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Journal of Chromatography A, 909 (2001) 79–85

JOURNAL OF CHROMATOGRAPHY A

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Miniaturized solid-phase extraction with resin disks

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Abstract

The properties and advantages of membrane disks for solid-phase extraction (SPE) are described. Miniaturization is a trend in SPE, as well as chemical analysis in general. A semimicro method is reviewed in which an extraction disk 4 mm in diameter is used for SPE. Even smaller scale separations are possible with a device in which a membrane 0.7 mm in diameter was incorporated into the needle of a 50-μl syringe. Aqueous samples containing 10 ppb of eight substituted benzenes were passed through the miniaturized-SPE syringe automatically using a single-syringe infusion pump. Elution volumes of 5 μl provided 500-fold concentrations and delivered average recoveries greater than 90% and an average relative standard deviation (RSD) of 4.6% for the analytes. Direct injection of the 5 μl eluate from the miniaturized-SPE syringe into a gas chromatograph also produced average recoveries greater than 90% and an average RSD of 6.2%.

Keywords: Solid-phase extraction; Membranes; Instrumentation; Benzenes

1. Introduction

There is a growing realization that faster and more efficient methods for sample pretreatment are essential. One study showed that more than 60% of analysis time was spent in sample preparation, compared to only about 7% for actual measurement of the sample constituents [1]. Solid-phase extraction (SPE) is a particularly attractive technique for isolation and preconcentration of target analytes. SPE is fast and effective, and can provide concentration factors of 100-fold or more. Very little chemical waste is produced. SPE is rapidly replacing older liquid–liquid extraction procedures of chemical analysis.

SPE can be divided into two major categories:

(Type I): Single-equilibrium methods in which each analyte partitions between a liquid or gaseous sample and a solid extractant.

(Type II): Multi-equilibrium methods in which the sample flows through a bed of a solid extractant. Since a typical SPE device may contain 20 or more theoretical plates [2], retention of analytes is generally more complete than with single-equilibrium methods. As the sample continues to be introduced into the SPE column, multiple equilibria occur in which competition for adsorption sites causes the more weakly retained analytes to be pushed further down the column. If the extraction capacity of the column were to be exceeded, a frontal analysis pattern would occur in which the analytes elute in order of their decreasing retention factors. However, sample breakthrough is to be avoided in SPE. After loading the sample onto the column, the analytes are usually eluted all together by an appropriate eluent.

Type II methods usually give nearly complete

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0021-9673/01/$ – see front matter
PII: S0021-9673(00)01014-1

extraction of the desired analytes. Type I methods often give a lower but fixed percentage extraction. Miniaturization is a growing trend in chemical analysis. In SPE, type I methods are already done on a micro scale [solid-phase microextraction (SPME)]. A current challenge is to do type II extractions quickly and efficiently on a smaller scale.

To do ''flow-through'' SPE on a smaller scale, the bed of solid extractant must also be made smaller. The particle size also needs to be made smaller to speed up the rate of mass transfer of analytes from the liquid sample to the solid extractant. Perhaps the best way to accomplish this is to use a resin-loaded membrane such as the 3M Empore series. These can be 0.5–1.0 mm thick and contain extractant particles that are 5 to 10 μm in diameter. The extractant particles constitute approximately 90% of the mass, the remaining 10% being fibrils that hold the membrane together. Liquid samples flow smoothly through the membranes with only a small amount of applied pressure or suction. The loaded particles are immobilized sufficiently that channeling does not occur, and analytes are absorbed evenly near the upper surface of the membrane.

Although the bed height of SPE devices is considerably less when membrane disks are used instead of cartridges or packed columns, the plate height of the disks would be much smaller. This is because the extractive particle size in disks is ~10 μm compared to ~50 μm or more packed columns [3]. High percentage recoveries are obtained with particle-loaded disks [2–7] and the volume of solvent needed for elution of adsorbed analytes is considerably less than with SPE devices packed with loose particles.

It is of course desirable to use SPE sorbents that will strongly retain the analytes of interest. In general, polymeric sorbents such as a polystyrene–divinylbenzene (PS–DVB) retain organic analytes more strongly than silica-based materials. However, it is important to have intimate contact between the predominately aqueous sample and the surfaces of the solid-extractant particles. Poor interfacial contact is made with the apolar surfaces of common extractants unless the SPE devices are first treated with methanol before each run. It is much better to avoid this difficulty by using extractant particles with a more polar surface. The surface of PS–DVB particles can be modified by an organic reaction [2] to incorporate acetyl, hydroxymethyl or a low concentration of sulfonic acid groups [5,6] into the outer benzene rings. Materials that incorporate a more hydrophilic co-polymer into the sorbent appear to accomplish the same goal. The Oasis sorbents from Waters are co-polymers of polyvinyl pyrrolidone and a cross-linked styrene.

A miniaturized device will be described and evaluated for performing type II SPE. A concentration factor if 500 is feasible and only 5 μl of eluting solvent is required.

2. Experimental

2.1. Extraction assembly

A 50-μl Hamilton gas-tight syringe with removable needle (Model 1705) (Hamilton, Reno, NV, USA) was modified to perform miniaturized SPE (M-SPE). The actual SPE apparatus housed inside the removable needle chamber is illustrated in Fig. 1. To prepare the syringe for SPE, the top half of the needle inlet of a 22 S-gauge needle was removed and a 0.25-mm deep cone was machined. Positioned above this cone was a 55-μm stainless steel mesh screen on which the membrane rested. The dimensions of the membrane were 1.2 mm in height×0.7 mm in diameter. All of these components were held in place by a PTFE ferrule that is a standard part of the Hamilton removable needle syringe series.

Samples to be analyzed were added to a 5-ml syringe that was attached to the M-SPE syringe by a modified clip. A gray GC septum was placed between the two syringes to provide a pressurized seal and prevent any leakage when pressure was applied.

2.2. Reagents and chemicals

Analytes in this study were >99% pure and used as received from Mallinckrodt (Paris, KY, USA), Fisher Scientific (Fair Lawn, NJ, USA), and Aldrich (Milwaukee, WI, USA). Sample spikes were prepared using laboratory distilled water passed through a Barnstead Nanopure II filtration system (Sybron Barnstead, Boston, MA, USA). Acetonitrile (HPLC-grade) was used as received from Fisher Scientific. Empore sulfonated PS–DVB membrane disks, avail-

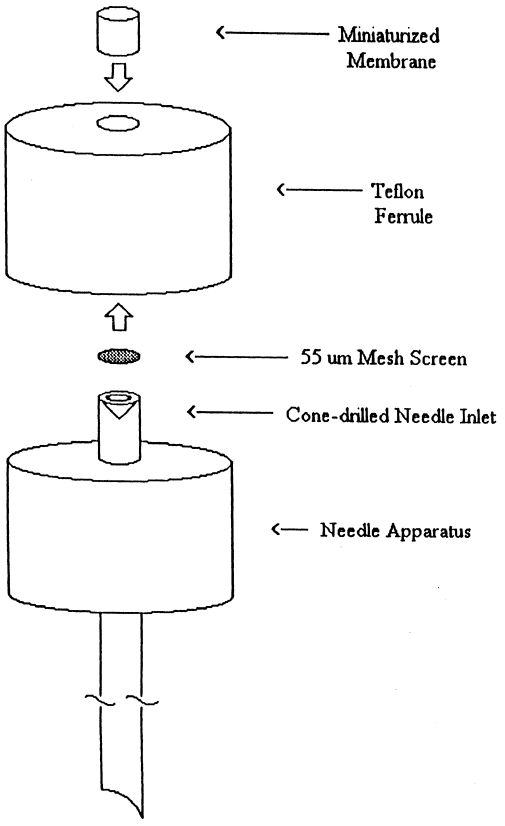

Fig. 1. Miniaturized-SPE assembly inside removable needle chamber.

able as SDB–RPS, were obtained from 3M (St. Paul, MN, USA).

2.3. Internal standard, standard, and spike preparation

Mesitylene (1,3,5-trimethylbenzene) in acetonitrile at a concentration of 5 ppm was used as an internal standard (I.S.). All other standards and spikes were created from a 1000 ppm stock solution of eight substituted benzenes in acetonitrile. The 1000 ppm stock solution was made by adding 10 mg of each substituted benzene to 10 ml of acetonitrile. A 5 ppm standard of the substituted benzenes in I.S. was prepared from the stock solution and used for the 500-fold concentration experiments. Sample spikes for the 500-fold concentration work were made by adding 2.5 μl of the 1000 ppm stock solution to 250 ml of a water–methanol (96:4) mixture. The final concentration of each substituted benzene in the spikes was 10 ppb.

2.4. Procedure

The M-SPE syringe was prepared for sample loading by manually plunging a 20-μl aliquot of acetonitrile and a 35-μl aliquot of water through the syringe (step 1). For the 500-fold concentration studies, 2.5 ml of the 10 ppb spiked sample solution was drawn into the sample syringe (step 2). After a sample was acquired, the sample syringe and M-SPE syringe were connected using the binder clip and septum (step 3).

Sample extractions were automated by placing the sample syringe/S-SPE syringe combination onto a Series 74900 single-syringe infusion pump (Cole-Parmer, Vernon Hills, IL, USA; step 4). Using the syringe pump, sample flow-rates could be precisely manipulated. For these experiments a flow-rate of 100 or 200 μl/min was maintained. After samples were extracted onto the miniature membrane, the syringes were uncoupled, and any remaining water was manually expelled from the M-SPE syringe.

Analytes were eluted off of the miniature membrane by drawing up 5 μl of internal standard solution into the M-SPE syringe (step 5). The eluting solution was allowed to be in contact with the membrane for 2–5 min. A second elution pass was completed when the concentrated mixture was plunged into a lined autosampler vial for later analysis (step 6). Direct injection of the 5 μl eluate into a gas chromatograph was also attempted and is described in more detail below.

2.5. Gas chromatography

All samples were manually injected into a Shimadzu GC-14A gas chromatograph with a flame ionization detection (FID) system and C-R4A Chromatopac data analysis system (Kyoto, Japan).

Analytes were separated on a Supelco SPB-5 column of 15 m×0.32 mm I.D. and 1.0 μm film thickness (Bellefonte, PA, USA). The GC conditions were as follows: 2 μl injections, 70°C initial temperature, 2 min initial time, 10°C/min program rate, 140°C final temperature, 1:60 split flow ratio, 200°C

injector temperature, 250°C detector temperature. Peak area ratios were determined using the mesitylene internal standard. Recoveries were calculated as the average of multiple trials using peak area ratios from sample injections compared to standard injections which were not subject to SPE.

3. Results and discussion

3.1. Semimicro SPE

It is a fairly easy matter to reduce the scale of SPE with a resin-loaded membrane as the solid extractant. Disks of any desired size can be cut from larger disks. These can be inserted into a suitable holder and an efficient SPE accomplished by passing a sample through the system.

One such device [7] used a disk approximately 4×0.5 mm cut from a larger Empore membrane and fitted snugly into the fluorocarbon hub of a 22-gauge stainless steel needle. The sample reservoir was a 5-ml, or larger, gas-tight syringe with a Luer lock that fits into the needle assembly. After pushing a 1- to 6-ml sample through the system only 20–50 μl of ethyl acetate or methylene chloride was needed to elute the adsorbed analytes. Using a 4×0.5 mm membrane containing lightly sulfonated PS–DVB particles, average recoveries of 95% were obtained for some 23 test compounds (0.17 to 1.0 ppm each). The scope of SPE could be expanded by using disks of two or more types.

3.2. Miniaturized solid-phase extraction

The goal of this research was to design a device that would provide essentially total extraction of sample analytes on a very small scale and would permit injection of the entire amount of eluted analytes directly into a gas or liquid chromatograph. This technique, dubbed M-SPE, employs an Empore membrane 0.7 mm in diameter and 1.2 mm thick housed inside the needle chamber of a 50-μl syringe (Fig. 1).

Employing a membrane of this size permits elution with 5–10 μl solvent while still maintaining recoveries greater than 90%. Because of the decreased solvent requirements, direct injection of the eluate becomes possible, resulting in a method where there is little or no wasted solvent. Other benefits of the M-SPE design include readily achieved high concentration factors and a simple, semi-automated procedure in which small samples can be processed easily. A detailed description of the device and general operating procedure are detailed in the Experimental section.

Preconcentration of aqueous samples containing 10 ppb concentrations of several substituted benzenes was used to test the efficacy of the M-SPE procedure. A 2.5-ml sample was passed through the device at a flow-rate of 100 μl/min. Then the analytes were eluted by 5 μl of acetonitrile containing an internal standard into an autosampler vial. A 2-μl sample of the eluate was injected manually into a GC system for chromatographic analysis. Table 1 shows the average recoveries of four trials for a 500-fold preconcentration of seven substituted benzenes using M-SPE. The average recovery for these experiments was 92% with an average relative standard deviation (RSD) of 4.6%. Chromatograms in Fig. 2 show an injection of a 5 ppm standard solution of the substituted benzenes and a 500-fold preconcentrated sample injection whose original concentration was 10 ppb. These chromatograms illustrate the value M-SPE to concentrate trace organics into an accessible detection range. The recovery of toluene (peak 2) could not be calculated in these experiments because impurities concentrated from the filtered water supply.

Another set of extractions was performed with a flow-rate of 200 μl/min maintained with a syringe infusion pump. The extraction time was cut in half to

Table 1
Recoveries of substituted benzenes after a 500-fold concentration[a]

Compound	Recovery (%) (n=4)
Benzene	83
Chlorobenzene	96
Ethylbenzene	96
Anisole	99
Bromobenzene	94
Propylbenzene	89
Butylbenzene	87

[a] Percentage recovery based on the average of four trials. Average relative standard deviation for all of the compounds was 4.6%.

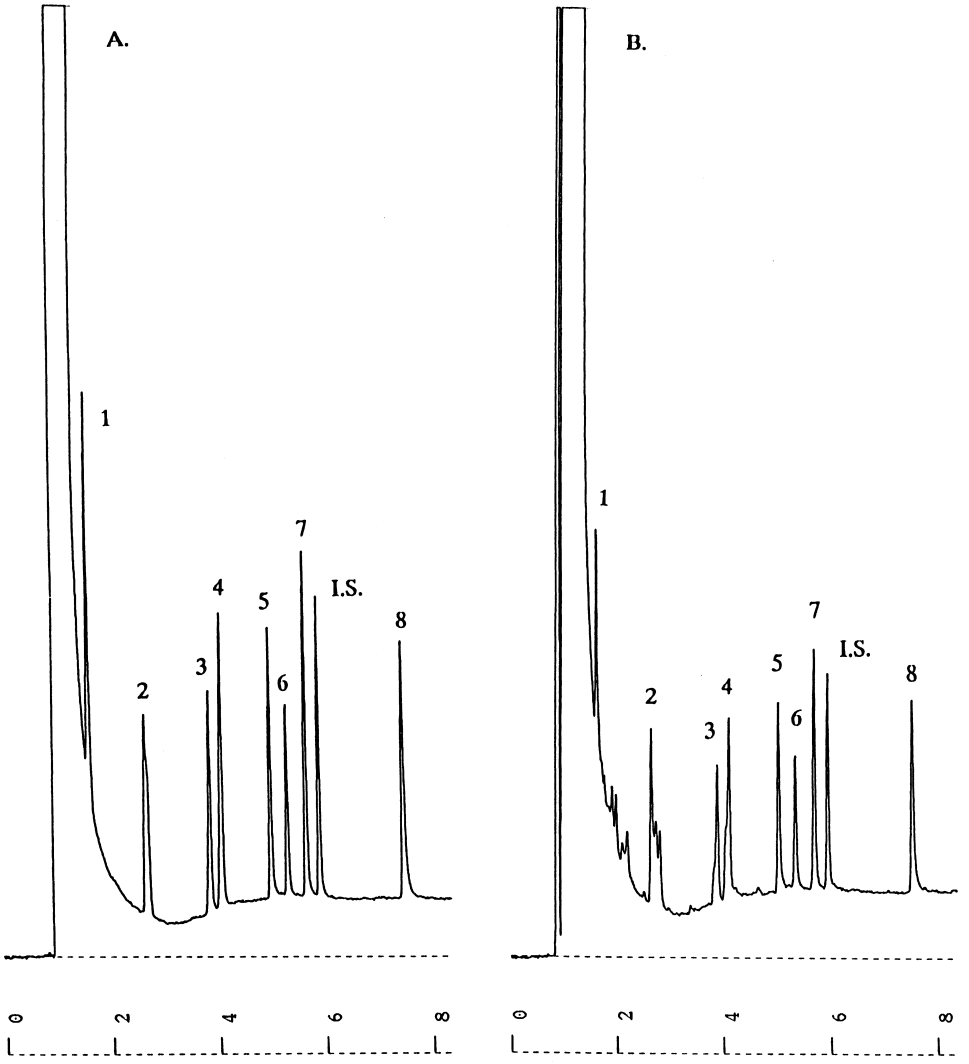

Fig. 2. Comparison of GC chromatograms. (A) 5 ppm standard. (B) 10 ppb sample after 500-fold concentration by M-SPE. Peak identification: 1=benzene, 2=toluene, 3=chlorobenzene, 4=ethylbenzene, 5=anisole, 6=bromobenzene, 7=propylbenzene, I.S.= mesitylene, 8=butylbenzene. Time scale in min.

12.5 min for 2.5 ml samples. A 500-fold concentration was again obtained with an average recovery of 91% for four trials.

Another goal of this research was to design a system that could transfer extracted analytes directly to a GC or LC system for chromatographic analysis. This would shorten sample preparation times and eliminate errors that might occur during transfer of eluate from one container to another prior to injection into a chromatographic instrument.

Essentially the same procedure as before was used, except that the 5 μl of eluting solution was expelled directly into a gas chromatograph. Only one modification was made to the previous GC conditions. Because 5 μl of solution were now being injected, instead of 2 μl, an increased split flow ratio was required. A split flow ratio of 1:100 was adopted to maintain the sensitivity needed for low parts per million detection while splitting enough solution to avoid overloading the column. By making this small

Table 2
Recoveries of substituted benzenes using direct injection of 500-fold concentrated samples[a]

Compound	Recovery (%) (n=4)
Benzene	91
Chlorobenzene	101
Ethylbenzene	96
Anisole	92
Bromobenzene	101
Propylbenzene	95
Butylbenzene	78

[a] Percentage recovery based on the average of four trials. Average relative standard deviation for all of the compounds was 6.2%.

adjustment, the M-SPE syringe could be inserted into an injection port and solutions could be plunged into the GC system in the same manner as normal injections without any modifications to the GC hardware or injection technique.

Table 2 shows the average results of four trials using the direct injection technique. The average recovery for these experiments was 93% with an average RSD of 6.2%. Fig. 3 demonstrates the high quality of chromatography that can still be achieved using direct injection from the M-SPE syringe. Peak heights were reduced slightly using this technique, but the use of an internal standard permitted quantitative data to be obtained with only a small decline in the reproducibility of the results.

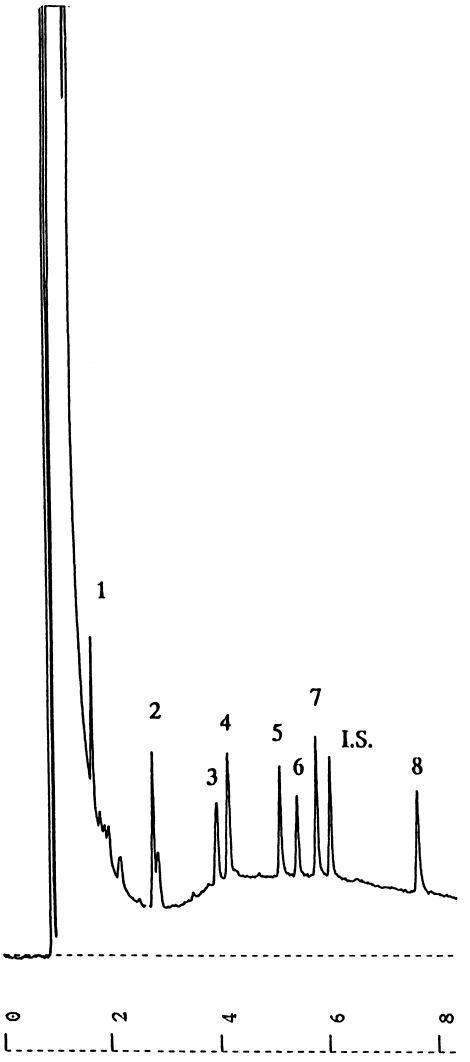

Fig. 3. Direct injection from M-SPE syringe into a GC system. A 10-ppb sample after 500-fold concentration using M-SPE. 1:100 split flow ratio. Peak identification: 1=benzene, 2=toluene, 3=chlorobenzene, 4=ethylbenzene, 5=anisole, 6=bromobenzene, 7=propylbenzene, I.S.=mesitylene, 8=butylbenzene. Time scale in min.

4. Conclusions

A device is described for performing flow-through SPE on a much smaller scale. By using a membrane disk only 0.7 mm in diameter, concentration factors of 500 or more can be obtained with essentially total extraction of sample analytes. A 5-μl volume of eluting solvent provides complete desorption of extracted analytes. If desired, the analyses can be eluted directly from the membrane disk into a gas chromatograph in a single step.

Acknowledgements

The authors thank the 3M Company of St. Paul, MN, USA, for the gift of the SDB–RPS membranes used in this research. This work was performed in the Ames Laboratory, US Department of Energy, under contract W-7405-Eng-85.

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