Solid-phase sample preparation of natural waters with reversed-phase disks

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ABSTRACT

An investigation of sample preparation for natural waters using the Empore disk was conducted. The Empore disk is a new solid-phase sample preparation technology which was developed for rapid isolation of organic contaminants from aqueous matrix. In order to increase the volume of water that could be prepared, it was found that in-line or off-line filtration prior to the extraction step was required. The appropriate filters were identified. When more than 10 liters of natural water were analyzed a non-specific interference to capillary gas chromatography–electron-capture detection determination of analytes was present. The use of the Empore disks offered some advantages in ease and specificity of elution that was not available with solvent extraction and other solid-phase sample preparation technologies.

INTRODUCTION

Assessment of risk from organic chemicals in natural waters requires determination of numerous analytes from diverse and complex matrices. Such matrices vary with geography and within a geographical location the matrix can vary with depth of the water column. In a given location the matrix can also vary with time as a result of local and/or upstream weather and seasonal change. Finally the matrix can vary as a result of human activities. Moreover, at this point in time it is still unclear which compounds constitute any given ecological problem. There is, however, sufficient evidence that organic chemicals in water do pose a real ecological hazard. Methods are, therefore, required to permit a thorough investigation of the nature of compounds that put the environment at risk.

Analytical methods that are to be applied to resolution of these problems should have a number of characteristics. Simplicity and rapidity are obligatory as these permit analysis of the large number of samples necessary for appropriate risk analysis. Some applications require independent determination of organics present in the water and those associated with the particulates, whereas for other applications determination of total organics in the sample is sufficient.

The sample preparation step of analytical methods is the most time consuming and most difficult to automate. One approach to simplification, reduction of costs and automation is solid-phase sample preparation (SPSP). The standard configuration for an SPSP apparatus is that of a semi-preparative column or cartridge packed with reversed-phase chromatographic support [1–6]. A more recent SPSP material is based on reversed-phase particles en-

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meshed in a PTFE network that is configured as a disk [7,8]. The disk permits a faster flow-rate by increasing the cross-sectional diameter of the stream. Efficient adsorption from the fast flowing water is a result of: (i) reduction in linear velocity of the water flow; (ii) reduction of the particle size to 10 μm; (iii) packing these particles densely so that the mean free path of dissolved analyte to an adsorptive surface is small.

While the Empore disk has been used successfully in a number of applications, to date, there has been no extensive study regarding the feasibility of using these disks for the preparation of larger volumes of samples from natural waters. As part of our programme in monitoring contamination of the Great Lakes of North America we investigated determination of analytes concentrated from 10 or more litres of water. Isolation and analysis of organics in more than 10 000–20 000-fold concentration from water is a requirement for this monitoring programme.

Monitoring of natural waters requires that methods of sampling be applicable to samples of differing complexity with some samples being free of particulates and others having a very heavy particle load. A second requirement is that work-up procedures can separate analytes of choice from contaminating materials. We investigated methods for: reducing the deleterious effects of particulates; increasing volume of sample that could be extracted by Empore disks; improving selectivity of sample preparation.

EXPERIMENTAL

Chemicals

Reversed-phase disks with octylsilica (C_8) and octadecylsilica (C_{18}) enmeshed in PTFE were supplied by Dr. C Markell, 3M Corporation (St. Paul MN, USA) or purchased from Varian Associates (Georgetown, Canada). The National Laboratory for Environmental Testing (NLET) at the Canadian Centre for Inland Waters (CCIW) (Burlington, Canada) supplied a solution containing standard organochlorine contaminants in methanol which was used for spiking water. Niagara River water was supplied courtesy of the Water Quality Branch (Ontario Region) and the NLET. Samples from Hamilton Harbour were supplied by the Laboratory of Dr. K.L.E. Kaiser of CCIW and were collect-

ed were collected in July 1991 and October 1991. Water was stored at ambient pH and at 4°C; samples collected in July were analyzed within 2 weeks whereas the October samples were stored and analyzed used over a period of 5 months. Extracts from the October samples were stored in dichloromethane at 4°C; this extract was also made available courtesy Dr. K. Kaiser. It was not possible to analyze the CH_2Cl_2 extracts and the Empore disk extracts simultaneously because samples were collected and prepared by liquid–liquid extraction as part of a routine monitoring programme. Timing of collection for this study could not be deferred to compensate for research and development on the use of Empore disks as this would have required collection of samples during the winter during which time the Harbour is frozen.

Diethyl ether, 2,2,4-trimethylpentane (isooctane), hexane and dichloromethane were all analytical grade and purchased from BDH (Toronto, Canada). n-Pentane was purchased from Caledon Labs. (Georgetown, Canada). Individual standards were purchased from the National Water Research Institute as solutions of calibrated concentrations in isooctane. Pre-filters used included the following: nylaflo (nylon 6 fibres) 0.2 μm and 0.45 μm (Gelman Sciences, Ann Arbor, MI, USA); multigrade glass microfibre filters (GMF) both 1 μm and 2 μm, 47 mm and 90 mm were from Whatman (Maidstone, UK). The Whatman glass microfibre filter GF/A, 1.6 μm, 90 mm was obtained courtesy of Dr. C. Markell, 3M Corp.

Sampling sites and techniques

The origin of all samples obtained was the western region of Lake Ontario which is the lowest of the Great Lakes.

The Niagara River site. The Niagara River flows from Lake Erie and enters Lake Ontario on the south western shore. Samples from this river were collected at the exit of the river into Lake Ontario at the Niagara-on-the Lake station in July 1991 as part of a monitoring programme of Environment Canada. According to the monitoring protocols established by Environment Canada, samples from the Niagara River were centrifuged by a Westphalia centrifuge prior to liquid–liquid extraction of organics from the water so clarified. This removed particulates greater than 10 μm.

Tap water. Tap water was obtained at the McMaster University Medical Centre which is supplied by the Hamilton municipal water supply. The intake pipes for this water supply is in Lake Ontario is approximately 1 km from the western shore of the lake. Water was collected directly from the tap as needed.

Harbour water. Hamilton Harbour is a partially enclosed water and the westernmost region of Lake Ontario. On the southwest, the harbour is bounded by the city of Hamilton, which is heavily industrialized. A substantial portion of the harbour front is occupied by large steel making installations. On the northwest, the harbour is bounded by a natural swamp fed by waters from a farming area and by runoff from a large open botanical garden. On the east the harbour is bounded by a large sand bar which has a ship channel connecting the harbour to Lake Ontario. Water at this site was collected without any pre-treatment and was sampled at the approximate geographical centre of the harbour.

Instrumentation

Gas chromatographic (GC) analyses were performed on a Hewlett-Packard 5790 gas chromatograph equipped with a capillary column and used under the following conditions: the column was purchased from Supelco (Mississauga, Canada) and was 30 m × 0.32 mm inner diameter, the phase was DB-5 with 0.25 μm film thickness. For the majority of analysis, the initial temperature was 130°C and was raised at 4°C/min to a final temperature of 290°C. The pressure of the hydrogen carrier gas was 9 p.s.i. (1 p.s.i. = 6894.76 Pa) and the linear velocity was 0.62 m/s at 160°C. The flow-rate for argon–methane make-up gas was 48 ml/min. Detection was by electron-capture detection (ECD) with the output recorded on a 3392A Hewlett-Packard integrator at an attenuation of 3 or 4.

Procedure

The SPSP procedure is presented in schematic form in Table I and consisted of two basic steps: filtration/extraction and elution.

Filtration. A standard filtration apparatus was used [7], which was connected to a water aspirator (less than 20 p.s.i.). If the water was to be filtered prior to extraction of the analytes onto an Empore disk, the filter and disk were arranged in one of two

TABLE I

SIMPLIFIED SOLID-PHASE EXTRACTION METHOD WITH EMPORE MEMBRANES

Prepare membrane by wetting with methanol
Adsorb analytes onto membrane from environmental water
Dry the membrane placing in dessicator for 1 hour
Elute analytes by soaking the membrane in 5 ml of diethyl ether for 10 min and washing with two 2.5-ml aliquots of ether
Concentrate analytes by evaporating ether under a stream of nitrogen to 2 ml. Add 0.5 ml of isooctane containing external standard: evaporate to 0.5 ml of isooctane.
Analyze 1-μl aliquot of extract by GC–ECD
Calculate yields

configurations. In the on-line configuration, the filter was placed above and in direct contact with the Empore disk, and the water was passed through the combined filters. In the off-line configuration water was filtered through the pre-filter and the filtrate was collected: this filtrate was then passed through the Empore disk.

Isolation of analytes from the extraction disk. The disk was placed in a desiccator containing $CaCO_3$ for 1 h. This eliminated the need to dry the solvents after eluting from the disk and prior to evaporation and concentration. Analytes were eluted from the 47-mm disk by placing the disk in a beaker and leeching the disk with 5 ml of diethyl ether for 20 min. The disks were then washed with two aliquots of 2.5 ml diethyl ether. All eluates were combined. For the 90-mm disk the volumes increased to 20 ml of diethyl ether for the leeching step and to 5 ml for the subsequent washing steps. To the combined ether eluates either 0.5 or 1.0 ml of isooctane was added as a keeper solvent; the isooctane contained 0.1 μg/ml of pentafluorobenzyl nonadecanoate as an unextracted internal standard. The solution was concentrated to 0.5 or 1.0 ml of isooctane under a stream of nitrogen at 30°C, and a 1 μl aliquot was injected directly onto the capillary column.

Spiking of standards into water. Standards were added to a volume of water and stirred for 2 h before extraction. Spiking for determination of recoveries from all matrices were determined at 1 μg/l concentrations.

Calculation of recoveries. For the extracts containing organochlorines, the ratio ($R1$) of the area of each component to the area of a known amount

of pentafluorobenzyl nonadecanoate was determined for each component. A same amount of pentafluorobenzyl decanoate in toluene was added to the spiking solution and the ratio was calculated for each component in the spiking solution ($R2$). To calculate yields, the ratios were compared and reported as a percentage $R1/R2 \times 100$.

Recoveries of 2,4-dichlorophenoxy acetic acid (2,4-D) and 2,4-dichlorophenol were based on the recovery of ^{14}C-labelled analogues. An aliquot of the 30 000 counts radiolabelled compound (equivalent to 0.83 nmol) was added to 1 l tap water, which had been previously adjusted to pH 2.2 with HCl. The spiked water was then filtered through the disk; the disk was not dried, but was extracted immediately with 5 ml of methanol and a 1-ml aliquot was transferred to a counting vial after 10 min. *NOTE: Drying of the disk was not used in this experiment because the analytes are volatile. For this reason, in order to minimize possible losses due to sample preparation, the analytes were eluted from the disk with methanol which could be mixed directly with the scintillation fluid for counting.* A 10-ml volume of scintillation cocktail (Ready Safe, Beckman, Fullerton, CA, USA) was added and radioactivity was determined by scintillation counting.

Fractionation. Isolation of organochlorine compounds from 10–11 l of Hamilton Harbour water required an additional purification step preparatory to determination by capillary GC–ECD. This was due to the presence a non-specific interference (NSI) to such determination that appeared to be related to a yellow coloured material that co-eluted from the adsorption disk with this class of analytes in the ether extract.

Two techniques were studied in order to separate analytes from the coloured material and/or the NSI. In the first, the organic analytes in the diethyl ether eluate from the Empore disk were first transferred to iso-octane during the concentration stage. This isolate was then transferred to a Florosil column consisting of 2 g of Florisil packed into a silanized glass pipette sealed with a silanized glass wool plug. The organics were then eluted with increasing concentrations of diethyl ether in isooctane or *n*-pentane. In the second technique, selective elution from the disk was attempted with the solid phase being leeched with 10, 25 or 50% diethyl ether in *n*-pentane to directly fractionate the yellow material and/or NSI from the organochlorincs *in situ*.

RESULTS AND DISCUSSION

Samples and matrices

In order to expand the possible applications of the Empore disks we investigated these disks as an SPSP phase in analysis of large volumes of natural water samples with matrices of varying complexity. The sample with the simplest matrix was obtained at the Niagara Rivers site which had markedly reduced particulate load as a result of centrifugation prior to collection of the water fraction. A somewhat more complex matrix was obtained by using tap water from the Medical Centre of McMaster University; tap water was used without any pretreatment. Samples with higher concentration of particulates were obtained by collecting water from Hamilton Harbour at two different seasons; midsummer (July) and mid-fall (October). The sample collected in July was turbid indicating an elevated particulate load and the sample collected in October was clear indicating a considerable reduction in the amount of particles present.

The investigations focused on the following questions. What is the magnitude and time course in the reduction in flow-rates through an Empore disk resulting from particulates in the sample? How can this reduction in flow-rates be minimized? What are the recoveries that could be achieved? Do natural components affect the subsequent chromatographic analysis and if adverse effects are found how can these be minimized?

TABLE II

THE EFFECT OF WATER SOURCE ON EXTRACTION TIMES USING A 47-mm EMPORE DISK

ND = Not determined.

Water source	Collection time for 1 l (min)	Collection time for 2 l (min)
Niagara River	12	ND
Tap	20	90
Hamilton Harbour (October 1991)	60	840
Hamilton Harbour (July 1991)	300	ND

Effect of matrix on the adsorption step of SPSP with Empore disks

As expected, water that is relatively free of particulates passed through a 47-mm Empore disk quite rapidly (Table II). Sample preparation time for 1 l of Niagara River Water was only 12 min because particulates had been removed by the Westphalia Centrifuge. Preparation of 1 l of tap water required a somewhat longer time because no laboratory treatment had been used to remove any organic or inorganic particulates remaining from treatment for converting lake water into drinking water [7].

Relative to these particulate free waters, the time for passing 1 l of untreated Hamilton Harbour water through a 47-mm Empore disk increased 3- to 15-fold depending on the particulate load. This volume of water collected in the fall of the year could be passed through a 47-mm Empore disk in 60 min (a reasonable time frame) whereas water collected in the summer required 300 min (Table II). With the sample collected from Hamilton Harbour October 1991 preparation of 2 l required more than 14 h to pass through a 47-mm Empore disk and this is prohibitive. (Due to the high particle load, it was not considerable practical to prepare 2 l of sample collected in July 1991). Increase in preparation time for 2 l rather than 1 l (although not as dramatic) was also observed for tap water. The exponential rate in decrease of flow through an Empore disk has been reported [7] and was reproducible in our hands; the relative standard deviation of time for collection of a given volumes on 6 different Empore disks was less than 14% for times of greater than 6 min and less than 18% for times of less than 4 min (see also Table IV).

Development of methods to increase the volume of sample prepared

The exponential curve in Fig. 1 was attributed to progressive occlusion by particulate matter of the disk with increased volume of water passed through the disk. Such a mechanism was also proposed by Hagen et al. [7]. Consequently, in order to increase the amount of sample that can be prepared in reasonable times, we investigated filtration prior to the adsorption step on the disk. This approach, rather than acidification [7], was selected because the intent of these studies was to develop methods for the wide diversity of sample types and problems en-
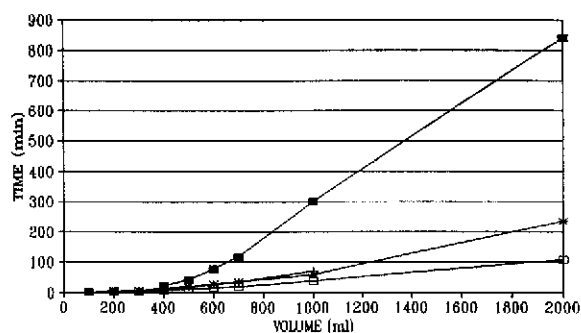

Fig. 1. Hamilton Harbour water collected in October 1991 was extracted under the following conditions: ■ = no filter octylsilica Empore disk alone; + = 0.45 μm filter on-line with octylsilica Empore disk; ∗ = 2.0 μm GMF filter on-line with octylsilica Empore disk; □ = 1.0 μm GMF filter on-line with octylsilica Empore disk.

countered in environmental monitoring and research. In this case it was important to consider a number of factors.

First, the chemical and physical composition of materials in natural waters that could occlude disk pores is highly variable. These materials can be of inorganic [7], organic or microbiological origin and the use of acidification would only be effective in dissolving the inorganic particulates. Second, although acidification is used as a preservation technique and is indeed obligatory for some analyses (e.g. isolation of acid herbicides), lowering of the pH may not be applicable to the wider range of problems. It should be recognized that under acid conditions the inorganic matrix and acid labile organic compounds are destroyed. In the former case compounds are liberated that otherwise might not be readily accessible to the environment and in the latter instance analytes of interest might be destroyed. In either case there is a possibility that important information may be lost.

Given the very heterogeneous nature of matrices and variety of problems found in environmental samples an empirical approach was required to develop filtration procedures for increasing the amount of natural waters that could be prepared on an Empore disk. A large number of filters were studied to determine compatibility both with the aqueous sample and any organic solvents that might be necessary for elution. Under these con-

straints only two classes of filter proved acceptable: glass fibre and nylaflo.

In the studies on preparation of 1–2-l samples, the filters were placed above and in direct contact with the Empore disk and the sample was passed through the combined filter/disk unit. This technique was termed on-line filtration. Both the chemico-physical nature and the pore size were important in determining the effectiveness at increasing the volume of sample that could be prepared in one h. Within the class of GMF filters, the pore size, as expected, had a significant effect (Fig. 1). Relative to the 2-μm GMF filter, one with 1 μm pore size afforded a two-fold decrease in the time to prepare 2 l of Hamilton Harbour water. Relative to the unfiltered case there was an eight-fold decrease in the time to prepare the 2 l of this sample when the sample was prepared via the 1-μm filter. The size of the pores was not the sole determinant of efficacy when different types of filters were used. Despite the four-fold differences in pores size, use of the 2-μm GMF and the 0.45-μm nylaflo filters gave comparable results for an on-line filtration (Fig. 1).

Increased efficiency with decreased pore size suggested that use of nylaflo filters with 0.2-μm pores might further increase the volume of waters that can be prepared in the 1–2-h time frame. Moreover, a possible source for occlusion of filter pores are microbes and since the 0.2-μm size is a sterilization filter, any microbiological matter would be trapped prior to passage through the Empore disk. A 47-mm nylaflo filter with 0.2-μm pore size was tested with the sample of Hamilton Harbour water collected in October 1991. Further reduction in pore size, rather than increasing flow rate, resulted in a filtration time of 130 min for 2 l of this sample. The long filtration time may be explained by the excessive blocking of the small pores of the pre-filter or by viscous drag through the small pores.

The substantially longer filtration times indicated that a different approach was required if the 0.2-μm pore nylaflo filters were to be used to remove particulates prior to the adsorption step on an Empore disk. An off-line filtration technique was studied in which the sample was first passed through the 0.2-μm nylaflo filter and the resulting filtrate was then passed through an Empore disk. The flow characteristics of this filtrate through the Empore disk were substantially improved. For instance, 2 l

TABLE III

TIMES REQUIRED FOR UNFILTERED WATER TO FLOW THROUGH A 0.2-μm NYLAFLO FILTER

Diameter of 0.2-μm pore size nylaflo filter (mm)	Volume (l) of October sample of natural water filtered through nylaflo filter	Time required (min)
47	2	131
90	2	9
90	5	128

of unfiltered sample collected from Hamilton Harbour in October 1991 required 840 min to pass through the 47-mm Empore disk whereas the filtered water required only 70 min (Tables III and IV).

These data suggested that efficient application of the sterilization filter would be to use larger-diameter filters prior to the adsorption onto the 47-mm Empore disks. A smaller Empore disk was considered preferable as this would minimize the volume of solvent needed for elution of analytes. In order to test this technique a 2-l sample of Hamilton Harbour water collected October 1991 was filtered water through a 90-mm nylaflo filter with 0.2-μm pore size and this step was complete in only 9 min.

A doubling of the diameter of the filter should increase the flow-rate by only four-fold yet in this instance there is a 15-fold decrease in the time required to collect 2 l. It should be recognized that these studies were conducted with natural waters that contained a particulate load. In such instance

TABLE IV

TIMES REQUIRED FOR WATER INITIALLY FILTERED THROUGH NYLAFLO TO FLOW THROUGH AN EMPORE DISK

Diameter of empore disk (mm)	Volume of pre-filtered water (l) passed extracted on an Empore disk	Time required (min)
47	2	70
90	5	21

TABLE V

LARGE VOLUME FLOW THROUGH 90-mm DISK

Filter type and pore size	Filtration time (min) for 2 l	Filtration time (min) for 5 l	Filtration time (min) for 10 l	Filtration time (min) for 11 l
GMF 2 μm	5	27	90	ND
GMF 1 μm	6	21	56	ND
GMF 1 μm	7	23	63	75
GF/A 1.6 μm	9	17	33	ND

the flow-rate decreases exponentially with volume filtered [7] (Fig. 1 and Table V). Accordingly, whereas with a 47-mm filter, the flow-rate is markedly decreased with filtration of more than 1 l a similar decrease in flow-rates is not observed with a 90 mm filter until more than 5 l are collected (Table V). Filtration of 2 l through the 90-mm filter thus occurs in throughout region of the volume filtered vs. time curve where for this size filter the flow-rate is very fast and so a sixteen-, rather than four-fold decrease in filtration time is observed.

Again the filtrate from the 90-mm nylaflo filter with 0.2-μm pore size could be passed through a 47-mm Empore disk in 70 min; thus the total time for adsorption step for 2 l of sample was 79 min as opposed to 209 min if both the filter and Empore disk were 47 mm in diameter. Thus, if small pore sizes are required (e.g. to remove microbes) then acceptable sample preparation times can be achieved filtering the sample through a larger-diameter filter prior to extraction which could be done on a smaller-diameter Empore disk.

Use of either of the GMF or the nylaflo filters produced some losses. The GMF filters are thick and this resulted in an approximate 10% loss of sample due to wicking and resultant leakage through the sides [7]. This problem may have been due to a poor design of the holder where clamping pressure is applied on one side only and the opposite side is thus not sufficiently compressed. The nylaflo filters are thinner and not subject to the same losses. These filters, however, (but not those of the GMF group) retained analytes with higher molecular masses such as DDT and DDE and reduced the yields by approximately 20-30%.

These results suggest that the GMF and nylaflo filters could be used for different applications. For

instance, the nylaflo class would be suitable for determination of total analyte (e.g. water soluble and particulate bound). In this case the filter (containing both particulates and some organics adsorbed on the nylaflo filter) and Empore disk (containing a substantial part of the organics dissolved in water) would be extracted together to recover total organics in water and sediment. The GMF filters (with better designed holder) would be suitable for a independent analysis of the compounds in the liquid and solid phases. In this case, the filter and the Empore disk would be separated and the two fractions independently extracted. The isolate from the filter would contain analyte initially bound to particulates and the isolate from the Empore disk would contain analytes that were initially dissolved in water.

In order to increase the amount of water collected the size of both the filtration and Empore disks was increased to 90 mm. With a 1-μm GMF filter on-line with the Empore disk, 10 l of sample collected from Hamilton Harbour in October 1991 could be passed through combined filter/Empore disk in 1 h (Table V). When a similar glass fibre filter, the 1.6-μm GF/A was used, the time for preparation of 10 l of the same water decreased to 33 min. By allowing the collection of 10 l use of the 90-mm disks can readily produce concentration factors of 10 000–20 000 fold. The data in Table V where two experiments on filtration through a 1-μm GMF filter again shows that filtration times are reproducible and also demonstrates the exponential decrease in flow-rates with volume collected.

Drying procedure for an Empore disk

In addition to decreasing the time for the adsorption step [7], it also proved simpler to dry and Em-

TABLE VI

SUCCESSIVE WASHES FOR RECOVERY OF INDIVIDUAL COMPONENTS: PERCENT OF TOTAL RECOVERY IN 30 min

Normalized to final recovery = (absolute recovery in fraction/final absolute recovery) × 100.

Component	Recovery in 10 min: absolute recovery (normalized to final recovery)	Recovery in 20 min: absolute recovery (normalized to final recovery)	Recovery in 30 min
Methoxychlor	77	96	100
Heptachlor	37 (76.3)	46 (94)	49 (100)
Mirex	32 (76.9)	39 (97)	41 (100)
Aldrin	28 (79)	35 (98)	36 (100)
Cummulative recoveries normalized to final recovery	(78)	(96.5)	(100)

pore disk than to dry a cartridge. Drying of the solid phase is required in all of SPSP techniques for two reasons: (i) a coating of water on the solid phase can reduce efficiency of the desorption for analytes adsorbed on the solid phase by water-immiscible solvents; (ii) whether by desorption or physical displacement water "co-elutes" from the solid phase with the eluting solvents which must be dried prior to further sample work-up or instrumental analysis. Drying solid phase in the column configuration requires filtering air through the SPSP phase at 100 ml/min to remove water from the solid phase [2]. Such a high flow-rate can cause losses of the more volatile constituents and in addition requires extra manipulation: first to link the gas lines to the cartridge, and then to detach the cartridge for subsequent isolation of analytes. The disk configuration, however, exposes a large area to the atmosphere and as a result the Empore disks can be dried by simply placing in a desiccator for 1 h.

Recoveries

Several recovery studies were performed by adding aliquots of the organochlorine spike to environmental water, tap water or distilled water and extracting using SPSP. Initial recoveries were high but with unacceptable variability; relative standard deviations were 32.4% (for spikes in the 0.5–5.0 ng/ml range), 30.9% (for spikes in the 2.5–25 ng

range), 25.2% (for spikes in the 5–50 ng range) (data not shown). The reason for the unacceptable R.S.D.s stems from the loss of components in the concentration and evaporation step. By using a more controlled evaporation and using 0.5 ml of iso-octane as a keeper solvent, reproducibility became more acceptable with R.S.D.s ranging from 8.6 to 18.8% (Table VI) in the 100 ng/l concentration range.

The studies on recovery identified three classes of compounds: those recovered in high yield; those obtained in low yield due to breakthrough and those obtained in low yield for causes (as yet undetermined) other than breakthrough. Half of the compounds studied were recovered in yields of 70% or more (Table VII). One compound, 2,4-dichlorophenol, was recovered in low yield but this was due to breakthrough as shown by the fact that the filtrate contained the "missing" portion of the analyte. Three of the 12 compounds (heptachlor, mirex, aldrin) were recovered in yields below 50% but the reduced yield was not due to breakthrough. Less than 2% of these three analytes initially spiked into the water was recovered from a re-analysis of the water that had passed through the disk. Losses on glass-ware of these three compounds was considered and so the containers used to make up the spiked aqueous solutions were washed with methanol but no analytes were recovered in this wash.

It was also considered possible that the low re-

TABLE VII

PERCENT RECOVERIES OF INDIVIDUAL COMPONENTS FROM TAP OR DEIONIZED WATER

Methods: A = 10 min leech time with recoveries being determined by GC–ECD; B = 30 min leech time with recoveries being determined by GC–ECD; C = elute analytes with methanol and determine recoveries by scintillation counting.

Component	Yield (%)	R.S.D. (%)	No. of trials	Method
Dieldrin	94	18.8	5	A
p,p'-DDE[a]	74	17.5	5	A
Endrin	132	16.2	5	A
p,p'-TDE[b]	100	8.62	5	A
o,p'-DDT[c]	100	8.62	5	A
Methoxychlor	90	9.64	5	A
Methoxychlor	103	ND	1	B
Heptachlor	48.7	9.64	5	B
Mirex	40.5	3.01	4	B
Aldrin	36.6	6.5	5	B
2,4-D[d]	69.2	11.2	5	C
2,4-Dichlorophenol	36.1	6.34	5	C

[a] p,p'-Dichloro-2,2-bis(p-chlorophenyl)ethylene.

[b] Tetrachlorphenylethane.

[c] o,p'-1,1,1-Trichloro-2,2-bis(p-chlorophenyl)ethane.

[d] 2,4-Dichlorophenoxyacetic acid.

covery of heptachlor, mirex and aldrin may have been due to insufficient time for elution of the analytes from the disk. The effect of elution time on recovery of analyte was therefore determined. A leeching technique was used to simplify control of the contact time between eluting solvent and solid phase. To this end the kinetics of the extractions were studied for four selected compounds (methoxychlor, heptachlor, aldrin, mirex) that had been adsorbed onto an Empore disk from tap water. It was found that an average of 78% of the total amount of analyte ultimately recovered was present in isolate after the first 10 min. An additional 18% were recovered by leeching the disk for another 10 min and only a 4% gain for the total 30-min leech (Table VII). This pattern of recovery was independent of whether the analyte was finally obtained in high or low yield. Similar results were obtained by leeching the disk in a large volume of diethyl ether and removing small aliquots at 10-min time intervals: 78% of the analyte ultimately recovered was present in the diethyl ether after the first 10 min of leeching and 94% was present after 20 min. How-

ever, while the second method requires less manipulation, the first method of leeching and decanting the eluant from the disk provides more consistent recoveries with R.S.D.s for the four separate analytes of less than 5%. These data suggested that losses did not result from insufficient extraction time. If low yields for compounds such as mirex were due to slow elution from the disk then the fraction recovered with longer extraction times would have been higher.

A 1-l volume of water (Hamilton Harbour) collected in July was extracted using a 47-mm Empore disk and the analytes were eluted from the solid phase by leeching with diethyl ether for 20 min. This provided sufficient amounts of analyte for detection of organic electrophores (Fig. 2). Several of these compounds could be identified on the basis of their retention times (min) in comparison to a spiking solution containing these components: penta- and hexachlorobenzene (7.26, 7.76), heptachlor (11.58), aldrin (13.03), γ-chlordane (16.07), α-chlordane (16.56), and endrin (18.71). Several components with retention times of 8.12, 21.42 and 25.69 min
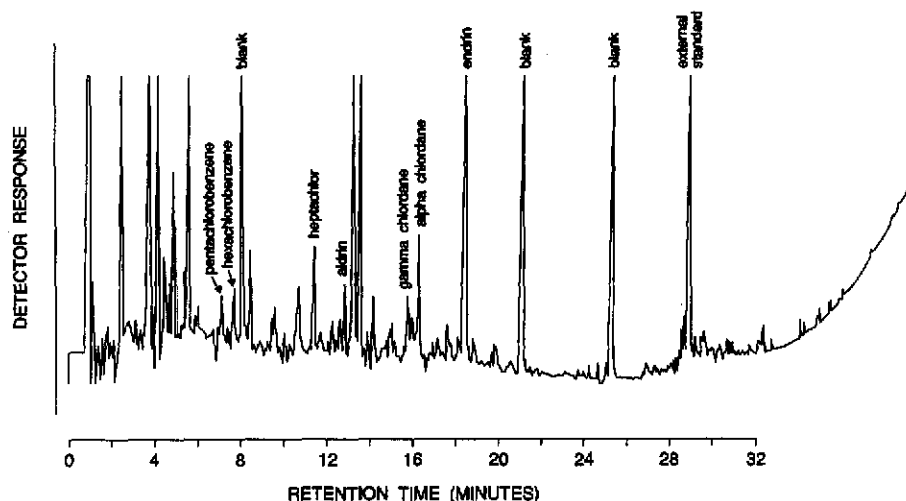
Fig. 2. Gas chromatographic trace with electron-capture detection of 1 l extract of Hamilton Harbour water on a 47-mm octylsilica Empore disk.

were also present in the extraction of 1 l of distilled or doubly distilled water demonstrating that these were contaminants from laboratory equipment or from the Empore disk. The remaining peaks could only be tentatively identified as organochlorines or phthalates on the basis of being detected by ECD.

Removal of non-specific interferences

When larger volumes of Hamilton Harbour water were extracted, elution of analytes with diethyl

ether resulted in a co-elution of standard organo-chlorine analytes with highly pigmented material and materials that caused considerable NSI in the capillary GC–ECD trace (Fig. 3). It is not yet clear whether the pigmented and the NSI material are one and the same. An investigation of this problem demonstrated another possible advantage to using the Empore disk; the feasibility of selective elution.

The initial approach to separate NSI from analytes was use of semi-preparative normal phase col-
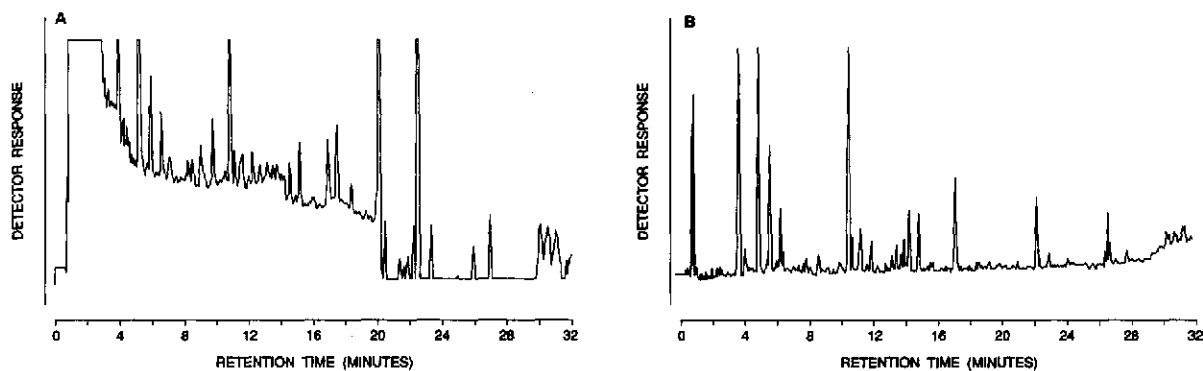

Fig. 3. (A) Capillary GC–ECD trace of a 10-l extract of Hamilton Harbour water (October 1991) using a 90-mm octylsilica Empore disk with a 1.0-µm GMF filter. Organics adsorbed on the disk were extracted with diethyl ether and concentrated with no further clean-up. (B) 10-l extract Hamilton Harbour water (October 1991) using a 90-mm octylsilica Empore disk with a 1.0-µm GMF filter. Organics adsorbed on the disk were extracted with diethyl ether and separated by elution from a Florisil column with diethyl ether–isooctane (5:95, v/v).
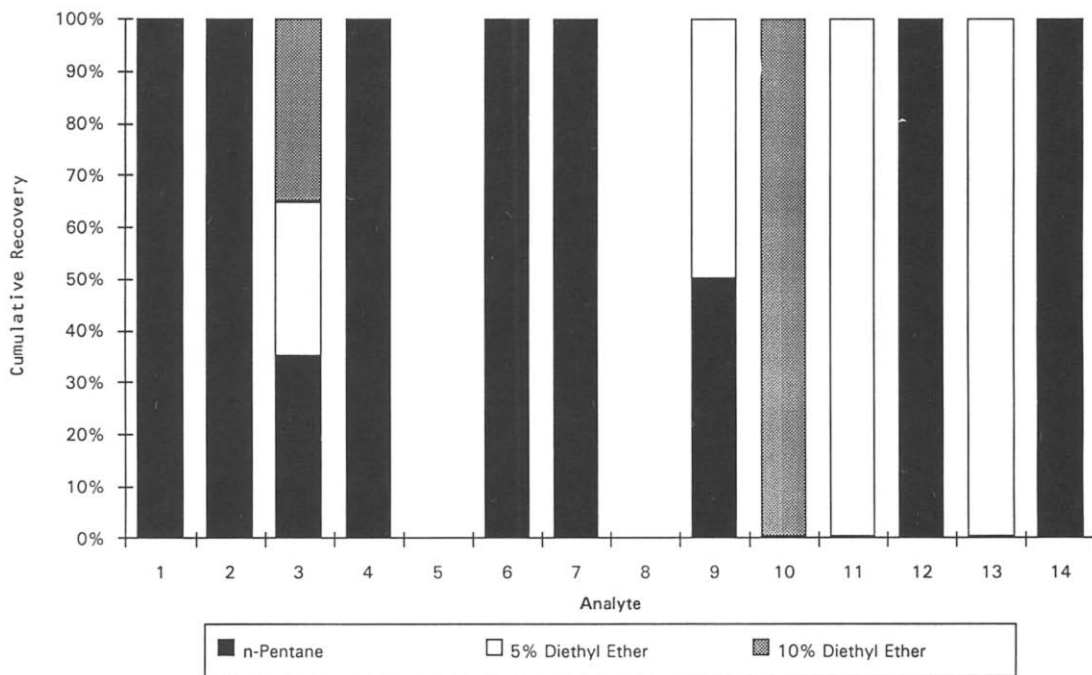
Fig. 4. Cumulative recoveries from a Florisil column by elution with n-pentane, 5 and 10% diethyl ether in n-pentane. Compounds: 1 = hexachlorocyclohexane; 2 = hexachlorobenzene; 3 = lindane; 4 = heptachlor; 5 = heptachlor epoxide; 6 = γ-chlordane; 7 = α-chlordane; 8 = dieldrin; 9 = p,p'-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p'-DDE); 10 = endrin; 11 = β-endosulphan; 12 = tetrachlorphenylethane (p,p'-TDE) + o,p'-1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (o,p'-DDT); 13 = methoxychlor; 14 = mirex.
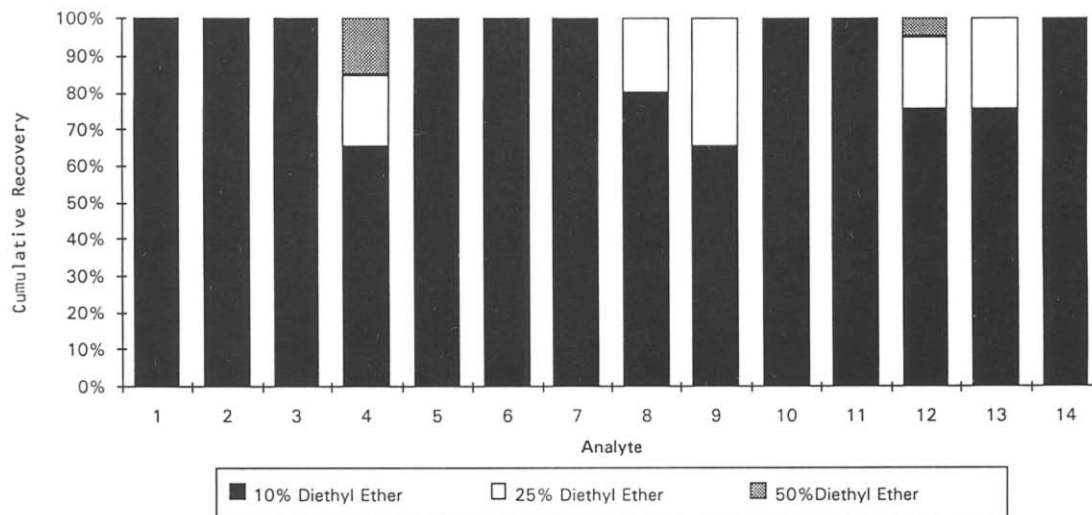

Fig. 5. Cumulative recoveries by leeching adsorbed analytes directly from an Empore disk with 10, 25 and 50% diethyl ether in n-pentane. Compounds: 1 = hexachlorocyclohexane; 2 = hexachlorobenzene; 3 = lindane; 4 = heptachlor; 5 = heptachlor epoxide; 6 = γ-chlordane; 7 = α-chlordane; 8 = dieldrin; 9 = p,p'-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p'-DDE); 10 = endrin; 1 = β-endosulphan; 12 = tetrachlorophenylethane (p,p'-TDE) + o,p'-1,1,1 trichloro-2,2-bis(p-chlorophenyl)ethane (o,p'-DDT); 13 = methoxychlor; 14 = mirex.

umn chromatography. The organics were first eluted from the Empore disk and concentrated in isooctane or hexane. This isolate was transferred to a column of Florisil for separation. (This phase has retentivity intermediate between silica and alumina). Elution with 10% diethyl ether in pentane recovered 12 of the 14 target organochlorine analytes in varying yield (Fig. 4). In order to recover the additional two analytes elution with 25% diethyl ether in pentane and then with 50% diethyl ether in pentane was attempted but the NSI began to elute with the former solvent system and more NSI was recovered with the latter eluent. These data suggested that normal-phase chromatography was not completely effective in separating analytes from the coloured material and/or the NSI.

The second separation technique was based on selective elution from the disk. In this case solvent of differing polarities were used to elute the compounds directly from the disk. The data in Fig. 5 shows that leeching the Empore disk with 10% diethyl ether in pentane recovers all of the compounds but does not optimally recover each analyte. Nevertheless all compounds spiked into the sample are isolated in greater than 65% of the final yield of any given analyte. More important, 10% diethyl ether in pentane does not elute either the coloured material and/or the NSI. In contrast, while 25% diethyl ether in pentane does give a more complete recovery this solvent system also elutes a sufficient amount of pigment and/or NSI is eluted so as to compromise the capillary column GC–ECD analysis.

Comparison of liquid–liquid extraction and SPSP with an Empore disk

Liquid–liquid extraction and SPSP of large volumes of Hamilton Harbour water were compared using recovery of ambient compounds as the basis for comparison. Despite the fact that a direct comparison (i.e. comparison of recoveries of analytes from two aliquots of the same water sample of water prepared by the same technician on the same day) could not be made a substantial fraction (8/13) of ambient compounds were recovered in comparable yield (Table VIII). Three compounds were recovered in greater yield by liquid–liquid extraction but this may have been due to losses on storage. The higher recovery for heptachlor with liquid–liquid extraction may, however, may accurately reflect the fact that recoveries for this compound by SPSP were low. One compound was extracted in higher yield by SPSP possibly reflecting a more efficient extraction. These data suggest that SPSP with the Empore disk is a viable alternative to liquid–liquid extraction for the recovery of ambient organic analytes from natural waters.

TABLE VIII

COMPARISON OF RELATIVE RECOVERIES OF ANALYTES FROM NATURAL WATERS USING USING LIQUID–LIQUID EXTRACTION (LLE) OR SOLID-PHASE SAMPLE PREPARATION (SPSP)

Component	$R1$ for LLE	$R1$ for SPSP	Ratio of recoveries for LLE/SPSP
1	19.6	5.84	3.36
2	5.05	17.8	0.28
3	3.29	3.44	0.96
4	29.1	25.7	1.09
5 (hexachlorobenzene)	2.71	4.01	0.68
6	4.38	4.39	1.00
7	4.66	20.5	0.23
8	1.8	2.58	0.70
9	3.4	1.39	2.45
10 (heptachlor)	14.1	1.42	9.93
11 (aldrin)	5.69	5.80	0.98
12 (α-chlorodane)	2.84	3.39	0.84
13	4.34	3.77	1.15

CONCLUSIONS

Optimal use of the Empore disk for the analysis of natural waters requires that the problems of particulate matter and non-specific interferences be resolved. It proved possible to use filters set in-line or off-line with the Empore disks to remove particulate matter prior to extraction and thus relatively large volumes (10–11 l) of natural water could be treated. Non-specific interferences could also be separated by selective elution from the disk whereas semi-preparative chromatography on Florisil does not separate all analytes from NSI. Finally, the recoveries from the Empore disks are comparable to that obtained by liquid–liquid extraction using dichloromethane.

ACKNOWLEDGEMENTS

REFERENCES

1 W. K. Wang and S. D. Huang, *J. Chromatogr.*, 483 (1989), 121–129.
2 W. E. Johnson, N. J. Fendinger and J. R. Plimmer, *Anal. Chem.*, 63 (1991) 1510–1513.
3 C. H. Marvin, I. D. Brindle, C. D. Hall and M. Chiba, *Anal. Chem.*, 62 (1990) 1495–1498.
4 M. W. F. Nielen, U. A. Th. Brinkman and R. W. Frei, *Anal. Chem.*, 57 (1985) 806–810.
5 B. Crathorne, M. Fielding, C. P. Steel and W. D. Watts, *Environ. Sci. Technol.*, 18 (1984) 797–802.
6 D. R. Green and D. LePape, *Anal. Chem.*, 59 (1987) 699–703.
7 D. F. Hagen, C. G. Markell, G. A. Schmitt and D. D. Bleuins, *Anal. Chim. Acta*, 236 (1990) 157–164.
8 A. Kraut-Vass and J. Thoma, *J. Chromatogr.*, 538 (1991) 233–240.