Methods and materials for solid-phase extraction

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Abstract

Solid-phase extraction (SPE) with porous solid particles goes back to the early 1970s. However, SPE has become more popular recently due to the availability of more efficient particles and the need to replace liquid extraction procedures that require the use of large quantities of organic solvents. Chemical introduction of acetyl or hydroxymethyl groups into polymeric resins improves the efficiency of SPE by providing better surface contact with aqueous samples.

Lightly sulfonated resins display excellent hydrophilicity and improved extraction efficiencies of polar organic compounds over underivatized resins. It is shown that the degree of sulfonation has a major effect on retention of such compounds. Sulfonated resins can also be used for group separation of neutral and basic organic compounds. The advantages of performing SPE with resin-loaded membranes is discussed.

1. Introduction

Sample preparation is often the most time-consuming step in a chemical analysis. One survey found that sample preparation accounted for 61% of the time typically required to perform analytical tasks [1]. Considerable attention is now being paid to solid-phase extraction (SPE) as a way to isolate and concentrate desired components from a sample matrix. In addition to being fast, efficient and easily automated, SPE is a clean analytical procedure. It is rapidly replacing liquid-extraction procedures that require the use of large quantities of polluting organic solvents [2,3].

In liquid–liquid extraction a temporary emulsion is formed by agitating two immiscible liquids, such as water and an organic solvent. Mass transfer of the solute takes place rapidly because of the large interfacial area of the emulsion. In

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SPE the extractant is a porous, particulate solid which has a large surface area available for interaction with the liquid sample solution. The porous solid particles are usually packed into a small tube and the aqueous sample passed through. The principles and applications of SPE have been reviewed [4].

The use of porous, polymeric resins for SPE goes back to the early 1970s. Columns filled with Rohm and Haas XAD resins were used for concentration, identification and quantitative measurement of trace organic pollutants in drinking water. Excellent recoveries were obtained for 85 different organic compounds in one paper [5], which was recently identified as one of the twenty most-cited papers published in the *Journal of Chromatography* [6]. However, practical use of this method was undoubtedly hindered by the necessity of grinding, sizing and purifying the early XAD resins. Now, with the ready availability of pure bonded-phase silica particles and, more recently, of pure polymeric

resins of appropriate particle size, SPE is becoming the preferred method for isolation of organic components from predominately aqueous samples. In this paper, we review some of our most recent research in methods and techniques for SPE.

2. Experimental

2.1. SPE with chemically modified resins

Amberchrome 161 resins (Supelco, Bellefonte, PA, USA) with an average size of ca. 40 μm were derivatized as described previously [7]. For SPE small columns were packed with ca. 100 mg of resin. The C_{18} silica SPE column was obtained from Alltech (Deerfield, IL, USA). This column was 55 × 6 mm I.D., packed to a bed height of 8–10 mm with resin of 40 μm particle size. Derivatized and underivatized Amberchrome 161 resins were packed dry to a bed height of 12 mm into empty 55 × 6 mm I.D. plastic columns obtained from P.J. Cobert Assoc. (St. Louis, MO, USA). Each column contained polyethylene frits to support and cover the resins. Each SPE column was connected to a laboratory-made reservoir by an adaptor (P.J. Cobert Assoc.). The flow-rate of the sample solution from the reservoir was controlled by air pressure applied to the top of the reservoir.

The organic compounds eluted from SPE column with ethyl acetate were collected and then analyzed using an HP 5880A gas chromatograph with a flame ionization detector, an HP 5880A Series Level 4 integrator and an HP 7673A automatic sampler (Hewlett-Packard, Avondale, PA, USA). The gas chromatographic columns used were J & W fused-silica capillary megabore DB-5 and minibore DB-1 (Alltech) and Supelco fused-silica capillary columns.

Approximately 1 ml of methanol was added to the SPE column to serve as an "activating" solvent. Aqueous samples (ca. 20 ml) containing about 5 ppm (w/w) of each test compound were passed through the column at a flow-rate of about 5 ml/min. After washing with 1 ml of pure water, the SPE column was disconnected from

the reservoir and the adsorbed analytes eluted with 1 ml of ethyl acetate. A measured amount of internal standard was added to the effluent and the individual analytes determined by gas chromatography (GC) [7].

2.2. SPE with lightly sulfonated resins

Sulfonation of resins

Sulfonated resins were prepared from 8 μm polystyrene–divinylbenzene (PS–DVB) resins obtained from Sarasep (Santa Clara, CA, USA). For sulfonation, 2 g of resin was mixed with 5 ml of glacial acetic acid and 50 ml of sulfuric acid were added with stirring. Reaction times ranged from 0.5 to 90 min at temperatures ranging from ice temperature to 90°C [8]. After a given reaction time, the mixture was quickly added to ice water to quench the sulfonation reaction. The resin was then filtered through a medium glass frit, and rinsed with deionized (DI) water, methanol, 2-propanol and finally acetone. After drying, the cation-exchange capacity was determined by acid–base titration.

Procedure for k determination

A small (20 × 2.1 mm I.D.) guard column (Supelco) was filled with approximately 20 mg of resin. This guard column was contained within a guard column holder and connected to the HPLC system via two injection loops; a 10-μl loop from the injector (Rheodyne, Berkeley, CA, USA) and a 50-μl loop to the detector. The HPLC system consisted of a Gilson Model 302B HPLC pump equipped with a Model 802B Gilson manometric module (Gilson, Middleton, WI, USA) and Model LP-21 Scientific Systems pulse dampener (Scientific Systems, State College, PA, USA) and a Kratos 783 UV–Vis detector (Applied Biosystems, Ramsey, NJ, USA). Retention times were measured with a Hitachi D-2000 Chromato-Integrator (EM Science, Cherry Hill, NJ, USA).

Samples (50 ppm) were prepared by diluting stock solutions in DI water. Depending on the absorbance of the analyte, 10–50 μl were injected. Phenols were detected at 270 nm and the carbonyl compounds at 205 nm. DI water at 0.5

ml/min was used as the eluent. The column dead time, t_0 was determined using the retention time of bromide (sodium bromide). This value included the travel time through the tubing (60 μl total volume) therefore 0.12 min were subtracted from the measured time to calculate the true t_0. This value was also subtracted from all analyte retention times.

Procedure for SPE

The apparatus for SPE consisted of a 30-ml glass syringe barrel fitted with a luer tip. A 1.5-ml polypropylene SPE column (P.J. Cobert Assoc.) was connected to the glass reservoir via a universal adapter. Loose 8-μm sulfonated resin and Empore membranes embedded with sulfonated resin were used as the SPE adsorbents. This was placed between two 20-μm polyethylene frits (P.J. Corbert Assoc.) in the column. The bed height measured approximately 1 cm. Positive pressure was used to force liquids through the adsorbents. Prior to use, the column was rinsed with 1 ml methanol and then with water.

Samples were prepared by adding a dilute methanol solution of several organic compounds to 15 ml of DI water. The final concentration of each compound in the sample was about 1 ppm. Air pressure was adjusted to provide a flow of 1–2 ml/min (30–60 p.s.i.; 1 p.s.i. = 6894.76 Pa). After loading, the glass reservoir was rinsed with 3–5 ml water and air was blown through the column to remove any remaining water. A 1-ml volume of ethyl acetate or methanol was used to elute the compounds into a GC vial. An internal standard was added to the vial, which was then analyzed by GC [8].

2.3. Group separation of neutral and acidic compounds

Preparation of anion-exchange resin

Amberchrome 161, ca. 40 μm was converted to a quaternary ammonium resin by the following procedure: 1 g of resin was wetted with 1 ml of glacial acetic acid and then reacted with 25 ml of a 2.2 M solution of paraformaldehyde in concentrated hydrochloric acid for 24 h at 70°C. After filtering and rinsing with water and metha-

nol, the resin was aminated by adding 25 ml of 25% trimethylamine in ethanol and stirring at room temperature for 24 h. The resin was then rinsed with methanol, water and acetone. The exchange capacity of the resin was determined by acid–base titration to be 0.9 mequiv./g.

Procedure for SPE and group separation

Approximately 100 mg of resin was packed into a column similar to that used for the previous SPE procedures. Prior to initial use, the anion-exchange column was cleaned with 1 ml each of acetonitrile and methanol. Then 5 ml of 0.1 M NaOH was added to ensure the resin was in the basic form. Aqueous samples containing ca. 0.5 to 5.0 ppm of each test compound were treated with dilute NaOH to raise the pH to approximately 11. The sample was then put into the reservoir and passed through the column at ca. 1 ml/min. The column and reservoir were then rinsed with ca. 5 ml of water.

The neutral fraction was eluted from the column with 1 ml of methylene chloride. This fraction was collected in a GC vial and spiked with 0.1 ml of an quinoxaline or toluene internal standard solution. The vial was capped, mixed with an orbital stirrer, and analyzed by GC. A 1-μl sample was injected with a split ratio of 1:40. Helium carrier gas was used at a flow-rate of 1 ml/min. The oven temperature was held initially at 50°C for 2 min, then ramped at 15°C/min to a final temperature of 225°C. A flame ionization detector was used. The acid fraction was then eluted with 1 ml of 0.1 M HCl in methanol. When the acid fraction contained phenols, the analysis was performed by GC as described for the neutral fraction. Recoveries were calculated by comparison of relative peak area to that of samples prepared and not subjected to SPE.

Samples containing carboxylic acids were eluted with 1 ml of 2 M HCl in methanol and analyzed by HPLC. A 5-μl aliquot of the sample was injected onto a 5-cm laboratory-prepared column of 1 mequiv./g sulfonated 5-μm PS–DVB resin. Separations were performed using an aqueous eluent of 10% acetonitrile, 1 to 2% butanol and 1 mM H_2SO_4. Quantitation was

achieved by comparison of peak areas to known standards.

3. Results and discussion

3.1. Chemical modification of resins

For analytical purposes, SPE is usually performed using a small column containing an appropriate solid packing, or with a membrane loaded with an appropriate solid material [9–11]. Following uptake of extractable solutes from a predominately aqueous sample, the adsorbed materials are eluted from the resin with a small volume of an organic solvent. The individual analytes are then determined quantitatively, usually by gas or liquid chromatography.

Chemically bonded silica, usually with a C_{18} or C_8 organic group, is by far the most commonly used material for SPE. However, cross-linked polystyrene and other porous polymeric resins are finding increasing use. Such polymers are more rugged and pH stable than silica materials. Our research has consistently shown that the percentage recoveries for many types of analytes are significantly higher with polymeric resins than with silica materials [12].

Chemically bonded silica and porous polystyrene resins have several shortcomings for use in SPE [13]. While silica itself is hydrophilic, the hydrocarbon chains make the surface hydrophobic. The consequence is poor surface contact with predominantly aqueous solutions. Porous polystyrene resins also have a hydrophobic surface [14]. Pretreatment of the SPE materials with an activating solvent (such as methanol, acetone or acetonitrile) must be used to obtain better surface contact with the aqueous solution being extracted. However, the activating solvent can be gradually leached out of the resin, thereby causing the extraction to become ineffective. This is particularly true if the SPE column inadvertently becomes dry, causing air to be sucked into the column [5].

A better strategy is to make the surface of a solid-phase extractant permanently hydrophilic through a chemical reaction. Sun and Fritz [7,15]

introduced an acetyl, hydroxymethyl, or cyanomethyl group into cross-linked polystyrene resins at a capacity of approximately 1 mmol/g. The modified resins were easily wetted by water alone and gave significantly higher recoveries for many of the organic analytes tested (Table 1). Each of the resin columns in this table was pretreated with 1 ml of methanol to aid in wetting the resin surface. When this step was omitted the average recoveries were as follows: silica C_{18} 26%, Amberchrome 35%, Amberchrome–CH_2OH 88%, Amberchrome–$COCH_3$ 93%.

Sulfonation of resins also increases their hydrophilicity and makes them wettable with water alone [16]. The degree of sulfonation was found to be very important [8,17]. The best performance for SPE was found to be with resins with about 0.6 mequiv./g of sulfonate groups.

The effect of sulfonate capacity of the resin on SPE was studied by measuring the capacity factor (k) of several solutes using a very small column packed with a sulfonated resin. A small volume of analyte solution (10–50 μl) was added to the top of the resin column and deionized water was passed through the column until an elution peak for the solute was obtained. The capacity factor was calculated from the well-known relationship

$$k = \frac{t_R - t_0}{t_0}$$

where t_R is the retention time of the peak and t_0 is the dead time. The value of t_0 was determined from the elution time of a non-sorbed solute (bromide) and a correction was applied for this solute to pass through the connecting tube.

A plot of k for phenol as a function of resin sulfonate capacity is given in Fig. 1. Curves of very similar shape were obtained for catechol, ethyl pyruvate and 2,3-butanedione, although the capacity factors of these are lower than for phenol. The following points are significant for the four solutes tested:

(1) In each case the plot has a maximum at approximately 0.6 mmol sulfonate/g resin. This value coincides with the onset of wetting of the dry resin by water without any pretreatment.

Table 1
SPE recoveries of phenolic and aromatic compounds with different sorbents

Compound	Recovery (%)			
	SiC_{18}	Amberchrome	Amberchrome–CH_2OH	Amberchrome–$COCH_3$
Phenol	6.3	90.7	94.0	99.7
p-Cresol	16.2	91.1	98.1	100.9
p-Ethylphenol	65.9	96.0	98.5	101.2
2-Nitrophenol	44.7	92.9	94.9	96.0
3-Nitrophenol	<5	81.0	84.9	92.5
4-Nitrophenol	<5	87.0	85.7	86.6
2,4-Dimethylphenol	70.8	94.7	97.3	100.2
4-tert.-Butylphenol	82.9	88.2	95.5	99.5
Anisol	77.9	90.6	94.1	98.1
Aniline	9.1	94.0	96.1	99.5
Benzylalcchol	10.2	91.5	98.2	99.2
Nitrobenzene	53.6	92.4	96.3	99.9
2,4-Dinitroflourobenzene	43.9	83.0	96.0	98.4
o-Hydroxyacetophenone	88.1	84.9	94.7	96.0
Isopentylbenzoate	83.8	71.8	89.2	95.2
Diethylphthalate	90.2	87.2	95.5	100.1
Average	47.1	88.6	94.3	97.6

(2) Increasing degrees of sulfonation cause a marked decrease in k.

(3) Unsulfonated resin and sulfonated resin 2.17 mmol/g were mixed in a proportion to give an average capacity of 0.6 mmol/g. However, the k for this mixture was low (ca. 50), indicating that the actual degree of sulfonation, rather than the average degree of sulfonation, is the critical factor.

SPE with sulfonated resin of the optimum capacity was found to be excellent with recoveries of typical analytes averaging higher than unsulfonated. Table 2 compares recoveries (average of three trials) of several analytes on sulfonated and unsulfonated SPE resin columns. The data indicate that it is not necessary to pretreat the column with methanol when the sulfonated resin is used. Recoveries with the unsulfonated resin average somewhat higher when the methanol pretreatment is included. However, the small particle size of the resins used still permits pretty good recoveries with unsulfonated resin and no pretreatment.

3.2. Use of resin-loaded membranes

For best efficiency a SPE column should be very short (a few millimeters) and should be packed with resins of quite small particle size (5–10 μm). However, some problems are often encountered. Smaller particles require a higher pressure to force the sample through the column. Incomplete retention of sample solutes can occur because of channeling through very short columns.

These difficulties can be largely avoided by using resin-loaded membranes of the type produced by the 3M Co. The sample solutes make intimate contact with the immobilized resins within the membrane, yet a high flow-rate is possible with only modest applied pressure [9–11]. These membranes can be used much like a piece of filter paper in a Buchner funnel. However, we elected to cut small disks from a larger membrane so that the disks would fit snugly into a 5 mm I.D. plastic tube. Tests with a red dye showed that the dye is adsorbed evenly over the
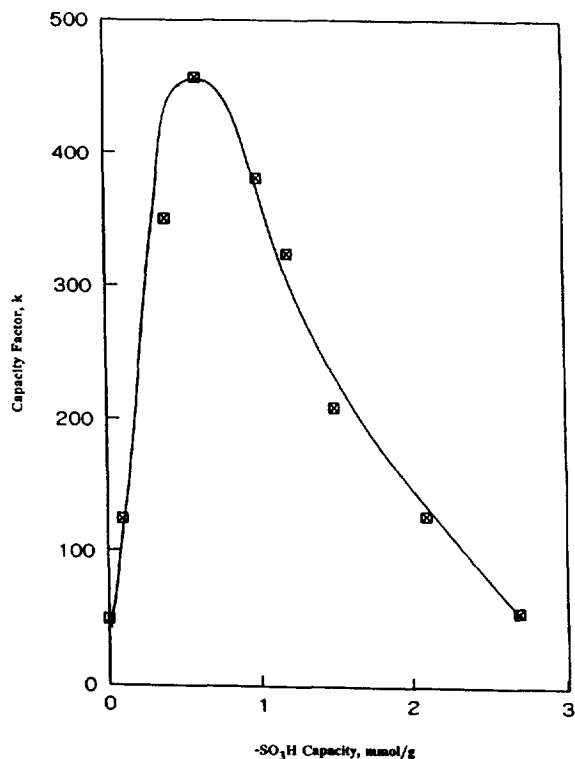
Fig. 1. Capacity factors of phenol on sulfonated resins (aqueous solution).

top area of the circle with no apparent channeling. These properties make it possible to use a very short SPE column with consequent elution of the adsorbed analytes by a very small volume of eluting solvent.

An extensive survey was made of SPE of 44 different analytes (1 ppm each) using sulfonated resin (0.6 mmol/g) incorporated in an Empore membrane [8]. The analytes included several compounds containing each of the following functional groups: alcohol, phenol, aldehyde, ketone, ether, ester, haloalkane, nitrile and nitro. The average recovery was 96% with a relative standard deviation of ca. 3%.

3.3. Simultaneous SPE and group separation of organic solutes

With samples containing many different organic compounds it is often desirable to simplify the analysis by a preliminary separation into neutral, basic and acidic groups. Sulfonation of a polymeric resin converts it into a cation exchanger. If the sulfonation is performed quickly under very mild conditions, the resulting resin retains organic solutes by two different mechanisms. Neutral compounds are retained by sim-

Table 2
Comparison between sulfonated (0.4 mequiv./g) and unsulfonated resins

Compound	Recovery (%)			
	Sulfonated		Unsulfonated	
	Not wetted	Wetted	Not wetted	Wetted
Anisole	94	93	83	89
Benzaldehyde	90	89	87	96
Nitrobenzene	96	95	88	96
Hexylacetate	94	94	84	82
Benzylalcohol	90	98	78	81
Phenol	98	95	77	89
Catechol	59	34	ND	ND
m-Nitrophenol	98	99	89	95
Mesityl oxide	98	97	93	99
tert.-2-Hexenyl acetate	93	90	79	89
Average ± R.S.D. (%)	95 ± 3.2%	94 ± 3.4%	84 ± 5.5%	91 ± 6.3%

Wetting solvent is methanol. Average of three runs. ND = Not detected.

ple adsorption, while protonated bases are retained by an ion-exchange mechanism. Simultaneous concentration of organic compounds thus becomes possible from aqueous samples, followed by selective elution of neutral and basic compounds.

The scheme for separating neutral and basic organic solutes into groups is as follows:

(1) The organic solutes are retained by passing an aqueous sample (adjusted to pH 2.0) through a small column packed with a macroporous resin particles with a sulfonate capacity of approximately 1.0 mmol/g.

(2) Neutral compounds are eluted with methylene chloride and the individual compounds determined by GC.

(3) Basic compounds are eluted by 2 M methylamine in methanol and the individual solutes separated and determined by GC.

(4) A solution of 2 M HCl in methanol is passed through to regenerate the resin. Full experimental details are given in a recent paper [16].

The purpose of the methylamine in the final elution step is to neutralize the protonated organic base cations so that the free base is formed, which is readily eluted by the methanol. The relatively low exchange capacity of the sulfonated resin, as well as the small amount of resin used (ca. 100 mg), permits neutralization of all of the resin protons with a small volume of $MeNH_2$ in methanol.

Initially, 2 M ammonia in methanol was used instead of 2 M methylamine. The ammonia-methanol eluent gave good recoveries of weak bases, such as pyridine and aniline, but the elution of stronger bases, such as alkylamines, was essentially zero. Methylamine is a much stronger base than ammonia and is able to deprotonate alkylammonium cations. The volatility of methylamine is also advantageous, causing the excess methylamine to elute well before the analyte bases in the GC analysis.

Excellent resolution of a number of test compounds into neutral and basic fractions was obtained using this procedure. The recoveries of test compounds were mostly 90–100%.

A similar procedure can be used to concentrate and separate neutral and acidic organic compounds into groups. In this case a macroporous anion-exchange resin of fairly low exchange capacity is used. The resin column is

Table 3
Average recoveries of neutral and acidic organic compounds concentrated from dilute aqueous solution by anion-exchange resin

Neutral compound	Recovery (%)	Acidic compound	Recovery (%)
Ethyl butyrate	101	p-Hydroxybenzoic acid	99
Chlorobenzene	87	Benzoic acid	96
Cyclohexanol	100	p-Nitrobenzoic acid	99
1-Bromohexane	90	1,2,4-Benzenetricarboxylic acid	105
Salicylaldehyde	99	Salicylhydroxamic acid	102
1-Octanol	100	2,4-Dihydroxybenzoic acid	99
Nonylaldehyde	100	Isophthalic acid	100
Triethyl orthopropionate	84	Phenol	87
1-Decanol	96	2-Chlorophenol	95
Benzyl alcohol	93	4-Chlorophenol	99
Octylaldehyde	92	2-Nitrophenol	99
Nitrobenzene	103	3-Nitrophenol	100
Toluene	93	p-Cresol	98
Anisole	96	2,5-Dimethylphenol	94
		4-Isopropylphenol	100

Neutral compounds were eluted with methylene chloride and acidic compounds were subsequently eluted with 1 M HCl in methanol.

treated with dilute sodium hydroxide to convert it to the OH⁻ form. The aqueous sample is also made basic to convert the acidic organic solutes to the anionic form. After passing an aqueous sample through the resin column, neutral solutes are eluted with 1 ml of methylene chloride and measured by GC. Then the acidic substances are eluted by 1 M HCl in acetonitrile or methanol. Excellent group separations of neutral/acidic compounds were obtained and the overall recovery of test compounds was also very high (Table 3).

Acknowledgements

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