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Analysis of *n*-Alkanes at Sub Microgram per Liter Level after Direct Solid Phase Microextraction from Aqueous Samples

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This work describes the application of the previously presented solid phase microextraction (SPME) fiber in direct mode for sampling of C_{10} – C_{20} *n*-alkanes from aqueous solution. The fiber has simple composition and is constructed from activated charcoal:PVC suspension in tetrahydrofuran. When the composition of the fiber was optimized that the optimum composition was 90:10 (activated charcoal:PVC) for direct mode, whereas it was 75:25 for sampling from the headspace of aqueous samples. This fiber is completely stable in contact with water. The extraction efficiency is improved in the presence of 0.1 M NaCl. The value is between 17.8 – 38.5% for the first extraction, which better than the efficiency of similar commercial fibers. After seven extractions, all analytes are removed from the aqueous samples nearly 100%. Single fiber repeatability and fiber-to-fiber reproducibility are good and both are less than 13% for all studied alkanes. Finally, direct mode SPME was used in the determination of *n*-alkanes in the range of sub μg L^{-1} without any additional preconcentration procedure. Gas chromatography along with flame ionization detection were used for separation and detection of the studied analytes.

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Introduction

The marine environment is subject to contamination by organic pollutants from a variety of sources. Organic contamination results from uncontrolled releases from manufacturing and refining installations, spillage during transportation, direct discharge from effluent treatment plants and run-off from terrestrial sources. In quantitative terms, crude oil is one of the most important organic pollutants in marine environments. It has been estimated that worldwide somewhere between 1.7 and 8.8×10^6 tons of petroleum hydrocarbons (mainly *n*-alkanes) impact marine waters and estuaries annually.[1] Therefore analysis of environmental samples which are polluted with crude oil or petroleum products is important.

Solid phase microextraction (SPME) as a simple and rather inexpensive method which involves no organic solvents and easy to use method, has gained widespread acceptance in many areas in recent years. It has been applied for the determination of a wide spectrum of analytes in a variety of matrices. The most widespread seems to be analysis of volatile and semi-volatile compounds in water. Examples in this area include determination of substituted benzene compounds,[2,3] volatile organic compounds,[4-6] polycyclic aromatic hydrocarbons and polychlorinated biphenyls,[7] pesticides,[8-13] phenols,[14,15] fatty acids,[16] as well as lead and tetraethyl lead.[17]

In direct extraction mode (as one of three SPME modes), the coated fiber is inserted into the sample and the analytes are transported directly from the sample matrix to the extracting phase. To facilitate rapid extraction, some level of agitation is required for transporting the analytes from the bulk of the sample to the vicinity of the fiber. For gaseous samples, the natural flow of air is frequently sufficient to facilitate rapid equilibration, but for aqueous matrices, more efficient agitation techniques, such as fast sample flow, rapid fiber or vial movement, stirring or sonication are required to reduce the effect of a "depletion zone" produced close to the fiber as a result of slow diffusional transport of analyte through the stationary layer of liquid matrix surrounding the fiber.[18]

In previous work, we proposed a new SPME fiber based on a solid sorbent (activated charcoal) in a polymeric matrix (PVC) coated on silver wire and we reported its application to the solid phase microextraction of aliphatic hydrocarbons from the gaseous phase and the headspace of contaminated soil samples prior to their analysis by capillary gas chromatography.[19] In succeeding studies, we used this fiber for the analysis of *n*-alkanes from aqueous samples in direct mode of SPME. Fiber efficiency in direct mode is comparable or better than its efficiency in headspace mode. Stability of the fiber in contact with water is excellent, so single fiber was used in all experiments.

Experimental

Chemicals and solutions

Activated charcoal (pa), polyvinyl chloride (PVC), undecane, dodecane, pentadecane, octadecane, nonadecane, eicosane and THF all were of analytical grade and were obtained from E. Merck (Germany). Decane, tetradecane, hexadecane and heptadecane were from Riedel-de Hean and tridecane was from Fluka. He and H2 (both with 99.999% purity) were prepared from Sabalan Co. (Tehran, Iran). About 3 mL of the analytes mixture standard solution was prepared by weight: decane 13%, undecane 8.74%, dodecane 8.62%, tridecane 8.66%, tetradecane

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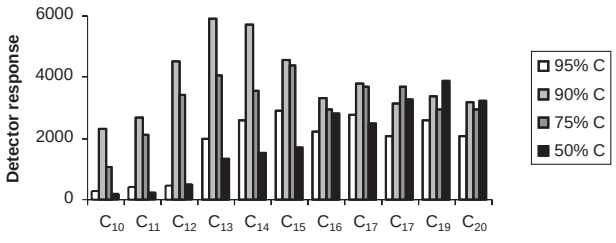

Fig. 1 Optimization of the fiber composition.

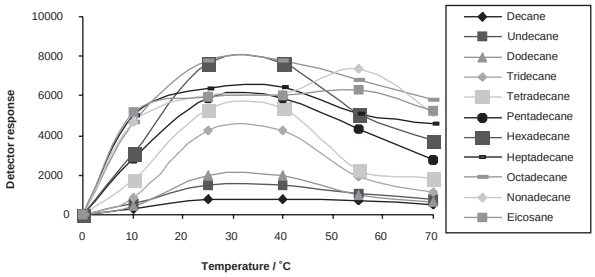

Fig. 2 Effect of temperature on the microextraction of *n*-alkanes by the SPME fibers.

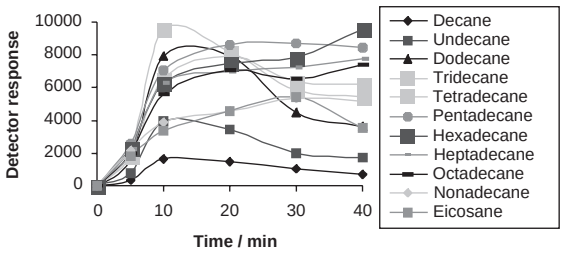

Fig. 3 Time of microextraction of *n*-alkanes by the SPME fibers.

8.61%, pentadecane 8.64%, hexadecane 9.13%, heptadecane 8.65%, octadecane 8.65%, nonadecane 8.63% and eicosane 8.67%. Working standard solutions were prepared by suitable dilution of the above solution with water.

Apparatus

The instrumentation consisted of a Sanayeh Teif Gostar GC 101B gas chromatograph (Tehran, Iran) equipped with an FID detector, a data processor and a split/splitless injector. A Machery Nagel Optima-17 (50% diphenyl) capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm) was used. Laboratory-made SPME devices were used in all experiments.

Fiber preparation and conditioning

A 0.01 g amount of polyvinyl chloride (PVC) was dissolved in 3 mL THF and then 0.09 g activated charcoal was poured into solution, which was well mixed. After a period of 1 – 2 h, THF was evaporated and a viscose suspension was formed. A 1-cm silver wire that was mounted in the laboratory-made SPME device was dipped into solution. After evaporation of THF at room temperature, a very firm porous coating was formed on the silver wire. For preparing thicker layers of the fiber, this procedure can be repeated several times. Then the fiber was conditioned in the injection port of the gas chromatograph at 250˚C for 15 min to remove fiber contaminations.

Direct SPME procedure from aqueous samples

A 0.1 μL volume of the mixture of standard *n*-alkanes was added into a 60-mL vial containing 55 mL water and mixed for a period of 10 min. Then, the fiber was dipped into bulk of the solution while it was stirred by a magnetic stirrer. After 20 min, the fiber was immediately transferred to the injection port of GC and the analytes were desorbed for 5 min at 250˚C.

GC conditions

The column beginning temperature was 30˚C, held for 5 min; then the temperature rise was programmed at 15˚C/min to 250˚C. Temperature of the injection port and detector was 250˚C. Helium was used as the carrier gas with an inlet pressure of 1.4×10^5 Pa and the make-up gas had a pressure of 10^5 Pa. The analytes were injected in the splitless mode into the GC.

Results and Discussion

Selecting optimum composition of coating

For this purpose, different compositions of activated charcoal: PVC coating were prepared and the extraction was performed by them. The coating compositions (95:5), (90:10), (75:25) and (50:50) were prepared by weight from activated charcoal: PVC. As shown in Fig. 1, fiber composition is less effective for the heavier alkanes but it is very effective for the lighter alkanes. Fibers with 95% and 50% C have poor extraction recoveries for C_{10} – C_{16} alkanes relative to other fibers. Thus the composition of 90:10 (activated charcoal:PVC) was used in the further studies.

Effect of temperature on the microextraction recoveries

The first factor considered was the effect of temperature. For this purpose, the sample was transferred into a special vial which was connected to a water bath; then the extraction was carried out at 10, 25, 40, 55 and 70˚C. Peak heights were plotted *vs*. temperature and are shown in Fig. 2. The results show that the extraction recoveries had reached to the maximum value and remained nearly constant at 25 and 40˚C. They decreased above 55˚C. However, elevated temperature can increase the partition coefficients and extraction rates between solution and coating, but some *n*-alkanes particularly lighter *n*-alkanes, rise to the headspace of aqueous samples and decrease the amount of them in the solution. Thus, 25˚C was selected as an optimum temperature and the rest of the experiments were performed at room temperature (25 ± 3˚C).

Optimization of the microextraction time

To evaluate the extraction rate, we added the mixture of *n*-alkanes into the water; then extraction was performed for 2, 5, 10, 20, 30 and 40 min from the bulk of the solution. Peak heights *vs*. the extraction time are presented in Fig. 3. As shown in this figure, after 20 min extraction of most analytes by the fiber is reached the maximum amounts. The number of sites on which adsorption can take place is limited. When all these sites are occupied, no more analyte can be adsorbed on the sorbent. In addition, a molecule with the lower affinity for the sorbent can be replaced by a molecule with the higher affinity. The lighter *n*-alkanes have higher diffusion coefficients in comparison with their higher homologues and the extraction rates for them are high. But after 10 min, adsorption of the heavier *n*-alkanes leads to desorption of the lighter *n*-alkanes. For this reason the extraction of four lighter *n*-alkanes reached

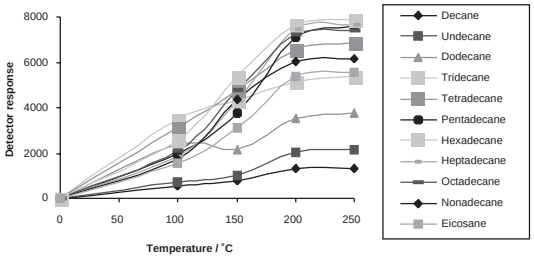

Fig. 4 Desorption temperature of *n*-alkanes from the SPME fiber. Desorption time, 5 min.

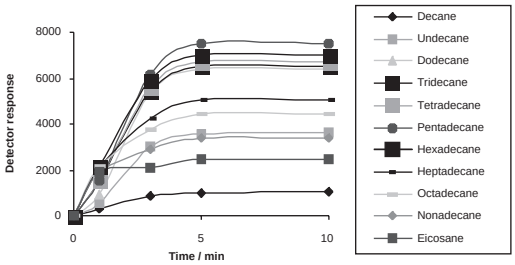

Fig. 5 Desorption time of *n*-alkanes from the SPME fiber. Desorption temperature, 250˚C.

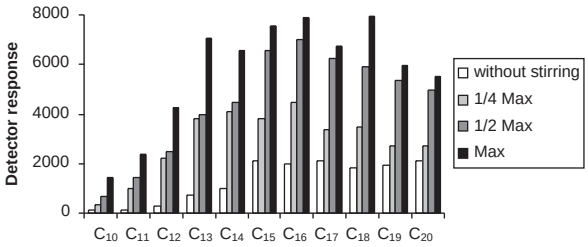

Fig. 6 Influence of stirring speed on the microextraction of *n*-alkanes by the SPME fiber.

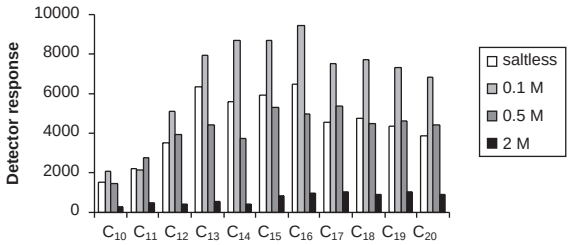

Fig. 7 Salting out effect on the extraction of *n*-alkanes by the fiber. Sodium chloride was used as a salting out agent at the mentioned concentrations.

the maximum at 10 min and then it gradually decreased. Because of this reasonable extraction efficiency of the fiber relative to the higher *n*-alkanes and the steady adsorption of four primary *n*-alkanes at 20 min, as well as to shorten the analysis time, 20 min was selected as an optimum time for the microextraction.

Selecting the desorption temperature

The adsorbed analytes were transferred into the column of GC in consequence of their thermal desorption in injection port. Thermally desorption was performed at 100, 150, 200 and 250˚C for 5 min. The obtained results are shown in Fig. 4. These results indicate that temperatures above 200˚C are suitable. In this study we used 250˚C as a desorption temperature. The fiber is completely stable in this temperature and no additional peaks were found in the chromatograms.

Optimization of the desorption time

The required time for the thermal desorption of the analytes was also studied. The fiber after the extraction was immediately transferred into the injection port of the gas chromatograph and was maintained for 1, 3, 5 or 10 min at 250˚C. As shown in Fig. 5 desorption is completed after 5 min and this process is relatively rapid. On the other hand, with trapping of the analytes in the initial part of the column peak broadening is decreased in spite of longer injection time.

Study of the stirring speed

Magnetic stirring method, in which a small rotor is put into the sample vial, was widely used in both headspace and direct solid phase microextraction (SPME). The agitation accelerates the transfer of analytes from matrix to the coating. In this study, effect of stirring speed was considered without the agitation and using 1/4 Max, 1/2 Max and Max speed of the magnetic stirrer in extraction of the analytes. The results are plotted as peak heights *vs*. the stirring speed and are shown in Fig. 6. This figure shows that extraction efficiencies improved by increasing stirring speed and the best results are obtained for the maximum stirring speed.

Salting-out effect

The salting out effect is widely used to increase the effectiveness of an organic solvent to extract organic compounds dissolved in water. In this study, sodium chloride is also added to the sample in order to increase the ionic strength and to enhance the amount of the analyte extracted by the fiber. The extraction was performed in the presence of different concentrations of NaCl (saltless, 0.1, 0.5 and 2 M). The results are shown in Fig. 7. This figure shows that salting out is more effective at 0.1 M NaCl. All *n*-alkanes except C_{11} are quantitatively extracted from this solution relative to other solutions. In higher concentrations, NaCl crystals deposited on the fiber decrease adsorption of the analytes by the fiber in the subsequent extractions. In this study, each fiber is immersed into distilled water for 10 min before it is used for sampling. This procedure is necessary to dissolve any sodium chloride deposited on the fiber at the previous extraction.

Reproducibility of the proposed method

We wanted to evaluate the quantitative characteristics of the present method, so the repeatability of the method was assessed by performing four repeated determinations on the standard solutions. The obtained results are shown in Table 1; these indicate that the relative standard deviations are less than 10% for all analytes. Also reproducibility studies (fiber-to-fiber repeatability) performed on the three home-made fibers show that RSD% is less than 13% for all compounds (Table 2). It is mentioned that commercial fibers rarely have RSD% better than that of the proposed fiber. On the other hand, this fiber is very stable and there is no need to use different fibers in analysis.

Studying recovery in the successive extractions

To evaluate the extraction recoveries, successive extractions

Table 1 Repeatability of the proposed method

Compound	Concentration/ μg L^{-1}	RSD, %[a]	Compound	Concentration/ μg L^{-1}	RSD, %
$C_{10}H_{22}$	170	7.70	$C_{16}H_{34}$	118	7.30
$C_{11}H_{24}$	113	8.00	$C_{17}H_{36}$	112	7.16
$C_{12}H_{26}$	112	9.10	$C_{18}H_{38}$	112	8.63
$C_{13}H_{28}$	112	5.00	$C_{19}H_{40}$	112	8.94
$C_{14}H_{30}$	112	5.61	$C_{20}H_{42}$	112	4.78
$C_{15}H_{32}$	112	9.54			

a. Relative standard deviation (n = 4).

Table 2 Fiber-to-fiber reproducibility

Compound	RSD,%[a]	Compound	RSD,%
$C_{10}H_{22}$	9.80	$C_{16}H_{34}$	8.18
$C_{11}H_{24}$	10.40	$C_{17}H_{36}$	10.29
$C_{12}H_{26}$	6.23	$C_{18}H_{38}$	10.90
$C_{13}H_{28}$	9.46	$C_{19}H_{40}$	13.00
$C_{14}H_{30}$	11.80	$C_{20}H_{42}$	12.62
$C_{15}H_{32}$	9.86		

Concentrations of the analytes are the same as in Table 1.
a. Relative standard deviation (n = 3).

Table 3 Quantitative characteristics of the proposed method

Compound	Calibration curve equation	R[a]	LOD[b]/ μg L^{-1}	LDR[c]/ μg L^{-1}
$C_{10}H_{22}$	D = 23.2C + 147[d]	0.992	0.3	1 – 50
$C_{11}H_{24}$	D = 78.8C + 212	0.998	0.2	0.6 – 30
$C_{12}H_{26}$	D = 70.3C + 321	0.993	0.2	0.6 – 30
$C_{13}H_{28}$	D = 68.4C + 437	0.999	0.1	0.3 – 30
$C_{14}H_{30}$	D = 79.8C + 341	0.991	0.1	0.3 – 30
$C_{15}H_{32}$	D = 81.4C + 229	0.997	0.1	0.3 – 30
$C_{16}H_{34}$	D = 85.5C + 374	0.990	0.1	0.3 – 30
$C_{17}H_{36}$	D = 115C + 437	0.991	0.1	0.3 – 30
$C_{18}H_{38}$	D = 116C + 561	0.991	0.2	0.6 – 30
$C_{19}H_{40}$	D = 114C + 540	0.992	0.2	0.6 – 30
$C_{20}H_{42}$	D = 107C + 508	0.992	0.2	0.6 – 30

a. Correlation coefficient.
b. Limit of detection.
c. Linear dynamic range.
d. D and C are detector response and concentration of the analytes (μg L^{-1}), respectively.

Table 4 Results obtained from the analysis of real samples

Compound	C ± SD[a]	Compound	C ± SD
$C_{10}H_{22}$	1.36 ± 0.27	$C_{16}H_{34}$	0.73 ± 0.08
$C_{11}H_{24}$	22.7 ± 1.25	$C_{17}H_{36}$	0.87 ± 0.03
$C_{12}H_{26}$	1.65 ± 0.12	$C_{18}H_{38}$	0.74 ± 0.12
$C_{13}H_{28}$	0.76 ± 0.07	$C_{19}H_{40}$	0.85 ± 0.17
$C_{14}H_{30}$	0.98 ± 0.05	$C_{20}H_{42}$	1.66 ± 0.26
$C_{15}H_{32}$	0.84 ± 0.10		

a. Mean concentration (μg L^{-1}) ± standard deviation (n = 3).

were performed on the single solution by the proposed fiber. The recoveries obtained at different extractions are shown in Fig. 8. This figure shows that, after seven extractions, all

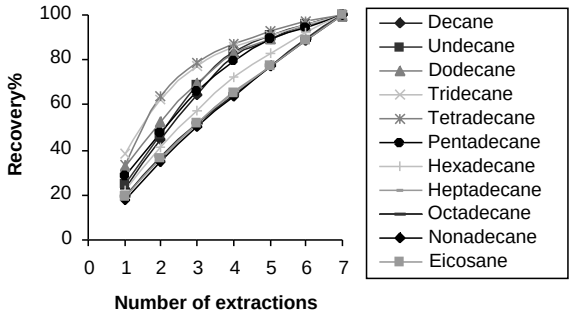

Fig. 8 Accumulative recoveries of *n*-alkanes in the successive extractions performed on the single solution.

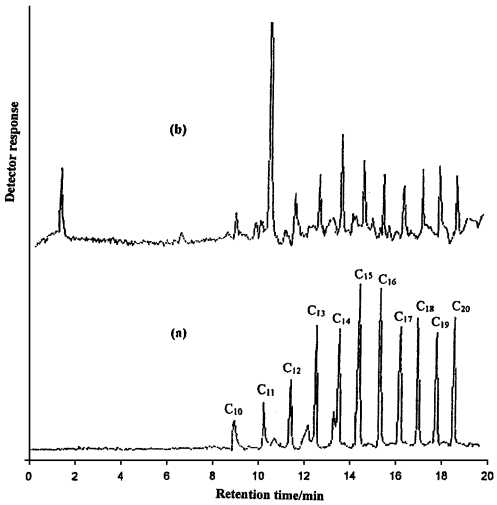

Fig. 9 Typical chromatogram of a) standard solution; C_{10}, 25 and others 15 μg L^{-1}; b) contaminated rainwater. Analytes adsorbed on the SPME fiber were injected to the GC.

analytes are qualitatively removed from the aqueous solution. The amount of analytes extracted in the first extraction (17.8 – 38.5) indicates that the proposed fiber has very high capacity with respect to the commercial fibers.

Quantitative characteristics of the proposed method

The linearity of the detector response to the component concentrations was tested using standard solutions having the variable concentrations at μg L^{-1} level dissolved in 0.1 M NaCl solutions. The calibration curve equations, correlation coefficients, limits of detection and linear dynamic ranges are summarized in Table 3. The correlation coefficients higher than 0.991 for all compounds, very low detection limits (0.1 – 0.3 μg L^{-1}) and relatively extended linear ranges of the calibration curves are main the advantages of the proposed method. The upper limits of the linear ranges are limited by saturating of the fiber and of aqueous solutions in the higher concentrations.

Analysis of real samples

To evaluate the efficiency of this method, we collected the rainwater contaminated with oil materials that was collected from opposite of a repair shop. The proposed method was used in the determination of some *n*-alkanes in this sample. The obtained concentrations of the analytes are listed in Table 4. A typical chromatogram of the sample along with a chromatogram of the standard solution is shown in Fig. 9. The concentration

of undecane was high and therefore its concentration was determined after dilution by ten times. All studied compounds were found in the contaminated rainwater.

Conclusions

In this study, the previously proposed fiber based on activated charcoal was used for the sampling of *n*-alkanes (C_{10} – C_{20}) in direct mode from the aqueous samples. The higher alkanes which can not be quantitatively introduced into the headspace are adsorbed on the fiber in direct mode and then transferred to the analyzing system. High capacity, stability and good reproducibility are the main advantages of the proposed fiber. Water has no evil effect on the adsorption behavior of the fiber. Sodium chloride in the concentration of 0.1 M improves the extraction recovery of alkanes by the SPME fiber. By the proposed method, the studied analytes were successfully determined in the range of sub μg L^{-1} using a capillary gas chromatograph equipped with a flame ionization detector.

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