase in the carcinogenic potential of the particulate phase [5].

Glass fiber filters (GFF) or quartz-fiber filters (FFQ) in active samplers are mainly used for sampling of PAH in PM. After collecting, the PAH are traditionally extracted from these filters by the Soxhlet method [6] with the use of large amounts of toxic solvents during long periods of time. Generally, the method requires a clean-up step with alumina or silica gel after extraction to reduce contamination and the evaporation of excess solvent in a pre-concentration step [7]. Methods such as sonication [8], microwave-assisted extraction (MAE) [9], supercritical fluid extraction (SFE) [10], and pressurized liquid extraction (PLE) [11] have been proposed to improve the performance of the PAH extraction and to minimize the use of solvents. Also, some of these methods require the pre-concentration of the extracted solutions before the analysis. This step increases the run time and can cause a decrease in analytical reproducibility. Therefore, alternative procedures reduce the number of steps by eliminating the use of solvents to avoid exposure and environmental contamination. The solid phase microextraction (SPME) is a simple method that meets these requirements since it combines sampling and pre-concentration in one step, does not require the use of solvents, and permits desorption directly into the injector of the chromatographic system [12]. This method has been used successfully in the analysis of PHA in complex environmental matrices [13].

* Corresponding author. Tel.: +55 3134095725; fax: +55 3134095700.
E-mail address: zenilda@ufmg.br (Z. de Lourdes Cardeal).

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The objective of the present work was to demonstrate that a new simple and low cost device for direct immersion (DI) and cold fiber (CF) SPME-GC/MS can be used for the determination of PHA in particulate material.

2. Experimental

2.1. Reagents and supplies

A US Environmental Protection Agency (EPA) PAH Mix, containing naphthalene, acenaphthene, acenaphthylene, anthracene, benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*ghi*]perylene, chrysene, dibenz[*a,h*]anthracene, fluoranthene, fluorene, indeno[1,2,3-*d*]pyrene, naphthalene, phenanthrene, and pyrene at 2000 μg/mL in methanol:methylene chloride (1:1) was purchased from Supelco (Bellefonte, PA, USA). Standard solutions of 1000 μg/L were prepared by appropriate dilution in HPLC-grade methanol Sigma–Aldrich (St. Louis, MO, USA). Standard reference material, SRM1649b urban dust, was obtained from the National Institute of Standards & Technology NIST (Gaithersburg, MD, USA). HPLC-grade methylene chloride and acetone from Sigma–Aldrich (St. Louis, MO, USA) were used to prepare standard solutions of PAH; ultra-pure Milli-Q water Millipore (Molsheim, France) was used.

2.2. Particulate matter samples

Quartz filters (20.3 cm × 25.4 cm and 47 mm thickness from Whatman International (Maidstone, Kent, England)) were used to collect aerosol samples in the outdoor environments. These filters were pre-heated at 500 °C for 24 h prior to use to reduce their water and organic matter blank values. After cooling, the filters were weighed and stored at 4 °C in aluminum foil until the time for analysis. Real samples of PM10 (particulate matter with aerodynamic diameter < 10 μm) were collected using a high-volume Model VFC-PM10 sampler from Thermo Scientific Inc. (Franklin, MA, USA) which utilizes a volumetric flow controlled system (a dimensional venturi device) for sampling large volumes of air. Samples of Total Suspended Particles (TSP) were collected using a high-volume Model VFC-TSP sampler from Thermo Scientific Inc. (Franklin, MA, USA). The particulate phase samples were collected in November 2009, at Divinopolis, Minas Gerais, Brazil (20°9′S; 44°53′W) in two different environmental locations. The average ambient temperature was of 23 ± 3 °C with a mild breeze and 70% relative humidity. The sampling of PM10 was near a steel industry that uses coke and coal in its production process. The flow was 62 m^3/h with a final collected volume of 1488 m^3 of air. Sampling of TSP was near a steel industry that only uses coal in its production process. The sampling flow was 90 m^3/h with a final volume of 2170 m^3. Particles were collected for 24 h on quartz filters. After sampling, the filters were stored in the dark in aluminum foil at −20 °C and transported to the laboratory.

2.3. Instruments

A Finnigan Trace DSQ GC/MS equipped with an ion trap spectrometer from Thermo Scientific Inc. (West Palm Beach, FL, USA) and a capillary column (30 m length × 0.25 mm I.D. × 0.25 μm film thickness) containing 5% diphenyl, 95% dimethylpolysiloxane HP-5MS Agilent Technology Inc. (Santa Clara, CA, USA) was used. The oven temperature program began at 35 °C, was held for 2 min, raised to 270 °C at 8 °C/min, and held for 20 min. The carrier gas was helium at a flow rate of 4.5 mL/min. The injector was operated at 270 °C in splitless mode for 1 min, followed by a 1:20 split ratio (RD). The mass spectrometer was operated in electron impact mode (EI) with an energy of 70 eV. The ion source temperature was 200 °C, and the GC/MS interface temperature was 290 °C. The analysis was performed in full scan mode (range 50–300 *m/z*), scan time of 3 scan/s. The quantification was achieved in selected ion monitoring (SIM) mode using the ion fragments presented in Table 3. Signal acquisition and data processing were performed using the Xcalibur software Thermo Finnigan Inc. (West Palm Beach, FL, USA). A USC 1600 ultrasonic bath from Unique (Indaiatuba, SP, Brazil) with a 40-kHz frequency and 135-W power was used to facilitate the dispersion of PAH that are less soluble in aqueous media.

Table 1
Experimental variables 2^3 two-levels full factorial design for PAH extraction.

Variable	Level		
	Low	Centre	High
Extraction temperature (°C)	30	50	70
Time of equilibrium in ultrasonic bath (min)	0	5	10
extraction time (min)	20	40	60

2.4. Cold-fiber SPME device

The device scheme for the CF-DI-SPME is presented in Fig. 1. A copper tube with a length of 70 cm, O.D. of 2.4 mm, and I.D. of 1.6 mm was used to transfer liquid nitrogen from a Dewer flask to the SPME device. One extremity of the tube was inserted into the Dewer flask through a rubber stopper, and the other end, composed of a 3-cm-long spiral with 2 mm I.D., held the needle of a manual SPME holder containing a 100 μm polydimethylsiloxane (PDMS) fiber. The SPME device and fiber were obtained from Supelco (Bellefonte, PA, USA). The rubber stopper (4 mm top diameter and 3 mm bottom diameter) was used to cap the Dewer flask containing 0.5 L of liquid nitrogen. Another copper tube (10 cm length, 6.4 mm O.D. and 4.7 mm I.D.) was used as a valve controlling the nitrogen pressure in the Dewer flask; 0.5 L of liquid nitrogen permitted a 3 h cooling period. By closing the valve, the liquid nitrogen evaporated slowly and passed through the spiral at a constant rate, absorbing heat from the manual SPME holder and, hence, the fiber. When the valve was open, the nitrogen was purged from the Dewer flask and no longer passed through the spiral. Thus, the cooling process in the spiral was terminated. For the extraction, the cooled fiber was immersed in 20 mL Pyrex vials sealed with silicone/PTFE septa and aluminum caps containing an aqueous solution of PAH. The vial was placed in an aluminum block with controlled temperature and constant stirring.

2.5. Experimental design

To optimize the SPME method, all experiments were performed with quartz filter disks (diameter of 0.5 cm) pre-heated at 500 °C for 24 h and spiked with 10.0 μg/L of PAH solution in 50% (v/v) methylene chloride and acetone. These spiked filters were stored at −20 °C for 24 h prior to testing. A study was conducted using a 2^3 two-level full factorial design (FFD) [14] to investigate the variables: extraction temperature (T), time of equilibrium in an ultrasonic bath (t_{eq}), and extraction time (t_{ex}) with direct immersion of the SPME fiber. The experimental values of these variables are presented in Table 1. The minimum and maximum levels of the parameters were selected based on the properties of the PAH analyzed. Extraction temperatures above 70 °C cause losses of low molecular weight PAH. On the other hand, temperatures below 30 °C make it difficult to extract the PAH. In the same way, 150 μL of a solution of 50% (v/v) methylene chloride and acetone was employed as a modifier during the equilibrium time. Acetone was selected to compose the modifier because it has been reported that solvents improve PAH extraction in environmental samples [15]. Moreover, studies show that the combination of acetone with other solvents tends to faci-

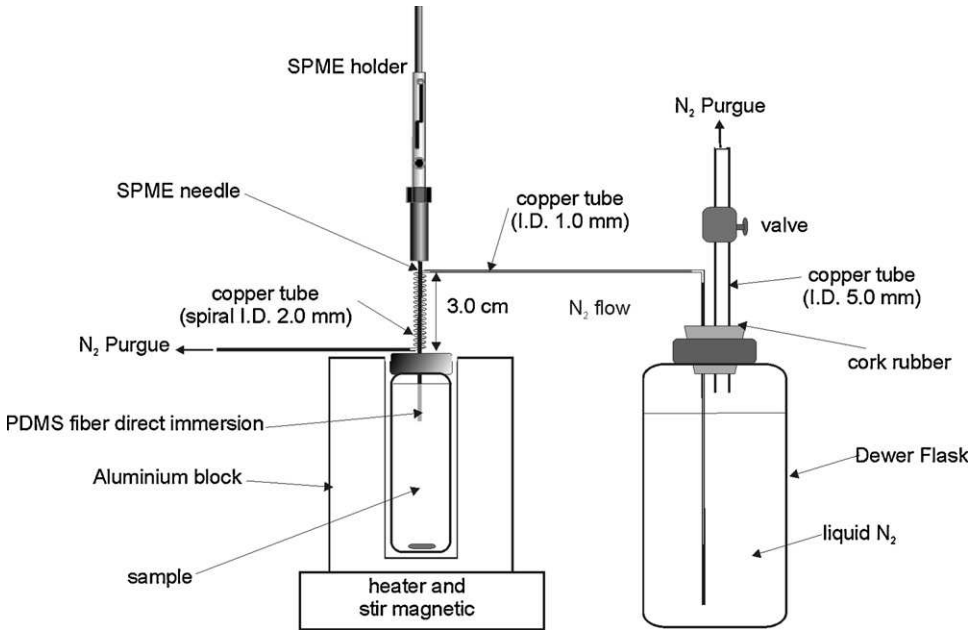

Fig. 1. Schematic of the device for DI-CF-SPME.

litate the solvation of analytes [16]. The volume of modifier was set at 150 μL to avoid damaging the fiber used in the extraction. The Doehlert design (Table 2) was applied using the most significant variables with the objective of reaching the optimum region of the investigated area. The Doehlert design can be applied from two variables, requires fewer experiments, and has a higher efficiency (φ) than designs such as Central Composite and Box-Behnken. The efficiency value can be determined by dividing the number of coefficients of the quadratic equation (p) by the number of experiments (N) required for the design [17]. A Doehlert design with two factors ($k = 2$) requires seven experiments to be completed. The experimental response (Y) as a function of variables (X_1 and X_2) is given by Eq. (1):

$$Y = b_0 + b_1(X_1) + b_2(X_1)(X_1) + b_3(X_2) + b_4(X_2)(X_2) + b_5(X_1)(X_2) \quad (1)$$

where b_0 is the constant term, b_1 and b_3 are coefficients of the linear terms, b_2 and b_4 are coefficients of the quadratic terms and b_5 is the coefficient of interaction between the two factors. Since p is 6 in this case, the efficiency value is 0.77. On the other hand, for Central Composite designs with the same number of factors, an efficiency value of 0.67 is obtained. The model fits were validated using the analysis of variance (ANOVA). The p-values smaller than 0.05 were considered significant. All the statistical analyses were performed using the statistical package Statistica 8.0 for Windows Statsoft Inc. (Tulsa, OK, USA).

Table 2
Experimental planning for PAH extraction according to Doehlert design.

No.	Coded values		Real values	
	X_1	X_2	Extraction temperature (°C)	Extraction time (t_{ex}, min)
1	−0.5	−0.87	40	20
2	+0.5	−0.87	60	20
3	+1	0	70	40
4	+0.5	+0.87	60	60
5	−0.5	+0.87	40	60
6	−1	0	30	40
7	0	0	50	50
8	0	0	50	50
9	0	0	50	50

3. Results and discussion

3.1. Experimental design and optimization procedure

The Pareto chart analysis of the effects showed different behaviors for each of the analytes. The PAH with lower molecular weights presented a non-significant equilibrium time, temperature, and extraction time (for a p-value < 0.05), however, the higher temperatures and longer extraction times significantly increased the responses of benzo[*a*]anthracene, benzo[*a*]pyrene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, indeno[1,2,3-*cd*]pyrene, dibenzo[*a,h*]anthracene and benzo[*ghi*]perylene. The main interaction observed for these analytes occurred between the temperature and extraction time. For this reason, these two parameters were used in the Doehlert design with the equilibrium time in the ultrasonic bath set at 5 min to reduce the total time of analysis.

The experimental conditions of the Doehlert design for real and coded values of each factor are shown in Table 2. For simplicity, the responses are not shown in this table. To assess the effect of each variable on the response, they were coded to eliminate the influence of their different magnitudes on such evaluation. The central point was achieved three times to obtain an estimation of the experimental error. An estimation of the coefficients ($b_0 - b_5$) of a second-degree polynomial model indicated by Eq. (1) was performed with the experimental responses. The level of significance for each regression was calculated by ANOVA and showed that the models were well fitted. The fractions of the explained variations (R^2) ranged from 0.971 for the benzo[*a*]anthracene to 0.989 for benzo[*ghi*]perylene. This fact indicated that a good capability for prediction existed under the experimental conditions employed. The optimum values for each independent variable were calculated using the adjusted models since they represent the point of the function where the extraction of each PAH is maximized and, therefore, should be used in the experimental procedure for the extraction of the analytes.

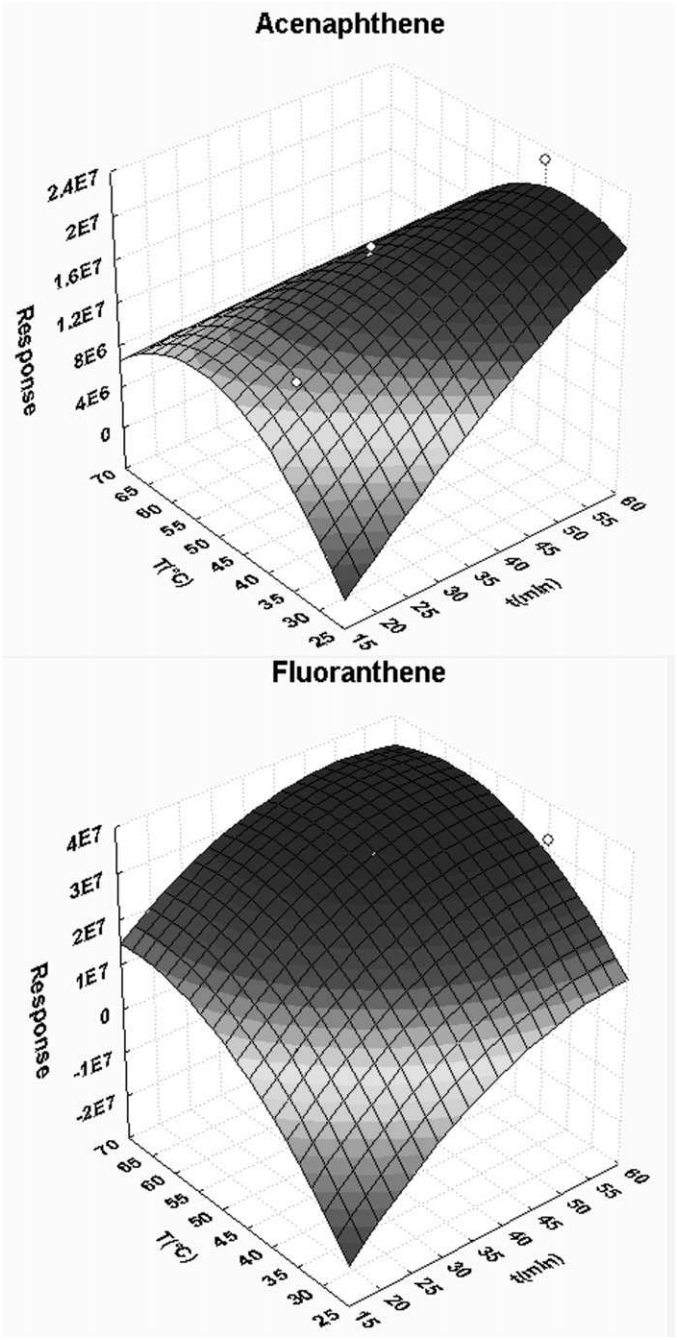

Fig. 2. Response surfaces obtained from the regression models of Doehlert design for acenaphthene and fluoranthene.

The response surfaces obtained from the regression models for acenaphthene, and fluoranthene are presented in Fig. 2. The best responses were presented by the lower molecular weight PAH for the extraction temperature in range of 30–50 °C and an extraction time of 40–60 min. The responses for PAH of higher molecular weights were optimum at temperatures near 70 °C and at all the extraction times. To achieve the optimum conditions for extraction of all the 16 PAH studied within the experimental domain, it was necessary to establish a compromise between the temperature and extraction time at the maximum levels, 70 °C and 60 min, respectively. Fig. 3 displays an overview of the method using optimized extraction conditions. A comparative study was performed with the new CF-DI-SPME device to evaluate the effect of cooling the fiber on the responses. The results of experiments conducted

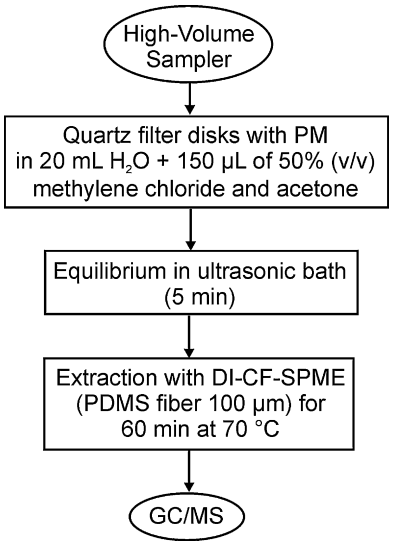

Fig. 3. Overview of the DI-CF-SPME-GC/MS method using optimized extraction conditions.

with three replicates of quartz filter disks employed as a blank matrix spiked with 10 μg/L PAH solution are presented in Fig. 4. The response area for all PAH was larger when the CF-mode DI-SPME was used than with the DI-SPME mode under the same conditions, but without cooling. The sorption process that occurred between the analytes and the fiber was exothermic, so the removal of excess thermal energy to the CF-DI-SPME device favored the mass transfer to the fiber, as was demonstrated by Ghiasvanda and Pawliszyn [18].

3.2. Quality control and quality assurance

The quality control and quality assurance method was performed according to EURACHEM guidelines [19]. Quartz filter disks were used as a blank matrix. Detection limits (LOD) and quantification limits (LOQ) were calculated from the mean and standard deviation of 10 blank measurements with 95% confidence. Linearity was established with quartz filter disks spiked with all the standard

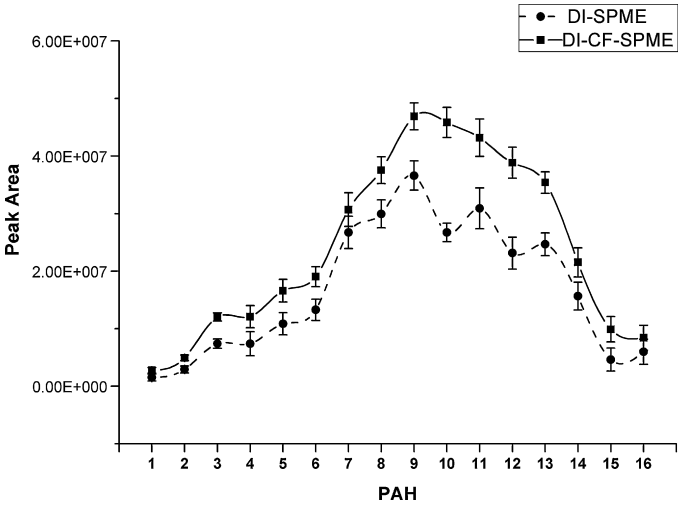

Fig. 4. Effect of the extraction mode using DI-SPME and DI-CF-SPME for 1, naphthalene; 2, acenaphthylene; 3, acenaphthene; 4, fluorene; 5, phenanthrene; 6, anthracene; 7, fluoranthene; 8, pyrene; 9, benzo[*a*]anthracene; 10, chrysene; 11, benzo[*b*]fluoranthene; 12, benzo[*k*]fluoranthene; 13, benzo[*a*]pyrene; 14, indeno[1,2,3-*cd*]pyrene; 15, dibenzo[*a,h*]anthracene; 16, benzo[*ghi*]perylene.

solutions (0.01–120 ng). Seven concentration levels were analyzed with three measurements at each concentration level. Because of the heteroscedasticity of the instrumental responses, the linear models for the calibration curves were constructed by the least squares method weighted by the experimental variance. The accuracy of the method was tested with samples of standard reference material, the SRM 1649b urban dust. The accuracy of the analytical method is acceptable if:

$$|X_m - X_{CRM}| \leq U$$

where X_{CRM} is the certified value, X_m is the mean experimental value of the replicates, U is the expanded uncertainty of the difference between mean experimental and certified values, corresponding to a confidence interval of approximately 95% [20]. The statistics tests were performed using the Origin 8.0 software from Origin Lab Co. (Northampton, MA, USA).

3.3. Method validation

Using the optimized conditions, some analytical performance was studied to validate the method. The results for linearity, detection limits, and quantification limits are presented in Table 3. A linear relationship was observed between the peak area and analyte concentration over the full range of the calibration curve, with determination coefficients above 0.990 (p value < 0.05).

All the analytes presented a LOD for the PAH between 0.02 and 1.16 ng, the range of LOQ was 0.05–3.86 ng. To assess the trueness, four aliquots of 5.00 ± 0.01 mg of SRM 1649b urban dust were extracted and analyzed under the same conditions (Table 4). The accuracy was expressed by the difference between the experimental mean and the certified value, and the precision was represented by the relative standard deviation (RSD). The method presented a good precision. The RSD for all of the PAH was 17.2 or less. Values of 8.3–25.4% RSD were observed in a study of the between-days reproducibility with three aliquots of 5.00 ± 0.01 mg of SRM 1649b urban dust analyzed on three consecutive days.

The accuracy measured was in agreement with SRM1649b certified values, except for benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, and benzo[*ghi*]perylene, which presented absolute differences higher than the expanded uncertainty for these compounds. Recovery efficiencies for all the PAH were higher than 88%.

Table 3
Linearity and limits of detection (LOD) and quantification (LOQ) of the DI-CF-SPME-GC/MS method.

PAH[a]	Quantitation ions	Linearity (r^2)	LOD (ng)	LOQ (ng)
Naphthalene	128	0.993	0.02	0.05
Acenaphthylene	152	0.991	0.31	1.02
Acenaphthene	153	0.992	0.35	1.18
Fluorene	166	0.990	0.40	1.34
Phenanthrene	178	0.993	0.92	3.06
Anthracene	178	0.992	0.62	2.05
Fluoranthene	202	0.995	0.37	1.24
Pyrene	202	0.991	0.33	1.09
Benzo[*a*]anthracene	228	0.994	0.30	1.01
Chrysene	228	0.994	0.57	1.89
Benzo[*b*]fluoranthene	252	0.992	0.43	1.44
Benzo[*k*]fluoranthene	252	0.993	1.16	3.86
Benzo[*a*]pyrene	252	0.995	0.75	3.46
Indeno[1,2,3-*cd*]pyrene	276	0.997	1.02	3.40
Dibenzo[*a,h*]anthracene	278	0.992	0.38	1.28
Benzo[*ghi*]perylene	276	0.994	1.13	3.77

[a] Compounds are listed in sequence of elution.

Table 5
PAH concentrations (ng/m^3) of samples of particulate material collected in the Divinopolis, MG, Brazil, urban site (n = 3).

PAH	PM 10Mean (RSD, %)	TSPMean (RSD, %)
Phenanthrene	0.62(9)	0.51(18)
Anthracene	0.37(27)	0.45(19)
Fluoranthene	0.67(12)	0.56(8)
Pyrene	1.30(7)	1.14(11)
Benzo[*a*]anthracene	0.81(5)	0.90(4)
Chrysene	0.89(8)	0.80(12)
Benzo[*b*]fluoranthene	1.38(10)	1.04(14)
Benzo[*k*]fluoranthene	nd[a]	nd[a]
Benzo[*a*]pyrene	1.29(13)	1.01(11)
Indeno[1,2,3-*cd*]pyrene	1.18(9)	1.32(12)
Dibenzo[*a,h*]anthracene	nd[a]	nd[a]
Benzo[*ghi*]perylene	1.50(12)	1.26(14)

[a] nd (not detected) is assigned for PAH having concentrations lower than the limit of quantification.

3.4. Application of the method to real samples of PM10 and TSP

The application of the validated DI-CF-SPME-GC/MS method for the determination of PAH to real samples was achieved through the analysis of samples from environmental air (n = 3) from two different locations in Divininópolis, MG, Brazil. This study is part of a Brazilian project to assess environmental exposure to carcinogenic chemicals in a city with many steel industries. The results, reported in Table 5, indicate that this method achieved good reproducibility when applied to real samples of PM10 and TSP. Phenanthrene and anthracene were the compounds that exhibited high RSD values in the PM10 and TPS samples because their concentrations were very close to the LOQ. These compounds have high vapor pressures so their concentrations are relatively low in the particulate phase [21]. In general the results for PAH in PM10 and TPS were similar to

Table 4
Quantification of PAH in NIST SRM1649b urban dust.

PAH	Mean (ng)[a]	Certified (ng)[a]	RSD (%)[b]	Difference (ng)[c]	Recovery (%)
Phenanthrene	21.91 ± 2.92	22.345 ± 0.266	13.31	−0.43 (1.46)	98.05
Anthracene	2.82 ± 0.15	2.886 ± 0.079	5.36	−0.06 (0.08)	97.71
Fluoranthene	34.05 ± 3.60	34.81 ± 0.68	10.56	−0.76 (1.83)	97.82
Pyrene	26.31 ± 2.82	27.125 ± 0.164	10.71	−0.82(1.41)	96.99
Benzo[*a*]anthracene	11.54 ± 0.62	11.862 ± 0.272	5.40	−0.32 (0.34)	95.43
Chrysene	16.54 ± 1.64	17.055 ± 0.249	9.93	−0.51(0.83)	96.98
Benzo[*b*]fluoranthene	33.05 ± 5.01	33.96 ± 1.13	15.16	−0.91(2.57)	97.32
Benzo[*k*]fluoranthene	9.74 ± 1.56	9.91 ± 0.471	16.05	−1.71(0.81)	98.28
Benzo[*a*]pyrene	12.44 ± 2.04	14.00 ± 0.96	16.38	−1.56 (1.12)	88.85
Indeno[1,2,3-*cd*]pyrene	14.82 ± 2.24	16.78 ± 0.96	15.12	−1.96 (1.22)	88.31
Dibenzo[*a,h*]anthracene	1.60 ± 0.25	1.62 ± 0.02	15.87	−0.04 (0.13)	97.76
Benzo[*ghi*]perylene	19.68 ± 3.39	22.323 ± 0.295	17.23	−2.65 (1.70)	88.16

[a] Expanded uncertainty at the 95% of confidence.
[b] Four 5 mg samples of SRM1649b.
[c] Difference = (experimental value − certified value). The expanded uncertainty at the 95% of confidence of difference for each PAH is given within parentheses.

average values for other urban areas [22–24]. Using benzo[*a*]pyrene as an indicator representing the PAH family, the concentrations of 1.29 ng/m^3 determined for PM10 and 1.01 ng/m^3 for TSP were above the current target set by the European Union.

4. Conclusions

A simple and inexpensive device has been proposed for CF-DI-SPME extraction of PAH at trace levels in particulate matter. The use of an experimental design enabled the optimization of extraction conditions. The DI-CF-SPME-GC/MS method, validated and tested on NIST SRM1649b urban dust, demonstrated the high accuracy and reproducibility and low LOD and LOQ for quantification of PAH in atmospheric aerosol samples collected on quartz fiber filters with high volume samplers. Future modifications of this method for in situ sampling are possible because of the portability of the device. The determination of volatile organic compounds in the environment, plants, drugs, and foods may represent additional applications of the method.

Acknowledgements

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References

[1] Polynuclear Aromatic Hydrocarbons. Part I. Chemical Environmental and Experimental Data (IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans (IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, vol. 32), International Agency for Research on Cancer (IARC), Lyon, 1983.
[2] US EPA, Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air, 2nd ed., Office of Research and Development, National Risk Management Research Laboratory, Center for Environmental Research Information, 1999, Cincinnati, OH, <http://www.epa.gov/ttn/amtic/airtox.html>.
[3] Directive 2004/107/EC 15-12-2004, Off. J. Eur. Union, L 23 (December 15, 2004) 13.
[4] M.F. Simcik, T.P. Franz, H. Zhang, S.J. Eisenreich, Environ. Sci. Technol. 32 (1998) 251.
[5] T. de Kok, H.A.L. Driece, J.G.F. Hogervorst, J.J. Briede, Mutat. Res., Rev. Mutat. Res. 613 (2006) 103.
[6] R. Westerholm, A. Christensen, M. Tornqvist, L. Ehrenberg, U. Rannug, M. Sjogren, J. Rafter, C. Soontjens, J. Almen, K. Gragg, Environ. Sci. Technol. 35 (2001) 1748.
[7] A. Albinet, E. Leoz-Garziandia, H. Budzinski, E. Villenave, J. Chromatogr. A 1121 (2006) 106.
[8] S.G. Riddle, C.A. Jakober, M.A. Robert, T.M. Cahill, M.J. Charles, M.J. Kleeman, Atmos. Environ. 41 (2007) 8658.
[9] S. Karthikeyan, R. Balasubramanian, S.W. See, Talanta 69 (2006) 79.
[10] M. Shimmo, H. Adler, T. Hyotylainen, K. Hartonen, M. Kulmala, M.L. Riekkola, Atmos. Environ. 36 (2002) 2985.
[11] L. Turrio-Baldassarri, C.L. Battistelli, L. Conti, R. Crebelli, B. De Berardis, A.L. Iamiceli, M. Gambino, S. Iannaccone, Sci. Total Environ. 327 (2004) 147.
[12] C.L. Arthur, J. Pawliszyn, Anal. Chem. 62 (1990) 2145.
[13] B. Tang, U. Isacsson, Energy Fuels 22 (2008) 1425.
[14] Y.Z. Hu, D.L. Massart, J. Chromatogr. 485 (1989) 311.
[15] I.J. Barnabas, J.R. Dean, I.A. Fowlis, S.P. Owen, Analyst 120 (1995) 1897.
[16] P. Bruno, M. Caselli, G. Gennaro, M. Tutino, Talanta 72 (2007) 1357.
[17] A.M.G. Campaña, L. Cuadros, A.L. Gonzalez, F.A. Barrero, M.R. Ceba, Anal. Chim. Acta 348 (1997) 237.
[18] R.H.S. Ghiasvanda, J. Pawliszyn, J. Chromatogr. A 1124 (2006) 35.
[19] The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics, EURACHEM Guide, LGC, Teddington, 1st English ed., 1998, http://www.eurachem.ul.pt/.
[20] Application Note 1, Comparison of a Measurement Results with the Certified Value, Joint Research Centre Institute for Reference Materials and Measurements (IRMM), Geel, Belgium, 2005.
[21] K. Ravindra, E. Wautersc, R. Van Griekenb, Sci. Total Environ. 396 (2008) 100.
[22] A.M. Mastral, J.M. Lopez, M.S. Callén, T. Garcia, R. Murillo, M.V. Navarro, Sci. Total Environ. 307 (2003) 111.
[23] A. Gutierrez-Daban, A.J. Fernandez-Espinosa, M. Ternero-Rodriguez, F. Fernandez-Alvarez, Anal. Bioanal. Chem. 381 (2005) 721.
[24] M.A. Barrero Mazquiarán, L.C.O. de Pinedo, Atmos. Res. 85 (2007) 288.

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An optimized method for determination of benzene in exhaled air by gas chromatography–mass spectrometry using solid phase microextraction as a sampling technique

Leiliane C.A. Amorim, Joana P. Carneiro, Zenilda L. Cardeal *

Department of Chemistry, ICEx, Department of Clinical and Toxicology Analysis, Faculty of Pharmacy, Federal University of Minas Gerais, Av. Antônio Carlos 6627, 31270-901 Belo Horizonte, M.G., Brazil

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ABSTRACT

The determination of benzene in exhaled air has contributed for the increase in the use of breath analysis in biological monitoring. This paper describes SPME as a sampling technique for determining benzene in exhaled air by GC–MS. A system was developed to generate a gaseous benzene standard by a permeation method to accomplish the breath analyses. The method presented good resolution, repeatability (the mean of %RSD values for intra-day measurements was 6.3), sensitivity (2.4 and 3.1 ppb for LOD and LOQ, respectively), and linearity of response (R^2 = 0.994). After optimizing the conditions, analyses of real samples were performed on two groups (exposed and not exposed to benzene). The results presented an average of 8.2 ppb for the control group and 25.3 ppb for the exposed group.

1. Introduction

Benzene is a ubiquitous pollutant and an important organic compound present in the steel and petroleum industries. It is a natural product of petroleum refining and is used as an intermediate in the production of a wide variety of chemical substances. It is a by-product of the production of coke for steel manufacturing [1]. Its toxicological importance is a result of having been classified as a member of group I – carcinogenic to humans – by the International Agency on Cancer Research since the 1980s [2]. However, the risk of exposure to benzene is not only an occupational risk; the general population is involved. A significant contribution to non-occupational exposure from tobacco smoke exists [3,4], as well as the significant emission from engine exhausts, it being an important component in gasoline.

Gasoline contains 1–5% benzene, the amount varying in different countries [5]. As a result, workers from the petrochemical industry, automobile mechanics and other occupational groups exposed to automobile emissions run a higher risk of contracting leukemia [6]. In addition, the risk is amplified when gasoline is used without exposure control in homes, as a solvent and within many occupational places. Some workers are exposed by multiple routes; some wash their hands with gasoline and even siphon gasoline by mouth. Dermal routes may be the source, since as much as 80% of the benzene levels measured in blood following repair work involves direct contact with gasoline [7]. Therefore, it is necessary to determine the risk by environmental and biological monitoring. Several biomarkers of benzene exposure are sufficiently specific and sensitive for routine use among low-exposure subjects, including non-metabolized benzene in the exhaled breath [5].

In humans, a spectrum of blood dyscrasias, including pancytopenia, aplastic anemia, thrombocytopenia, granulocytopenia, lymphocytopenia, myeloid leukemia and acute leukemia, can result from exposure to benzene. The level, timing and pattern of exposure are extremely important factors in determining the incidence and severity of hematological and bone marrow changes. Furthermore, the stage of stem cell development affected will determine which effects to observe [1].

Benzene has become one of the most intensely regulated occupational agents in the world. With a rapidly increasing number of reports of its hematological effects since 1930, there has been a reduction in exposure limits. These effects have been identified at ever-lower levels, accompanied by a societal concern for improved standards of occupational health. Over the past 25 years, benzene exposure limits have been extensively revised and reduced to the point that, currently, most developed countries have full-shift exposure limits in the range of 0.5–1.0 ppm [8]. The American Conference of Governmental Industrial Hygienists (ACGIH-USA) has established benzene occupational exposure limits of 100, 50, and 25 ppm since 1946. The limit was reduced to 10 ppm in 1977. After 20 years (1997), the threshold limit value (TLV) recommended was

* Corresponding author. Tel.: +55 31 34095725; fax: +55 31 34095700.
E-mail address: zenilda@ufmg.br (Z.L. Cardeal).

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reduced to 0.5 ppm, and this limit holds to the present day [9]. Many other countries either used or followed the ACGIH TLV and have been influential in setting the standards for exposure controls worldwide.

The analysis of benzene in exhaled air has been proposed and used as a biomarker for the assessment of occupational exposure and has been the object of several studies. A correlation between the levels in exhaled air and the exposure in the workplace atmosphere was observed [3,4,6,10–17]. Benzene present in exhaled breath is related to the blood concentration and the absorbed dose. This biomarker can provide direct information about the body burden, and inferences can be made from occupational exposure.

The determination of benzene in the exhaled air offers some advantages by being a selective and sensitive biomarker for evaluating recent exposure. It is easily accepted by the workers because it is not an invasive sampling method. In addition, the matrix (air) is extremely simple, compared to other biological fluids. On the other hand, there is a lack of data on which to base the analysis of exhaled air; mainly because it is not a common practice in biological monitoring. In spite of the fact that the toxickinetics of a large part of those substances are known, they provide little information about the relationship between the concentration in the exhaled air and exposure. This fact is especially true for the normal variation of the concentration of contaminants in the workplace environment [18]. The analysis of exhaled air also presents a challenge because of the low concentrations of the chemical substances present, which require a highly sensitive analytical technique. The results can also be affected by habitual smoking.

The sampling can be performed during the workday, at the end of the day (30 min after termination of the work shift) and the following morning to evaluate the occupational exposure. The standardization and the interpretation of the results should take the collection schedule into account because the time of collection is critical for analytical reliability, especially because the benzene in exhaled air has a short half-life. The concentration at the end of the day is greatly affected by the variations in exposure during work. The concentration of benzene in exhaled air during the work period contemplates the moment of sampling. Some authors suggest that the determination of benzene in exhaled air that was collected on the morning following the exposure reflects the integral exposure of the previous day and displays a better correlation with occupational exposure [19].

Interest in the VOC analysis of exhaled air, be it clinical interest or interest in the biomonitoring of occupational exposure to chemical substances, has grown in recent years. However, the concentration of foreign compounds in human breath is extremely low, a fact that explains why they are not detected. The SPME technique has demonstrated an enormous potential in the VOC analysis of exhaled air, and it has been applied for analysis of chemical substances present in human expiration in the nanomolar range [20]. The technique is extremely attractive because it combines the sampling and pre-concentration of the analyte in a single process and permits the direct desorption in a chromatography system. SPME is a fast, selective and relatively inexpensive method for sample preparation [21].

The present study sought to optimize a simple and sensitive method for determination of benzene by gas chromatograph–mass spectrometry using SPME for active sampling. Active sampling involves the collection of exhaled air as the individual expels breath over a fiber that is attached to a simple mouthpiece [20]. An SPME device, modified as described by Grote and Pawliszyn [22], was used. The SPME fiber, with a protective Teflon tube, was inserted directly into the mouth of the subject. A homemade permeation device was developed and used to generate the gaseous benzene standard.

2. Experimental

2.1. GC–MS system

The chromatographic system used was a thermo electron trace gas chromatograph (GC), equipped with a POLARIS Q model ion trap mass spectrometer with EI and CI ionization modes; a 30 m × 0.25 mm i.d. × 0.25 μm film capillary column containing 5% diphenyl, 95% dimethylpolysiloxane-HP-5 MS (Hewlett-Packard) as the stationary phase and *X CALIBUR* data acquisition. The oven temperature was programmed for 30 °C for 1 min, 10 °C/min to 60 °C, then 30 °C/min to 150 °C, and 220 °C for 10 min after the run to clean any contaminants that might be released by the SPME fiber from the column. The flow rate of the helium carrier gas was 1.0 mL/min.

The mass spectrometer was operated in the electron impact (EI) mode with ionization energy of 70 eV. The ion source was set to 200 °C and the GC–MS interface to 275 °C. In addition to analyses in the scan mode, full scan (mass range 50–90 m/z) and selected ion monitoring (SIM) was applied to quantitative analysis.

2.2. Permeation device to generate benzene standard

The gaseous benzene standard was generated by a permeation device designed to continuously release material at a fixed rate at 35.0 °C, the temperature being precisely controlled (±0.1 °C). An accurately metered dilution flow (Fig. 1) was provided. Synthetic air was used as a diluent in the permeation system for generation of the gaseous benzene standard, and the flow measurements were performed in quintuplicate. The pressure was maintained at 5.0 (±0.2) psi during all the experiments.

The standard emission device is an inert polymeric tube that contains the analyte in its liquid form. This permeation tube for benzene was purchased from VICI Metronics, Inc. and was certified traceable to N.I.S.T. standards with the following characteristics: 3.5 cm long; permeation rate of 19.8 ± 2.0 ng/min at 35.0 °C.

2.3. SPME method

The solid phase microextraction (SPME) was performed with a manual holder with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS)-50/30 μm fiber (purchased from Supelco, PA, USA). The fiber was conditioned with an injector temperature of 250 °C for 4 h to remove fiber contaminants. A blank of the SPME fiber was analyzed by GC before each standard extraction and sample analysis to check the memory effect and also to condition the SPME fiber for the next injection. After sampling and extraction, the SPME fiber was desorbed in the hot injection port at 250 °C during 3.0 min. The split ratio was 1:20.

The extraction procedure for the gaseous benzene standard consisted of: (1) introduction of the SPME device into the sampling bulb as shown in Fig. 1 and (2) subsequent exposure of the fiber during a period of 30 s. An extraction time of 30 s was determined as a function of the concentration range of interest for construction of the analytical curve.

2.4. Analytical curve

The concentration was changed by varying the diluent flow rate to create a range of concentrations while the device was kept at a constant temperature set point. The concentration of benzene obtained in ppm by volume was computed using the following formula:

$$C = \frac{KP}{F}$$

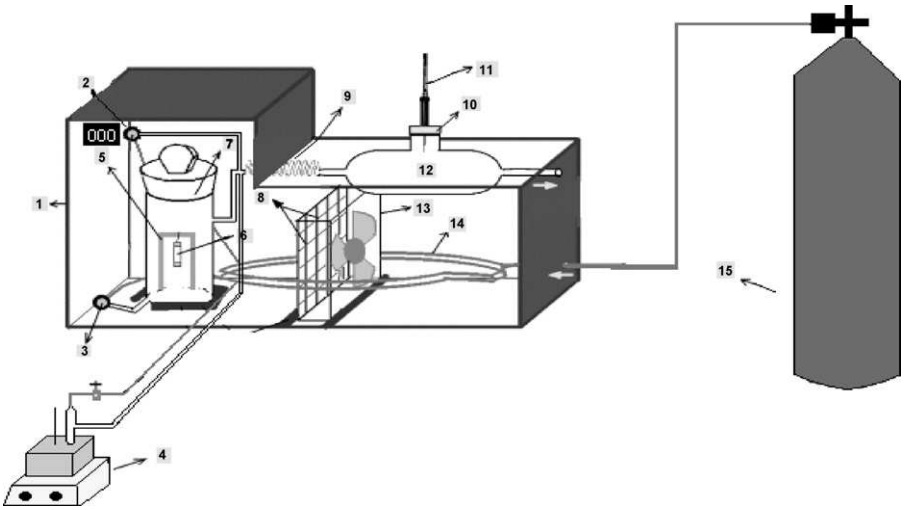

Fig. 1. Steams generation system for the permeation method. The arrows indicate the gas flow sense: (1) wood box; (2) dilution valve; (3) control flow valve of the gas of the permeation camera; (4) generating system of humidity; (5) metallic supports; (6) permeation tube; (7) permeation camera; (8) resistance; (9) serpentine; (10) Teflon septum with sealing wax and aluminum; (11) SPME device; (12) sampling bulb; (13) mini fan; (14) copper tube and (15) synthetic air.

where: C is the concentration in ppm by volume, F the dilution flow rate in mL/min, P the permeation rate in ng/min, K the molar constant = 24.46/MW and MW is the molecular weight of benzene.

The analytical curve was constructed for a certain concentration range as a function of its applicability for biological monitoring of occupational exposure. Concentration levels for the environmental presence of benzene were included. The standard flow rate for the gaseous generation system for a concentration range of 6.0–53.0 ppb was adjusted and measured in quintuplicate.

2.5. Sampling procedure

The sampling of exhaled air was based on the procedure described by Grote and Pawliszyn [22], which consisted of the direct exhalation onto the SPME fiber inserted into a Teflon tube. The Teflon tube was made for that purpose from a compact piece of Teflon. The subjects were trained to perform a slow exhalation to completely empty the lungs so as to obtain the same amount of sample from all the subjects.

A small opening was made near the end of the tube to allow the substitution of the air present in the tube before the individual exhaled and to facilitate exhalation during the sampling procedure. The internal diameter on the right side of the tube was smaller than that on the left side so as to permit the perfect fit of the SPME (Fig. 2).

The collection of exhaled human breath involved the following procedure:

- The volunteers inhaled through their noses and held their breath for about 5 s.
- They exhaled for approximately 5 s without the presence of the SPME fiber.

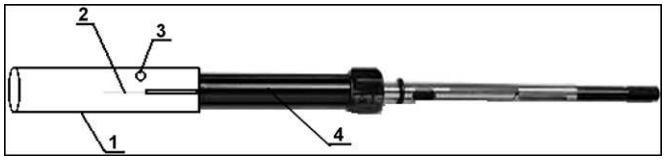

Fig. 2. Adaptation of SPME device goes breath sampling: (1) Teflon tube; (2) exposed fiber; (3) hole and (4) SPME device [22].

- They exhaled as slowly as possible directly onto the exposed SPME fiber during the total 30-s extraction time with a clip compressing the nose.

3. Study protocol and volunteers

Two groups of 25 subjects, "not exposed" and "exposed to benzene from gasoline", participated in this study. There were subjects in the exposed group who worked at gasoline stations and laboratories for the quality control of gasoline. The non-exposed or control group included volunteers among employees, teachers and students from the Federal University of Minas Gerais. Non-smokers were selected for both groups. All the samples were collected at the end of morning or in the middle of the work shift.

4. Results and discussion

Some SPME parameters were studied. The choice of an appropriate coating is essential for the SPME method. The sensitivity of each type of fiber varies according to the molecular weight and the polarity of the analytes to be extracted [22]. Four SPME fibers with different polymeric phases were investigated to optimize the method for determination of benzene: (1) 100 μm polydimethylsiloxane (PDMS); (2) 70 μm carbowax/divinylbenzene (CW/DVB); (3) 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB); and (4) 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS). The fiber chosen was the 50/30 μm DVB/CAR/PDMS, which is specific for gaseous samples; it presented a larger area (response) than the other phases.

The development of SPME methods requires a study to establish optimal analyte extraction conditions. For this purpose, other SPME parameters were studied in addition to the type of fiber. Another parameter determined was the effect of the length of the fiber inside of the tube. The SPME device allows the adjustment of the length of the needle where the fiber is collected. This test assumed that the efficiency of extraction of the analytes depends on the distance to which the fiber is inserted into the mouth. Moderate exhalations of 20 s were made using 2.0 and 4.0 cm extensions of exposed fiber. This experiment showed that the length of the exposed fiber in the tube affects the degree of extraction of the ana-

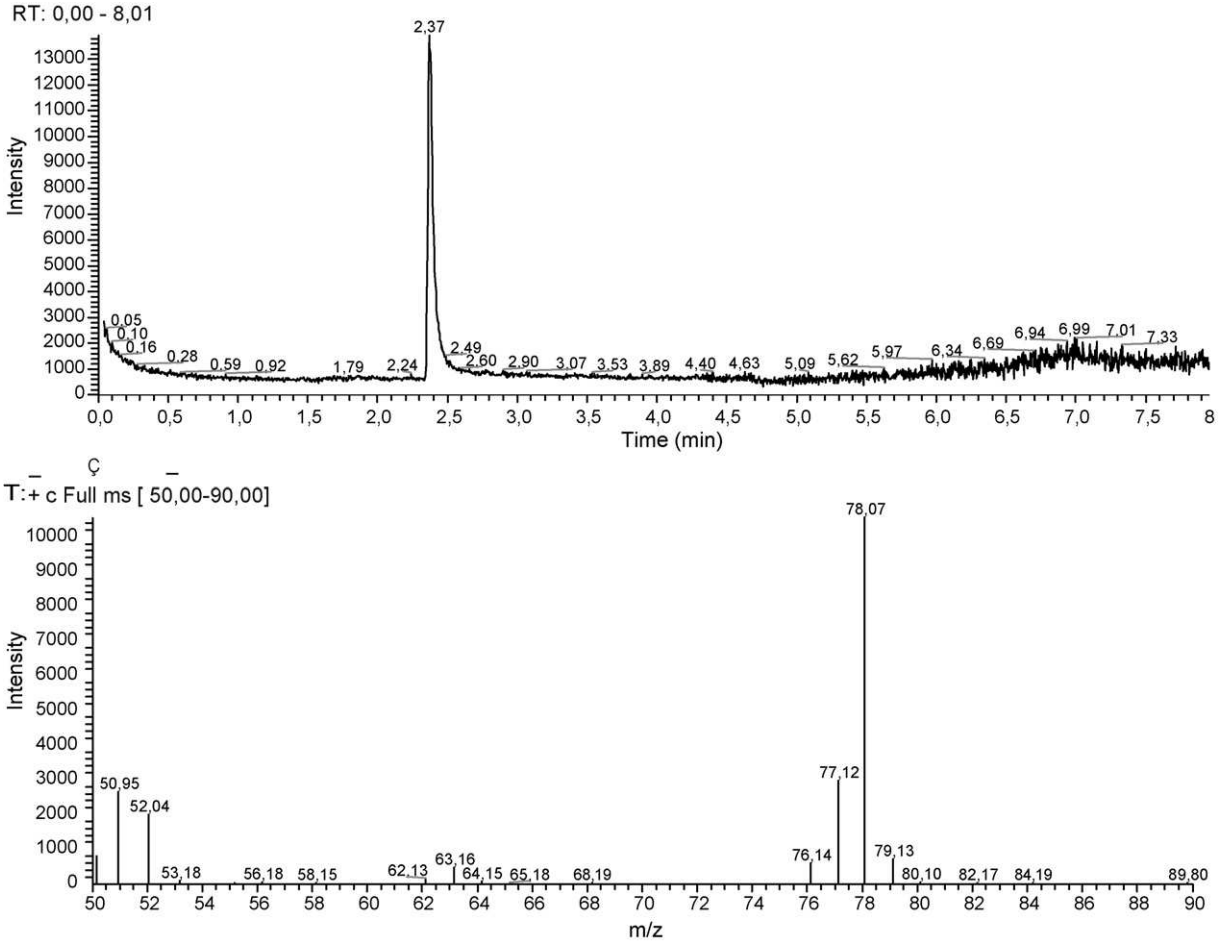

Fig. 3. GC–EI-MS selected-ion-monitoring (*m*/*z* 51 and 78) chromatogram and mass spectrum of benzene for gaseous standard (30.8 ppb).

lytes. The standard deviation obtained from the repetitions with the fiber having a length of 4.0 cm and closer of the mouth was larger (20%) than the standard deviation obtained for the repetitions with the 2.0 cm fiber. Thus, the 2.0-cm-long fiber was chosen.

The extraction and desorption times were evaluated by monitoring the peak areas observed at 20, 30, 45 and 60 s for absorption and 0.5, 1.0, 2.0 and 3.0 min for desorption. The optimum times were 30 s for extraction and 3.0 min for desorption and were considered to be the most reasonable with regard to the sampling of the breath and the sensitivity of standard gas analysis. Method validation was then performed. Chromatograms of gaseous benzene standard and exhaled air from workers exposed to benzene are shown in Figs. 3 and 4.

Knowledge of the equilibration time for the permeation system was important for the construction of the analytical curve. The study evaluated the stability of the system after the adjustment of the flow rate, which was necessary for construction of the analytical curve. The flow rate was measured five times (%RSD = 0.98). Fig. 5 presents the study for establishing the equilibration time of the permeation system for the construction of the analytical curve. A 50/30-μm DVB/CAR/PDMS fiber was used to extract benzene every 0.5–3.5 h. The procedure was repeated for three consecutive days. According to the data, the system reached equilibrium in approximately 1 h, which was considered to be a satisfactorily short time frame.

The precision of the method was evaluated in three different concentrations using optimized conditions. Seven replicate extractions were performed with benzene concentration levels of 6.0, 19.0 and 41.0 ppb. The relative standard deviations (%RSD) observed

Table 1
Results of breath analysis of benzene from the non-exposed volunteers and workers exposed to gasoline

Sample	Benzene concentration (ppb)	
	Control group	Exposed group
1	6.4	30.7
2	9.4	71.2
3	9.9	14.9
4	15.0	15.8
5	9.6	18.7
6	5.3	11.8
7	9.5	20.9
8	8.3	26.6
9	4.9	11.5
10	13.5	18.3
11	7.3	26.5
12	12.5	10.9
13	<LOD	13.5
14	2.3	15.2
15	2.8	11.6
16	<LOD	15.8
17	3.7	22.2
18	8.5	22.7
19	7.8	22.3
20	11.5	35.3
21	14.6	52.2
22	3.2	49.4
23	11.5	39.2
24	8.0	28.4
25	4.2	28.1
Minimum concentration	2.3	10.9
Maximum concentration	15.0	71.2
Media	8.2	25.3

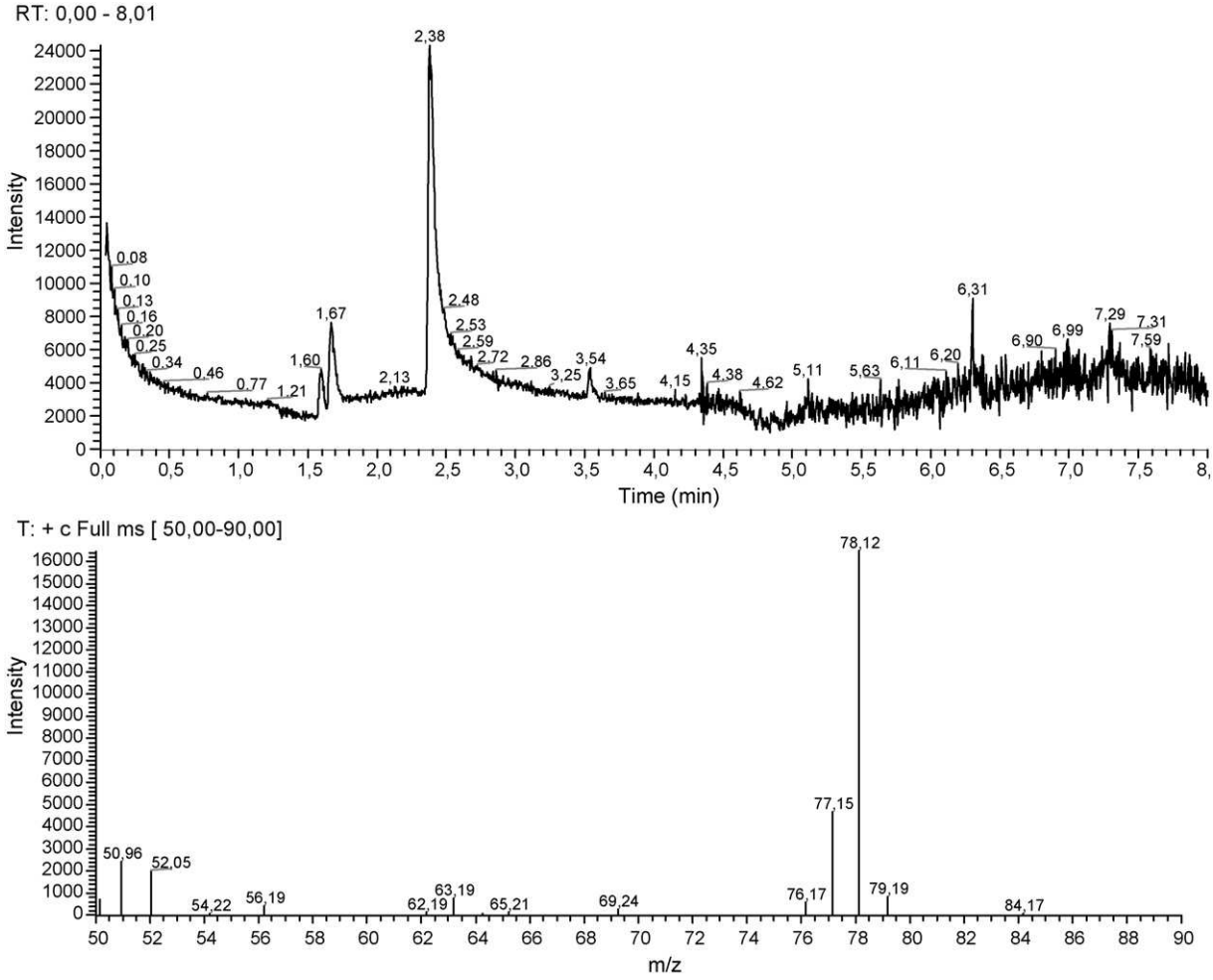

Fig. 4. GC–EI-MS selected-ion-monitoring (*m*/*z* 51 and 78) chromatogram and mass spectrum of benzene in exhaled air from worker exposed to gasoline (52.2 ppb).

were 11.0, 4.6 and 3.2, respectively. The mean of the %RSD values for intra-day measurements (6.3) were satisfactory when compared to other studies with SPME [22–24].

As for sensitivity, the limit of detection (LOD) and the limit of quantification (LOQ) were calculated according to the recommendations of the EURACHEM Guide (2002) [25]. This method used the equations LOD = mean of sample blank + 3*S*, and LOQ = mean of sample blank + 10*S*, where *S* is the standard deviation for 10 repetitions of the extraction procedure with the sample blank. The generation system was maintained for 24 h without a benzene permeation tube prior to the experiment. The results obtained were 2.4 and 3.1 ppb for LOD and LOQ, respectively.

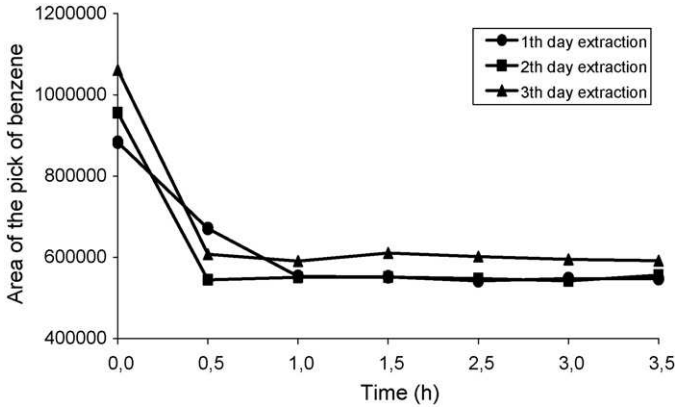

Fig. 5. Study of benzene flow rate equilibrium time of the permeation system.

The application of the SPME method to real samples was achieved through the analysis of workers exposed to gasoline and volunteers who were not exposed, as described in Item 3. This study has approved by the Ethical Committee of the Federal University from Minas Gerais, in accordance with the World Medical Association's "Ethical Principles for Medical Research Involving Human Subjects". The results for the control group and the exposed group are presented in Table 1.

The results for the real samples indicated a significant difference between the groups. Application of the one-way ANOVA test, Brown–Forsythe's Test for equal variance, the Bonferroni Test and the Scheffe Test showed that the population means were significantly different at the 0.05 level. However, both groups presented a large variability in benzene levels of the breath, indicating that the exposed group was not homogeneous. However, it is consistent with findings in other occupational studies [6]. The control group could be considered to be an environmentally exposed group similar to those studied by Perbellini et al. [12].

5. Conclusions

This work describes an alternative method for analysis of benzene and other volatile organic compounds (VOC) using SPME for active sampling of exhaled air. The method proposed in this study was proven to be suitable for evaluating occupational and envi-

ronmental exposure through biological monitoring. This procedure was validated and was found to be precise, linear and sensitive in the range of concentrations of interest to the occupational and environmental fields.

The advantage of this method is the short time in which an analysis can be completed. It requires approximately 10 min, after which more samples can be analyzed. In addition, the active sampling onto SPME fiber decreases contamination and loss of sample, thereby permitting the determination of low levels of benzene. Finally, this method presents the advantages of being solvent-free, of low cost and fast. However, this method requires that the sample not be collected in the workplace because of contamination by the work environment. If the analysis requires, the sample is stable for 30 min when absorbed on the SPME fiber [26]. The applicability of this method to determine other VOC at low concentrations needs to be evaluated.

References

[1] IPCS, Environmental Health Criteria 150-Benzene, World Health Organization, Geneva, 1993.
[2] IARC, Monographs on the evolution of the carcinogenic risk of chemicals to humans, Lyon, 1988.
[3] W.K. Jo, K.W. Pack, Environ. Res. 83 (2000) 180.
[4] S.M. Gordon, L.A. Wallace, M.C. Brinkman, P.J. Callahan, D.V. Kenny, Environ. Health Perspect. 110 (2002) 689.
[5] WHO, Biological Monitoring of Chemical Exposure in the Workplace, WHO, Geneva, 1996.
[6] P.P. Egeghy, L. Nylander-French, K.K. Gwin, I. Hertz-Picciotto, S.M. Rappaport, Ann. Occup. Hyg. 46 (2002) 489.
[7] J. Laitinen, J. Kangas, K. Pekari, J. Liesivouri, Chemosphere 28 (1994) 197.
[8] A.C. Capleton, L.S. Levy, Chem. Biol. Interact. 153 (2005) 43.
[9] ACGIH, Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices, ACGIH, Cincinnati, 2005.
[10] C.D. Money, C.N. Gray, Ann. Occup. Hyg. 33 (1989) 257.
[11] C.N. Ong, B.L. Lee, J. Chromatogr. B: Biomed. Appl. 660 (1994) 1.
[12] L. Perbellini, A. Princivalle, M. Cerpelloni, F. Pasini, F. Brugnone, Int. Arch. Occup. Environ. Health 76 (2003) 461.
[13] P.P. Egeghy, L. Hauf-Cabalo, R. Gibson, S.M. Rappaport, Occup. Environ. Med. 60 (2003) 969.
[14] F. Brugnone, L. Perbellini, G.B. Faccini, F. Pasini, B. Danzi, G. Maranelli, L. Romeo, M. Gobbi, A. Zedde, Am. J. Ind. Med. 16 (1989) 385.
[15] L. Perbellini, G.B. Faccini, F. Pasini, F. Cazzoli, S. Pistoia, R. Rosellini, M. Valsecchi, F. Brugnone, Br. J. Ind. Med. 45 (1988) 345.
[16] C. Plebani, G. Tranfo, A. Salerno, A. Panebianco, A.M. Marcelloni, Talanta 50 (1999) 409.
[17] L. Wallace, T. Buckley, E. Pellizzari, S. Gordon, Environ. Health Perspect. 104 (1996) 861.
[18] P.O. Droz, M.P. Guillemin, J. Occup. Environ. Med. 28 (1986) 593.
[19] R.H. Lauwerys, P. Hoet, Guidelines for Biological Monitoring, Lewis Publishers, Boca Raton, 1993.
[20] M. Giardina, S.V. Olesik, Anal. Chem. 75 (2003) 1604.
[21] G. Vas, K. Vekey, J. Mass Spectrom. 39 (2004) 233.
[22] C. Grote, J. Pawliszyn, Anal. Chem. 69 (1997) 587.
[23] C. Prado, P. Marin, J.F. Periago, J. Chromatogr. A 1011 (2003) 125.
[24] H. Yu, L. Xu, P. Wang, J. Chromatogr. B 826 (2005) 69.
[25] EURACHEMGuide, "Guide to Quality in Analytical Chemistry, an AID to Accreditation", CITAC/EURACHEM, 2002.
[26] J.A. Sales, Departamento de Química, Universidade Federal de Minas Gerais, Belo Horizonte, 2003, p. 80.

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DOI 10.1007/s00216-009-3206-x

ORIGINAL PAPER

Sampling of benzene in environmental and exhaled air by solid-phase microextraction and analysis by gas chromatography–mass spectrometry

Helvécio C. Menezes · Leiliane C. A. Amorim · Zenilda L. Cardeal

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Abstract Benzene is classified as a Group I carcinogen by the International Agency for Research on Cancer (IARC). The risk assessment for benzene can be performed by monitoring environmental and occupational air, as well as biological monitoring through biomarkers. The present work developed and validated methods for benzene analysis by GC/MS using SPME as the sampling technique for ambient air and breath. The results of the analysis of air in parks and avenues demonstrated a significant difference, with average values of 4.05 and 18.26 μg m^{-3}, respectively, for benzene. Sampling of air in the occupational environment furnished an average of 3.41 and 39.81 μg m^{-3}. Moreover, the correlations between ambient air and expired air showed a significant tendency to linearity (R^2=0.850 and R^2=0.879). The results obtained for two groups of employees (31.91 and 72.62 μg m^{-3}) presented the same trend as that from the analysis of environmental air.

Keywords Benzene · Environmental analysis · Breath analysis · SPME · GC/MS

Introduction

Benzene is a primary pollutant in the petrochemical and steel industry. Its production and consumption have caused worldwide concern for health risks resulting from exposure, mainly because of its carcinogenic effect. The petrochemical industry, processing of coke and some chemical syntheses are also activities that contribute to emissions in the external environment [1]. In addition to the burning of cigarettes [2], the use of carpets, adhesives, and cardboard [3], and the use of coal and biomass for heating [4, 5] can contribute to the contamination of indoor environments. Workers involved in the production, transportation, and marketing of fuels are exposed to various levels of benzene, whose average content in gasoline is 3% *v/v*. This level can vary with the origin of the gasoline, the regulations of each country, and the adulteration with aromatic solvents [6].

Benzene is classified as a Group I carcinogen by the International Agency for Research on Cancer [7] and is listed by the World Health Organization [8] as a top priority compound. Studies have shown that in addition to being a risk factor for leukemia, it can cause significant hematological changes in people exposed to 3.19 mg m^{-3} (101.3 kPa and 298 K) or 1 ppm [9]. Prolonged exposure to benzene causes various effects on the human body, especially myelotoxicity, genotoxicity, and its carcinogenic action. Other effects on various organs such as the central nervous system and the endocrine and immune systems are known [10].

The occupational exposure limit of 1.60 mg m^{-3} recommended by the American Conference of Governmental Industrial Hygienists Threshold Limit Value—Time-Weighted Average (TLV-TWA) [11] has decreased significantly in recent years. The National Institute for Occupational Safety and Health set the exposure limit recommended exposure limit (REL)-TWA to 0.32 mg m^{-3}, and the short-term exposure limits (STEL) is 3.19 mg m^{-3} for 15 min of exposure [12]. The gradual increase in the concentration of benzene in urban atmospheres has shown that non-occupational exposure should receive special attention because the number of people exposed is much greater than in occupational environments. The limit of 5 mg m^{-3} for

H. C. Menezes · L. C. A. Amorim · Z. L. Cardeal (✉)
Chemistry Department, ICEx, Federal University of Minas Gerais,
Avenida Antonio Carlos 6627,
31270-901 Belo Horizonte, MG, Brazil
e-mail: zenilda@ufmg.br

benzene was initially set by the European Community as an average yearly value [13], to be reduced to zero by 2010.

The assessment of the risk of exposure to benzene can be achieved by monitoring the environmental and occupational air for this compound, as well as the use of biological indicators of exposure and the determination of the effects through biological monitoring. Among the biological exposure indicators, the presence of unchanged benzene in urine and breath and of *trans*, *trans*-muconic acid and phenylmercapturic acid metabolites in urine [14] are indicated. The other biomarkers such as benzene adducts with albumin or hemoglobin are suggested as indicators of a biological effect [15].

Among the methods for biological monitoring of unchanged benzene, the determination in exhaled air has the advantage of being a non-invasive technique, in addition to the fact that air is a less complex matrix than blood and urine. Some studies have shown significant correlations between the concentrations of benzene in the environment and in the breath of mechanics [16], and active and passive smokers [17].

Several techniques can be used for sampling benzene in air, such as the use of metal containers, sampling bags, adsorbent bulbs, and tubes with activated charcoal [18]. These techniques are efficient but they have many steps. The method TO-15 of the U.S. Environmental Protection Agency (EPA) uses canister as benzene sampling technique and GC/MS (gas chromatography with mass spectrometry detection) as analysis method, with detection limit of 0.92 μg m^{-3} [19]. Studies using GC/MS or GC/FID (gas chromatography with flame-ionization detection) presented typical detection limits of 1.0 μg m^{-3} for environment air [20] and 1.5 μg m^{-3} for exhaled air [16, 17, 21], values larger than those found in the present study. In those studies, the quantification limits were not usually reported, and the calibration method merely mentioned.

One technique that has proved to be very efficient for sampling of volatile compounds in air is SPME (solid-phase microextraction). It combines sampling and pre-concentration in a single step, with subsequent desorption directly into the analytical instrument.

In air sampling by SPME, the extraction can be performed by direct exposure of the fiber to the matrix. The partition equilibrium with the transfer of a portion of the analyte to the fiber occurs. It is necessary to use gaseous standards that simulate the environment of interest for the determination of volatiles in the air by this technique. The generation of standards by permeation has advantages such as versatility, minimization of the adsorption of the analyte by the system, ease of operation, and the ability to include a wide range of concentrations in a single dilution stage. The present study used a constructed system [22] to generate benzene standards by permeation, with SPME sampling in ambient air and exhaled air. After direct desorption in the injector, determinations by gas chromatography coupled to mass spectrometry were performed. The optimized method is simple and sufficiently sensitive for the analysis of benzene in breath and environmental air.

Experimental

Generating the benzene standard

A system for the generation of gaseous standards, as shown in Fig. 1, was used. The air was conducted to a UHP-10ZA Brand Domnick Hunter scrubber after compression to 345 kPa and then passed through a spiral copper pipe (1/8 in.) for preheating. After the dilution control valve, the air entered the permeation chamber and passed through a permeation tube of polymer material containing liquid benzene. The tube, manufactured by Vici Metronics, Inc. and certified for traceability by NIST standards, was 3.5 cm in length, and the rate of permeance was 19.8±2.0 ng min^{-1} at 35.0 °C. The tube was subjected to a constant flux with the temperature controlled by a thermostat to 35.0±0.1 °C. The temperature control system was connected to a resistance and a small fan for even distribution of heat throughout all the components inserted into the space bounded by heat insulation (dotted rectangle of Fig. 1). The mixture of air with benzene was homogenized in a spiral glass tube before reaching the sampling bulb where the SPME fiber was then exposed. The flow was measured with a Supelco Optiflow 650 digital flowmeter. The system pressure was maintained at 99±1 kPa in all the experiments by a flow control valve.

The dilutions required for the construction of an analytical curve for benzene in ambient air with a 15-min extraction time and a curve for benzene in breath with an extraction time of 30 s were obtained through the control of airflow in the permeation chamber. The concentrations of benzene for both curves were calculated from the expression:

$$C\left(\mu\text{g m}^{-3}\right) = \frac{10^3 \times Q(\text{ng min}^{-1})}{F(\text{mL min}^{-1})} \quad (1)$$

where C is the concentration of benzene at 101.3 kPa and 298 °K, Q is the permeation rate, and F is the corrected flow (mL min^{-1}) at 101.3 kPa and 298 °K. For each concentration level of the analytical curves, the flow was altered, and, after the 150 min equilibrium time, the flow was measured in replicate (n=7), followed by extraction with the SPME fiber and quantification by GC-MS for the areas of each peak. The readings of the blank were obtained after withdrawing the permeation tube and passing diluent air for 24 h before performing the extraction.

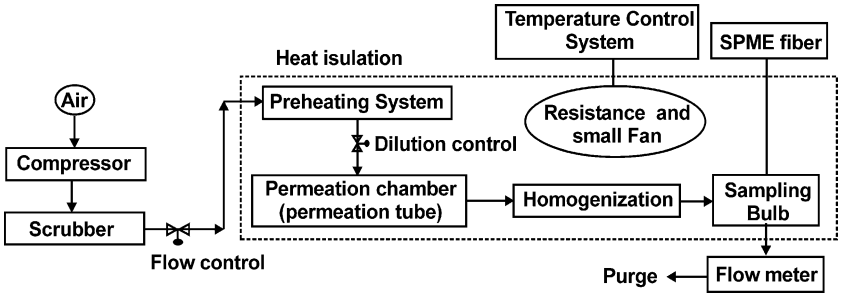

Fig. 1 Fluxogram of the system for vapor generation by the permeation method

Solid-phase microextraction method

For the solid-phase microextraction (SPME), a handheld fiber (manufactured by Supelco, PA, USA) with 50/30 μm divinylbenzene/Carboxen/Polimetilsiloxano (DVB/CAR/PDMS) was used. The extraction of the gaseous benzene standard was achieved by exposure of the fiber in the sampling bulb (Fig. 1) for a period of 15 min. This sampling time was used when attempting to determine the STEL. A 30 s sampling period was chosen for breath, sufficiently long to collect the alveolar portion of expiration and not cause respiratory discomfort to the volunteers

GC/MS system

A Thermo Electron Trace gas chromatographic system coupled to a POLARIS Q model ion trap spectrometer and equipped with the capillary column (30 m×0.25 mm id×0.25 μm film) containing 5% diphenyl, 95% dimethylpolysiloxane HP-5 ms (Agilent Technology Inc.) was used. The temperature program began with an oven temperature of 30 °C for 1 min; the oven was heated at a rate of 10 °C min^{-1} to 60 °C, then at 30 °C min^{-1} to 120 °C, where it was held for 1 min; the oven was then heated at 100 °C min^{-1} to 250 °C and held for 3 min. The helium flow was 1.5 mL min^{-1}. The injector was maintained at 250 °C in splitless mode for 3 min, followed by a split in the ratio of 1:20. The mass spectrometer was operated in electron impact mode at 70 eV. The temperature of the ion source was 200 °C and that of the GC-MS interface was 250 °C. The analysis was performed in full scan mode (range 50–90 m z^{-1}), with quantification in selected ion monitoring mode using the m z^{-1}=51 and 78 fragments.

Sample collection

The urban air samples were obtained during the period from June–July, 2008, with an average temperature of 22±3 °C, in Belo Horizonte, a city located in southeastern Brazil (19° 55′ 57″ S, 46° 56′ 32″ W). It has 2.4 million inhabitants and 1.1 million vehicles in circulation. Fifteen sampling points distributed in avenues, 15 points in parks, 45 points in two groups of occupational environments—teaching and research laboratories of the Department of Chemistry/UFMG and gasoline stations—were selected. A high traffic density (>10,000 vehicles per day) was considered for the selection of avenues. All the parks were selected within the urban perimeter of the city. For the choice of occupational environments, factors related to the type of activity, location, and the availability of administrative facilities to cooperate with the investigation were considered. For each sampling, the fiber was exposed for 15 min in the respiration zone (1.70±0.20 m above ground level). After the collection, the fiber was withdrawn and wrapped in aluminum foil at 3±2 °C until the time of analysis. The maximum period between collection and analysis did not exceed 2 h so that no volatilization of the analyte occurred. For collection of the breath samples, a nose clip was placed on the nose of the person, who inspired and held his breath for 5 s, expired for 5 s, and then expired for 30 s directly onto the SPME fiber inserted into a Teflon tube. After collection, the sample was preserved in a manner similar to that described for the ambient air. This study used two groups of volunteers, all male and nonsmokers. The first group (n=15) was composed of workers exposed to low levels of benzene; employees of restaurants, coffee shops, offices, park guards, and teachers. The second group (n=30) was composed of workers in jobs involving the resale of fuel and who were directly exposed to benzene. For each collection of exhaled air, a simultaneous sampling of the ambient air of the workplace by exposure of the fiber during 15 min in the respiration zone during 15 min was performed. The analytical protocol for the determination of benzene in air samples is schematically depicted in Fig. 2.

Statistical analysis

The Shapiro–Wilk test was used to verify the normality of the data and the Mann–Whitney test was employed for comparisons between the means of the data that did not follow normality. Because of the heteroscedasticity of the instrumental responses, the linear models for the calibration curves were constructed by the least squares method

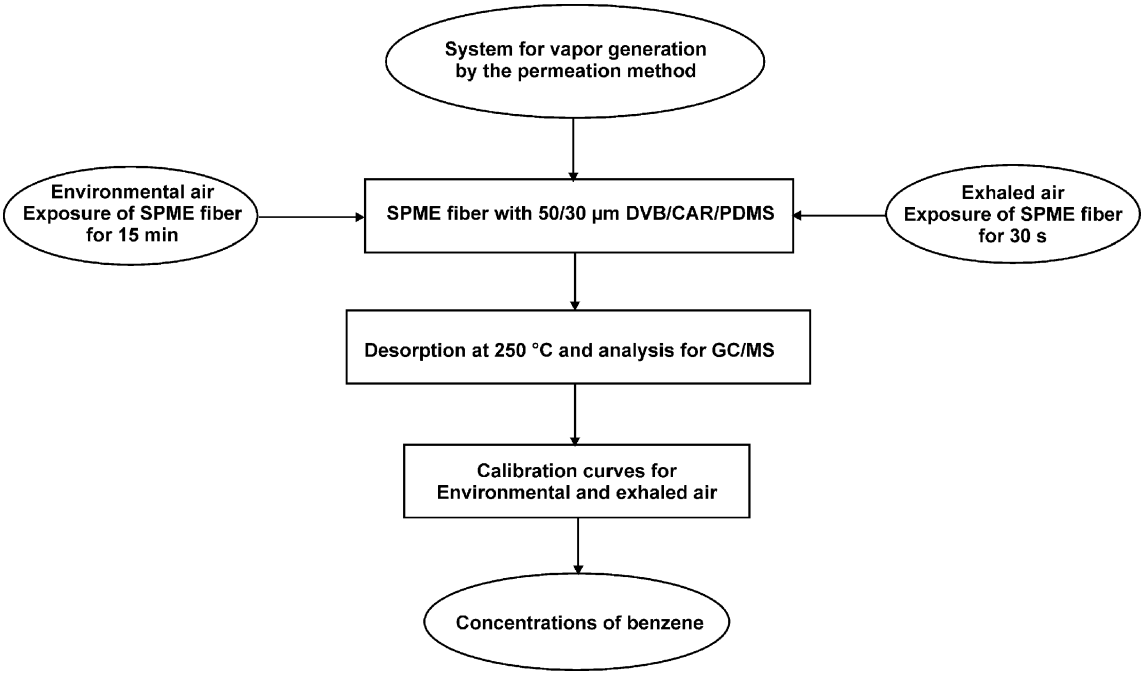

Fig. 2 Flow diagram representing the analytical protocol

weighted by the experimental variance. p values below 0.05 were considered significant. The tests were performed using the Origin 8.0 software (OriginLab Corp).

Results and discussion

The calibration curves were constructed in seven levels of analyte concentration with three steps for each level. For benzene in ambient air, the curve (y=1,429.118x+0.004) presented a linear range of 0.82±0.01 to 317.00± 0.02 μg m^3, R^2=0.996 (p value <0.0001). The limits of detection (LOD) and quantification (LOQ) were calculated by the expressions:

$$LOD = X_b + 3\,s \quad (2)$$

$$LOQ = X_b + 10\,s \quad (3)$$

according to the recommendation of the Eurachem Guide [23] where X_b and s are the mean and standard deviation, respectively, of ten consecutive measurements of the blank. The LOD was (0.24±0.01) μg m^{-3} and the LOQ was (0.80±0.01) μg m^{-3}. In assessing the intra-assay precision (repeatability), ten replicates at the concentration levels of 16.7, 127.5, and 256.2 μg m^{-3} were analyzed on the same day. The coefficient of variation (RSD) presented a range of 1.50–4.20%, with a mean of 2.85%. These values were lower than the results obtained in other studies for the assessment of benzene in ambient air [24, 25]. Five replicates at three concentration levels were analyzed on three consecutive days to assess the intermediate precision. The values of RSD obtained in this study varied from 2.58 to 6.11%, with a mean of 4.34%.

For the analysis of benzene in breath, the curve (y= 65.691x+0.006) presented a linear range from 3.22 to 187.00 (±0.01) μg m^{-3}, R^2=0.998 (p value <0.0001). The LOD and LOQ were (0.95±0.01) μg m^{-3} and (3.22±0.01) μg m^{-3}, respectively. Replicates (n=10) at the concentration levels of 15.00, 85.40 and 159.50 μg m^{-3} were used to study the repeatability. The %RSD results obtained were 2.90–7.40%, and the mean was 5.15%. These values were considered favorable in comparison to other studies that analyzed the benzene in exhaled air [26]. Values of 5.78–8.43 μg m^{-3} for %RSD, with an average of 7.11 μg m^{-3}, were observed in a study of the intermediate precision with five replicates and three levels of concentration analyzed on three consecutive days.

The results for the concentrations of benzene in the ambient air of avenues, parks, and occupational environments and in the breath of individuals exposed occupationally are presented in Fig. 3, and the location of sampling points of parks (a) and avenues (b) are presented in Fig. 4. All the samples presented concentrations above the LOQ established for the methods employed.

The average concentration of benzene encountered in the parks was 4.05±1.74 μg m^{-3}, with a range of 2.55 to (7.98±0.01) μg m^{-3}. The parks that presented the highest levels of benzene (samples 4a, 6a, and 9a) are situated in regions with an average population and traffic density, except for sample 9a, which presented a relatively high

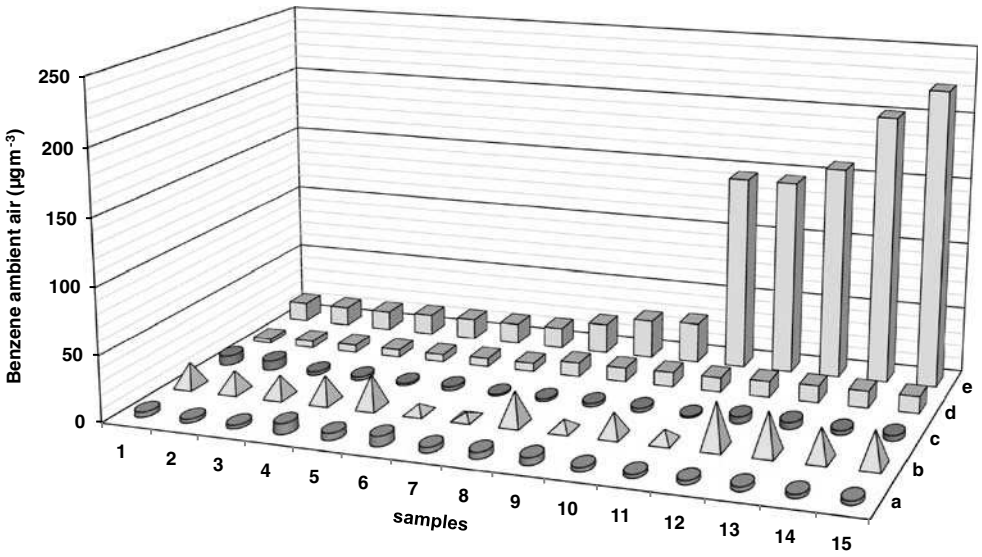

Fig. 3 Concentration (micrograms per cubic meter) of benzene in the environmental air of **a** parks, **b** avenues, **c** laboratories, **d**,**e** fuel resale stations

benzene level compared to the other parks, even though it is located in a region far from the urban center. This result can be attributed to factors such as the geographic and topographic position and the direction of the wind at the moment of sampling because this park is located at a higher altitude than the rest of the city, and the wind was blowing from the urban center towards the park at the moment of sampling.

All the conditions described above possibly facilitated the transport of pollutants, especially those that are more

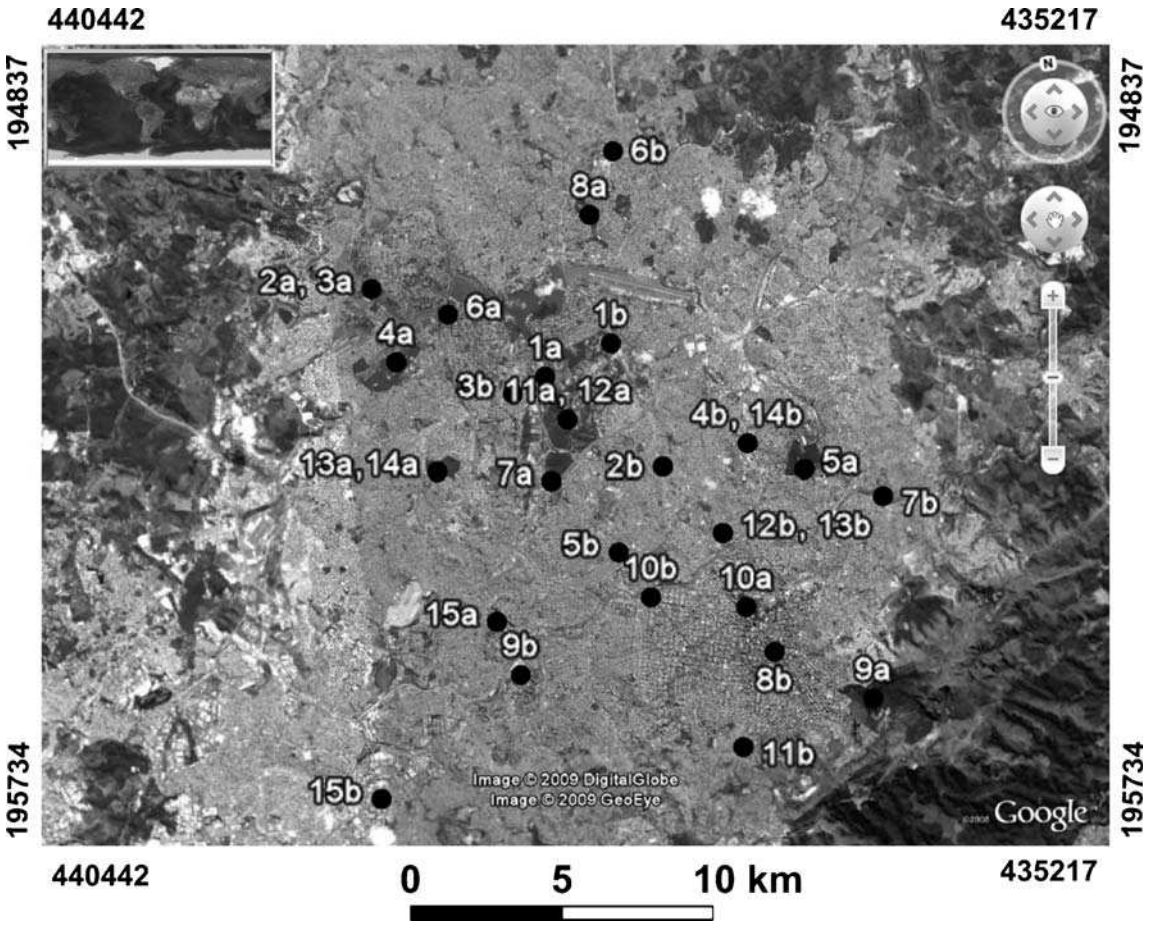

Fig. 4 Map with the location of sampling points in parks (**a**) and avenues (**b**) of the city of Belo Horizonte

volatile, such as benzene. The high level of benzene presented by sample 6a is probably related to the existence of a stream that ran through the park and whose water presented a strong odor characteristic of solvents resulting from the discharge of industrial wastes above the sampling point. Sample 4a was obtained from a zoo in which vehicles are allowed to circulate. This fact contributed to an increase in the environmental benzene level.

The mean level encountered in avenues was 18.26± 17.90 μg m^{-3}, and the range was 4.15 to (33.52±0.01) μg m^{-3}. The highest values for the avenues were presented by samples 12b and 13b because they correspond to the points situated at the entrance and the exit of a tunnel. Sample 15b also presented a relatively high level of benzene, probably because it was obtained on a road located in an industrial area. Sample 5b, although not obtained from an industrial area, presented a benzene level near that of sample 15b, mainly because of the slow vehicle traffic characteristic of the region where the sampling was performed. Sample 7b, despite being from a point near the center of the city, contained a relatively low concentration of benzene. This result was probably obtained because the avenue is located near a park with a large green area. The number of vehicles, the use of catalytic converters, traffic speed, location, topography, and weather are among the factors that may affect the observed variability in the benzene levels in urban environments. The results observed in this study and other recent studies [27, 28] show that the general population is exposed to a basal concentration of benzene.

The median for the parks was significantly lower ($p<$ 0.0001) than that of the avenues. This observation underlines the importance of green areas for improvement of air quality in urban compartments. The values encountered for the parks and avenues are above the limit recommended by the policy of the European Community. Although Brazil does not have regulations for benzene levels in ambient air, the identification and implementation of more effective measures to reduce benzene emissions into the urban atmosphere are necessary. Replacement of fossil fuels, regular maintenance of the fleet in circulation, rationalization of the use of vehicles, and replacement of the current concept of focusing on individual transport mobility by a model that encourages public transport are some of the actions required to reduce emissions of benzene in the concentrated populations.

With regard to occupational environments, the average benzene level in laboratory air was 3.41±1.98 μg m^{-3}. Among the samples, three (1c, 2c, and 13c) presented higher levels of benzene, probably because of the poor natural ventilation and the absence of collective protection equipment in these environments. The average level was 39.81±63.30 μg m^{-3} at retail gasoline stations, with a median significantly ($p<0.0001$) greater than those of laboratory environments. The relatively high levels of benzene samples 13e, 14e, and 15e correspond to fueling stations with an intense movement of vehicles and with poor or nonexistent ventilation (sample 15e). Samples 11e and 12e were obtained at stations with a small movement of vehicles, but they were receiving fuel from distribution tanker trucks at the time of sampling. This fact indicates that, despite all the care taken in this type of operation, leakage of benzene to the atmosphere still occurs. The large variability observed for retail gasoline stations is probably a result of fluctuations in the ventilation conditions, volatilization of fuel, and the number of vehicles refueling.

The exhaled air of workers and/or students in research and educational laboratories contained an average benzene concentration of 31.91±41.50 μg m^{-3}, ranging from 3.20 to (153.20±0.01) μg m^{-3}, while those workers who worked in retail gasoline stations had an average of 72.62± 52.78 μg m^{-3}, with a range of 16.30 to (197.60±0.01) μg m^{-3}. The values observed for workers in retail gasoline stations were significantly higher than those in the other group with lower exposure. In other studies, values for benzene values on the order of 67.0 μg m^{-3} [29] and

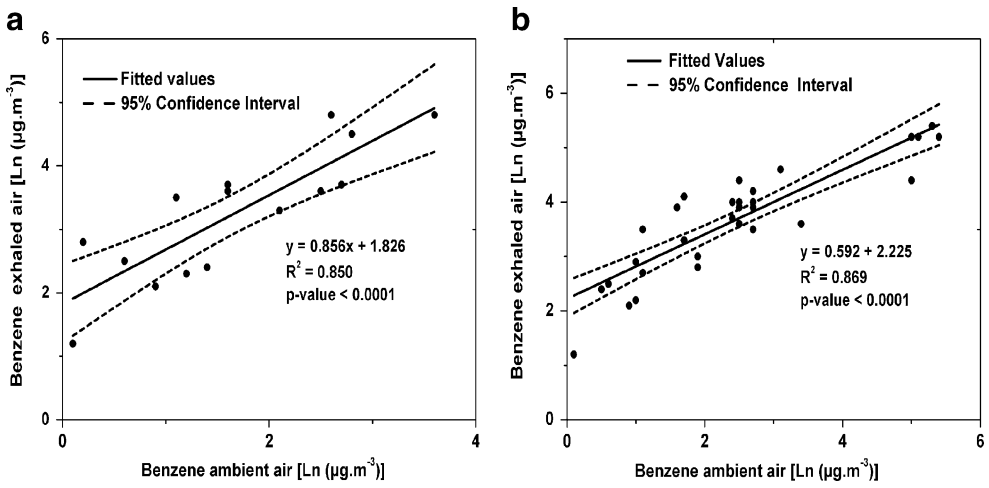

Fig. 5 Correlation between the Ln for the concentration of benzene in exhaled air and in the air of offices and laboratories (**a**) and of fuel resale stations (**b**)

40.3 μg m^{-3} [30] have been observed in the exhaled air of workers.

Correlations between ambient air and breath indicate a significant linear trend (Fig. 5), both for laboratory environments (R^2=0.850, β=0.856, p<0.0001) and for the resale posts (R^2=0.879, β=0.592, p<0.0001). Other studies have encountered a correlation of 0.77 (p<0.0001) between benzene in the occupational environment and benzene in exhaled air at self-service fuel resale posts [30] and a correlation of 0.546 (p<0.0001) for workers exposed to aviation fuel [31]. In using the analysis of benzene in exhaled air as a biomarker, one must consider that the relatively short half-life of this substance in the blood reflects only recent exposure and invalidates the evaluation of an old exposure. The high basal concentrations of benzene can cause an overestimation of exhaled benzene. Genetic and physiological differences and individual habits can also contribute to an increase in the benzene background. However, these factors are present when using other benzene biomarkers.

Conclusions

An alternative method for the analysis of benzene using SPME for passive sampling of environmental and occupational air is presented. The method was simple, non-invasive, fast, and relatively non-expensive for the analysis of exhaled air as an exposure biomarker. This procedure was validated and was found to be precise, linear and sensitive in the range of concentrations of interest for environmental and biological monitoring. The results obtained in the environmental analyses of parks and avenues demonstrated the ability of the method to measure the levels of benzene in different urban compartments. The great variability observed in the occupational atmospheres of the fuel retail posts is in agreement with other studies and with the observations of the predominant conditions at each moment of sampling. The significant correlation observed between benzene in occupational air and exhaled air demonstrates that these parameters show promise for the monitoring of the environment, and as benzene biomarkers for evaluating occupational exposure.

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References

1. Jo WK, Lee JW, Shin DC (2004) Int Arch Occ Env Hea 77:113–120
2. Lee SC, Li WM, Ao CH (2002) Atmos Environ 36:225–237
3. Lai HK, Jantunenc MJ, Nünzlid N, Kulinskaya E, Colvilea R, Nieuwenhuijsen MJ (2007) Atmos Environ 41:9128–9135
4. Abalak R, Bruce N, McCraken JP, Smith KR, Gallardo T (2001) Environ Sci Technol 35:2650–2655
5. Sinha SN, Kulkarni PK, Shah SH, Desai NM, Patel GM, Mansuri MM, Saiyed HN (2006) Sci Total Environ 357:280–287
6. Skrobot VL, Eustáquio VRC, Pereira RCC, Pasa VMD, Fortes CPF (2008) Energ Fuel 21:3394–3400
7. IARC(1982) Monographs on the evaluation of the carcinogenic risk of chemicals to humans. Some industrial chemicals and dyestuffs vol 29. http://monographs.iarc.fr/ENG/Monographs/vol29/volume29.pdf. Accessed nov 2008
8. WHO (2006) Development of WHO Guidelines for Indoor Air Quality. http://www.euro.who.int/Document/AIQ/IAQ_mtgrep_Bonn_Oct06.pdf. Accessed jan 2009
9. Lan Q, Zhang L, Li G, Vermeulen R, Weinberg RS, Dosemeci M, Rappaport SM et al (2004) Science 306:1774–1776
10. Crump KS (1996) Environ Health Persp 104:1437–1441
11. ACGIH(2008) Threshold limit values for chemical substances and physical agents and biological exposure indices for 2008. http://www.techstreet.com/cgi-bin/detail?product_id=1604745. Accessed feb 2009
12. NIOSH (2005) Pocket guide to chemical hazards. Department of Health and Human Services-Centers for Disease Control and Prevention. http://www.cdc.gov/niosh/npg/. Accessed dec 2008
13. ECOJ(2000) Directive 2000/69/EC of the European Parliament and of the Council of 16 November 2000 relating to limit values for benzene and carbon monoxide in ambient air. http://eur-lex.europa.eu. Accessed dec 2008
14. Navasumrit P, Chanvaivit S, Intarasunanont P, Arayasiri M, Lauhareungpanya N, Parnlob V, Settachan D, Ruchirawat M (2005) Chem Biol Interact 153:75–83
15. Rappaport SM, Waidyanatha S, Qu Q, Shore R, Jin X, Cohen B, Chen LC et al (2002) Cancer Res 62:1330–1337
16. Egehy PP, Velez R, Rappaport SM (2002) Environ Health Persp 108:1195–1202
17. Jo WK, Pack KH (2000) Environ Res 83:180–187
18. Wang DKW, Austin CC (2006) Anal Bioanal Chem 386:1089–1098
19. EPA (1999) Compendium of methods for determination of toxic organic compounds in ambient air. Center of Environmental Research Information. US Environmental Protection Agency. http://www.epa.gov/ttn/amtic/airtox.html. Accessed sept 2009
20. Demeestere K, Dewulf J, Witte BD, Langenhove HV (2007) J Chromatogr A 1153:130–144
21. Gordon SM, Wallace LA, Brinkman MC, Callahan PJ, Kenny DV (2002) Environ Health Persp 110:689–698
22. Amorim CAL, Carneiro JP, Cardeal ZL (2008) J Chromatogr B 865:141–146
23. EURACHEM (2002) Guide to Quality in Analytical Chemistry an Aid to Accreditation Geel: Cooperation on International Traceability in Analytical Chemistry and A Focus for Analytical Chemistry in Europe. http://www.eurachem.org/guides/. Accessed Dec 2008
24. Bruno P, Caputi M, Caselli M, Gennaro G, Rienzo M (2004) Atmos Environ 39:1347–1355
25. Larroque V, Desauziers V, Mocho P (2006) J Environ Monit A 8:106–111
26. Yo H, Xu L, Wang P (2005) J Chromatogr B 826:69–74
27. Gonzalez-Flesca N, Nerriere E, Leclerc C, Le Meur S, Marfaing H, Hautemaniere A et al (2007) Atmos Environ 41:2549–2558
28. Iovino P, Salvestrini S, Capasso S (2008) Chemosphere 73:714–718
29. Plebani C, Tranfo G, Salerno A, Panebianco A, Marcelloni AM (1999) Talanta 50:409–412
30. Egeghy PP, Nylander-French L, Gwin KK, Hertz-Picciotto I, Rappaport SM (2002) Ann Occup Hyg 46:489–500
31. Egehy PP, Hauf-Cabalo L, Gibson R, Rappaport SM (2003) Occup Environ Med 60:969–976

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An evaluation of solid phase microextraction for analysis of odorant emissions from stored biosolids cake

M. Visan*, W.J. Parker

Department of Civil and Environmental Engineering, Carleton University, 1125 Colonel By Drive, Ottawa, Ont., Canada K1S 5B6

Abstract

Odors are a common occurrence at wastewater treatment plants, biosolids processing facilities and biosolids recycling locations. Accurate, objective measurement techniques are needed to monitor emissions, to develop new waste handling procedures and to reduce the production of the volatile gases. The objective of this study was to evaluate the use of solid phase microextraction for measuring common odorants that are found in biosolids facilities. The odorants were collected and concentrated by solid phase microextraction (SPME) and then quantified by gas chromatography with detection by mass spectrometry. A 75-μm Carboxen-Polydimethylsiloxane coating was used for the analysis of trimethylamine, dimethyl sulfide, dimethyl disulfide and methyl mercaptan. Gaseous standards were generated for individual compounds and for dry and wet mixture from permeation apparatus. The differences in sensitivity between fibers, the competition between analytes and water vapor for the active sites on the fiber and the lack of production of artifacts make SPME suited for qualitative analysis and enables quick screening for the identification of compounds with adverse organoleptic characteristics.

Keywords: SPME; Volatile organic sulfur compounds; Trimethylamine; Adsorption

1. Introduction

A failure to acknowledge the potential for odors and to work to prevent odor emissions can result in complaints, shutdowns, expensive retrofits and non-acceptance of the finished product. To control odors, they must first be measured. From the large number of analytical method available for collection of chemical compounds responsible for biosolids odor, SPME-gas chromatography was chosen for examination in this study. SPME is a simple, effective adsorption–desorption technique, which eliminates the need for solvents or complicated apparatus for concentrating volatile or non-volatile compounds in liquids or gases (SUPELCO, Bulletin 923, 1998).

SPME integrates sampling and preconcentration in one step. A chemically modified fused silica fiber is exposed in the headspace of the sample or in the liquid sample. Ideally, equilibrium is reached between the odor matrix and fiber, but for accuracy and precision, consistency in sampling time is more important than full equilibrium (SUPELCO, Bulletin 923, 1998). The extraction process is governed by the kinetics of diffusion in the surrounding medium and/or the polymer fiber coating. Several studies (Pelusio et al., 1995; Haberhauer-Troyer et al., 1999; Payne, 2000; Kim et al., 2002; Murthy et al., 2001) have reported the use of SPME in combination with GC-MS to analyze low concentrations of volatile sulfur compounds and trimethylamine.

*Corresponding author. Tel.: +1-6135900285.
E-mail address: mvisan@rogers.com (M. Visan).

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However, Haberhauer-Troyer et al. (1999) found that SPME has a number of limitations, including the production of artifacts. Methyl mercaptan was readily oxidized to dimethyl disulfide, isopropyl mercaptan and isobutyl mercaptan were oxidized into disulfides and dimethyl sulfide was oxidized into dimethyl sulfoxide. In addition, the extraction efficiency was found to depend on humidity and there was low stability of volatile sulfur compounds on the fiber, which led to the occurrence of decomposition products.

This paper addresses a study of the applicability of solid phase microextraction in combination with gas chromatography/mass spectrometry (GC-MS) for determination of volatile sulfur compounds and trimethylamine from the headspace of dewatered biosolids. The influence of humidity and competition for adsorption sites as well as the effect of the needle material upon artifact formation was investigated.

2. Methodology

To evaluate SPME as a method for odor measurement, gas standards were prepared and used to make calibration curves. In the development of calibration curves, potential SPME limitations that were suggested in the literature were assessed. One of the SPME variables studied in this project was the extraction time. The most important challenge with SPME reported in the literature was the occurrence of artifacts and competition on the porous fiber coating. Hence, the experimental program focuses on these variables. The impact of needle coating on the formation of artifacts and competition on the porous fiber was evaluated by testing standards containing a range of odorant concentrations in dry and wet nitrogen, individually and in mixtures.

2.1. Preparation of standard gases

Gas standards were generated using certified Teflon membrane permeation devices (Kin-Tek Laboratories, Inc. La Marque, TX, USA). The permeation devices that contained methyl mercaptan, dimethyl sulfide, dimethyl disulfide and trimethylamine were placed individually and in combinations in a thermostated glass chamber (Kin-Tek Laboratories, Inc.). A base flow of high purity (99.99%) nitrogen gas was maintained through each permeation chamber at 50 mL/min, and the concentration of the target compounds was varied using additional dilution gas. The temperature inside the chamber was measured with a temperature probe. The flow was measured at room conditions with an SKC-UltraFlo device and corrected afterwards for standard conditions of 0 °C and 1 atm. For humidification of the standards a Perma Pure Inc. Nafion membrane humidifier was used.

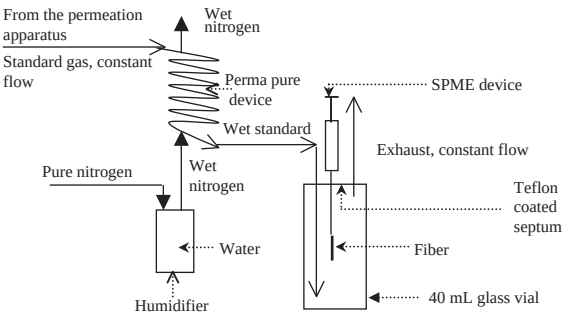


Fig. 1. Apparatus for sampling of gaseous wet standards.

The SPME fibers were exposed to the gas standard in a sampling apparatus (Fig. 1) located in a temperature-controlled room (23–24 °C). The SPME fiber was exposed for 5 min to a continuous flow of the standard that passed through a 40 mL amber glass vial. The vial had a Teflon-coated septum through which the needle of the SPME device was inserted. For each change of flow and each change in concentration, approximately 20 min was allowed to pass before sampling, to ensure a constant concentration in the vial. When the wet standards were used the dry standards and wet nitrogen were allowed to reach equilibrium for approximately 1 h. After this period of time the standard gas became saturated with water vapor.

2.2. Equilibrium time testing

The biosolids employed in this study were obtained from the City of Ottawa, Robert O. Pickard Environmental Center (ROPEC), where wastewater treatment sludge is treated in a process that includes mesophilic anaerobic digestion of primary sludge and waste activated sludge and high solids centrifuge dewatering. ROPEC has a high solids centrifuge dewatering system that uses cationic polymers to enhance the dewaterability of the biosolids. Samples were collected from a hopper that collected cake immediately after the centrifuge. The biosolids were put into 500 mL Nalgene bottles for transporting to the laboratory. During the time required for transporting the biosolids samples were kept at atmospheric temperature. Two bottles were collected each time and the biosolids were immediately transported to the laboratory. Approximately 10 g of biosolids were transferred into 40 mL amber glass serum vials. The biosolids occupied approximately one half of the vial volume. The vials were then sealed with a Teflon septum and plastic cap. Over the test period the samples

were located in a temperature-controlled room (23–24 °C).

The literature reveals a wide range of exposure imes for gas phase sampling. For the extraction of volatile sulfur compounds from air, SUPELCO recommends a 5 min exposure time at ambient temperature and a 10 min exposure time at 40 °C. For the collection of volatile sulfur compounds from air Haberhauer-Troyer et al. (1999) used a 20 min exposure time and for the collection of volatile sulfur compounds and trimethylamine from a biosolids headspace Kim et al. (2002) and Murthy et al. (2002) used an exposure time of 1 h.

In this study a Carboxen-PDMS fiber was exposed to the headspace of the biosolids sample for 5, 10, 15, 20 and 25 min. The exposure time ranging from 5 to 25 min was chosen because of two reasons. First, it was considered that for longer exposure time the source of volatile sulfur compounds and trimethylamine would change and second SPME-GC was found to be time consuming. The fiber was subsequently desorbed into the gas chromatograph at 250 °C, for analysis. This experiment indicated that the fiber is very sensitive to the test conditions. Any contact between the fiber and a solid (sample or jar walls) resulted in failure of the fiber.

2.3. Generation of artifacts

The formation of artifacts such as dimethyl disulfide (DMDS) from methyl mercaptan (MM) is often catalyzed by metallic surfaces. In the SPME procedure the only metal that the gases might contact is the stainless steel needle that support SPME fiber. Hence, it was hypothesized that coating the SPME needle with an inert coating material might reduce the formation of artifacts.

The impact of the SPME needle coating on the formation of artifacts was evaluated by testing a standard containing a range of concentrations of methyl mercaptan in dry and wet nitrogen. The SPME fiber was exposed for 5 min to a continuous flow of the standard that passed through a 40 mL vial. In each case the fiber was subsequently desorbed into the gas chromatograph at 250 °C, for analysis.

The impact of needle coating on production of the artifacts was evaluated by testing three sets of fiber assemblies obtained directly from SUPELCO (Sigma-Aldrich, St. Louis, MO, USA). All three fibres were coated with Carboxen-PDMS that were from the same batch. The differences among the assemblies were in the way in which the needle was treated:

1. Assembly 1: Carboxen-PDMS, standard—untreated needle.
2. Assembly 2: Carboxen-PDMS, SilcosteelR—treated needle.
3. Assembly 3: Carboxen-PDMS, SulfinertTM—treated needle.

2.4. Competitive adsorption

Different combinations of standards were used to study the competition for sorption sites on the fiber. Initially, combinations of dry standards of volatile sulfur compounds including MM and dimethyl sulfide (DMS), MM and DMDS, DMS and DMDS and a mixture of DMS, DMDS and MM were examined. Upon completion of these tests a mixture of MM and trimethylamine (TMA) was tested. Finally, dry and wet mixtures of all standards available were tested. At least duplicate samples were analyzed and the coefficient of variance was calculated for duplicates.

To evaluate competition onto the adsorption sites the SPME fiber was exposed for 5 min to a continuous flow of the standards that passed through a 40 mL vial and was subsequently desorbed into a gas chromatograph for analysis.

2.5. SPME procedure and gas chromatography

The 75 μm Carboxen-Polydimethylsiloxane was exposed for 5 min to the target compounds. Immediately after extraction the needle was manually introduced into the splitless injector of the gas chromatograph. By exposing the fiber to the carrier gas stream, the analytes were thermally desorbed and transferred onto the GC column. The needle with the exposed fiber was left in the injector for 5 min at 250 °C. GC-MS analyses were carried out with an Agilent GC (6890N) that was equipped with an HP 1–50 m × 0.32 mm ID–0.52 μm film column and a mass spectrometer as detector (5973N). The carrier gas was helium at a volume rate of 1.0 mL/min. To separate the analytes, the oven temperature was initially held at 35 °C for 15 min and then ramped at 8 °C/min to 70 °C where it was held for 15 min and then ramped at 15 °C/min to 130 °C where it was held 2 min. The scans were performed in SIM mode, as shown in Table 1. A characteristic gas chromatogram is presented in Fig. 2.

Table 1
Optimized SIM for target compounds from compounds

Group	Start time (min)	Target qualifier ions	Compounds
1	4.20	47, 45, 48	Methyl mercaptan
2	5.00	58, 59, 42	Trimethylamine
3	5.95	62, 47, 45	Dimethyl sulfide
4	19.59	94, 79, 45	Dimethyl disulfide

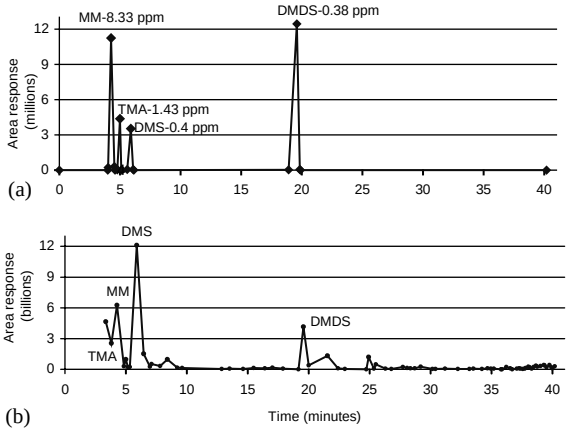


Fig. 2. Typical gas chromatographs result: (a) characteristic chromatogram for a mixed standard and (b) characteristic chromatogram for a biosolids sample.

3. Results and discussion

3.1. Equilibrium time measurements

In order to determine the equilibrium time that would be employed in this study, a Carboxen-PDMS fiber was exposed to the headspace of a biosolids sample for 5, 10, 15, 20 and 25 min. Fig. 3 presents the area counts for a variety of compounds as a function of sampling time. From Fig. 3, one can see that the sorption process was fast at the beginning and after that it was relatively slow. The difference between 25 and 5 min was between 8.42% (MM) and 231% (TMA).

The responses for most of the compounds were less after 10 min than after 5 min and after 10 min they increased. This behavior was consistently observed in duplicate samples. The above behavior was observed in the summer of 2002. In the winter of 2002–2003 a new experiment was performed to determine if this behavior was repeatable. For this experiment the area responses for all compounds of interest increased from 5 to 25 min exposure time. To verify this latter result, an additional run with an exposure time of 5 min was performed at the end of the experiment. The area responses for this last run for methyl mercaptan and dimethyl disulfide were higher than for the 15- and 10-min exposure times, respectively.

The observed behavior may have been due to either dynamic competitive adsorption among the wide range of compounds in the biosolids headspace or time-varying concentrations due to biological activity in the biosolids. In this study the exact cause of the behavior was not identified. The responses for the 25-min exposure times were consistently higher for all the compounds than the responses for 5 min, but in the same range of magnitude. Given the similarity in responses

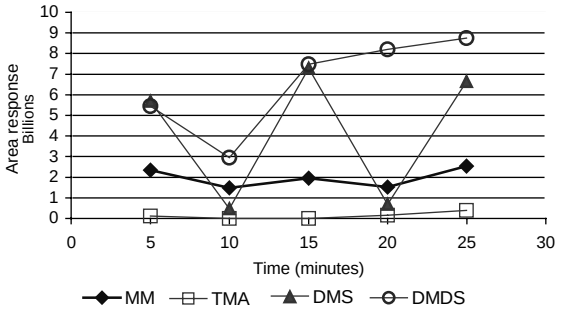


Fig. 3. Responses in headspace vs. exposure time.

and the need to efficiently analyze a number of samples within a limited time span, the 5 min sampling interval was selected for the remaining experiments.

3.2. Generation of artifacts

From the literature it was found that the most important limitations in SPME use were the potential generation of artifacts and competition on the porous fiber coating. In these experiments the impact of the SPME needle on the production of artifacts, the effect of different needle treatments on the production of artifacts and the competition between odorous chemical compounds and water vapors were examined.

DMDS was examined as a potential by-product of methyl mercaptan oxidation. When MM was analyzed alone some oxidation of methyl mercaptan to dimethyl disulfide was observed. It is believed that the oxidation happens in the presence of metallic surfaces and/or water vapors (Chin and Lindsay, 1994; Lomans et al., 2002).

The potential generation of DMDS by oxidation of MM was evaluated by generating a series of MM standards in wet and dry gases and by measuring the actual MM and DMDS concentrations. The concentration of MM and DMDS that were measured in the dry and wet gas are presented in Table 2. In this table the expected concentration represents the concentration calculated with the diffusion coefficients that were provided for each permeation tube. The concentrations from the calibration curves represent the concentrations measured using the calibration curves that were developed for each standard gas separately.

From Table 2 it can be seen that a relatively small fraction of the MM was converted to DMDS. Comparing the values measured for DMDS for dry and wet gas it was found that the presence of water vapor did not influence the generation of DMDS. The quantities of DMDS were the same as for dry MM. However, the area responses for the wet methyl mercaptan standard gas were half the area responses for dry methyl mercaptan. Because the production of artifacts seemed

Table 2
Production of dimethyl disulfide as an artifact of dry and wet methyl mercaptan

Methyl mercaptan				Dimethyl disulfide	
Expected concentration (ppm)		Concentration from calibration curves (ppm)		Concentration from calibration curves (ppm)	
Dry	Wet	Dry	Wet	Dry	Wet
2.27	0.879	2.18	0.889	0.01	0.01
2.66	1.58	2.78	1.59	0.01	0.01
3.91	4.99	4.25	5	0.03	0.03
7.98	—	8.07	—	0.05	—
16.64	17.3	16.50	17.31	0.08	0.08

Table 3
Measured area responses for MM and concentration values for DMDS with three different assemblies

	Expected MM concentration (ppm)	Area response for MM ($\times 10^6$)			DMDS concentration (ppm)		
		F1	F2	F3	F1	F2	F3
Dry standard gas	2.83	39.7	42.0	51.9	0.04	0.04	0.05
	4.03	54.4	56.3	59.5	0.04	0.05	0.05
	8.02	114.7	133.3	118.9	0.06	0.08	0.08
Wet standard gas	1.17	1.23	2.58	1.21	0.01	0.01	0.01
	2.21	1.11	2.46	1.36	0.01	0.01	0.02
	7.36	1.75	2.68	1.57	0.04	0.04	0.05

Where F1-Assembly 1: Carboxen-PDMS, standard—untreated needle, F2-Assembly 2: Carboxen-PDMS, Silcosteel^R—treated needle, F3-Assembly 3: Carboxen-PDMS, Sulfinert™—treated needle.

to be the same for both wet and dry standards, this decrease in area response would appear to be due to competition between methyl mercaptan and water vapor for sites on the porous fiber coating.

The standard gas did not come into direct contact with any metal surfaces (fittings, inlet of gas chromatograph) and, hence, the only metal surface that came in direct contact with MM was the needle of the fiber. The next part of the research was conducted to study the effect of the presence of the metal associated with the SPME fiber assembly on the production of artifacts. This was accomplished by testing needles that had been coated with inert substances.

The area responses obtained for MM and the concentrations of DMDS that were obtained with the different fiber assemblies are presented in Table 3. In Table 3 the expected concentrations represent the values that were calculated with the diffusion coefficients that were provided for each permeation tube. The area responses were those generated by the GC-MS. The DMDS concentrations were calculated from the calibration curve that was developed for DMDS alone. From Table 3 it can be seen that the DMDS concentrations measured with the Silcosteel and Sulfinert treated assemblies were either equal to or in some cases slightly higher than for the standard assembly. The same trend (an increase in area response for these two fibers) was also observed for MM. It would appear that the Silcosteel and Sulfinert treated needles slightly enhanced the sensitivity of the assemblies in concentrating sulfur compounds. The sensitivity seemed to be higher for the Silcosteel treated needle.

Comparing the area responses for the MM dry standard gas measured with the standard fiber employed in the above experiment with the area responses measured with a Carboxen-PDMS fiber that was obtained from a different batch, it was found that the values measured with the former fiber were higher than the values measured with the latter fiber by approximately (21–28)%. To check these values, and implicitly the calibration curve, new injections were performed. The additional tests were performed with the original assembly (F4) that was used for the development of the individual calibration curve. The data from these tests are not shown.

For fiber F4 the differences between the initial and repeat calibration curves were less than 10%, demonstrating that the calibration curves were correctly developed and the GC-MS was functioning consistently. Fiber F1 differed from fiber F4 by 20–30%. Similar

Table 4
Mixture of dimethyl sulfide with dimethyl disulfide and methyl mercaptan

Methyl mercaptan		Dimethyl sulfide		Dimethyl disulfide	
Conc. expected (ppm)	Conc. measured (ppm)	Conc. expected (ppm)	Conc. measured (ppm)	Conc. expected (ppm)	Conc. measured (ppm)
3.17	3.16	0.15	0.13	0.133	0.16
3.3	3.28	0.186	0.14	0.164	0.2
5.69	4.55	0.271	0.18	0.239	0.26
8.52	6.84	0.410	0.27	0.358	0.39

differences were observed in the responses of the two different fibers when wet standards were tested. Because of these differences it was determined that new calibration curves have to be made when a new fiber is used. This increases the amount of time required for analyses using SPME for this application.

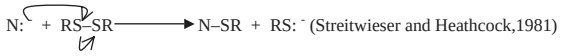
3.3. Competitive adsorption

After the calibration curves for individual dry and wet MM, DMS, DMDS and TMA standards were completed, the analysis of gas mixtures was assessed. To study the competition for sorption sites on the fibers, different combinations of standards were used. Initially, combinations of dry standards of volatile sulfur compounds including MM and DMS, MM and DMDS, DMS and DMDS and a mixture of DMS, DMDS and MM were examined. Upon completion of these tests a mixture of MM and TMA was tested. The expected concentrations were calculated using diffusion coefficients provided for each permeation tube. The measured concentrations represent the concentrations that were determined from the individual calibration curves.

The results indicated that binary competition among the volatile sulfur compounds was relatively minor. When MM was mixed with either DMS or DMDS, which are heavier molecules, its measured concentration was slightly lower than expected, on the basis of the single species calibration curve. The MM concentration was 11% less in the presence of DMS and 20% less than expected in the presence of DMDS. Approximately 1.5% of the MM was oxidized to DMDS. When DMS competed with DMDS, the response of DMS decreased by 21%, while competition was insignificant for DMDS.

Table 4 presents the measured and expected concentration values for mixture that contained all the volatile sulfur compounds used in this study are presented. From Table 4, it can be seen that tertiary competition among volatile sulfur compounds was relatively small. In the competition for sites on the fiber DMDS seemed to slightly displace the other volatile sulfur compounds (MM and DMS). The values measured for MM and DMS were somewhat lower than the expected values from the individual calibration curves. These differences increased from 0.3% to 20% for MM and from 13% to 34% for DMS as the concentration increased. DMDS seemed to be not affected by competition on the fiber, with the measured values slightly higher than the calculated ones. Production of DMDS by the oxidation of MM might explain the slightly higher values for the measured DMDS.

The final dry gas combination that was tested involved MM in a mixture with TMA. TMA has a basic and nucleophilic nature (Baboul et al., 1998) and the sulfur–sulfur bond of disulfides is susceptible to cleavage by nucleophilies as follows (Streitwieser and Heathcock, 1981):



(Streitwieser and Heathcock,1981)

It was expected that DMDS produced by the oxidation of MM would react with TMA producing a slight decrease in MM and TMA concentrations. The data obtained for this test are presented in Table 5. In Table 5 the expected concentrations were calculated from the diffusion coefficients provided for each permeation tube. The measured concentrations were determined from the individual calibration curves.

From Table 5 it can be seen that the presence of MM had a substantial impact on the measured concentration of TMA. In addition, the measured MM was approximately 30% less than the expected values over the range of concentrations examined. The decrease of TMA was very high with values that were 64–87% less than the expected values. Another interesting observation was that DMDS was generated at a constant and low concentration (lower that the value measured for MM without TMA) for the entire range of the MM concentrations. A small reduction of trimethylamine could be explained through the reaction mentioned above. However, the observed reductions of both TMA and MM were larger than would be predicted by the

Table 5
Mixture of methyl mercaptan and trimethylamine

MM (ppm)			TMA (ppm)			DMDS (ppm)	
Expected	Measured		Expected	Measured		Measured	
	Mean	CV (%)		Mean	CV (%)	Mean	CV (%)
3.32	2.53	5.34	0.46	0.06	9.09	0.02	11.2
4.95	6.64	2.2	0.69	0.16	3.2	0.02	0
8.48	6.42	4.4	1.18	0.42	3.6	0.02	0

above-mentioned reaction. It seems reasonable to assume that TMA reacted with MM. The product of the reaction could not be measured by the analytical method used. The constant value for dimethyl disulfide, the identification of the reaction products and the mechanism of reduction would require further investigations.

Upon completion of the preliminary competition tests, mixtures of all four compounds in dry and wet gases were prepared. Not all the compounds were present at equal concentrations in the mixture. Based on the literature review MM was found to have the higher concentrations in the biosolids headspace followed by DMS and DMDS. The ratio between MM and DMS/DMDS concentrations was chosen to be around 20/1. TMA is not usually reported as being present in the headspace of mesophilic digested cake and its concentration was based upon the availability of permeation tubes. The minimum and maximum values of the concentration of target compounds were dictated by the performances of the permeation tubes.

Figs. 3–6 present the calibration curves for the individual compounds alone and in the dry and wet mixtures. Fig. 4 includes an additional calibration curve for wet MM alone.

From Fig. 4 it can be seen that the response for DMS in the wet mixture was approximately half the response in the dry mixture and this was half the response of the individual standard gas. When DMS was tested either in a mixture of MM and DMS or a mixture of MM, DMS and DMDS substantial competition was not observed. The reduced concentration of DMS in the four-compound-mixture would therefore appear to be due to the presence of TMA. The addition of water vapor to the mixture appeared to provide additional competition for DMS on the fiber.

In Fig. 5 it can be seen that the response of MM in the dry mixture was slightly lower than for the individual standard alone. When water vapors were added to the standard gas either for the mixture or individual compound the responses were reduced by a factor of 2. The differences between the area responses for MM in

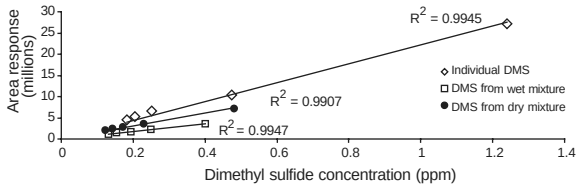


Fig. 4. Calibration curves for dimethyl sulfide gas standard.

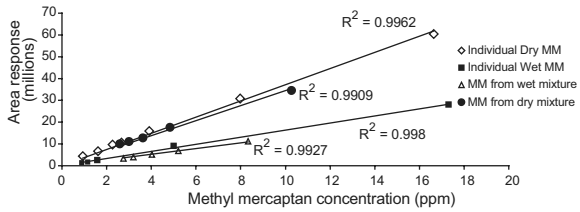


Fig. 5. Calibration curves for methyl mercaptan gas standard.

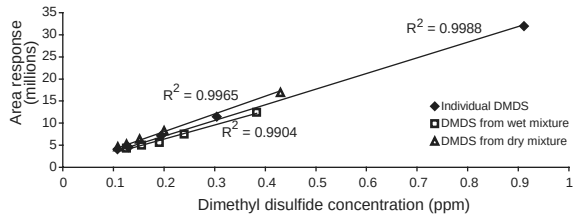


Fig. 6. Calibration curves for dimethyl disulfide gas standard.

the wet mixture and the wet MM alone were negligible. The reduced values for MM seemed to be primarily due to competition with water vapor. The competition with the other volatile compounds did not influence MM values.

The results obtained for MM in the four-compound-dry mixture were somewhat inconsistent with those that were observed in the two-way mixture of MM with TMA. In the binary mixture the values of MM were reduced by approximately 30% in the presence of TMA

over the entire range of concentrations, while in the four-compound-mixture MM was reduced from 1% to 20% over the concentration range. The results would suggest that TMA might have preferentially reacted with one or more of DMS and DMDS in the four-compound-mixture. This conclusion seems to be in concordance with the results from the DMS response presented above.

From Fig. 6 it can be seen that DMDS was the only compound that was not affected by competition. Furthermore, DMDS in the dry mixture had values that were higher than that for individual DMDS. This behavior likely resulted from oxidation of MM to DMDS.

From Fig. 7 it can be observed that the responses for TMA in wet and dry mixtures were comparable. These results indicate that water vapor is not a significant competitor for adsorption sites with TMA. There were however large differences between the responses for the mixtures and the TMA alone. The reduced TMA response in the presence of the other compounds was consistent with that observed in the binary mixture of TMA and MM. This response may have been due to either competition with the other compounds or as a result of chemical reactions with the other compounds.

From Figs. 4–7 it can be seen that SPME provides linear responses over a wide concentration of analytes. The results from this study suggest that for systems containing mixtures of reduced sulfur and nitrogenous compounds it will be difficult to obtain an exact quantitative measurement of these compounds with SPME. The presence of water vapor appeared to have the greatest impact on the analytical results, while competitive adsorption and chemical reactions also had an impact. For the purposes of characterizing the pattern of odor generation from dewatered biosolids in a confined headspace, it is reasonable to assume that the headspace will be saturated with water vapor. Hence, use of calibration standards in water-saturated air should provide reasonably quantitative estimations of contaminant concentrations in biosolids headspace. In a real matrix with more compounds than the ones used in this experiment and with heavier compounds at higher concentrations the impact of competition may increase.

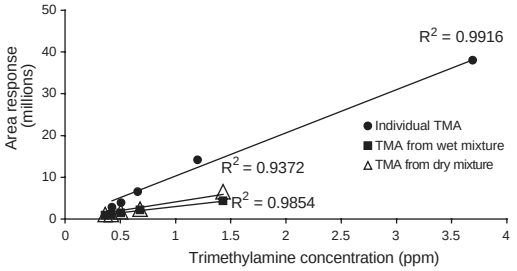


Fig. 7. Calibration curves for trimethylamine gas standard.

4. Conclusions

There were differences between fibers and hence it is necessary to make a calibration curve for each new fiber. For this reason their use is somewhat time consuming. Treatment of the needle in the SPME assembly with inactivating coatings caused slight differences in responses. It would appear that the Silcosteel and Sulfinert treated needles enhanced the sensitivity of the assemblies in concentrating sulfur compounds. The sensitivity seemed to be slightly higher for the Silcosteel treated needle.

The production of artifacts (DMDS) when analyzing gas-phase mercaptans by SPME was found to be insignificant, and seemed to not be influenced by water vapor. It was found that the responses of MM, DMS, DMDS and TMA were significantly affected by competition with each other and with water vapor. In the latter case their responses in wet gases decreased by more than a half of the dry gas values. Because of the significant disappearance of TMA (either from dry or wet mixture) it was assumed that other compounds were formed as a result of the reaction between volatile sulfur compounds and TMA.

From the above-mentioned reasons the application of SPME for analyzing biosolids odor is only somewhat quantitative. SPME can however be used with success for quick screening of odor compounds.

Acknowledgments

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References

Baboul, A.G., Schlegel, H.B., Glukhovtsev, M.H., Bach, R.D., 1998. Computational study on nature of transition structure for oxygen transfer from dioxirane and carbonyloxide. J. Comput. Chem. 19, 1353–1369.

Chin, H.W., Lindsay, R.C., 1994. Ascorbate and transition-metal mediation of methanethiol oxidation to dimethyl disulfide and dimethyl trisulfide. J. Food Chem. 49, 387–392.

Haberhauer-Troyer, C., Rosenberg, E., Grasserbauer, M., 1999. Evaluation of solid-phase microextraction for sampling of volatile organic sulfur compounds in air for subsequent gas chromatographic analysis with atomic emission detection. J. Chromatogr. A 848, 305–315.

Kim, H., Nochetto, C., McConnell, L.L., 2002. Gas-phase analysis of trimethylamine, propionic and butyric acids, and sulfur compounds using solid-phase microextraction. Anal. Chem. 74, 1054–1060.

Lomans, B.P., Pol, A., Op Den Camp, H.J.M., 2002. Microbial cycling of volatile organic sulfur compounds in anoxic environments. Water Sci. Technol 45 (10), 55–60.

Murthy, S., Kim, H., McConnell, L.L., Peot, C., Ramirez, M., Strawn, M., 2001. Characterization of wastewater and solids odors using solid phase microextraction at a large wastewater treatment plant. IWA Speciality Conference, Acapulco, Mexico.

Murthy, S.N., Sadick, T., Kim, H., McConnell, L., Peot, C., Bailey, W., Novak, J., Glindemann, D., 2002. Mechanisms for odour generation during lime stabilization. IWA Biennial Conference, Melbourne, Australia.

Pelusio, F., Nilsson, T., Montanarella, L., Tilio, R., Larsen, B., Facchetti, S., Madsen, J., 1995. Headspace solid-phase microextraction analysis of volatile organic sulfur compounds in black and white truffle aroma. J. Agric. Food Chem. 43, 2138–2143.

Payne, R., 2000. SPME resolves difficult matrix problems. The Reporter, vol. 18.4.

Streitwieser, A., Heathcock, C.H., 1981. Introduction to Organic Chemistry. Macmillan Publishing Co Inc., New york.

SUPELCO, 1998. Solid phase microextraction: theory and optimization of conditions. Bulletin 923, New York.

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Calibration procedure for solid phase microextraction—gas chromatographic analysis of organic vapours in air

D. Gorlo, L. Wolska, B. Zygmunt, J. Namieśnik *

Department of Analytical Chemistry, Chemical Faculty, Technical University of Gdańsk, 80-952 Gdańsk, Poland

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Abstract

A calibration procedure for solid phase microextraction—gas chromatographic (SPME-GC) analysis of organic vapours in air was described in which GC detector (MS in this case) signal is directly related to concentration of analytes of interest sampled by SPME. Gaseous standard mixtures used for the calibration were generated by means of a home-made permeation-type apparatus described elsewhere, W. Janicki et al., Chem. Anal., 38 (1993) 423 and modified to permit easy sampling of analytes on an SPME fibre. To establish sampling parameters, times for equilibrium partitioning of five selected organic compounds (carbon tetrachloride, toluene, chlorobenzene, *p*-xylene, *n*-decane) between gaseous mixtures and the fibre (fused silica fibre coated with 100 μm polydimethylsiloxane) were determined. For 10 min sampling time, the detector response and hence amount sampled on the fibre were linear functions of analytes concentration in a gaseous sample. © 1997 Elsevier Science B.V.

Keywords: Calibration; Gas chromatography; Microextraction; Organic vapours

1. Introduction

The dominant trend in present day environmental analytics can be related to the necessity to determine an increasingly wide range of pollutants on increasingly lower concentration levels in very complex matrices [2]. Hence, isolation and preconcentration of analytes of interest are usually necessary steps of the analytical procedure. Recently, a rapidly developing technique for this purpose is solid phase microextration (SPME) [3–10]. In the technique, sampling, isolation and enrichment are incorporated into a single step and use of solvents, which can be possible environmental pollutants, is eliminated. The technique can be applied to gases and relatively pure liquids by dipping an SPME fibre directly into an analysed medium; and to solid matrices and wastewater samples with grease, oil, and high molecular mass humic acids by analyte sampling from the headspace being at equilibrium with an original sample. The possible ways of sampling from different matrices are shown in Table 1.

In the case of SPME combined with gas chromatography, the fibre (with analytes extracted from the sample into stationary phase coated on it) is introduced into an injection port of a gas chromatograph. The analytes are thermally des-

* Corresponding author.

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Table 1
Sampling in the case of different matrices

Sample type	Sampling is done
Gas	By fibre exposition directly to an analyzed sample
Liquid	By fibre exposition directly to a liquid sample
	From a gaseous phase being at equilibrium with an original sample
Solid	From a gaseous phase at equilibrium with an analyzed solid sample
	From a liquid extract

orbed from the fibre and transferred by a carrier gas into a GC column for separation and then to a detector for quantitation.

However, SPME is an equilibrium method and analytical methodologies based on it require calibration. It seems that the most reliable calibration approach would be to subject standard gaseous mixtures of precisely and accurately known analyte concentration to the complete analytical procedure (starting from sampling to final analysis) identical to the procedure for a real sample.

Standard gascous mixtures used for calibration as well as for studies on isolation and enrichment of trace analytes from gases in the above approach should satisfy the following requirements [11–13]:

- concentration of an analyte of interest should be constant for a required period of time;
- concentration should be known with an accuracy much better than the accuracy of a calibrated instrument or method;
- mixtures should be available in quantities sufficient to perform planned studies;
- analyte concentration can preferably be calculated from such basic quantities as mass, temperature, pressure, etc.

For environmental analytics in general and for these studies in particular (mixtures of very low analyte concentration: ppm, ppb, ppt levels required), dynamic methods based on permeation of vapours of volatile organic compounds through membranes (mainly PTFE, polyethylene and silicone rubber) into a stream of diluting gas seem the most adequate. In such methods very low analyte concentrations can be obtained. These concentrations can be varied within a wide range by changing such parameters as membrane thickness and area, flow rate of diluting gas and temperature of a permeation vessel. The dynamic permeation methods has been extensively studied and used, among them the apparatus designed in our laboratory [1,14,15].

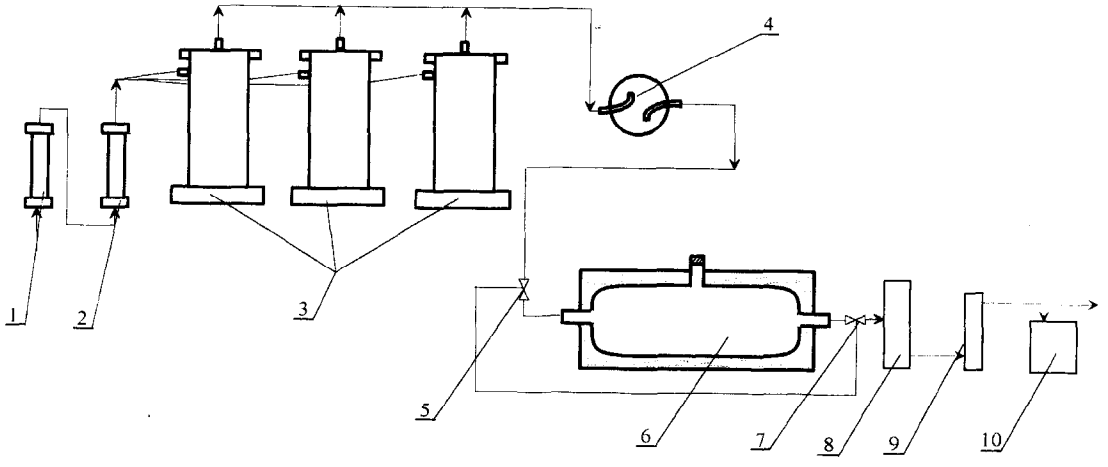

Fig. 1. The apparatus for generation of gaseous standard mixtures; 1- purifier, 2-drier, 3-generators, 4-preliminary mixing chamber, 5-teflon 3-way valve, 6-thermostated mixing glass chamber, 7-glass 3-way valve, 8-flowmeter, 9-rotameter, and 10-suction pump.

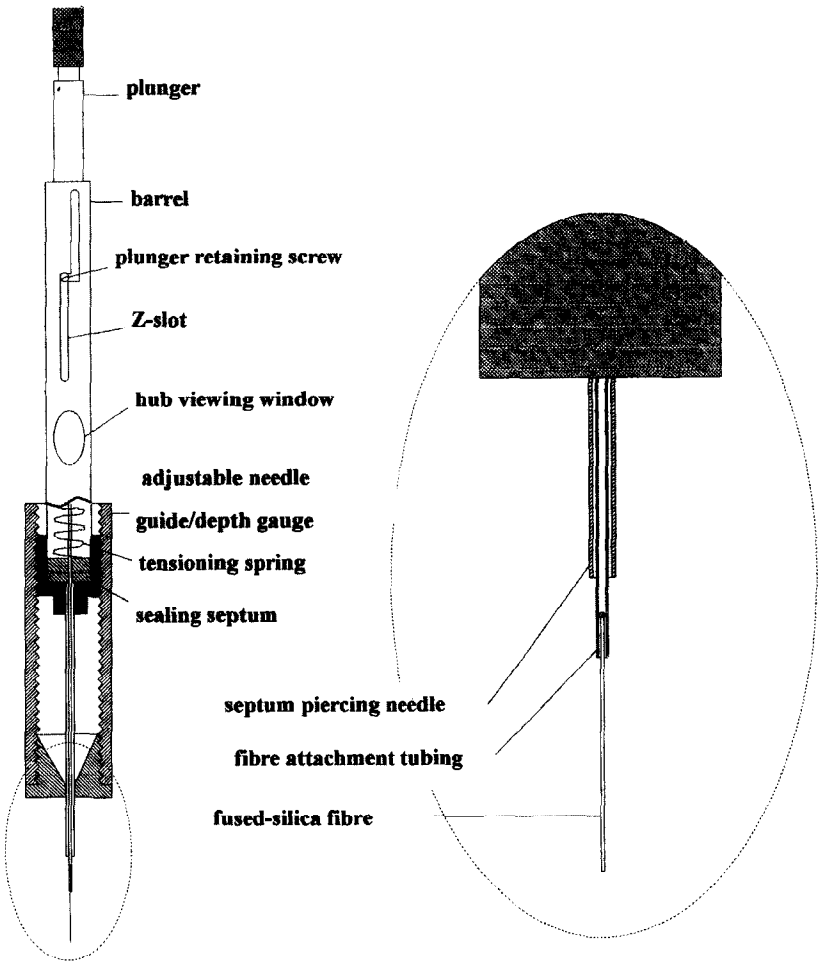

Fig. 2. SPME apparatus for manual sorption and injection [20].

The aim of this work was to develop a reliable method of SPME-GC-MS system calibration (output signal as a function of analyte concentration in gaseous sample) which can be used in analysis of different air samples (atmospheric, indoor and workplace air) for organic vapours. For the purpose we used the apparatus for permeative generation of standard gaseous mixtures described elsewhere [1] and modified to enable reproducible sampling of organic vapours present in the gaseous mixtures on an SPME fibre.

2. Experimental

2.1. Reagents and materials

n-Decane (pure) were from Merck, Germany, and carbon tetrachloride, chlorobenzene, toluene and *p*-xylene (all reagent grade) were from Polskie Odczynniki Chemiczne, Gliwice, Poland. PTFE foils of 50, 120 and 220 μm thickness (from Du Pont, USA) were used for the preparation of permeation membranes.

Table 2
Concentrations of organic compounds of interest in gaseous standard mixtures (purified air as a diluting gas)

Compound analyzed	Analyte concentration (mg m^{-3}) for different flow rates of diluting gas			
	25 cm^3 min^{-1}	50 cm^3 min^{-1}	100 cm^3 min^{-1}	150 cm^3 min^{-1}
Carbon tetrachloride	23.45	11.73	5.86	3.91
Toluene	2.33	1.17	0.58	0.39
Chlorobenzene	16.84	8.42	4.21	2.81
p-Xylene	1.22	0.61	0.3	0.2
n-Decane	0.75	0.38	0.19	0.13

2.2. Apparatus

The apparatus for the dynamic generation of gaseous standard mixtures with stop flow facility for sampling is given in Fig. 1. It consists of three generators (3) [1]. In each generator two or three permeation vessels are placed [1,16–19]. They are filled with liquid analytes of interest (carbon tetrachloride, toluene, chlorobenzene, *p*-xylene, *n*-decane). Diluting gas (atmospheric air purified from organic compounds) (1) is sucked by a suction pump (10) through purifier (1), drier (2) and generators (3) containing permeation vessels kept at a temperature of 28 ± 0.1°C. The stream of the gaseous mixture formed was then passed through a preliminary mixing chamber (4), Teflon 3-way valve (5), thermostated mixing glass chamber (6), glass 3-way valve (7), flowmeter (8) used for an accurate measurement of gaseous mixture flow rate and rotameter (9) with which flow rate is controlled. Mixing chamber (6) is equipped with a septum through which a needle of a SPME device can be inserted. During exposition the mixture flow through chamber (6) is stopped (the gas mixture by-passes the chamber).

GC-MS analysis was carried out with a Fisons GC-8000 gas chromatograph coupled with a Fisons MD-800 quadrupole mass spectrometer.

For SPME extraction an SPME holder for manual injection (SUPELCO, Bellefonte, PA, USA) with a 100 μm polydimethylsiloxane-coated fused-silica fibre (SUPELCO, Bellefonte, PA, USA) was used (Fig. 2) [20].

2.3. Calibration of permeation vessels

Permeation rates were determined by measuring vessel mass decrease (weighing) in a given time (1–30 days). When permeation rates became constant, permeation vessels were ready to use, and the concentration of a given analyte in a gaseous mixture was calculated from the permeation rate and diluting gas flow rate [1,19]. The concentrations of analytes in standard mixtures used in the studies are given in Table 2.

2.4. Analyte extraction (a) and injection into a gas chromatographic column (b)

(a) The membrane of the mixing chamber (Fig. 1) is pierced with an SPME syringe needle, the fibre is pushed out from the needle and exposed to a standard mixture for a given time and then withdrawn into the needle, whose tip is immediately closed by piercing it into a silicone septum to prevent the analytes from desorption.

Table 3
Parameters of fibre exposition

Exposition parameters	
Stationary phase	Polydimethylsiloxane
Thickness of stationary phase film	100 μm
Exposition temperature	25 ± 0.2°C
Mixture relative humidity	Dry air
Exposition time	0.5, 1, 2, 3, 10, 20 min
Flow rate of diluting gas (purified and dried air)	25, 50, 100, 150 cm^3 min^{-1}
Analyte concentration	as given in Table 2

Table 4
GC-MS parameters

Injection port	Splitless, (liner 1.2 mm I.D.)
Injector temperature	250°C
Desorption time	60 s
Analytical column	DB 5 MS 30 m × 0.32 mm I.D. 0.25 μm d_f (RESTEC, Bellefonte, USA)
Temperature program	30°C–2 min–5°C min^{-1} 50°C–15°C min^{-1} 230°C–10 min
Type of MS	SCAN
Ions for quantitation	Carbon tetrachloride 117, toluene 91, chlorobenzene 112, *p*-xylene 91, *n*-decane TIC
Ionisation	electron impact (70 eV)

(b) The device needle is quickly introduced into a splitless injector of a gas chromatograph, the fibre extended from the syringe for 20 s. At this time the analytes are desorbed and transferred into a GC column by carrier gas (parameters given in Table 3 and Table 4).

2.5. GC and MS parameters

The parameters of GC-MS are given in Table 4.

3. Results and discussion

Constancy of analyte concentration (after preconditioning) in gaseous standard mixtures, which is a basic requirement of the calibration method proposed, has been confirmed earlier [1]. Application of a mixing chamber of a special design makes SPME fibre exposition to a standard mixture simple and easy. Development of a calibration method requires the determination of minimum time necessary for equilibrium partitioning of analytes between a gaseous mixture and a fibre. For this purpose, the fibre was exposed to standard mixtures for different periods (0.5; 1.0; 2.0; 3.0; 5.0; 10 and 20 min). The fibre was exposed in the gaseous mixture at a temperature of 25 ± 0.2°C. Sorbed analytes were injected into a GC column and peak areas measured. It was found earlier that the injection parameters used (Table 4) ensure complete desorption of analytes from the fibre. In Fig. 3 plots of peak areas versus exposition time for different analytes concentrations are given. Each point is an average of five parallel measurements. For carbon tetrachloride, toluene, chlorobenzene, *p*-xylene and *n*-decane equilibration time ranges from 1.0 to 5.0 min (Table 5). The equilibration is relatively fast, and this step would extend the total time of an analytical procedure only slightly. A 10 min extraction time was selected for further experiments. For sampling at lower temperatures, e.g., outdoor air sampling on cold days, slightly longer extraction can be needed.

In the successive step, the relationship between SPME-GC-MS response and analyte concentration in the gaseous mixture (calibration curves) was studied. For each concentration five independent measurements were made.

The relatively high correlation coefficients (r) for linear regression ranging from 0.988 to 0.999 (Table 6) show that, in the examined concentration range, calibration curves can be assumed linear with high reliability. An example of a calibration curve is given in Fig. 4. Linearity of calibration curves, i.e., independence of partition coefficients on concentration, simplifies the analytical procedure. This was found for a fibre exposition temperature of 25°C, but should also be valid for other temperatures normally used during air sampling for organics.

However, partition coefficients (Kg) between the fibre coating and the gaseous phase are temperature dependent, which was quantitatively described for a number of organic compounds by Arthur et al. [8]. Therefore, the temperature of fibre extraction from the standard mixture (for calibration purposes) should be every time adjusted to a temperature of real sample extraction. Such an approach seems simpler and more accurate than the correction based on previously determined temperature variability of Kg. With the apparatus proposed this is easily done simply by changing the temperature of mixing chamber (6).

Humidity can affect sorption of organic compounds on the fibre but to small extent. As shown by Chai and Pawliszyn [10], the relative humidity reduces the amounts extracted at room temperature by less than 10% at up to 75%. The small

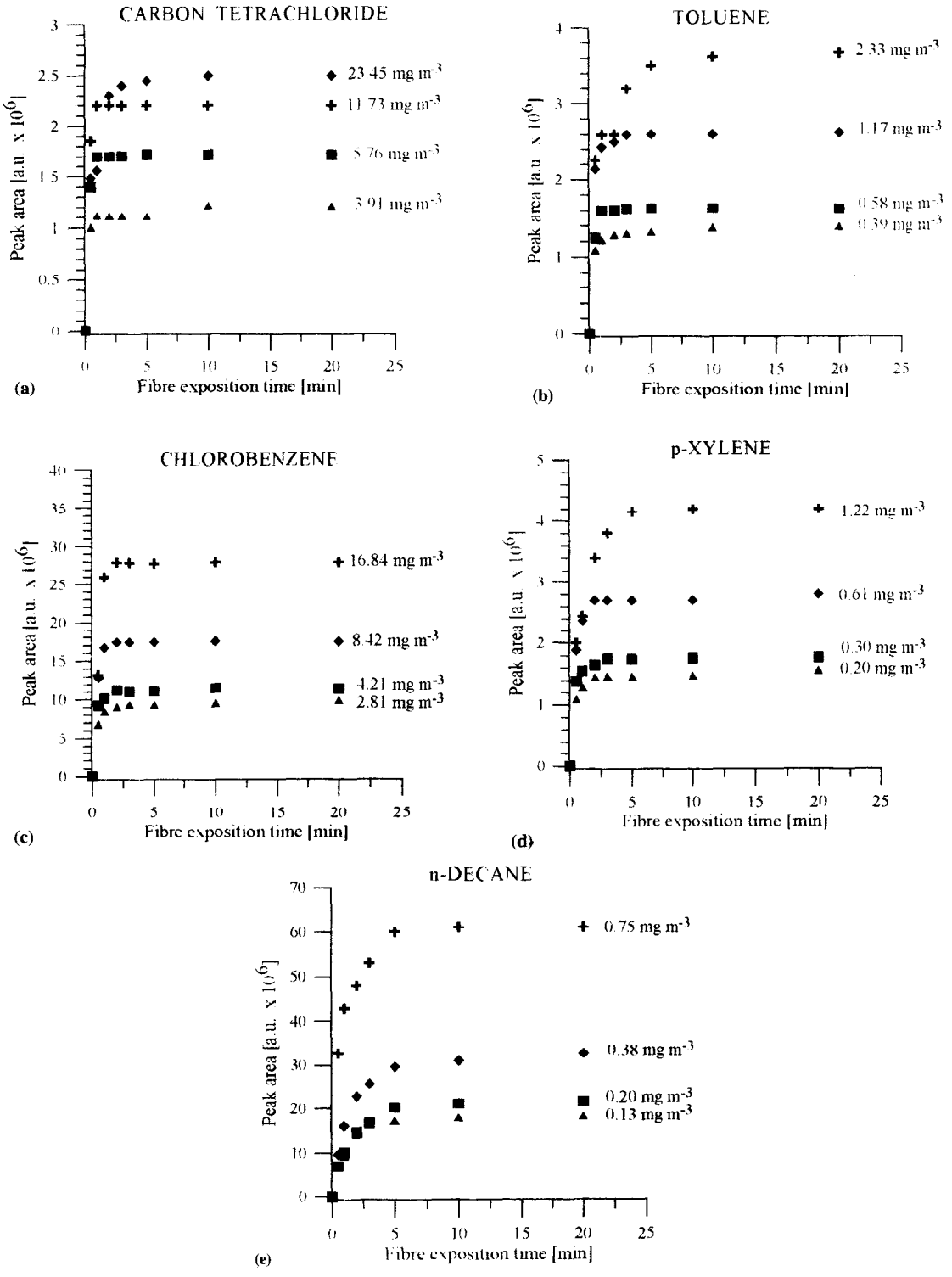

Fig. 3. Plots of GC-MS response (peak area in arbitrary units-a.u.) versus SPME fibre exposition time for different analyte concentrations in gaseous standard mixtures: (a) carbon tetrachloride; (b) toluene; (c) chlorobenzene; (d) *p*-xylene; and (e) *n*-decane.

Table 5
SPME equilibrium times for different organic compounds in gaseous mixture

Compound	Equilibration time [min] (concentration [mg m^{-3}])			
Carbon tetrachloride	1.0 (3.91)	1.0 (5.86)	1.0 (11.73)	2.5 (23.45)
Toluene	1.0 (0.39)	1.0 (0.58)	1.5 (1.17)	5.0 (2.33)
Chlorobenzene	1.5 (2.81)	1.5 (4.21)	1.5 (8.42)	1.5 (16.84)
p-Xylene	2.0 (0.20)	2.0 (0.30)	2.0 (0.61)	4.5 (1.22)
n-Decane	2.5 (0.13)	3.0 (0.20)	4.5 (0.38)	4.5 (0.75)

Table 6
Characteristies of calibration curves

Compound	*a* [a.u.]	S_a [a.u.]	*b* [a.u. mg^{-1} m^3]	S_b [a.u. mg^{-1} m^3]	Correlation coefficient *r*	Concentration range [mg m^{-3}]
Carbon tetrachloride	73×10^4	7.3×10^4	7.7×10^4	0.53×10^4	0.995	3.91 ÷ 23.45
Toluene	1.0×10^6	0.17×10^6	1.2×10^6	0.13×10^6	0.988	0.39 ÷ 2.33
Chlorobenzene	6.1×10^6	0.29×10^6	1.31×10^6	0.029×10^6	0.999	2.81 ÷ 16.84
p-Xylene	1.0×10^6	0.11×10^6	2.6×10^6	0.16×10^6	0.996	0.20 ÷ 1.22
n-Decane	0.3×10^7	5.2×10^7	8×10^7	1.2×10^7	0.980	0.13 ÷ 0.75

Each calibration curve consists of four calibration points and each point is an average of five measurements.
$y = bx + a$ (y—peak area in arbitrary unit (a.u.), x—analyte concentration in mg m^{-3}).

difference in humidity between a gaseous calibration mixture and an air sample analysed would influence the results but only slightly. The effect of humidity is nearly constant for the relative humidity range of 25–75%, which covers majority of indoor, outdoor and workplace air cases. Maintaining the humidity of standard gaseous mixtures within this range is relatively easy with the apparatus proposed.

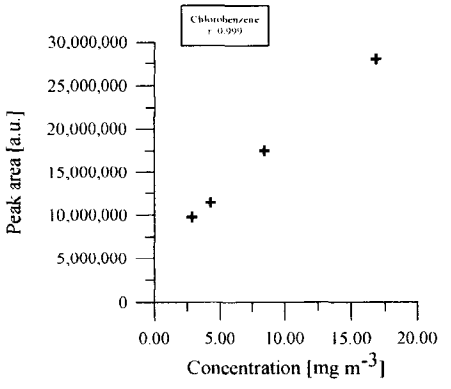

Fig. 4. The plot of SPME-GC-MS response versus concentration of chlorobenzene in a gaseous standard mixture.

4. Conclusions

The apparatus for the generation of standard gaseous mixtures with mixing and sampling chambers gives a mixture of uniform concentration and enables easy exposition of an SPME fibre to the mixture. Since the concentration can be easily controlled and calculated the easy and reliable calibration of SPME-GC system versus analyte concentration in gaseous sample is feasible.

The described calibration procedure should make the SPME technique a versatile, convenient and reliable tool for the determination of organic pollutants in various types of gaseous samples, such as indoor, atmospheric and workplace air. The determination would consist of two series of SPME-GC measurements: one for the sample studied and one for the standard mixture.

The analysis of the above types of samples using the SPME combined with the proposed method of calibration is relatively short and of

relatively low costs, since use of very pure solvents is eliminated, which is also important from environmental protection viewpoint.

Acknowledgements

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References

[1] W. Janicki, L. Wolska, T. Górecki and J. Namieśnik, Chem. Anal., 38 (1993) 423.
[2] J. Namieśnik, M. Biziuk, W. Chrzanowski, W. Wardencki and B. Zygmunt, Chem. Anal., 40 (1995) 115.
[3] C.L. Arthur and J. Pawliszyn, Anal. Chem., 62 (1990) 45.
[4] D. Louch, S. Motlagh and J. Pawliszyn, Anal. Chem., 64 (1992) 1187.
[5] C.L. Arthur, M. Chai and J. Pawliszyn, J. Microcol. Sep., 5 (1993) 1.
[6] C.L. Arthur, L.M. Killam, S. Motlagh, M. Lim, D.W. Potter and J. Pawliszyn, Environ. Sci. Technol., 26 (1992) 979.
[7] D.W. Potter and J. Pawliszyn, J. Chromatogr., 625 (1992) 247.
[8] C.L. Arthur, L.M. Killam, K.D. Bucholz and J. Pawliszyn, Anal. Chem., 64 (1992) 1960.
[9] Z. Zhang and J. Pawliszyn, Anal. Chem., 65 (1993) 1843.
[10] M. Chai and J. Pawliszyn, Environ. Sci. Technol., 29 (1995) 693.
[11] J. Namieśnik, Zeszyty Naukowe Politechniki Gdańskiej Nr 396, Chemia Zeszyt nr XXVIII, Gdańsk 1985.
[12] J. Namieśnik, J. Chromatogr., 300 (1984) 79.
[13] J.J. McKinley, Instrum. Technol., June 1974, 45.
[14] A.E. O'Keefe and G.C. Ortman, Anal. Chem., 38 (1966) 760.
[15] D.P. Lucero, Anal. Chem., 43 (1972) 1744.
[16] J.S.-Y. Ho, PH. A. Schlecht and P.C. Schlecht, Am. Ind. Hyg. Assoc. J., 42 (1981) 70.
[17] J.L. Perkins and A.D. Tippit, Am. Ind. Assoc. J., 46 (1985) 455.
[18] J. Namieśnik, L. Torres, E. Kozłowski and J. Mathieu, J. Chromatogr., 39 (1989) 281.
[19] J. Namieśnik, Chromatographia, 17 (1983) 47.
[20] Z. Zhang, M.J. Yang and J. Pawliszyn, Anal. Chem., 66 (1994) 844.