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Improved resins and novel materials and methods for solid phase extraction and high performance liquid chromatography

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Improved resins and novel materials and methods for solid phase extraction and high performance liquid chromatography

by

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Ronald Carl Freeze

has met the dissertation requirements of Iowa State University

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For the Major Program

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For the Graduate College

This work is dedicated to my wife, Ann, who has brought so much into my life. Not only did you give me so much of yourself, you also gave us Elizabeth and Anthony. I am happy to say you often made getting this degree difficult. Spending time at home with you and the kids was generally a highlight to my day, and many nights I found it impossible to come back to the lab. When times were difficult, you always provided me with encouragement and reminded me of what was really important. Thanks for creating and sharing so many of the most important moments of my life. I love you.

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ABSTRACT

Solid-phase extraction (SPE) has grown to be one of the most widely used methods for isolation and preconcentration of a vast range of compounds from aqueous solutions. By modifying polymeric SPE resins with chelating functional groups, the selective uptake of metals was accomplished. By reducing the size of the resin beads and optimizing reaction conditions, resins were produced which had excellent capacities both in the packed-column and the suspension mode. By using a suspension of resin and a batch equilibrium system, many problems associated with packed columns were eliminated. By reducing bead sizes to 1 μm, direct injection of the beads into an inductively coupled plasma (ICP) torch was possible. The resin, along with adsorbed metals, was vaporized in the ICP and detection of the metals was then possible using either mass or emission spectroscopy.

Drug analyses in biological fluids have received heightened attention as drug testing is on the increase both in sports and in the work environment. The analysis of drugs in biological fluids usually involves time consuming pretreatment steps for the removal of the drugs from the biological matrix before analysis with HPLC. Pretreatment steps such as liquid-liquid extraction or precipitation not only increase the overall analysis times, they also introduce an additional possibility for error in the final measurements. By using a direct-injection technique, biological fluids can be injected directly into the liquid chromatographic system with no pretreatment.

A new surfactant, a sulfonated form of Brij-30 (Brij-S) is shown to prevent the uptake of serum proteins on commercial HPLC columns by forming a thin coating on the silica C18 surface. Small analyte molecules are separated normally on these precoated

columns. Excellent separations of eight or more drugs with a wide range of retention times were obtained. The separations had sharper peaks and lower retention times than similar separations performed with the surfactant sodium dodecylsulfate (SDS). Quantitative recovery of a number of drugs with limits of detection near 1 ppm with a 5 μl injection volume were obtained. A gradient system resulted in sharper peaks and reduced retention times.

Finally, a method for solid-phase extraction in a syringe is introduced. The system greatly reduced the volume of solvent required to elute adsorbed analytes from the SPE bed while providing a semi-automated setup. SPE in a syringe consists of a very small bed of resin-loaded membrane packed into a GC or HPLC syringe. After extraction, elution was performed with just a few μl of solvent. This small elution volume allowed injection of the eluent directly from the syringe into the chromatographic system, eliminating the handing problems associated with such small volumes.

GENERAL INTRODUCTION

Dissertation Organization

This dissertation begins with a general introduction which contains a comprehensive review of related literature. The introduction is followed by three chapters. The first chapter is based partially on work to be published in a journal along with work done in our lab that has led to the development of a commercially available resin. Due to the proprietary nature of this work, discussion is limited to results obtained with the resin. The final two chapters are based on papers that have been or will be submitted to refereed journals in the field of analytical chemistry. Permission from the publishers extending reproduction and distribution rights has been obtained. A separate introduction, conclusions and references section is presented for each paper. Each paper is similar to the published version, although additional figures and tables have been added. The final section is general conclusions to provide continuity to the entire work.

Solid-Phase Extraction

Sample preparation steps before analysis are often the weakest link in an analytical measurement and require the majority of the overall analysis time. Preparation steps are often required to remove the analyte of interest from a complex matrix or for preconcentration of the analyte to lower detection limits. As analyses are pushed to provide lower detection limits with lower costs in less time, the preparation steps are generally the first area targeted. Common preparation techniques include solid phase extraction (SPE), liquid-liquid extraction (LLE), supercritical fluid extraction, and Soxhlet extraction. Of these methods, LLE is possibly the most widely used preparation technique,

though SPE has proven to be the most attractive choice when dealing with liquid samples. The advantages of SPE over LLE are well documented and include overall reduced costs due to smaller solvent volumes and shorter preparation time, ease of automation, higher extraction efficiencies, and elimination of emulsion problems (1-6).

Since Fritz *et al* (7) first described using a polymeric resin for extraction of phenols from aqueous solutions in 1974, the applications of solid phase extraction have expanded to cover a wide range of analytes (8-10). Bonded-phase silica particles are the most commonly used resins, though porous polymeric resins have also proven to be a strong alternative. Polymeric resins are more resilient to extremes in pH and solvent strength, and have higher surface areas which provide better uptake of organic compounds from solution. A representation of polystyrene/divinylbenzene is represented in Figure 1. SPE consists of a resin bed packed into a small extraction tube, usually made of a plastic. The resin is packed between two frits to hold the resin bed securely in place. The liquid sample is passed through the resin bed by applying either positive pressure or a vacuum to the column (11).

SPE usually involves four steps. The first step in SPE is conditioning of the column. Due to the hydrophobic nature of most SPE resin, a "mediating" solvent such as methanol or acetonitrile is required to wet the resin, allowing intimate contact between the aqueous sample and the resin bed. Once this solvent is passed through the SPE column, the excess is removed from the column with a water rinse. After preconditioning, care must be taken to ensure the column never goes dry. If the mediating solvent is evaporated from the column, reduced recoveries will be observed (12). While preconditioning is required for hydrophobic resins, by adding a polar group to the resin, this step can be

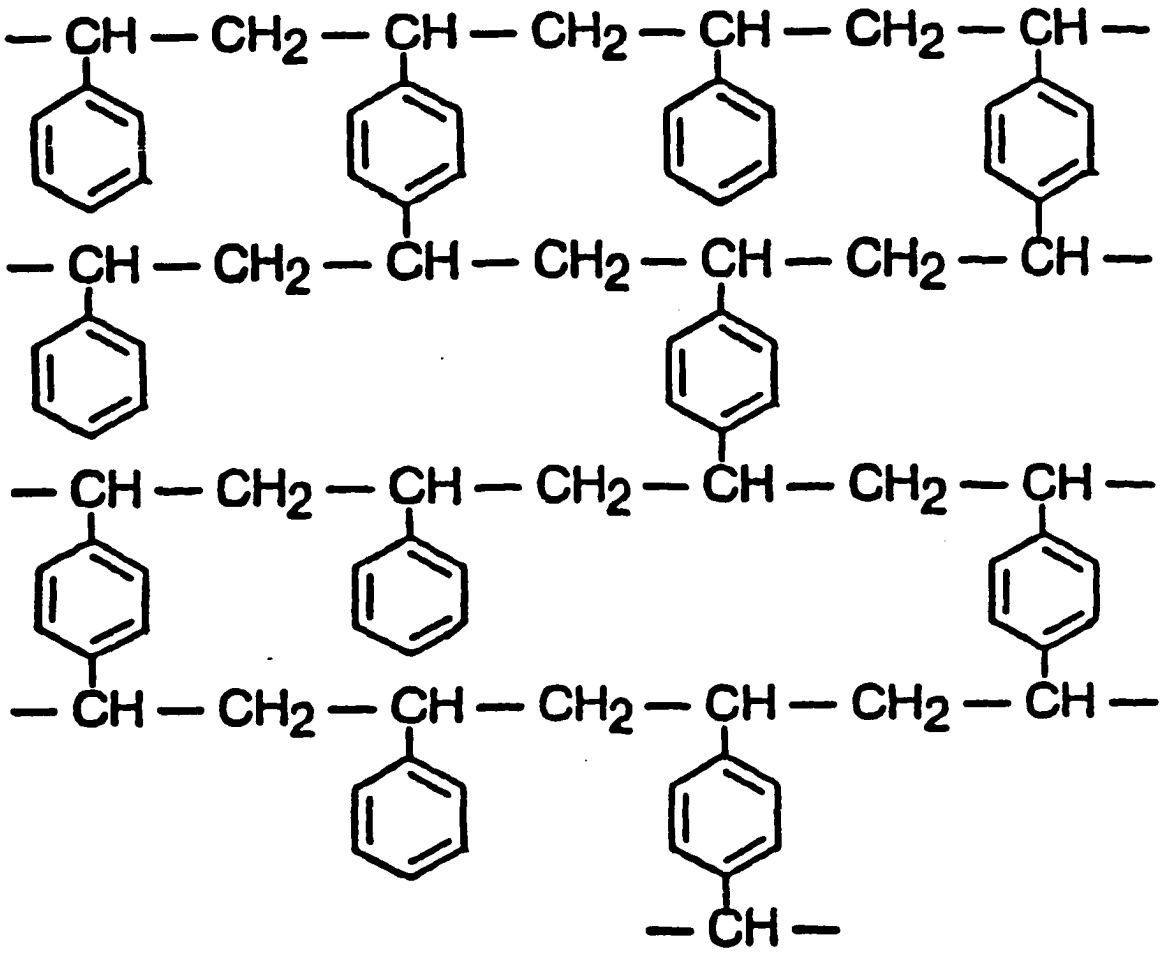

Figure 1. Chemical Structure of Polystyrene divinylbenzene (PS/DVB).

eliminated (13,14).

The first step in SPE consists of loading analytes onto the column. As the aqueous sample is passed through the column, the analyte makes intimate contact with the resin and is attracted to the resin surface. The attractive forces encountered depend on the resin being used and can include dispersive, dipole-dipole, ionic, hydrogen bonding, or even covalent interactions (15). Due to these forces, the analyte is extracted onto the surface of the resin as the rest of the sample matrix passes through the column. These encounters occur throughout the column, resulting in multiple extraction steps. Due to these multiple extractions, SPE is much better at quantitative extraction of the analyte from solution than LLE which has only a single extraction step (15).

The second step in SPE is to rinse the column to assure the unretained matrix is eluted from the column and therefore separated from the analyte. This step can also be utilized to remove some of the retained compounds from the adsorbent, resulting in an even more selective extraction (16). The final step is the elution of the analytes from the adsorbent. For organic analytes, eluting solvents can include methanol, acetonitrile, propanol, acetone, or other organic solvents. With solid phase extraction as little as 100 µl of eluent can be used to elute the compounds from the column, resulting in very large concentration factors. Concentration factors ranging from 10-1000 have been obtained, resulting in very low detection limits (17).

While much of the work involving SPE has focussed on the uptake of analytes from solution using bonded phase silica or polymeric resins (18-27), many resins have been synthesized for the uptake of inorganic analytes. Polymeric resins are easily functionalized by covalently bonding either chelating (28-50) or ion exchange groups (51-

54) to the resin. By first chloromethylating the resin, a methyl-chloride group is added to the ring and is an active site for further derivitization. Chelating resins involve the formation of chelating rings between the functional group on the resin and the metal of interest. These rings are very stable and are often specific for certain metals. A list of chelating resins and their references are listed in Table I. While very good uptake of metals was achieved by these resins, chapter two explores using state-of-the-art resins that have much smaller particle sizes, are carefully cross-linked, and that are more thoroughly chloromethylated than conventional resins.

Though SPE in the column mode is very effective for the uptake of metals, there are drawbacks to using packed resin beds. The primary disadvantages to column SPE include channelling, limited flow rates, insufficient equilibration time for quantitative uptake, incomplete elution, and memory effects from previous extractions. Many of these disadvantages are more pronounced in chelating resins. The formation of chelating rings is a very powerful extraction technique, but the kinetics of this process are often slow. As the sample passes through the column, the time for the formation of these rings is limited by flow rate. If the flow rate is too fast, uptake may be incomplete. Once the metal chelates are formed, elution of the metals from the column may be very difficult. An example of this is the need for warm 4 M HCl to elute chelated metals from dithiocarbamate resins (32). The metals are bound so tightly that the elution is achieved only by destroying the functionality of the resin.

The second section describes a new technique where very small resins are used for improved kinetics and higher capacities. Instead of packing the resin into a column, a resin slurry is injected directly into the sample. Once sufficient time is provided for

Table 1. Chelating resins available for solid phase extraction and related references for each.

Chelating Group	Structure	reference
iminodiacetic acid	R-N($CH_2COO^-Na^+$)$_2$	16-19
dithiocarbamate	R-NHCS_2^-	20-24
8-hydroxyquinoline	C_9H_6NOH	25-27
thiouronium	R-SC(NH_2)NH	28,29
hydroxamic acid	R-CONHOH	30-35
cation exchange	R-SO_3^-	36,37
anion exchange	R-N$(CH_3)_3^+$	38,39

uptake of the metals from solution, the resin is separated from the solution by filtration through a hollow fiber cartridge. This cartridge provides a very large filtration area and therefore a high filtration rate. Once the matrix is separated from the resin, the resin can be injected directly into an inductively coupled plasma (ICP) torch, vaporizing both the resin and the adsorbed metals. The metals that are concentrated on the resin are then detected by mass spectrometry or emission.

Direct Injection HPLC

The use of surfactants as mobile phase additives has been explored extensively (55). The addition of surfactants to the mobile phase results in additional interactions between the analytes and the surfactant in the mobile phase as well as between the analytes and the surfactant adsorbed on the surface of the stationary phase. While surfactants such as sodium dodecylsulfate (SDS) and cetyltrimethylammonium bromide (CTAB) have been used in ion-pair chromatography at concentrations below their critical micelle concentrations (56-58), most work has been done with surfactants above their critical micelle concentration in aqueous solutions. The surfactant is used in place of the organic solvent that is usually required in conventional hydro-organic chromatography. This technique, which was introduced by Armstrong and Henry in 1980, is called micellar liquid chromatography (MLC) (59-61). While many advantages over conventional HPLC were realized with MLC, this technique is often associated with lower separation efficiency than seen in conventional HPLC. This is due to peak broadening that is attributed to slow mass transfer of the analyte from the micelle to the stationary phase (62,63).

Though MLC suffers from lower separation efficiency than conventional HPLC,

the addition of even 1 - 2% of an organic solvent, such as propanol or acetonitrile minimizes this problem (63). Xue and Fritz found that even in mobile phases containing 70% organic solvent a dramatic decrease in retention times is seen when a surfactant is added (64). Xue and Fritz went on to show that by mixing two or more surfactants even greater reductions in retention times are obtained (65). In their study of a variety of surfactants, which are shown in Figure 2, it was found that while SDS is the most widely used additive for MLC, it was actually a poor surfactant when compared to other commercially available surfactants.

As new ways are being developed to use surfactants as mobile phase additives, many papers have explored the use of surfactants as a tool in direct injection of biological fluids onto HPLC columns (66-78). By using a surfactant in direct injection HPLC, the analytical column is protected from the proteins in serum due to a protective coating of surfactant which is deposited onto the stationary phase. This eliminates the need for lengthy and tedious pretreatment such as liquid-liquid extraction or precipitation steps which are otherwise required prior to analysis to remove the proteins from the biological fluids (79,80). The addition of a surfactant also eliminates the need for dilution of the biological sample or the addition of an internal standard (81). It has also been shown that surfactants free drugs which are bound by proteins allowing detection of the total free drug concentration (82,83). If both the bound and free forms of the drug exist upon analysis, peak broadening or even the appearance of a second peak is possible, raising detection limits and making quantification of drug concentrations more difficult. By freeing the bound drug, detection of the total drug concentration in biological fluids is made possible.

Two techniques that rival MLC for direct injection are the use of specialized

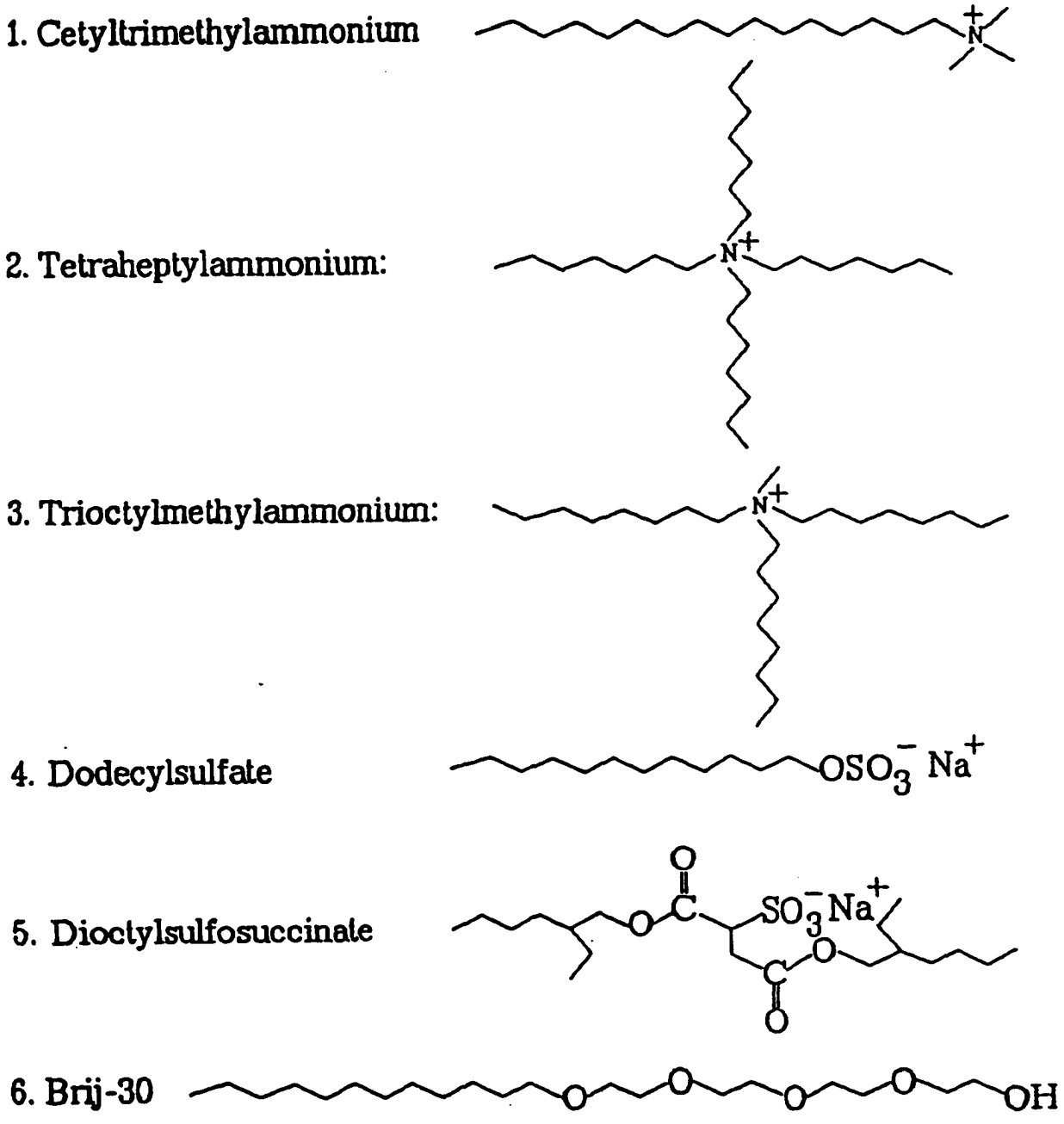

Figure 2. Structures of common mobile phase additives.

stationary phases and using a column switching setup. The area of specialized stationary phases for direct injection has been dominated by restricted access medium columns (84). Several reviews have been published describing this technique (85-88). Restricted access medium is a general term for packing material which employs a hydrophobic interior protected by a hydrophilic barrier. This barrier allows passage of small molecules to the hydrophobic interior while preventing the passage of large molecules, such as proteins (89,90). The drawbacks to these columns include difficulty in column to column reproducibility and a split peak phenomenon under certain conditions due to some of the drug still being bound in the protein (91-93).

Column switching offers the added advantage of preconcentration and a wider choice of eluents, but requires a more complex apparatus (94). Column switching utilizes two columns in series, a precolumn and an analytical column, connected by a switching valve. Once the proteins have passed through the precolumn and to waste, the mobile phase is switched and the retained analytes are eluted from the precolumn onto the analytical column (95-100). The amount of proteins passing through the analytical column is minimized, lengthening column life.

Many papers have explored the use of SDS in direct injection HPLC (101-104). SDS has the advantage over many surfactants of being soluble in aqueous mobile phases. Many of the surfactants that show promise at higher organic solvent levels are limited in their usefulness in direct injection HPLC due to their solubility in water. In direct injection HPLC the highest organic solvent level that can be used is about 20% due to denaturing and precipitation of proteins above this level (105). For this reason the use of MLC for direct injection has been restricted almost exclusively to SDS.

The third section of the dissertation introduces a new surfactant for use in direct injection HPLC. Through a simple sulfonation reaction which was performed in the lab, commercially available Brij-30 is sulfonated. While Brij-30 has a very limited solubility in water, requiring 30% acetonitrile levels to avoid precipitation of a 50 mM solution, the sulfonated form of Brij-30 (Brij-S) can be used in purely aqueous mobile phases at much higher concentrations. Brij-S was found to be comparable to SDS in solubilizing and eluting proteins, and was a much stronger surfactant than SDS when used in drug separations. Dramatic reductions in retention times of late eluting compounds, along with different selectivities for early eluting compounds were observed. Excellent recovery of drugs, good limits of detection, and feasibility of a gradient system were also demonstrated.

Solid-Phase Extraction in a Syringe

As the restrictions on hydrocarbon based solvents increase, a push to minimize the requirements for solvents in extraction techniques is underway. SPE provided a method with much smaller solvent requirements than liquid-liquid extraction techniques. Resin-loaded membranes further reduced the amount of solvent required, often using up to 90% less solvent than traditional SPE cartridges (106,107). This technique still utilized 1-10 ml of solvent, which could still be considered relatively high. Dianna Ambrose introduced semi-micro solid-phase extraction (SM-SPE) which employed very small membrane disks and allowed elution of analytes with just 20-50 µl of solvent (108). While these advances have lead to a system that uses a very small volume in the traditional SPE setting, still more advances were desired.

The recent introduction of solid-phase micro-extraction (SPME) provides some of the advantages of SPE with no eluting solvent required (109-111). In SPME a thin coating of stationary phase is deposited onto a fiber. In a two-step process, the fiber is first exposed to the analytes of interest. These analytes can be contained in either a solution or in the headspace above a solution. Once the fiber is exposed, the analytes partition into the stationary phase based on their partitioning coefficient. The second step of the process involves the thermal desorption of the analytes from the stationary phase into a gas chromatograph (GC) (112-118).

SPME has proven to be a strong alternative to conventional SPE and has developed into a commercially available system (119,120). SPME is similar to SPE in its simplicity, cost, and on-site sampling capabilities while eliminating the need for eluting solvents. The limitations of SPME are found in its extraction step. While SPE is based on exhaustive extraction of analytes from solution, SPME is based on equilibration for extraction. This technique results in less sensitive limits of detection and sometimes difficult calibration is required if quantification is desired. The desorption process has also been optimized for GC thermal desorption. Though a LC desorption system has been introduced (121-123), it relies on a special injection port and a microbore HPLC system.

The final chapter of this dissertation introduces the possibility of SPE in a syringe. In this system, given the name membrane-based micro solid-phase extraction (MMSPE), a much smaller membrane is utilized than any system previously explored. MMSPE allows extraction of analytes on a membrane that has a total volume of less than 0.5 μl and elution of extracted analytes with just a 1.0 - 5.0 μl of eluting solvent Overall recoveries similar to the high analyte recoveries obtained with conventional SPE are realized.

Finally, this solvent is injected directly into either a GC or HPLC system with no required modifications to the systems. Since the syringe in which the extraction is performed is also the syringe used for injection, no tedious collection step of the small elution volumes is required. This eliminates the possibilities of either evaporation of the small volume or partitioning of the analytes from solution into the headspace above the solution. With MMSPE 200-fold concentration factors were achieved with just 1.0 ml of sample solution. Due to the very small elution volume required and the fact that the entire elution volume is used in the analyses, these very high concentration factors are made possible.

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IMPROVED CHELATING RESINS FOR THE SELECTIVE UPTAKE OF METAL IONS

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Abstract

Chelating resins have proven to be very effective for the selective uptake of metals from a wide range of solutions. Generally these extractions are performed by solid phase extraction (SPE) in the column mode. When chelating resins were used in the batch mode, uptake efficiencies remained high due to the strong complexes formed between the resin and the metal of interest. By reducing the size of the resin beads and optimizing reaction conditions, resins were synthesized that had higher capacities and improved recoveries in the batch mode than conventional solid phase extraction resins. Resins which were synthesized and examined included an iminodiacetic acid resin, a resin for the uptake of rare earth metals, a dithiocarbamate resin, and a thiouronium resin. A new, commercially available instrument was used for much of the work. The DSX-100, a fully automated

extraction and analysis system from Cetac Technologies, concentrated metals onto chelating resins and injected the resins directly into an inductively coupled plasma (ICP) torch. Due to the small size of the resin, the beads and the adsorbed metals were vaporized in the torch, allowing analysis by either emission or mass spectrometry.

Introduction

The use of solid phase extraction (SPE) has proven to be a very powerful tool for the extraction and concentration of metals from a wide range of matrixes. Ion exchange resins take up ionic metals as either cations or as ionic complexes when an organic complexing agent is employed. While ion exchange resins are very effective, chelating resins provide many advantages over traditional ion exchange resins. Chelating resins are especially powerful due to their selectivity. This selectivity allows chelating resins to take up trace amounts of metals in the presence of much higher concentrations of interfering ions. With chelating resins, extraction and concentration of metals from matrixes such as extreme pH and salt brines is possible, something impossible with ordinary ion exchange resins (1,2).

While chelating resins are very good at taking up metals, they do have drawbacks. Many chelating resins suffer from slow kinetics in forming chelating rings with the metals of interest. This problem can limit the uptake of the metals by the resin. By switching from the traditional packed-column SPE to a batch mode system, or one where a resin suspension is added directly to the metal-containing sample, extraction time is no longer limited by the flow rate through the column. Once the metals are extracted, problems with elution of the metals from the resin can occur due to the strength of the chelating rings

(3,4). By producing resins of very small size, the beads can be injected directly into a torch for direct detection of the adsorbed metals, avoiding the elution step entirely.

In this work chelating resins as small as 0.2-1.0 μm were derivatized with chelating groups. Very good uptake of metals were achieved in the batch mode. With the commercially available DSX-100 system (Cetac Technologies, Inc.), these very small resins were filtered from the interfering matrix and injected directly into an ICP torch where they were vaporized along with the metals adsorbed on their surface. Metals were extracted from salt brines with both an iminodiacetic acid resin and a resin used for the extraction of actinided metals. The system was completely automated, minimizing the time and labor required for analysis.

Experimental

Reagents

Metal ion solutions were either used as received or made by dissolving the reagent grade metal salt in water. All water used was distilled and then further purified by a Barnstead Nanopure II system (Sybron Barnstead, Boston, MA, USA). All chemicals were obtained from Aldrich Chemical Company (Milwaukee, WI, USA) or Fisher Scientific (Pittsburgh, PA, USA).

Apparatus

The solid phase extraction column apparatus consisted of a 5 mm I.D. mini-column packed with the desired resin. The resin bed was held in place with a 20-μm frit placed above and below the bed. A circle of 0.2 μm filter paper was placed next to the lower frit

to prevent resin leakage. Resin bed depth within the SPE column was generally 4-6 mm. Once packed, the column was attached to a pressure source and 20 p.s.i. was applied to elute solutions from the column.

Batch mode equilibrations were performed by suspending a ten-fold excess of the desired resin in the corresponding metal solution. The solution was mixed and then sonicated for ten minutes to allow sufficient time for complexation. After sonication the mixture was then filtered to separate the resin from the solution. The filtrate was then analyzed for metal concentration and the percentage extraction was calculated.

Metal capacities and percentage recoveries for uranium and copper were calculated by colorometric-complexation with Arsenazo III and 4-(2-pyridylazo)resorcinol (PAR), respectively. The complexed metal concentrations were then determined by measuring the adsorption on a Varian DMS 100S UV-Vis Spectophotometer (Walnut Creek, CA, USA). Once resins were found to be of good quality in laboratory tests, they were sent to CETAC Technologies (Omaha, NE, USA) for further testing.

Two different resins were obtained from Sarasep (Santa Clara, CA, USA) for the derivatization reactions, a 0.5-1.0 μm macroporous chlormethylstyrene-divinylbenzene co-polymer and a 10 μm macroporous polystyrene-divinylbenzene resin. The resins were used as received with only an initial rinse with methanol and water. Blanks were run using only the resins with no metals present, and very low levels of metals were observed in these experiments.

DSX-100 instrument

The DSX-100 system is pictured in Figure 1. The analysis begins with the autosampler injecting a known amount of resin which is suspended in a buffer into the sample vial. Due to the very small resin sizes, no treatment of the resin was required to facilitate suspension. After equilibration the solution is drawn into the hollow fiber filtration device shown in Figure 2. The hollow fiber cartridge consists of a seven fiber bundle. The fibers are composed of a polysulfone, with a molecular weight cutoff of 1,000,000 amu for pore size. The resin is retained on the walls of the porous hollow fiber while the unretained matrix passes through the fiber walls to waste. The resin is then freed from the fiber walls with a burst of water. The resin is then either injected into the ICP torch or sent back into a fresh sample solution, increasing the concentration factors which are possible with the instrument. Normal ten-fold concentration was achieved when 1.0 mL of resin solution was injected into 9.0 mL of sample solution. By repeating this process ten times with the same resin, 100-fold concentration factors were achieved with no additional apparatus.

Resin synthesis

Before further derivatization, the 10 μm polystyrene-divinylbenzene resin was first wetted with acetic acid and then chloromethylated by reaction with paraformaldehyde and hydrochloric acid, as described by Barron and Fritz (5,6).

The iminodiacetic acid resin was produced by the reaction depicted in Figure 3. The chloromethylated resins were first swelled in dimethyl formamide for 24 hours. After swelling, a reaction mixture was used which consisted of 1.0 g of swelled resin, 2.0 g of

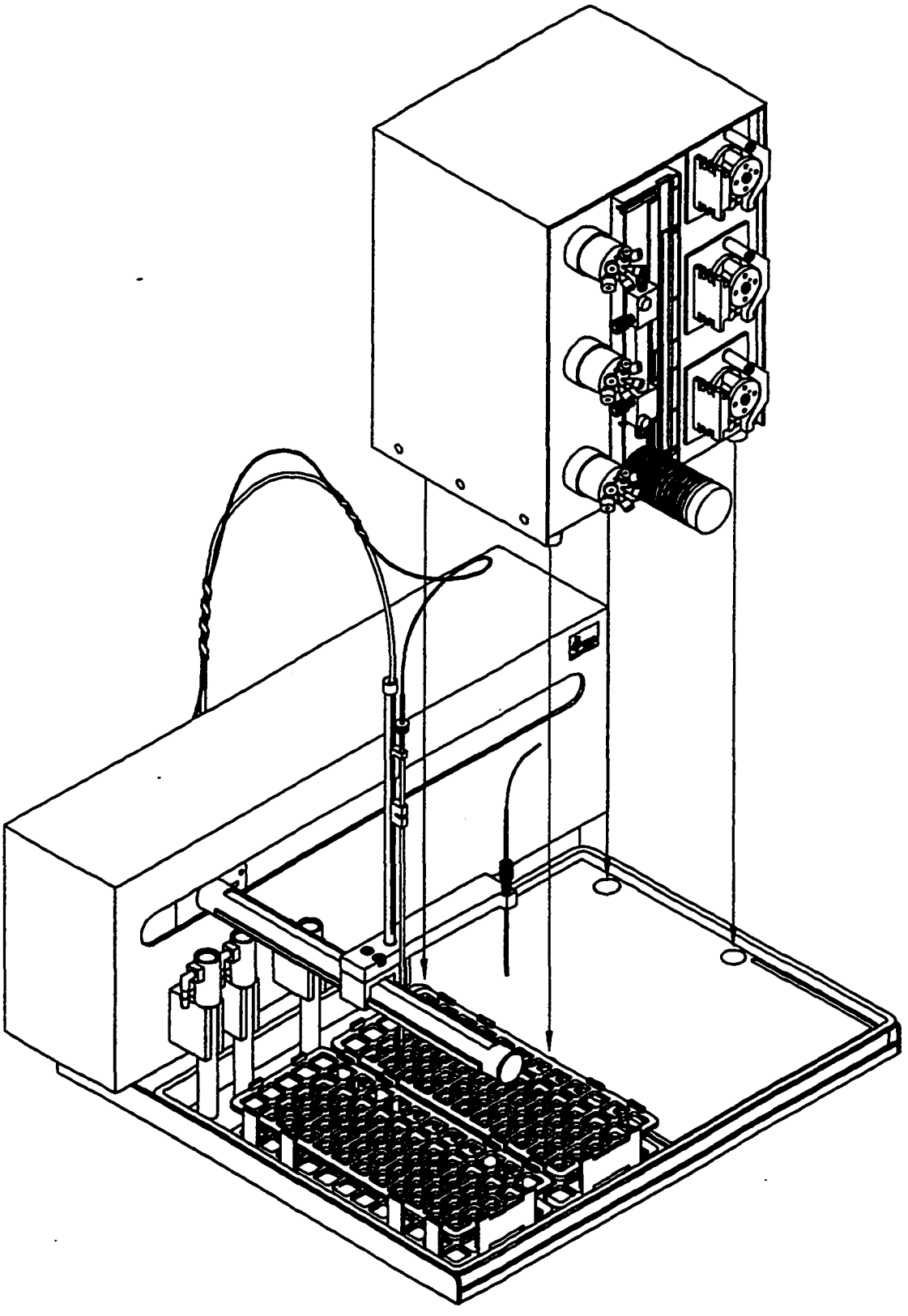

Figure 1. The DSX-100 System, showing the mounting setup in the right half of an autosampler tray.

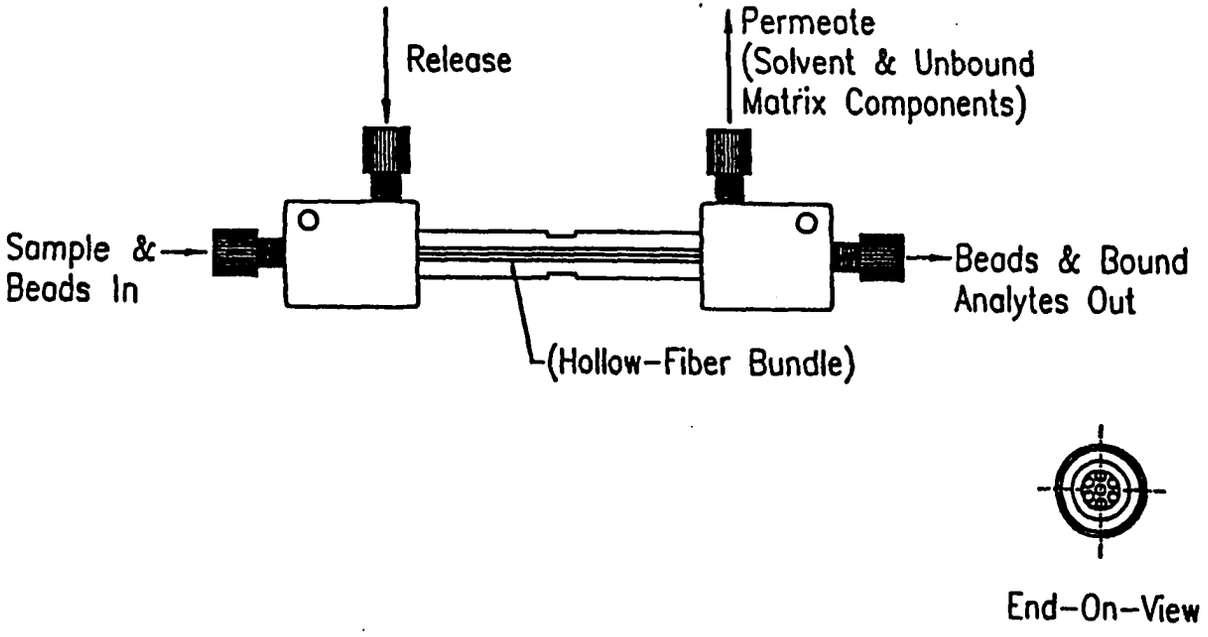

Figure 2. Hollow fiber filtration cartridge for the DSX-100 system.

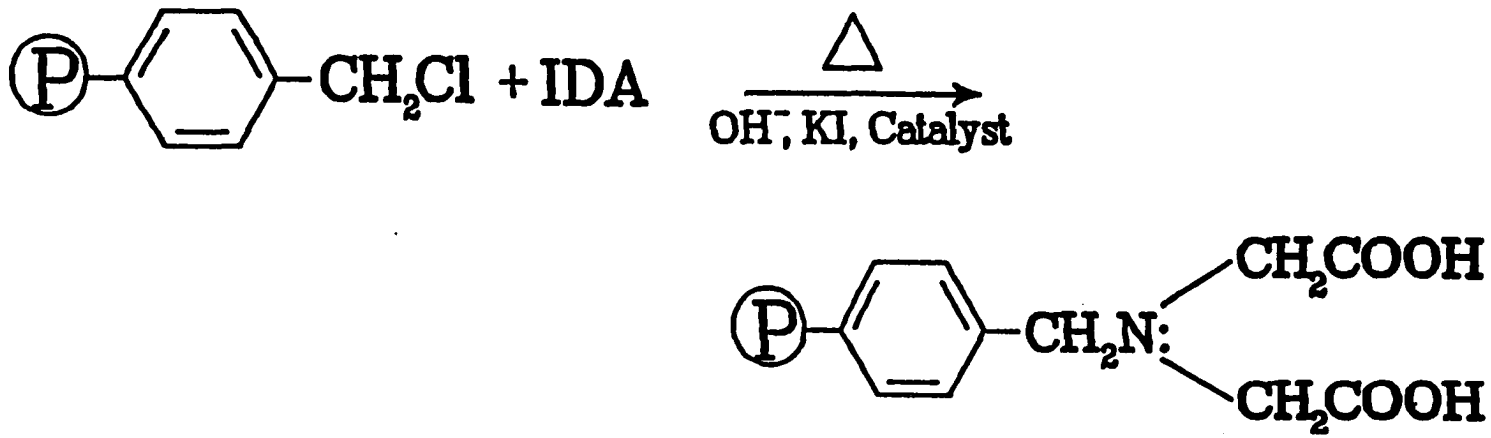

Figure 3. Iminodiacetic acid resin synthesis.

iminodacetic acid sodium salt, 3.0 g of potassium iodide, 1.0 ml of saturated sodium hydroxide, 5.0 ml of deionized water, and 3.0 ml of isopropylethylamine as a catalyst. After a 24 h reaction at 90 °C, the resins were filtered through a ground glass filter and rinsed with isopropanol, water and methanol. The finished resins were then oven dried at 50 °C for eight hours.

Due to the proprietary nature of the resin used to take up rare earth metals from acidic solutions, the reaction will not be discussed in detail. Results, which were obtained for the resin in our lab, at Cetac Technologies, and at Los Alamos National Laboratory will be discussed in the results and discussion section.

The dithiocarbamate resin was prepared by the procedure outlined by Fritz, Freeze, Thornton, and Gjerde and shown in Figure 4 (7). The first step was a reaction of 1.0 g of resin, 5.0 ml of ethylamine, and 1.0 ml of saturated sodium hydroxide to attach a secondary amine to the chloromethylated resin. After a 24 h reaction at 100 °C, the resins were rinsed and dried as discussed above. The aminated resins were then reacted with 10.0 ml of carbon disulfide and 1.0 ml of saturated sodium hydroxide in 10.0 ml of dimethyl formamide. After a 24 h reaction at room temperature, the resin was again rinsed and allowed to dry at room temperature.

For the thiouronium reaction, 2.0 g of the chloromethylated resin was first swelled in 10.0 ml of dioxane for 24 h. After swelling, the resin was reacted with 1.2 g of thiourea which had been dissolved in 20.0 ml of water (8). After a 24 h reaction at 90 °C, the reaction was filtered and rinsed. The reaction is depicted in Figure 5.

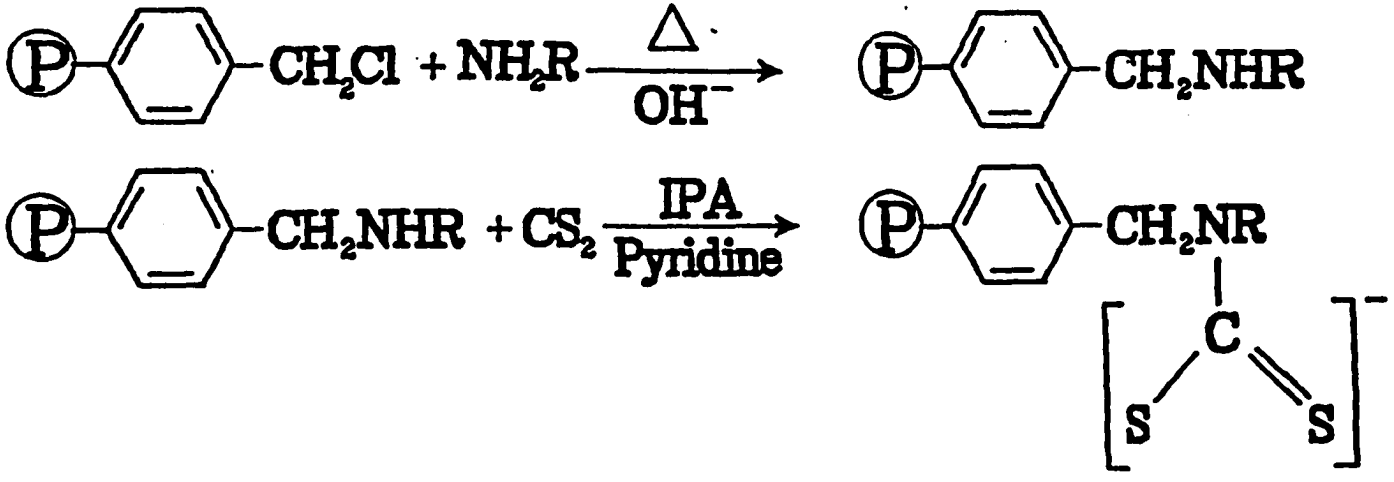

Figure 4. Dithiocarbamate resin reaction.

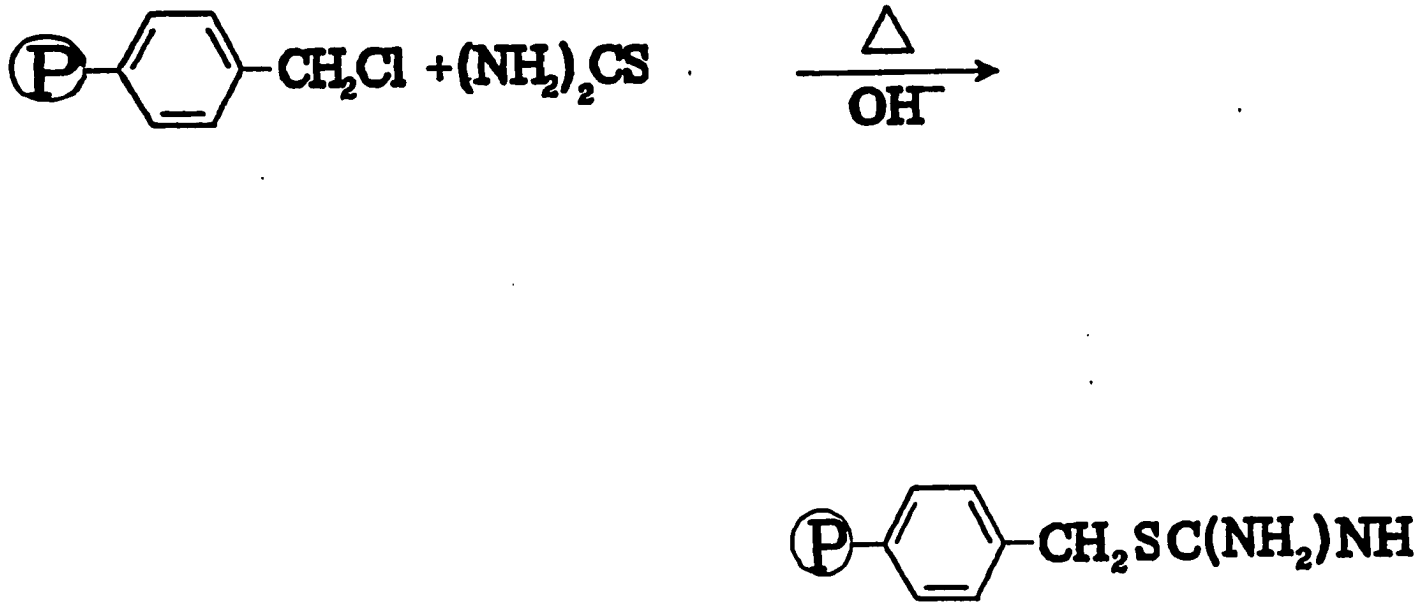

Figure 5. Thiouronium resin synthesis.

Results and Discussion

Iminodiacetic acid (IDA) resin

IDA resin is known to complex a wide variety of metals while discriminating against Group I elements (9-11). Using two different sizes of IDA resin, the capacities and percentage extractions were found for resin packed in a column. For capacity determinations, a 100 ppm solution of copper was passed through a column packed with ~100 mg IDA resin. The capacity of the resin was determined by collecting the effluent from the column and mixing it with PAR for a colorometric detection of copper. Percent extraction was calculated by collecting the first milliliter of effluent from the column and determining the concentration of copper contained in it. Due to the multiple equilibrations in the column mode, the percentage extraction was almost always 100% for the packed column tests. Once the capacity of the resin in a packed column was determined, the batch extraction was performed. To a 10.0 ml solution of 5 ppm copper was added a ten-fold excess of resin. The amount of resin required was calculated using the amount of copper in solution and the previously calculated capacity for the resin. All solutions were buffered at pH 7 with a 10 mM phosphate buffer. The percentage extraction using the resin suspension was also calculated by comparing the initial copper concentration to the concentration of copper in solution after the resin had been filtered off. This information is shown in Table 1. It was found that while a quantitative extraction and a capacity of 1.6 meq/g were obtained for the 10 μm resin in the column, just 39% of the copper was extracted in the batch mode. When using the 0.2 μm resin, a similar capacity of 1.8 meq/g was obtained for the resin packed in a column, but the extraction of copper in the batch mode jumped to 94%.

Table 1. Uptake of copper using iminodiacetic acid resin.

Resin	Capacity	Percent Extraction
10 μm		
Column	1.6 meq/g	100%
Suspension	N/A	39%
0.2 μm		
Column	1.8 meq/g	100%
Suspension	N/A	94%

* all solutions buffered at pH 7

Further work was performed with a similar resin at Cetac using the DSX-100 system. This resin employed an identical IDA group, but was prepared by a slightly different reaction. Figure 6 shows that when the resin was added to a seawater sample there was almost no uptake of the sodium. The sodium concentration was reduced from 27,000 ppm in seawater before DSX-100 treatment to only 30 ppm after treatment. This reduction of the sodium concentration allowed the use of ICP-MS or ICP-AES for the detection of the metal of interest.

The concentration and the matrix elimination effect of the IDA resin using the DSX-100 system is presented in Table 2. All solutions were buffered at pH 7 with a phosphate buffer. When seawater is analyzed by ICP-MS the high salt content degrades the signal obtained for metals by both limiting the focussing of the ion stream and salting up the torch and sampling cones. Deposition of the salt onto the cones also creates the need for regular cleaning of the instrument. This decrease in signal is shown in Table 2. When metals are measured in seawater, only ~50% of the original signal is obtained. When the metals were first extracted and preconcentrated with the IDA resin, the decrease in signal was eliminated and an increase in signal corresponding to the ten-fold concentration was achieved.

Figure 7 depicts the concentration of uranium from tap water using the DXS-100 system. Before treatment, the uranium 235 peak was difficult to measure at just 80 counts/second. After a 100-fold preconcentration step, the peak was increased to an easily measured 7700 counts/second. Again all solutions were buffered at pH 7 with a phosphate buffer.

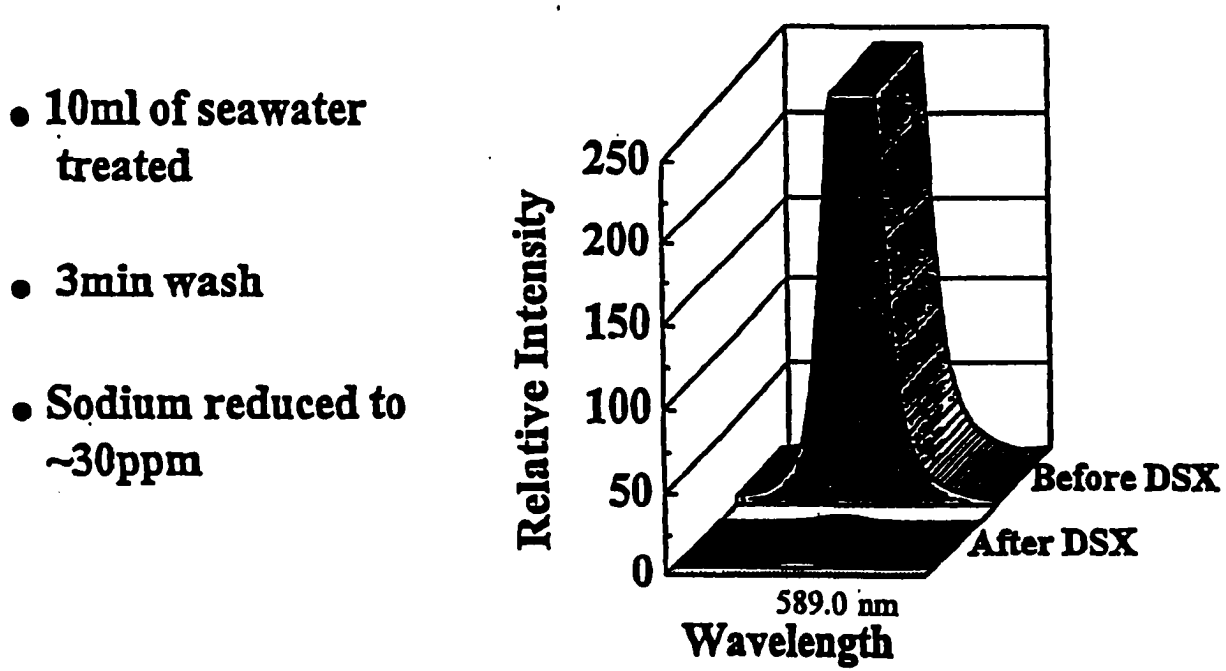

Figure 6. Matrix elimination of sodium using IDA resin. Sodium emission line measured at 589.0 nm.

Table 2. Recovery of metals from seawater using IDA resin.

Element	10 ppb sample	10 ppb sample in seawater	10 ppb sample after 10X preconc from seawater
Co	52,700	24,400	506,000
Cu	13,700	5,500	134,000
Zn	5,220	2,600	55,100
Cd	14,600	6,900	137,000
Pb	32,100	22,400	294,000
U	57,900	29,400	467,000

* All measurement recorded in counts/second

* All solutions buffered at pH 7 with a phosphate buffer

* Analyses done at CETAC TECHNOLOGIES, Omaha, NE

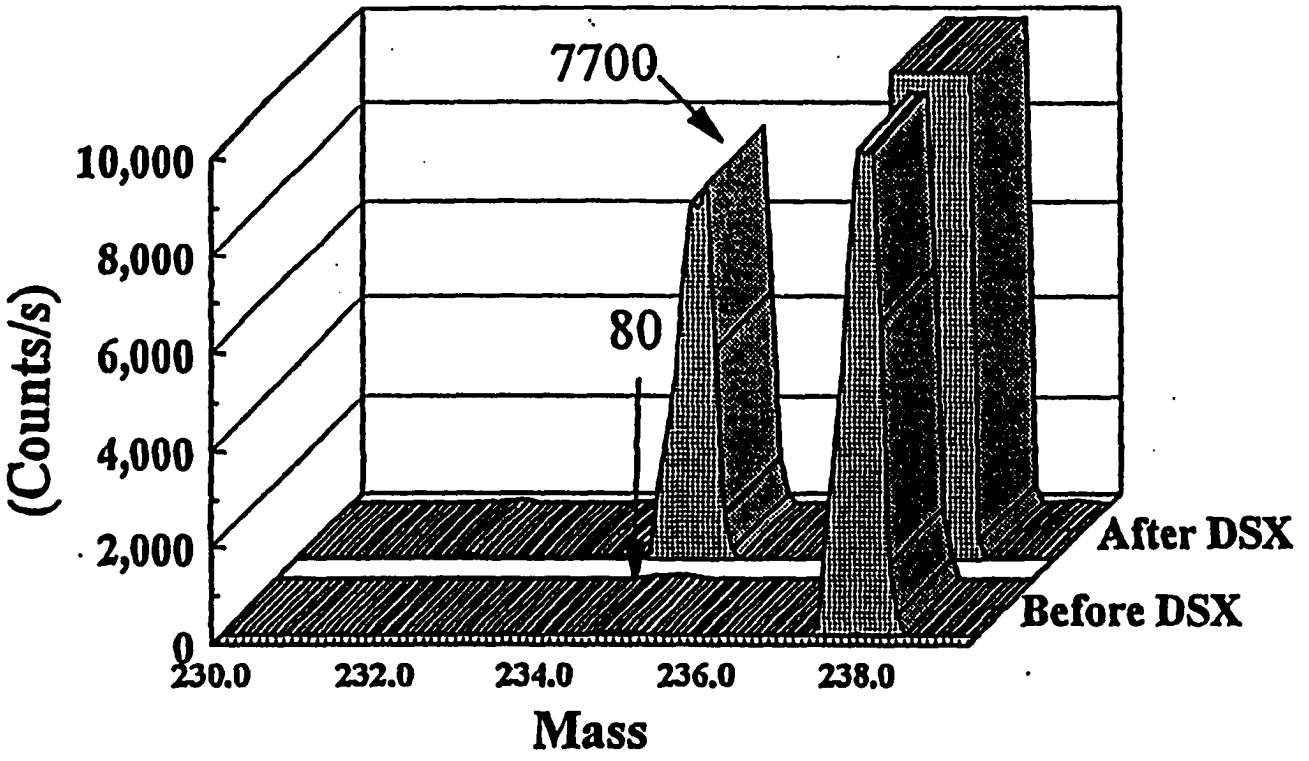

Figure 7. Concentration of uranium in tap water using IDA resin. Analysis performed on the DSX-100 instrument at Cetac, Technologies. Solutions were buffered at pH 7 and a ten-fold concentration step was performed.

Rare earth resin

While the IDA resin is a very powerful chelating resin, at acidic pH the chelating properties are limited (11). To obtain a resin for the uptake of uranium and other rare earth metals at acidic pH, a new resin was synthesized. As with the IDA resin, both a 10 μm and a 0.2 μm resin were produced and compared for their uptake of uranium. A 1000 ppm solution of uranium in 5% HNO_3 was used for the capacity and extraction experiments. Capacities and percentage extractions were calculated as described for the IDA resin. The results of the study are shown in Table 3. While an increase of only 0.40 to 0.43 meq/g was observed in capacity when going from the 10 μm to the 0.2 μm resin, a much larger change in percentage extraction in the batch mode was seen. An 82% extraction of uranium was achieved with the 10 μm resin while a 97% extraction was obtained with the 0.2 μm resin.

When the 0.2 μm resin was used with the DSX-100 system, extraction and concentration of both uranium and thorium was achieved. Table 4 shows a ten-fold concentration step produced a similar increase in signal for both metals. Iron in the sample solution greatly limited the uptake of both uranium and thorium. A solution to this problem was the use of EDTA in the sample as a masking agent. The EDTA binds iron very strongly, preventing its extraction onto the resin. The effectiveness of EDTA masking is shown in Table 4. When a sample was spiked with 20 ppm iron, recoveries of both uranium and thorium fell to near 10%. With the addition of 30 mg of EDTA to the 100 ml solution, signals recovered to >60% of the original signals obtained from solutions that contained no iron.

In work performed at Los Alamos National Laboratories, this resin was tested for

Table. 3. Uptake of uranium using CETAC resin.

Resin	Capacity	Percent Extraction
10 μm		
Column	0.40 meq/g	100%
Suspension	N/A	82%
0.2 μm		
Column	0.43 meq/g	100%
Suspension	N/A	97%

* Metals were concentrated from a 5% nitric acid solution

Table 4. Recovery of thorium and uranium using CETAC resin.

Sample	Th 232	U 235	U 238
resin blank	106	24	3290
Tapwater + Thorium	9340	190	25800
Tapwater + Thorium + 10X concentration	119000	2640	359000
Tapwater + Thorium + 20 ppm Fe + 10X concentration	15480	231	31324
Tapwater + Thorium + 20 ppm Fe + 30 mg EDTA + 10x conc.	113000	1630	220000

*All samples were 100 ml and all measurements were recorded as counts/second

*100 µl of a 10% resin suspension used

*Thorium was spiked at 2 ppb level

*Analyses done at CETAC TECHNOLOGIES, Omaha, NE

the uptake of uranium and thorium from concentrated salt brine. The recipe for one liter of salt brine is shown in Table 5. The brine was made to represent the complex matrix encountered in depleted salt brines. The focus of this work was to monitor leaching of uranium, thorium, and or plutonium from spent nuclear waste stored deep within these mines. While the concentration of salts in these areas are very high, detection of low levels of the rare earth metals, even at the sub-parts per billion range, is required. For these experiments, the brine solution was spiked to 0.1 ppb with the metals of interest. Just 2.0 μl of a 10% suspension of Cetac resin, or 0.2 mg of resin, was used for each milliliter of sample. In Figure 8, the extraction of uranium from the salt brine was tested with the DSX-100 system. The uranium concentration in the salt brine was 0.1 ppb. The relative standard deviation in recovery over eight trials was 7.5% with percentage recoveries near 80%. No definite trend in the signal was apparent, representing complete removal of the resin between analyses and no memory effects using the DSX system.

The effect of sample pH and loading capacity of the Cetac resin were also tested at Los Alamos. Figure 9 shows that the best recoveries for both uranium and thorium were obtained near pH 3.3, providing a 76% recovery for uranium and a 40% recovery of thorium. Finally, Figure 10 represents the loading capacities possible with the resin. Reasonable linearity was observed as the concentration of uranium and thorium was increased from 0 to 500 ppb. This concentration range extends well beyond those expected from the brine solutions.

Table 5. Recipe for 1 liter of salt brine used for uranium and thorium uptake studies.

Compound	Weight/1L of water
$MgCl_2$ - 6 H_2O	292.1 g
NaCl	100.0 g
KCl	57.2 g
Na_3SO_4	6.2 g
$Na_2B_4O_7$-10 H_2O	1.95 g
$CaCl_2$	1.66 g
$NaHCO_3$	0.96 g
NaBr	0.52 g
LiCl	125 mg
RbCl	27.25 mg
$SrCl_2$ - 6 H_2O	15 mg
KI	13 mg
$FeCl_3$ - 6 H_2O	12.5 mg
CsCl	1.25 mg
HCl, 1M	0.15 mL

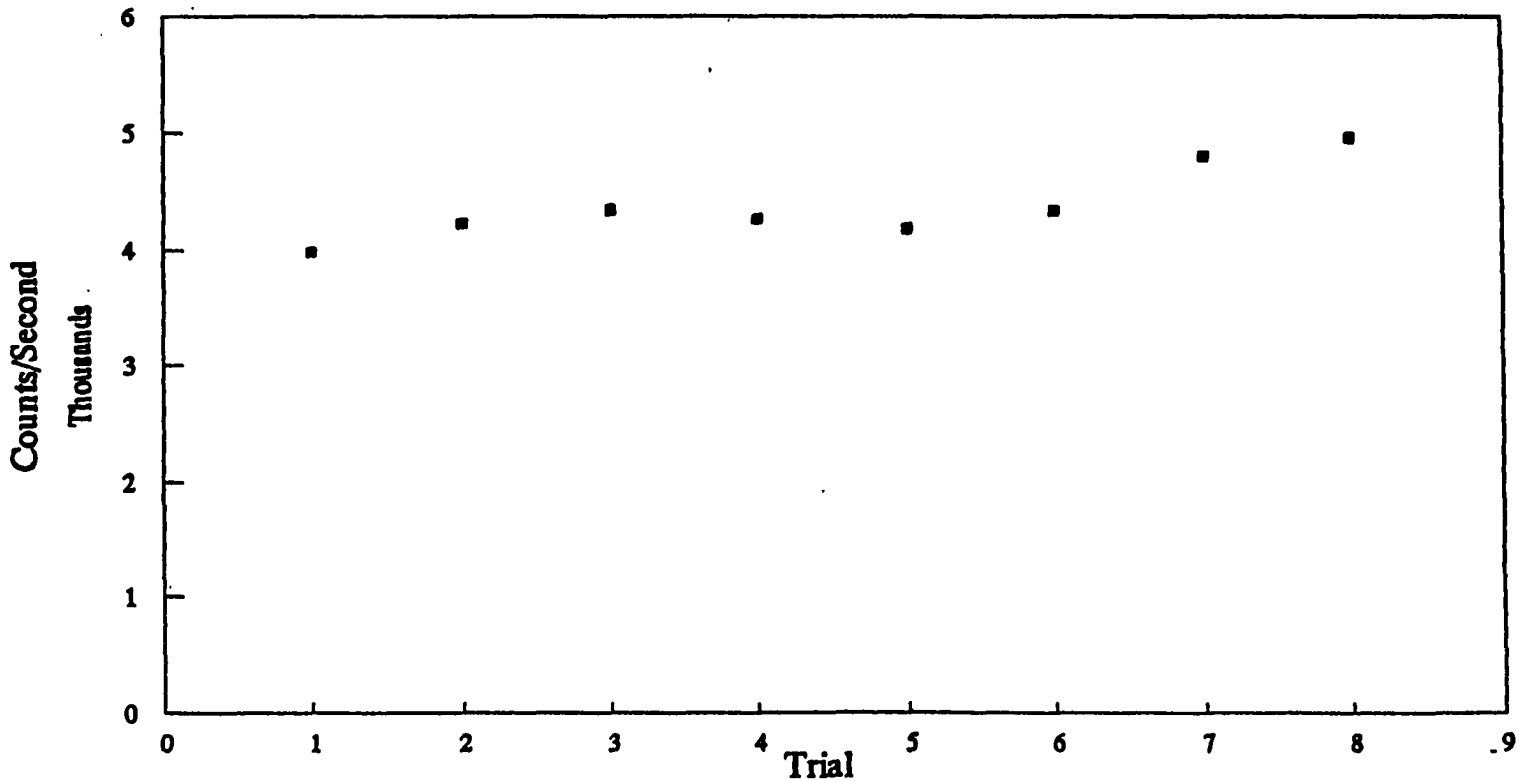

Figure 8. Reproducibility study for the CETAC resin using the DXS-100 system and a 0.1 ppb uranium-spiked brine. All analyses performed at Los Alamos National Laboratories. Solutions buffered at pH 3.3.

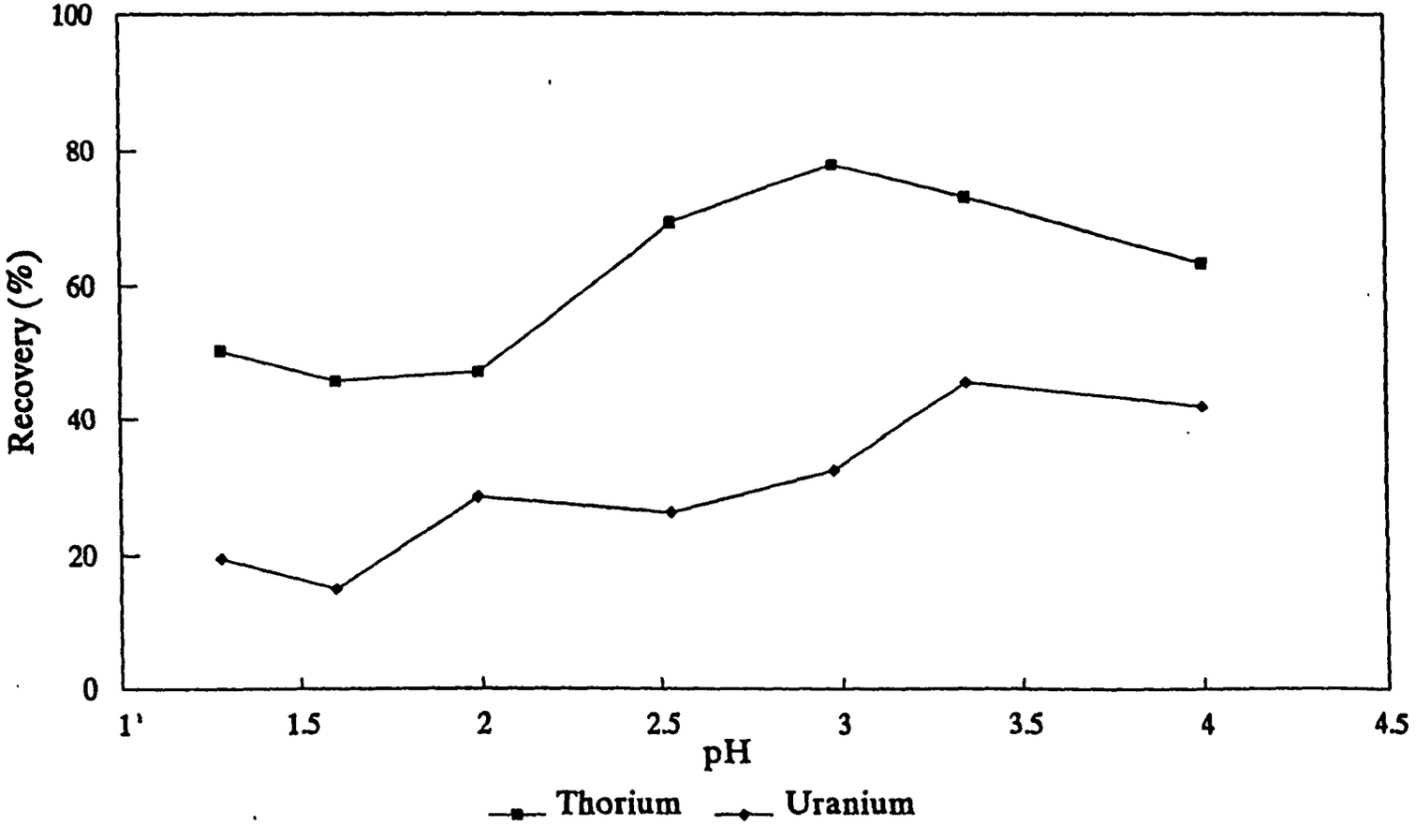

Figure 9. Effect of pH on recovery of 0.1 ppb uranium and thorium from salt brine, using DSX-100 system and CETAC resin. Analyses performed at Los Alamos National Laboratory using the DSX-100 system.

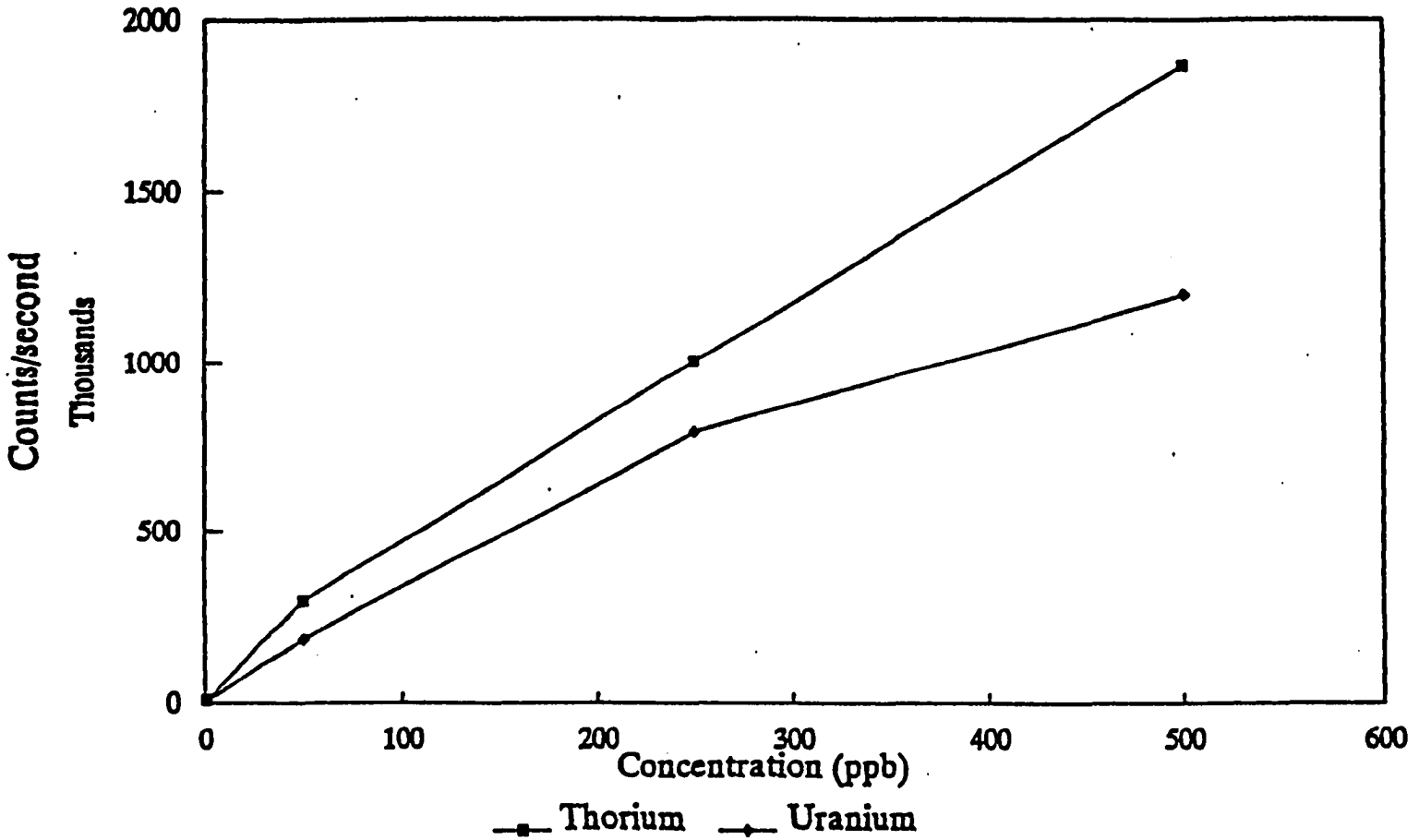

Figure 10. Capacity study of uranium and thorium using 0.2 µl of CETAC resin/ml of sample and the DSX-100 system.

Dithiocarbamate resin

One of the strongest chelating resins contains the dithiocarbamate (DTC) functional group. Not only does the DTC resin form very strong complexes, it also takes up a wide variety of metals (12). When used in the column mode, elution of retained metals from this resin often becomes the most difficult part of the analysis. Reported modes of elution from DTC resin include warm 4 M nitric acid or a solution of bromine in acetic acid (4,8). Both of these elution steps present not only problems in handling the eluted solution, but also difficulties in analyzing the metals contained in the powerful elution mixtures. These elutions steps also destroy the functionality of the resin, making multiple uses of the same resin impossible. By injection the DTC resin directly into an ICP, this elution problem is eliminated.

Copper was used as a representative metal for initial capacity and percent recovery studies. Extraction was performed at a buffered pH of 7 with the determination of capacity factors and percent recoveries performed following the method described for the IDA resin. Table 6 shows the much stronger complexes formed by the dithiocarbamate resin resulted in better recoveries of the metals in the batch mode. While a higher capacity was achieved with the smaller resin, both resin sizes gave very good recoveries in the batch mode.

Thiouronium resin

The final resin studied was one that has proven to be very good for the uptake of gold and platinum group metals while discriminating against other transition metals (13,14). The extraction of metals from acidic solutions is also possible with this resin.

Table 6. Uptake of copper using dithiocarbamate resin

Resin	Capacity	Percent Extraction
10 μm		
Column	0.34 meq/g	100%
Suspension	N/A	>99%
0.2 μm		
Column	0.52 meq/g	100%
Suspension	N/A	>99%

* Solutions buffered at pH 7

Due to the strong chelation properties of the resin, difficulties are encountered with elution. To obtain purified metals with the thiouronium resin, one published procedure utilized ignition of the resin, leaving the purified metals as residue (15). Again, the possibility of injecting the resin directly into an ICP torch avoids the elution problem.

Table 7 shows the uptake of ruthenium from a 5% solution of HCl. A 1000 ppm solution of ruthenium was passed through the resin bed which contained ~100 mg resin. Due to the very strong adsorbance of ruthenium at 458.8 nm, breakthrough was found by monitoring the adsorbance of the effluent from the column. The same procedure outlined for the IDA resin was employed for calculating the percent recoveries for the resin, with adsorbance at 458.8 nm being used for determining the ruthenium concentration. Like the dithiocarbamate resin, this resin also proved to have very good recoveries of metals in the batch mode for both the large and small resin sizes. Recoveries very close to 100% were obtained for ruthenium with both sizes of resin. Platinum, palladium, and gold were also tested and quantitative uptake of the metals was achieved. The results of these tests are shown in Table 8. The very high capacity obtained for gold can be explained in two possible ways. First, any free carboxylic acid groups on the resin will take up gold from solution. This uptake of the gold on a secondary site will produce elevated capacities for the resin. The second explanation for the elevated gold capacity is that the thiouronium resin has the ability to reduce gold to its metallic form (16). This metallic gold was observed on the resin as solid yellow spots.

Table 7. Uptake of ruthenium using thiouronium resin.

Resin	Capacity	Percent Extraction
10 μm		
Column	0.95 meq/g	100%
Suspension	N/A	97%
0.2 μm		
Column	Not Performed	Not Performed
Suspension	N/A	>99%

* ruthenium was contained in a 5% HCl solution.

Table 8. Extraction of platinum group metals with thiouronium resin.

Metal	Capacity	Percent Extraction
Pt^{+2}	0.64 meq/g	100
Pd^{+2}	0.84 meq/g	100
Rh^{+}	0.95 meq/g	100
Au^{+3}	3.8 meq/g	100

* Metals extracted from 5% HCl solutions.

* All extractions calculated based on a column extraction with 10 μm resin.

Conclusions

By going to smaller resin sizes and optimizing reaction conditions, better chelating resins can be produced. Higher capacities in both column and batch equilibrations were found, and much better uptake efficiencies were seen when using these very small resins for the uptake of trace amounts of metals in the batch mode. Although chelating resins often pose the problem of elution of tightly bound metals, injecting the resin directly into a plasma to be vaporized alleviates this problem. This allows the optimization of both capacity and complexation strength in chelating resins. An effective anion exchanger for more general separations was also found to be very effective using this procedure. The resins discussed cover a very wide range of metals and allow for selective uptake of many of the metals through the use of pH control or masking agents. These resins were prepared easily and effectively using only one or two step reactions.

Acknowledgements

We wish to thank Cetac Technologies, Omaha, NE, USA for their funding of this project along with Sarasep, Santa Clara, CA USA for providing all of the resin used in this work.

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IMPROVED DRUG SEPARATIONS IN BIOLOGICAL FLUIDS USING DIRECT INJECTION HPLC

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Abstract

The use of a new surfactant, a sulfonated form of Brij-30 (Brij-S), permits direct injection of biological fluids onto conventional HPLC columns. Brij-S is shown to prevent the uptake of serum proteins by forming a thin coating on the silica C18 surface. Smaller analyte molecules are separated normally on this precoated column. Excellent separations of eight or more drugs in human serum were obtained with this new surfactant. The separations had sharper peaks and lower retention times than similar separations performed with sodium dodecylsulfate (SDS). Brij-S achieved quantitative recovery of a number of

drugs with limits of detection near 1 ppm with a 5 μl injection volume. A gradient system resulted in sharper peaks and reduced retention times.

Introduction

The determination of drugs and drug metabolites in serum and urine is important in clinical chemistry and in pharmaceutical research. High performance liquid chromatography (HPLC) is generally the most popular method for separating and determining the individual drugs. The method usually requires a time-consuming sample preparation step to remove proteins and other matrix components that would interfere with the chromatographic separation by adsorption. Precipitation, ultrafiltration, liquid-liquid extraction, or solid-phase extraction have all been used for sample pretreatment prior to chromatographic separation.

The ideal way to determine drugs in biological fluids is by direct sample injection into a liquid chromatograph without any pretreatment whatsoever. Pinkerton (1) used various materials for HPLC that resist adsorption of protein matter from biological matrices. Perry (2) used an internal-surface reversed phase for direct injection with some success. A number of other approaches have been used with varying degrees of success (3-11).

Several workers have used a micellar mobile phase for direct injection of serum or urine samples into a liquid chromatograph. In these systems a surfactant is used to solubilize proteins in the sample and prevent precipitation or adsorption of the proteins onto the stationary phase of the column. In addition to solubilizing the proteins, the surfactant also displaces drugs which are bound by the proteins. The drugs can then

partition into the mobile phase, allowing detection of the total drug concentration in the sample (12). In general, this approach has been only partly successful. Often only two or three drug peaks have been observed and these tend to fall on the trailing edge of a large protein peak. Almost all of the work in this area has used a single surfactant, sodium dodecylsulfate (SDS).

Li and Fritz (13, 14) found that other surfactants were much better at improving separations than SDS. Dioctylsulfosuccinate (DOSS) and Brij-30 were found to be two of the best surfactants in their study. Although these surfactants were more powerful than SDS, separations were limited to mobile phases having acetonitrile concentrations above 40%.

This work introduces a new surfactant for use in direct injection HPLC. Commercially available Brij-30 is sulfonated in the lab to give the anionic surfactant $C_{12}H_{25}(OCH_2CH_2)_4SO_3^-$(15). This new anionic form of Brij-30, Brij-S, was found to have a much higher solubility in aqueous mobile phases than the non-ionic form. A mobile phase containing 50 mM Brij-S and a low concentration of acetonitrile was found to work extremely well for the direct injection of biological fluids onto an HPLC column (DI-HPLC). The protein peak passes rapidly through the chromatographic column and excellent separations of eight or more drugs can be obtained.

Experimental Section

Chromatographic system

The chromatographic system for the isocratic separations consisted of several components. A Dionex DXP pump (Dionex, Sunnydale, CA) was used to provide a flow of 1 ml/min. A Rheodyne injector (Rheodyne, Berkeley, CA) was used to make 5 μL sample injections. A Krotos Spectra Flow 783 UV absorbance detector (Kratos Analytical Instrument, Ramsey, NJ) was used for detection. Finally a Servago 123 chart recorder, along with a Shimadzu C-R3A Chromatopac integrator (Shimadzu, Kyoto, Japan) for the recovery studies, were used for recording the separations. For the gradient separations an HP 1100 diode array detector system with Chemstation software was used. All separations were performed on a Supelcosil LC-18 column (150 mm x 4.6 mm i.d.).

Reagents and chemicals

Acetonitrile (ACN) was of HPLC grade and used as obtained from Fisher (Fisher Scientific, Pittsburgh, PA). Sodium dodecylsulfate (SDS), Brij-30, sodium hydroxide, and chlorosulfonic acid were reagent grade and obtained from Aldrich (Aldrich Chemical Co., Milwaukee, WI). All drug and serum samples were obtained from Sigma (Sigma Chemical Co., St. Louis, MO). Stock solutions were used to prepare all sample solutions. Dehydrated serum was reconstituted as required with distilled deionized water that had passed through a Barnstead Nanopure II system (Sybron Barnstead, Boston, MA).

Chromatographic procedure

A flow rate of 1 ml/min was selected for all the chromatographic separations. The separation column was equilibrated with mobile phase until the baseline was stabilized. All samples were filtered through a 0.45 μm filter before injection. The eluted species were detected by a UV-visible detector at 254 nm. Capacity factor, k' was calculated according to the expression $k'=(t_r - t_0)/t_0$. The system dead time, t_0, used to calculate the capacity factor, k', was determined by the measuring the time between injection and the first baseline disturbance as described by Dolan (16).

Sulfonation reaction

The sulfonation of Brij-30 was performed in our lab. 18.66 g of Brij-30 was weighed into a reaction flask and cooled in an ice bath to 15 °C. An equimolar amount of chlorosulfonic acid (6.0 g) was added dropwise over 20 minutes, keeping the reaction temperature below 25 °C. After the chlorosulfonic acid addition, the reaction mixture was stirred for an additional five minutes at room temperature. The reaction mixture was then added to 3.2 g of sodium hydroxide dissolved in a 50 g ice slurry. The solution was stirred and 3 M sodium hydroxide was added to adjust the solution to pH 8. The solution was then diluted with distilled deionized water to make a 250 mM solution which was diluted as required for mobile phase preparation.

Results and Discussion

Composition of Brij-S

Sulfonated Brij-30 has been used successfully in capillary electrophoresis for separation of uncharged organic compounds (15) as well as in the present work for HPLC. Sulfonation of several batches of Brij-30 gave products with reproducible properties for both CE and HPLC. However, examination of the sulfonated Brij by mass spectrometry (MS) showed it to be mixture. If the formula of sulfonated Brij-30 is represented as $C_mH_{2m+1}(OCH_2CH_2)_nOSO_3^-$, the mass spectral scan in Figure 1 shows n to range from 0 to 12 with a maximum around n = 3 to 4 represented by the peaks at m/z = 397.7 and 441.7, respectively. The stated value of m is 12 but the MS shows that some of the product mixture has different values of m. Figures 2 and 3 show that by comparison, SDS and DOSS are relatively pure compounds. SDS has just one primary peak, while DOSS has the primary peak and one other peak, probably from the formation of a dimer.

Comparison of SDS, DOSS, and Brij-S

The comparison of SDS, DOSS, and Brij-S for the separation of organic compounds by HPLC using a mobile phase containing 30% acetonitrile supported results obtained by Li and Fritz at higher acetonitrile concentrations (13). DOSS was a stronger surfactant than SDS and the sulfonated form of Brij-30 had a similar eluting strength to underivitized Brij-30. Figures 4-6 show separations using SDS, DOSS, and Brij-S at 30% acetonitrile and buffered at pH 9.2 with a 30 mM phosphate buffer. While the SDS separation took 29 minutes to complete, both the DOSS and Brij-S separations were completed in about 22 minutes. The peak shapes obtained with DOSS and Brij-S were

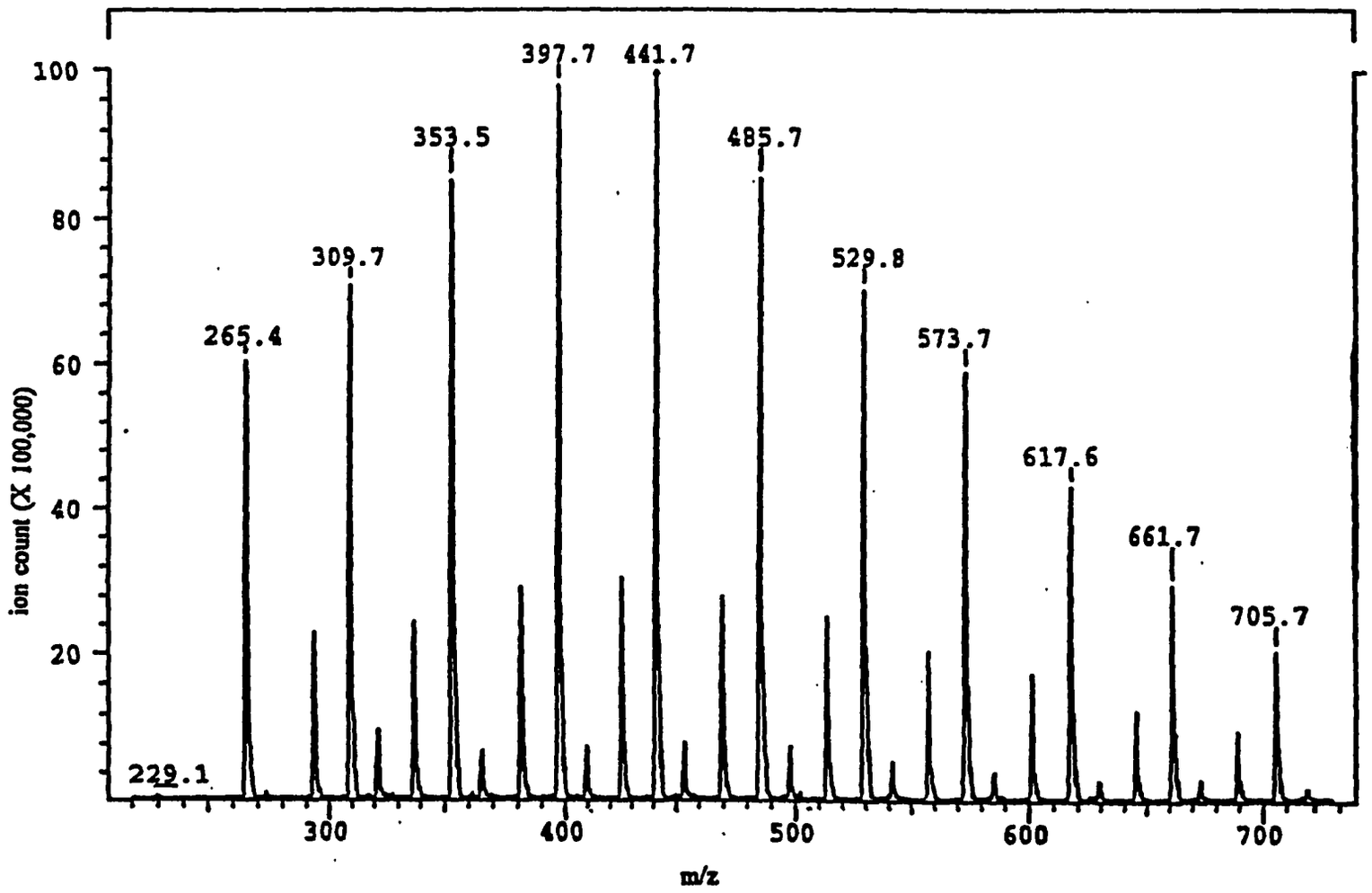

Figure 1. Mass Spectrum of Sulfonated Brij-30. Performed with electrospray ionization in the negative ion mode.

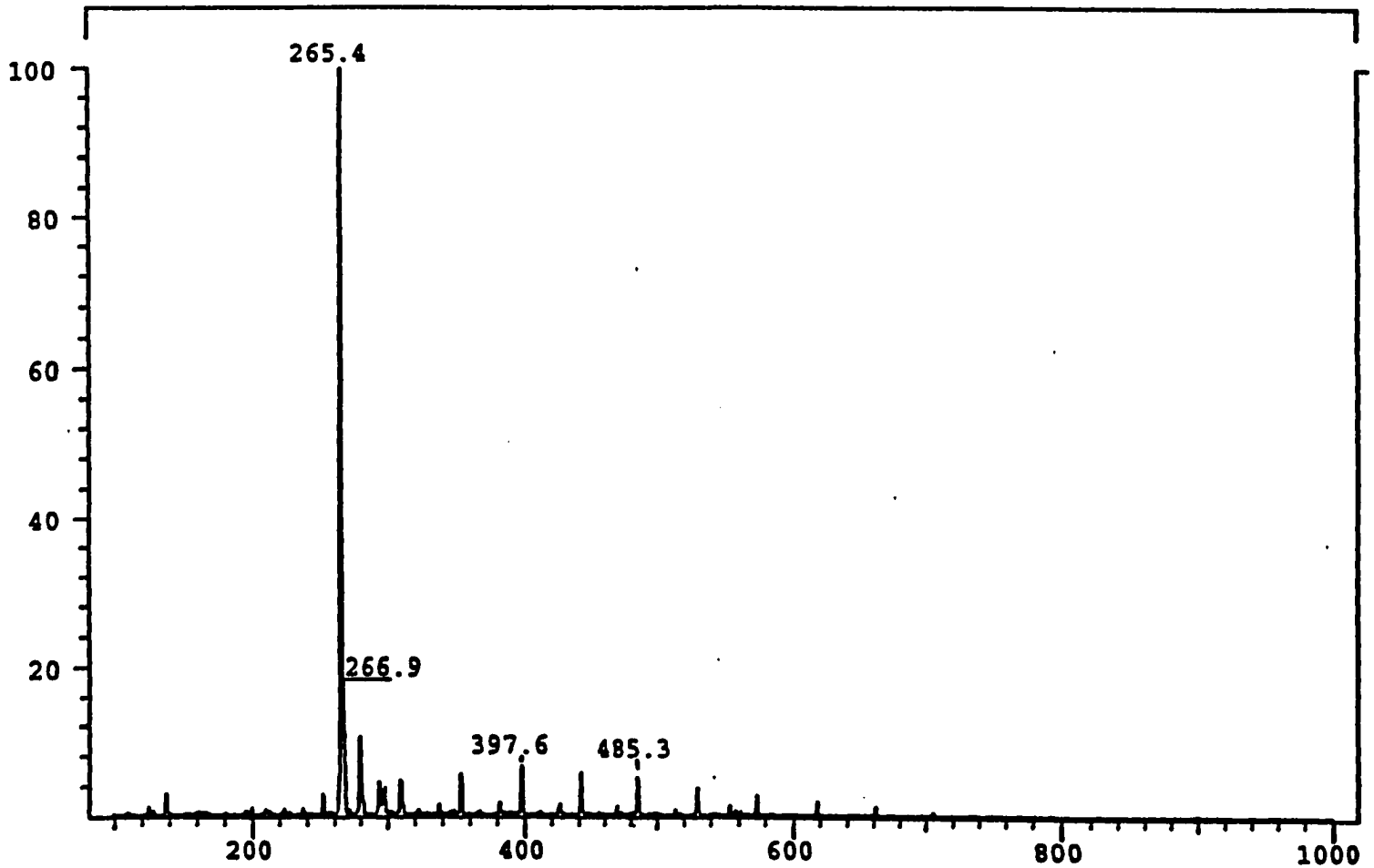

Figure 2. Mass Spectrum of sodium dodecy sulfate (SDS). Performed by electrospray MS in the negative ion mode.

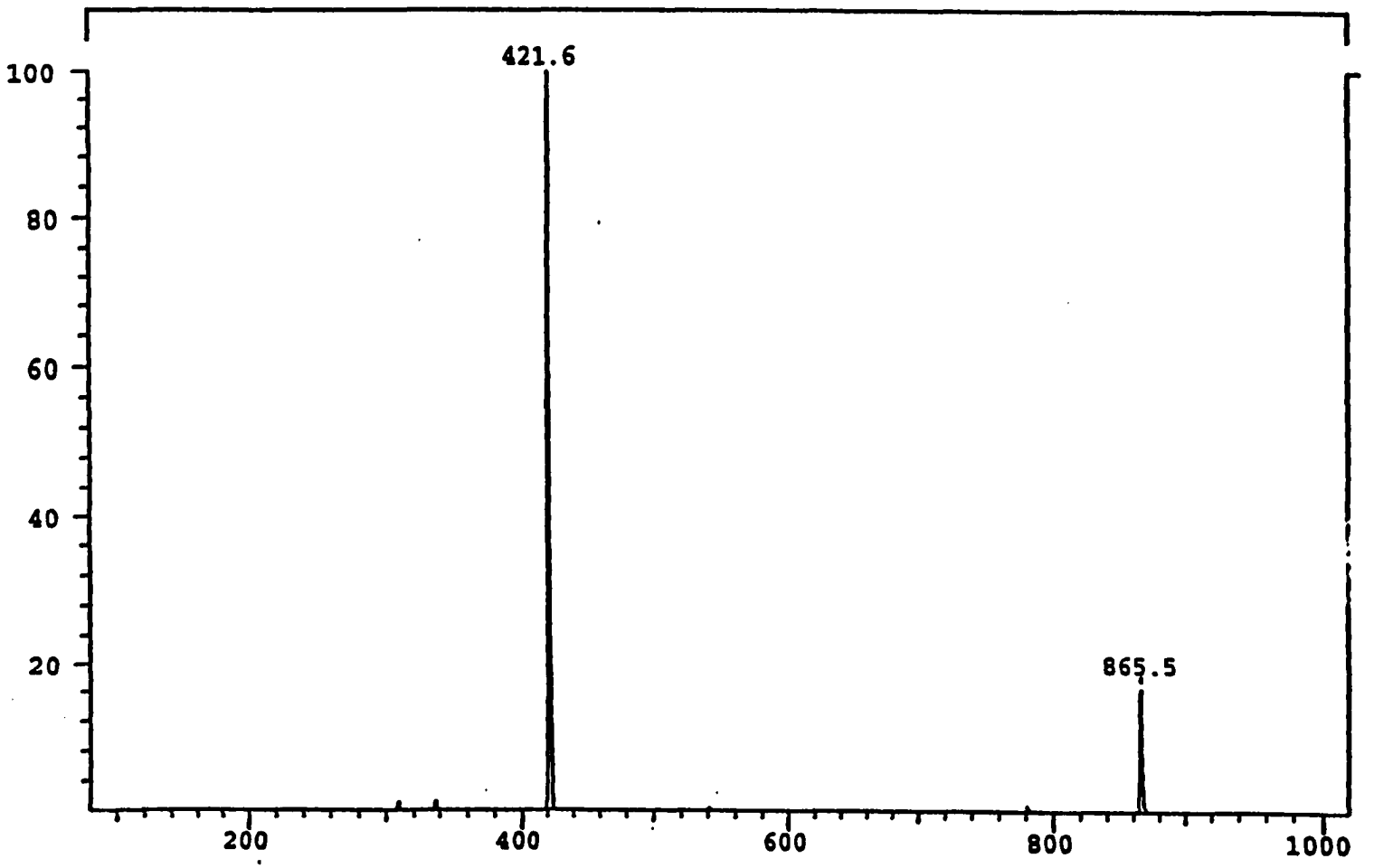

Figure 3. Mass spectrum of dioctylsulfosuccinate (DOSS). Performed by electrospray MS in the negative ion mode.

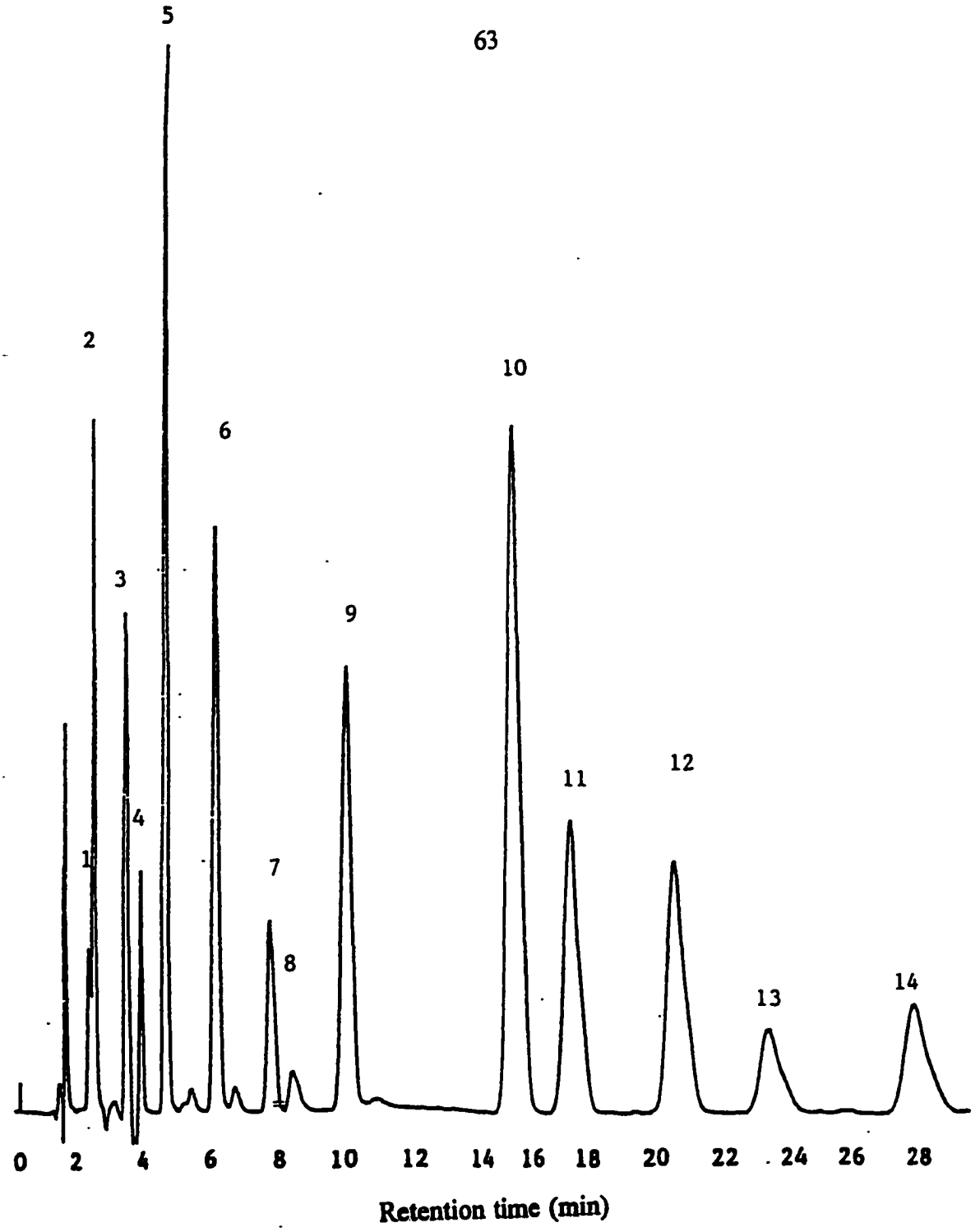

Figure 4. Chromatographic separation on Supelcosil LC-18 (150 mm x 4.6 mm I.D.) column. Eluent: 30% acetonitrile/water, 50 mM SDS, pH 9.2 with 0.3 M phosphate buffer. Flow rate, 1 ml/min; detection, UV at 254 nm. Peak identification: 1 = 4-nitrophenol, 2 = benzoylhydrazine, 3 = phenol, 4 = phenyl ethylalcohol, 5 = 3-nitroacetophenone, 6 = benzothiazole, 7 = benzyl acetate, 8 = butylbenzene, 9 = anisole, 10 = 4-bromo-nitrobenzene, 11 = phenetole, 12 = benzobromide, 13 = chlorobenzene, 14 = bromobenzene.

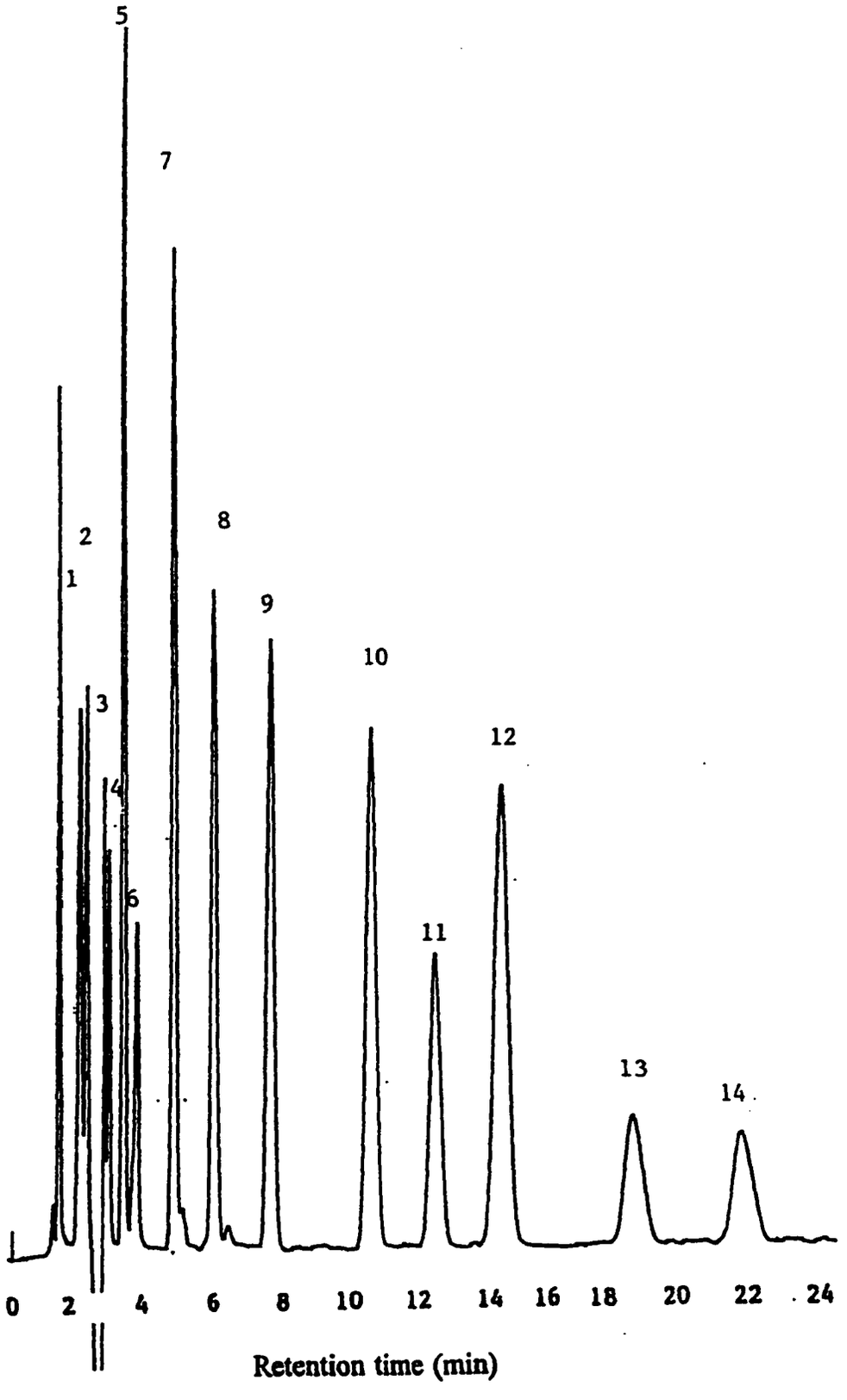

Figure 5. Conditions and numbering identical to Figure 4, but 50 mM DOSS used.

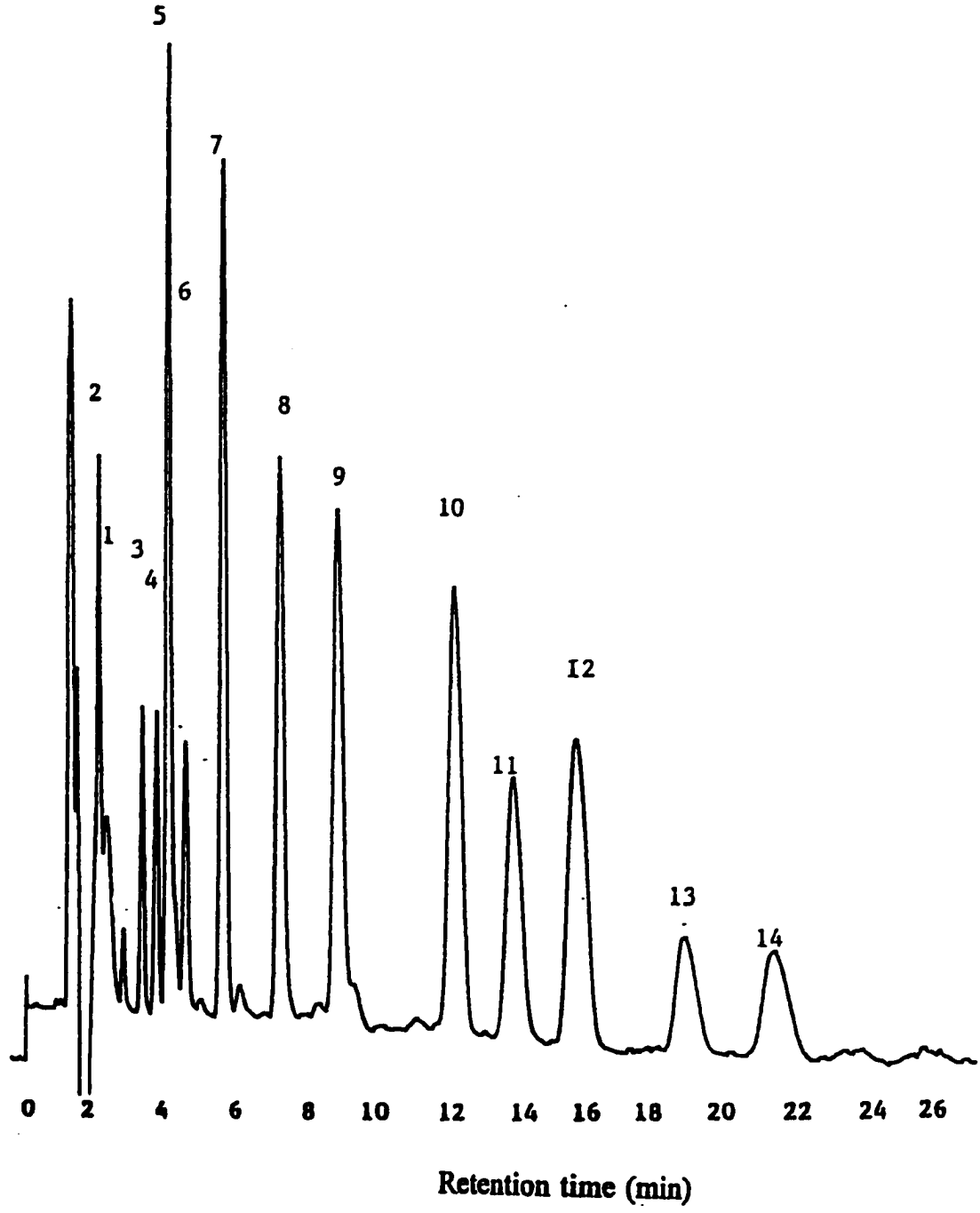

Figure 6. Conditions and numbering identical to Figure 4, but 50 mM Brij-S used.

similar and both surfactants gave improved peak shapes compared to those obtained with SDS. Finally, many peak shifts were observed in the separations performed with different surfactants, indicating complex formation between the surfactants and analytes in solution.

Table 1 summarizes data obtained from similar separations performed in 20% acetonitrile. When comparing retention times to a separation performed with no surfactant, all three surfactants decreased the capacity factors markedly. When a stronger mobile phase was used, the surfactants had less time to affect the separation and smaller differences between the separations obtained with the different surfactants were observed. Table 2 summarizes the results obtained with a 40% acetonitrile mobile phase. While DOSS and Brij-S again proved to be more powerful than SDS, it was apparent from the table that DOSS had a stronger effect on the earliest eluting peaks, while Brij-S was more effective at decreasing the capacity factors of the latest eluting compounds in the separation. This again showed that complexation between the analytes in solution and the surfactants is occurring.

Comparison of SDS to Brij-S for direct injection

A mobile phase containing 50 mM Brij-S in acetonitrile-water (10:90 v/v) at an apparent pH of 9.2 was used for preliminary direct injection experiments. The large organic matrix peak in both serum and urine was eluted cleanly in two minutes or less (Figure 7). Similar results were obtained using 50 mM SDS in 10% acetonitrile at apparent pH 9.2 (Figure 8). However, the mobile phase containing Brij-S gave separations for drugs in serum that were significantly faster, especially for the later-eluting peaks (Figures 9 and 10). This is consistent with findings by Li and Fritz that unsulfonated Brij-30 is a more effective

Table 1. Capacity factors obtained at 20% ACN with different surfactants.

Compound	No Surf	SDS	DOSS	Brij-S
Benzonitrile	10.2	6.0	2.5	4.6
Benzothiazole	12.8	8.9	3.0	6.7
Anisole	26	12.7	4.6	10.9
4-Bromonitrobenzene	na	18.9	11.8	14.3
Phenetole	na	21.1	14.5	16.4
Benzylbromide	na	24.6	16.5	18.4
Chlorobenzene	na	27.1	20.3	20.9
Bromobenzene	na	31.1	23.5	22.8

All additive concentrations were 50 mM. Separations done on a Supelcosil LC-18 column (150mm x 4.6 mm) buffered at pH 9.2 with 30 mM phosphate buffer. All values are capacity factors (k') where $k'=(t_r-t_0)/t_0$.

Table 2. Capacity factors obtained at 40% ACN with different surfactants.

Compound	No Surf	SDS	DOSS	Brij-S
Benzonitrile	4.3	3.1	2.2	2.5
Benzothiazole	4.6	3.4	2.7	3.0
Anisole	7.6	4.8	3.8	4.0
4-Bromonitrobenzene	12.1	6.8	4.8	5.2
Phenetole	12.1	7.7	5.5	5.8
Benzylbromide	14.2	8.2	5.8	6.0
Chlorobenzene	14.2	9.1	7.6	7.4
Bromobenzene	16.7	10.7	9.1	8.4

All additive concentrations were 50 mM. Separations done on a Supelcosil LC-18 column (150mm x 4.6 mm) and buffered at pH 9.2 with a 30 mM phosphate buffer. All values are capacity factors (k') where $k'=(t_r-t_0)/t_0$.

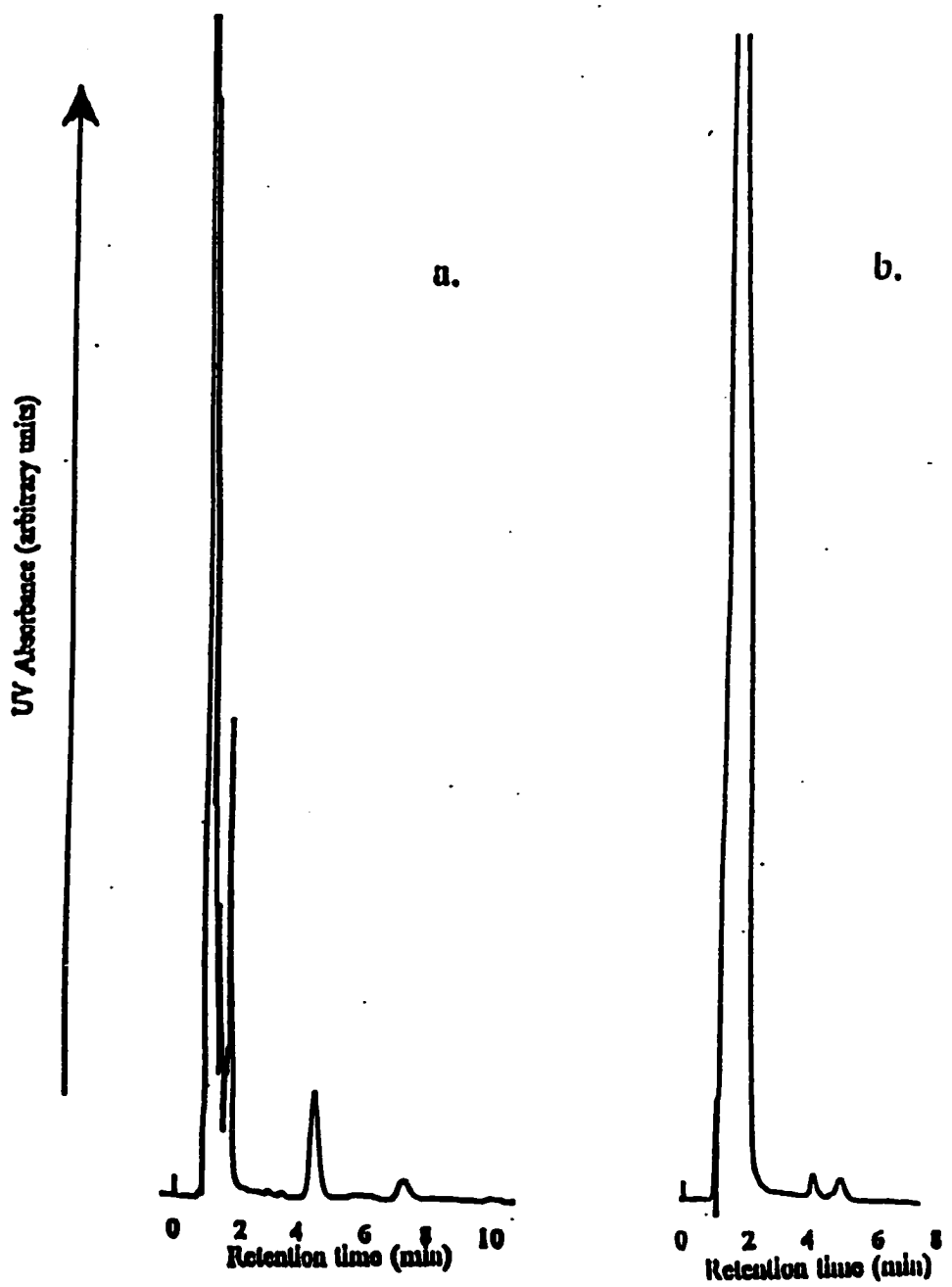

Figure 7. Elution of undiluted (a) serum and (b) urine from a Supelcosil LC-18 (150 X4.6 mm I.D.) column. Eluent, acetonitrile-water (10:90) buffered at pH 9.2 with a 0.03 M phosphate buffer and containing 50 mM Brij-S; flow rate, 1 mL/min; detection, UV at 254 nm.

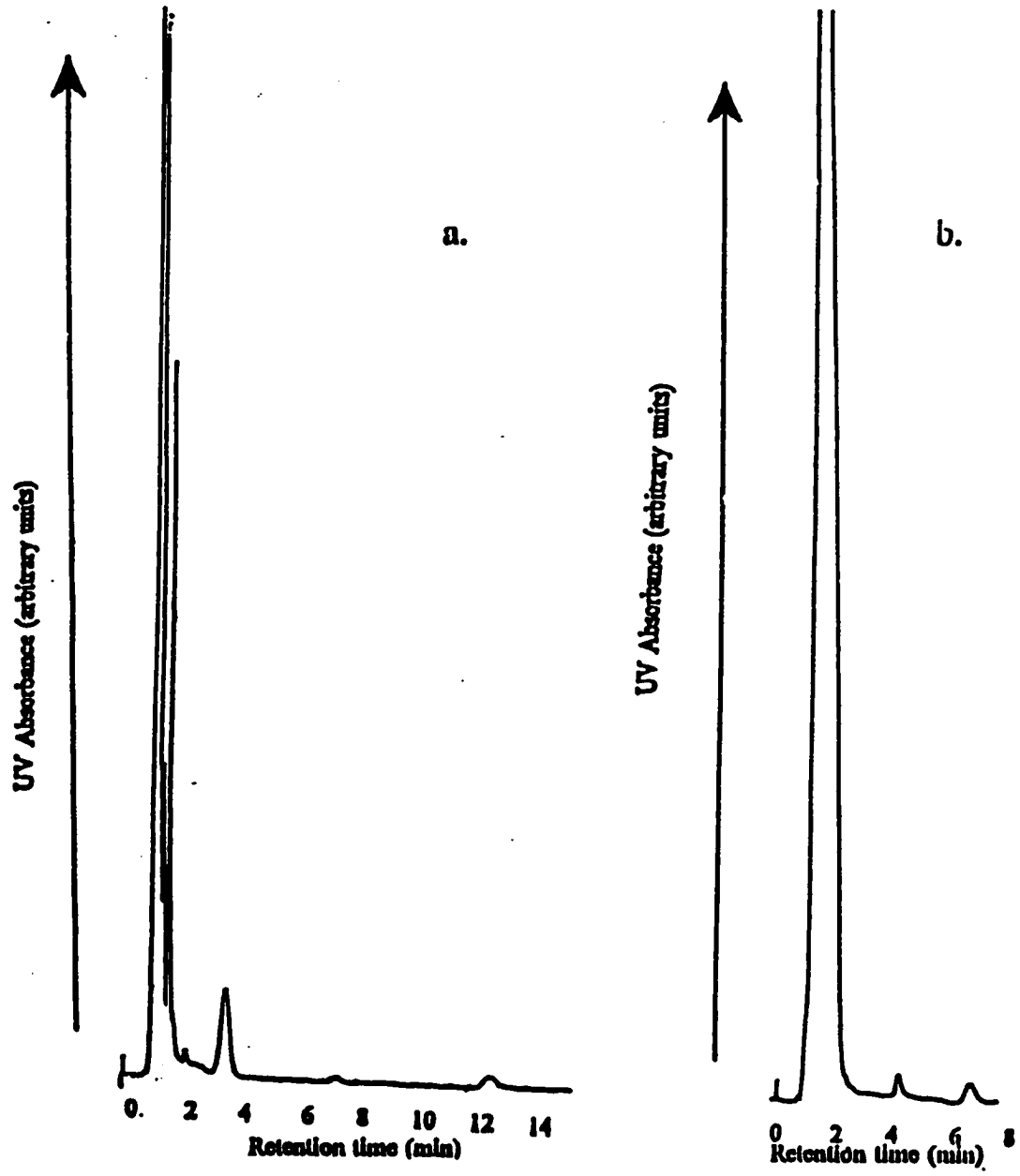

Figure 8. Elution of undiluted (a) serum and (b) urine from a Supelcosil LC-18 (150 X 4.6 mm I.D.) column. Eluent, acetonitrile-water (10:90) buffered at pH 9.2 with a 0.03 M phosphate buffer and containing 50 mM SDS; flow rate, 1 mL/min; detection, UV at 254 nm.

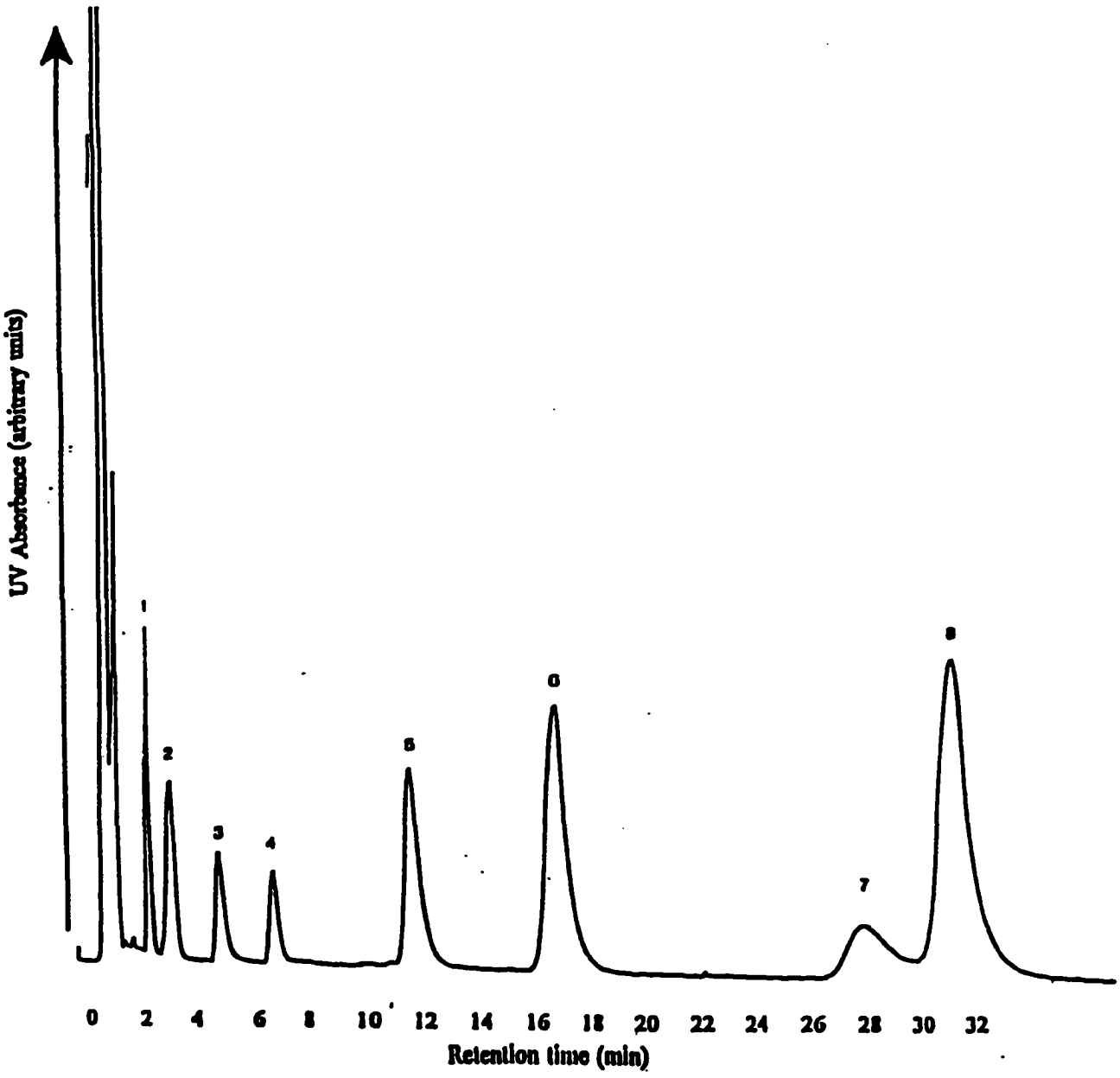

Figure 9. Separation of drugs from serum on a Supelcosil LC-18 (150X4.6 mm I.D) column. Eluent, acetonitrile-water (10:90) buffered at pH 9.2 with a 0.03 M phosphate buffer and containing 50 mM SDS; flow-rate, 1 ml/min; detection, UV at 254 nm. Peaks: 1=trimethoprim, 2=indomethacin, 3=chloramphenicol, 4=carbamazipine, 5=pindolol, 6=nifidipine, 7=lidocaine, 8=benzophenone. All unlabelled peaks are from serum.

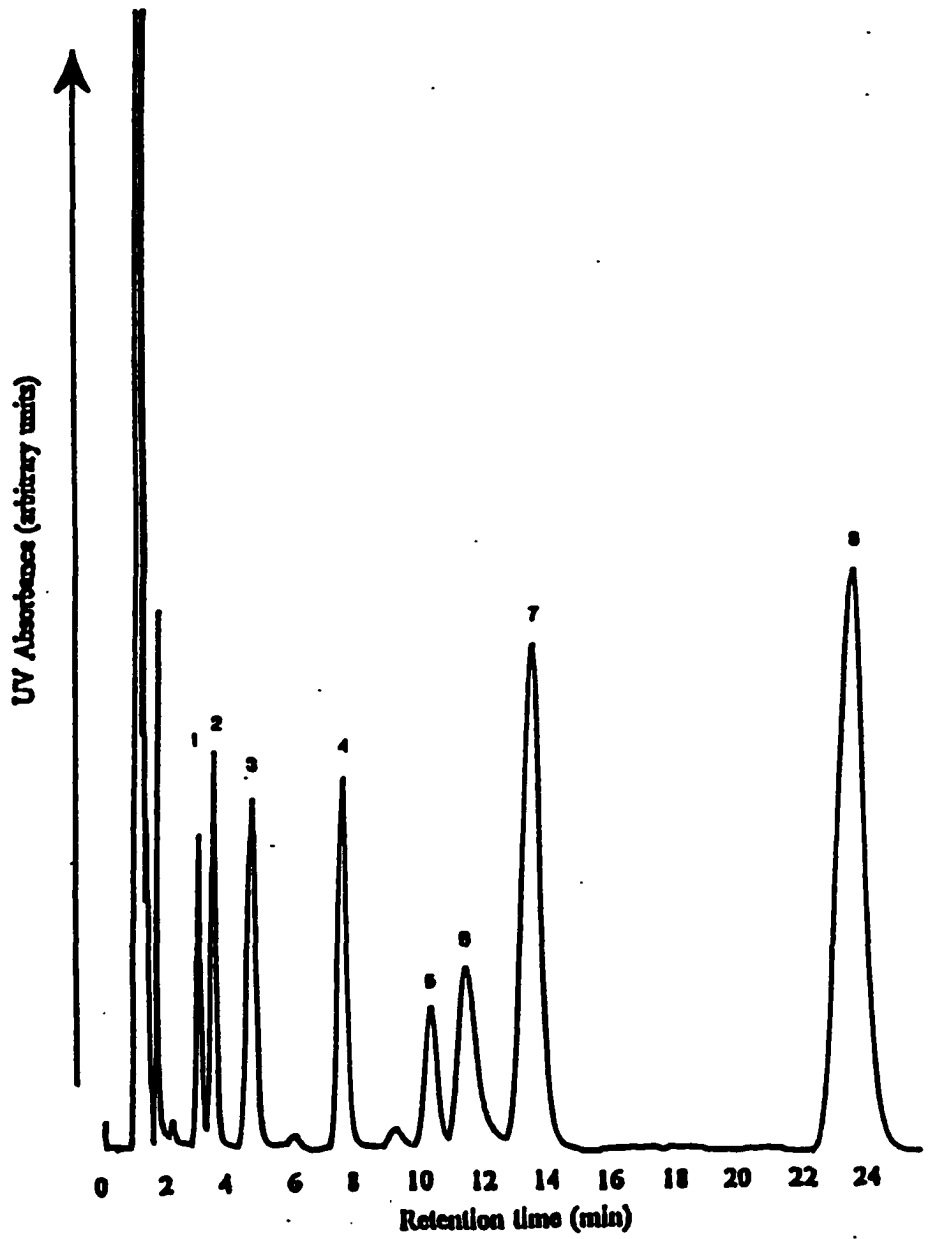

Figure 10. Separation of drugs from serum on a Supelcosil LC-18 (150X4.6 mm I.D) column. Eluent, acetonitrile-water (10:90) buffered at pH 9.2 with a 0.03 M phosphate buffer and containing 50 mM Brij-S; flow-rate, 1 ml/min; detection, UV at 254 nm. Peaks: 1=trimethoprim, 2=indomethacin, 3=chloramphenicol, 4=carbamazipine, 5=pindolol, 6=lidocaine, 7=nifidipine, 8=benzophenone. All unlabelled peaks are from serum.

mobile phase additive than SDS for HPLC in acetonitrile-water mobile phases (13, 14). Capacity factors for various analytes are compared in Table 3 for mobile phases containing Brij-S and SDS. The most dramatic difference is the retention times for lidocaine, which eluted in 11.7 min. with Brij-S compared to 28.6 min. with SDS.

Brij-S concentration

A range of Brij-S concentrations in 10% acetonitrile was tested for the direct injection of serum samples containing several drugs. Although it has been reported (17) that 1 mM SDS can quantitatively elute bovine serum from a μ-Bondapak C18 HPLC column, we were unable to reproduce these results using a Supelcosil LC-18 column, nor could we elute the serum protein using 1 mM Brij-S (Figure 11). Separation of 8 drugs in serum was accomplished with 20 mM Brij-S in 10% acetonitrile as the mobile phase (Figure 12), but the separation took approximately one hour. A much better and faster separation was obtained with 50 mM Brij-S (Figure 10). Elution with 100 mM Brij-S in 10% acetonitrile was faster still, but the first peaks were not resolved and higher column pressures were encountered (Figure 13). These results show not only that the Brij-S is needed for efficient elution of serum proteins, but also that Brij-S plays an important part in eluting drugs from the column.

Effect of solvent type and concentration

Much of the previous work on direct-injection HPLC of serum samples focused on drugs that elute very close to the protein peak (18-20). Relatively little attention was paid to the type and strength of organic solvent in the mobile phase because solvent strength

Table 3. Comparison of capacity factors for drugs in SDS and Brij-S.

Drug	k' in SDS	k' in Brij-S	% change in k'[a]
Trimethoprim	2.00	2.11	+5.5
Indomethacin	2.89	2.78	-3.8
Chloramphenicol	4.89	4.11	-16.0
Carbamazipine	7.00	7.33	+4.7
Pindolol	12.3	10.4	-15.3
Nifidipine	18.1	13.9	-23.3
Lidocaine	30.8	11.7	-62.1
Benzophenone	34.1	25.0	-26.7

Eluent for separations was acetonitrile-water (10:90) and pH 9.2 with a 0.03 M phosphate buffer. All separations performed an a Supelcosil LC-18 (150X4.6 mm I.D.) column.

[a] The percent change was calculated as (% change) = ((k' Brij-S) - (k' SDS))/(k' SDS).

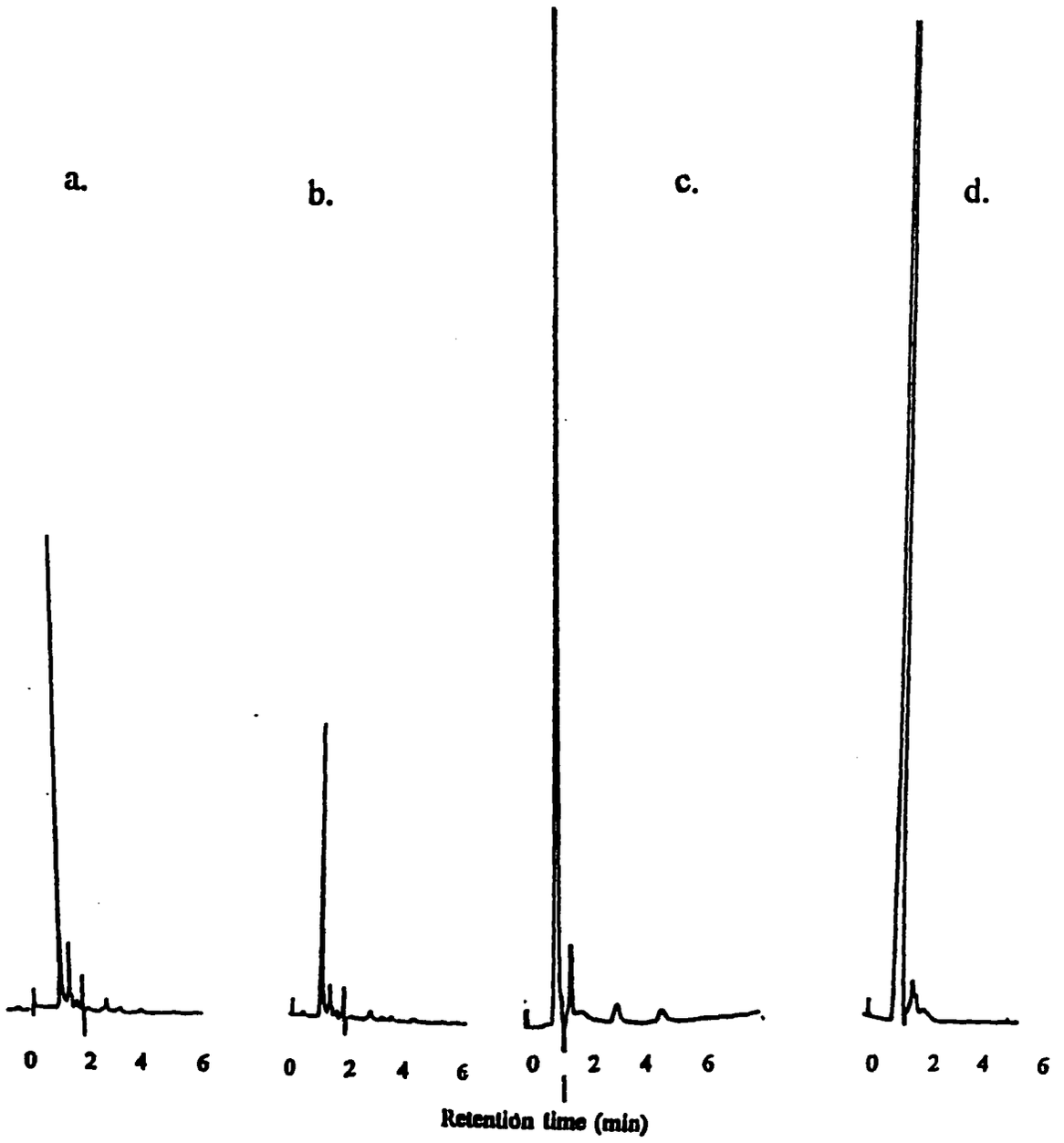

Figure 11. Elution of 50 mg/ml bovine serum albumin with (a) 1 mM SDS, (b) 1 mM Brij-S, (c) 20 mM Brij-S, and (d) 50 mM Brij-S on a Supelcosil LC-18 (150 X 4.6 mm I.D.) column. Eluent, acetonitrile-water (10:90) buffered at pH 9.2 with a 0.03 M phosphate buffer; flow rate, 1 mL/min; detection, UV at 254 nm.

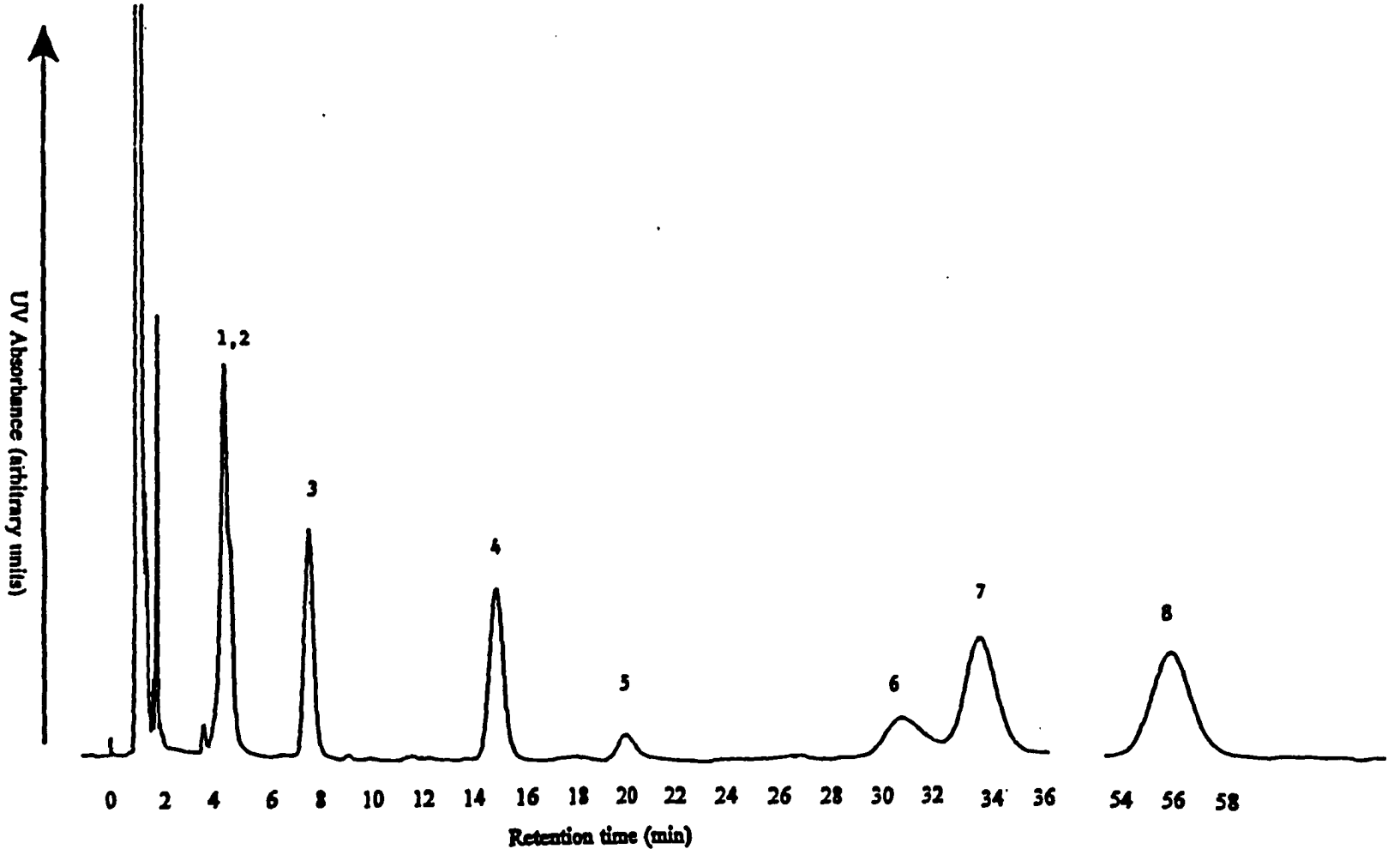

Figure 12. Separation of drugs from serum on a Supelcosil LC-18 (150X4.6 mm I.D) column. Eluent, acetonitrile-water (10:90) buffered at pH 9.2 with a 0.03 M phosphate buffer and containing 20 mM Brij-S; flow-rate, 1 ml/min; detection, UV at 254 nm. Peaks: 1=trimethoprim, 2=indomethacin, 3=chloramphenicol, 4=carbamazipine, 5=pindolol, 6=lidocaine, 7=nifidipine, 8=benzophenone. All unlabelled peaks are from serum.

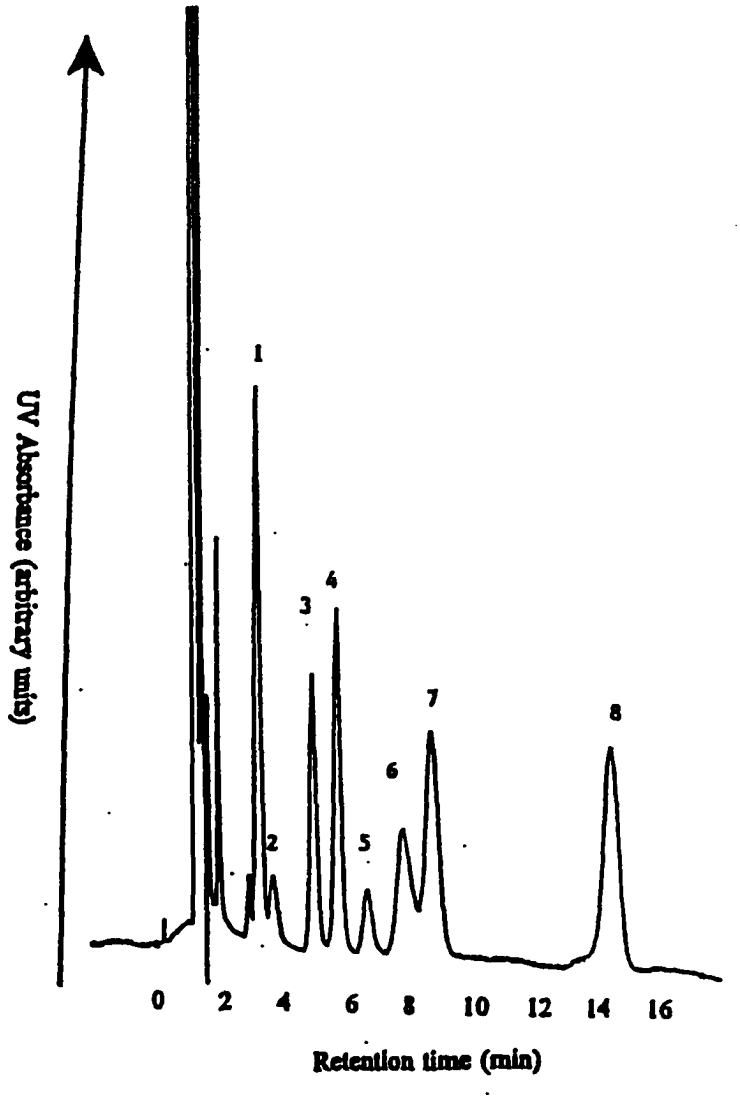

Figure 13. Separation of drugs from serum on a Supelcosil LC-18 (150X4.6 mm I.D) column. Eluent, acetonitrile-water (10:90) buffered at pH 9.2 with a 0.03 M phosphate buffer and containing 100 mM Brij-S; flow-rate, 1 ml/min; detection, UV at 254 nm. Peaks: 1=trimethoprim, 2=indomethacin, 3=chloramphenicol, 4=carbamazipine, 5=pindolol, 6=lidocaine, 7=nifidipine, 8=benzophenone. All unlabelled peaks are from serum.

is not very crucial for these early-eluting peaks. However, a broader range of drugs was studied in the present work, including many with longer retention times. Three different solvents were compared for elution of drugs from serum samples: methanol, n-propanol, and acetonitrile. The effect of acetonitrile concentration on the separations was also explored.

Table 4 summarizes the capacity factors obtained with 10% of methanol, propanol, or acetonitrile for the drug separations. Methanol was found to be the weakest solvent for elution of the drugs, while propanol and acetonitrile gave similar results. Though propanol and acetonitrile gave similar retention times, the peak shapes obtained with acetonitrile were slightly better than those obtained with propanol. For this reason, acetonitrile was used for the remainder of our work. The capacity factors obtained for separations performed with mobile phases containing 0, 10, and 20% acetonitrile are also listed in Table 4. While the aqueous separation provided good peaks for the earliest eluting compounds, the later peaks were broader, and the overall analysis time was extended by six minutes. With 20% acetonitrile, the first three peaks were not well resolved, but the later peaks were sharper and eluted sooner. The best separation for our drug mixture was obtained with 10% ACN as shown in Figure 10.

Effect of pH and buffer strength

Analysis of serum samples at pH values near pH 7 or higher has been recommended to avoid precipitation (18). We found that a pH of approximately 9 provided the best separations. Both peak shape and retention times were much better at pH 9 than at pH 7. Due to the basic nature of the drugs analyzed, a basic pH minimized

Table 4. Comparison of capacity factors for drugs separated in different solvents and solvent concentrations with mobile phases containing 50 mM Brij-S..

Drug	0% Solvent	10% MeOH	10% PrOH	10% ACN	20% ACN
Timethoprim	3.8	3.6	2.1	2.1	2.0
Indomethacin	5.8	4.7	3.4	2.8	2.2
Chloramphenicol	9.5	8.2	5.1	4.1	2.4
Carbamazipine	12.0	9.1	7.0	7.3	4.4
Pindolol	16.2	13.4	10.9	10.4	6.1
Lidocaine	18.4	15.7	11.6	11.7	8.8
Nifidipine	21.5	17.8	14.1	13.9	9.4
Benzophenone	32.2	27.8	25.2	25.0	17.0

Conditions for separations were pH 9.2 with a 0.03 M phosphate buffer. All separations performed an a Supelcosil LC-18 (150X4.6 mm I.D.) column.

the ionization of the drugs, thus limiting peak broadening and tailing. All mobile phases were buffered at the apparent pH of 9.2 with a 0.03 M phosphate buffer for a solution containing 10% acetonitrile and 50 mM of the appropriate surfactant. While buffer concentration was found to affect the solubility of serum under acidic conditions, no solubility problems were encountered at the slightly alkaline conditions used in our work.

Quantification of Drugs in Serum

To investigate whether Brij-S works as well as SDS in freeing bound drugs from proteins, the peak heights of drugs in serum were compared to peak heights of identical concentrations of drugs in an acetonitrile-water matrix. All analyses were performed with a 50 mM Brij-S, 90% water - 10% acetonitrile mobile phase at pH 9.2. Table 5 summarizes the data obtained from this study. Percentage recovery and the relative standard deviation (RSD) for five analyses are included in the table. Recoveries of all six drugs were near 100%, with RSDs below 2% for five trials. The high recoveries, along with the fact that all drugs provided a single, sharp peak, show that Brij-S works well in releasing any bound drugs from the proteins.

The detection limits for six drugs were also determined, along with the linear range of detection for the drug concentrations of 1 to 100 ppm (Table 6). The limits of detection were determined at a signal to noise ratio of three. The absolute LOD is based on the amount of drug present in a 5 μl injection volume. It was found that all detection limits were near 1 ppm, putting the absolute detection limits near 5 nanograms. The linear range for all drugs extended at least over two orders of magnitude in the range of 1 to 100 ppm

Table 5. Recovery of Drugs from serum. s.d. is the standard deviation, RSD (%) is the percent relative standard deviation, and n is the number of recovery tests performed.

Drug	Recovery	s.d.	RSD (%) (n=5)
Trimethoprim	99.2	1.79	1.81
Indomethacin	101.1	1.62	1.63
Chloramphenicol	99.2	0.66	0.67
Carbamazipine	98.6	1.95	1.97
Lidocaine	99.0	0.68	0.69
Benzophenone	101.8	0.92	0.93

Table 6. Statistical Study of seven drugs in serum. R is the correlation coefficient in the range of 100 to 1 ppm, LOD is the limit of detection, and absolute LOD is the amount of drug in the 5 μm injection volume calculated from the LOD.

Drug	R	LOD (ppm)[a]	abs. LOD (ng)
Caffeine	.9992	0.3	2
Ibuprofen	.9967	5	27
Trimethoprim	.9988	0.7	3
Chloramphenicol	.9987	2	11
Carbamazipine	.9999	0.6	14
Lidocaine	.9995	3	17
Benzophenone	.9992	0.6	3

[a] LOD is calculated at S/N=3 and with the slope of values obtained at 1, 5, and 10 ppm only.

with no noticeable deviation. All calibration curves had a correlation coefficient rangingfrom 0.997 - 0.999.

The use of a gradient

While column switching has proven to be a viable alternative to using surfactants in direct injection HPLC, by using a gradient some of the advantages demonstrated in column switching can be realized without the required column switching instrumentation. Once the protein peak has passed through the analytical column, an increase in the organic solvent concentration in the mobile phase can be used to sharpen and reduce the retention times of the later-eluting analyte peaks. Figure 14 shows a separation of eight drugs in serum that required approximately 25 min. by isocratic elution (10% acetonitrile, 50 mM Brij-S). Separation of a mixture by gradient elution was accomplished in just over 11 minutes (Figure 15).

A blank was analyzed using the same gradient as in Figure 15, but with no serum or drugs present. This blank run, shown in Figure 16, has a large peak due to Brij-S. The peak is not present in Figure 15 due to background correction, but traces of the large peak can be seen from 4 - 10 minutes in Figure 15 as a noisy baseline. Under the initial elution conditions, the surface of the silica C18 is believed to have been coated with Brij-S by a dynamic equilibrium. As the proportion of acetonitrile in the mobile phase was increased, the surface-adsorbed Brij-S partitioned back into solution, resulting in the broad peak shown in Figure 16.

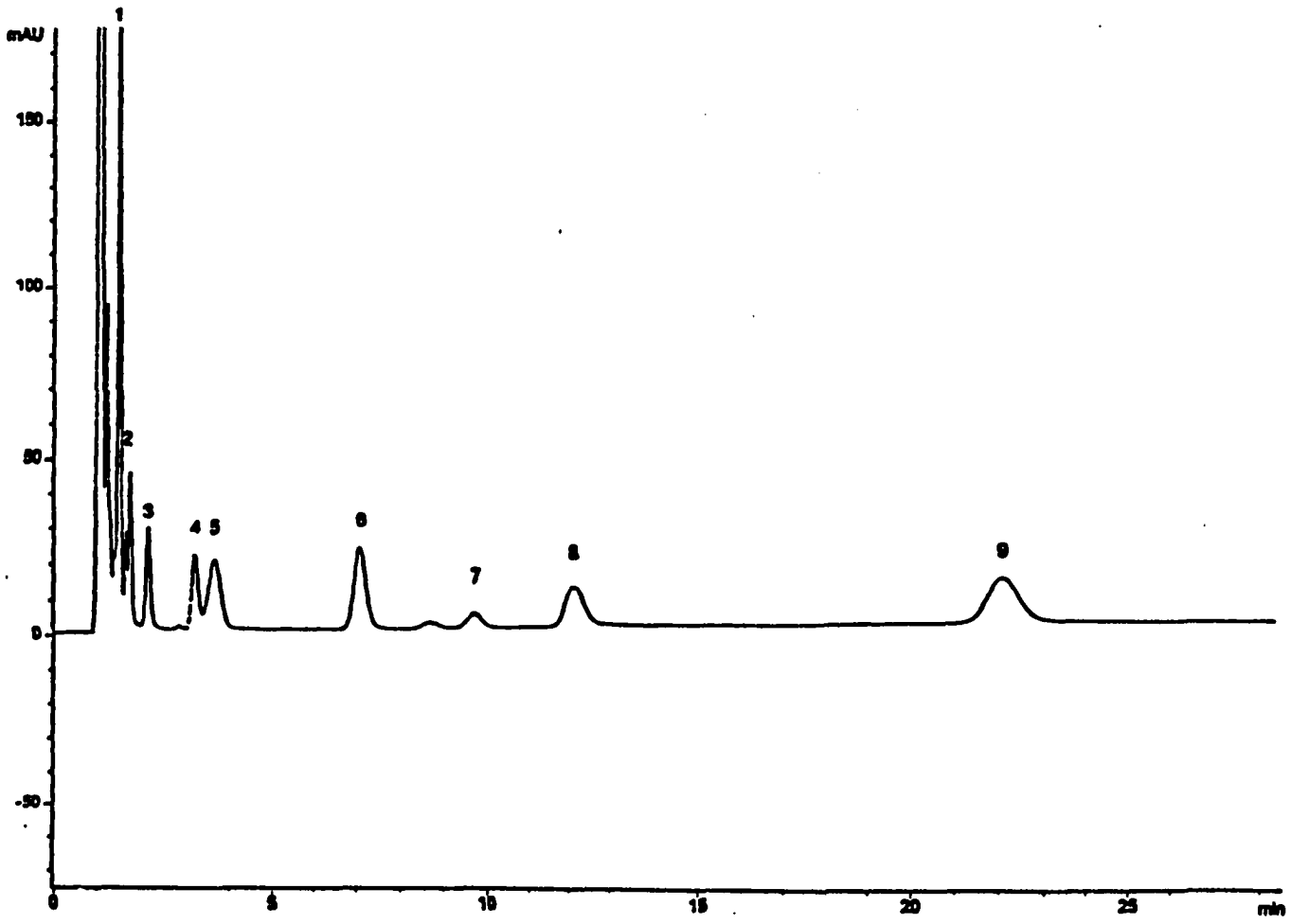

Figure 14. Separation of drugs from serum on a Supelcosil LC-18 (150X4.6 mm I.D) column. Eluent, acetonitrile-water (10:90) buffered at pH 9.2 with a 0.03 M phosphate buffer and containing 50 mM Brij-S; flow-rate, 1 ml/min; detection, UV at 254 nm. Peaks: 1=sulfapyridine, 2=caffeine, 3=ibuprofen, 4=trimethoprim, 5=indomethacin, 6=carbamazipine, 7=lidocaine, 8=nifidipine, 9=benzophenone. All unlabelled peaks are from serum.

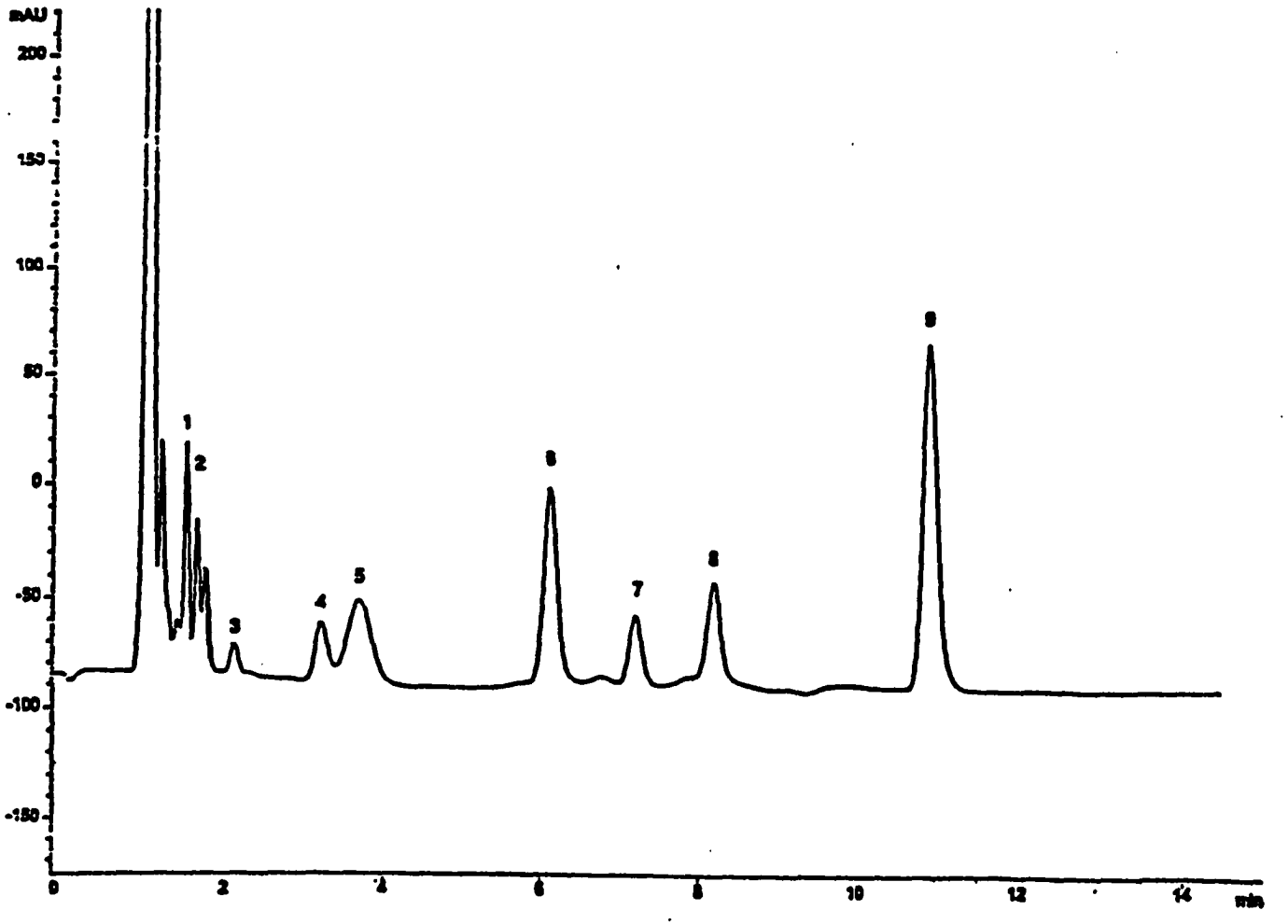

Figure 15. Separation of drugs from serum on a Supelcosil LC-18 (150X4.6 mm I.D) column. Eluent, acetonitrile-water (10:90) for four minutes, followed by a constant ramp to acetonitrile-water (40:60) at ten minutes, and then a constant level of acetonitrile-water (40:60) for the remainder of the run. The mobile phase was buffered at pH 9.2 with a 0.03 M phosphate buffer and containing 50 mM Brij-S; flow-rate, 1 ml/min; detection, UV at 254 nm. Peaks: 1=sulfapyridine, 2=caffeine, 3=ibuprofen, 4=trimethoprim, 5=indomethacin, 6=carbamazipine, 7=lidocaine, 8=nifidipine, 9=benzophenone. All unlabelled peaks are from serum.

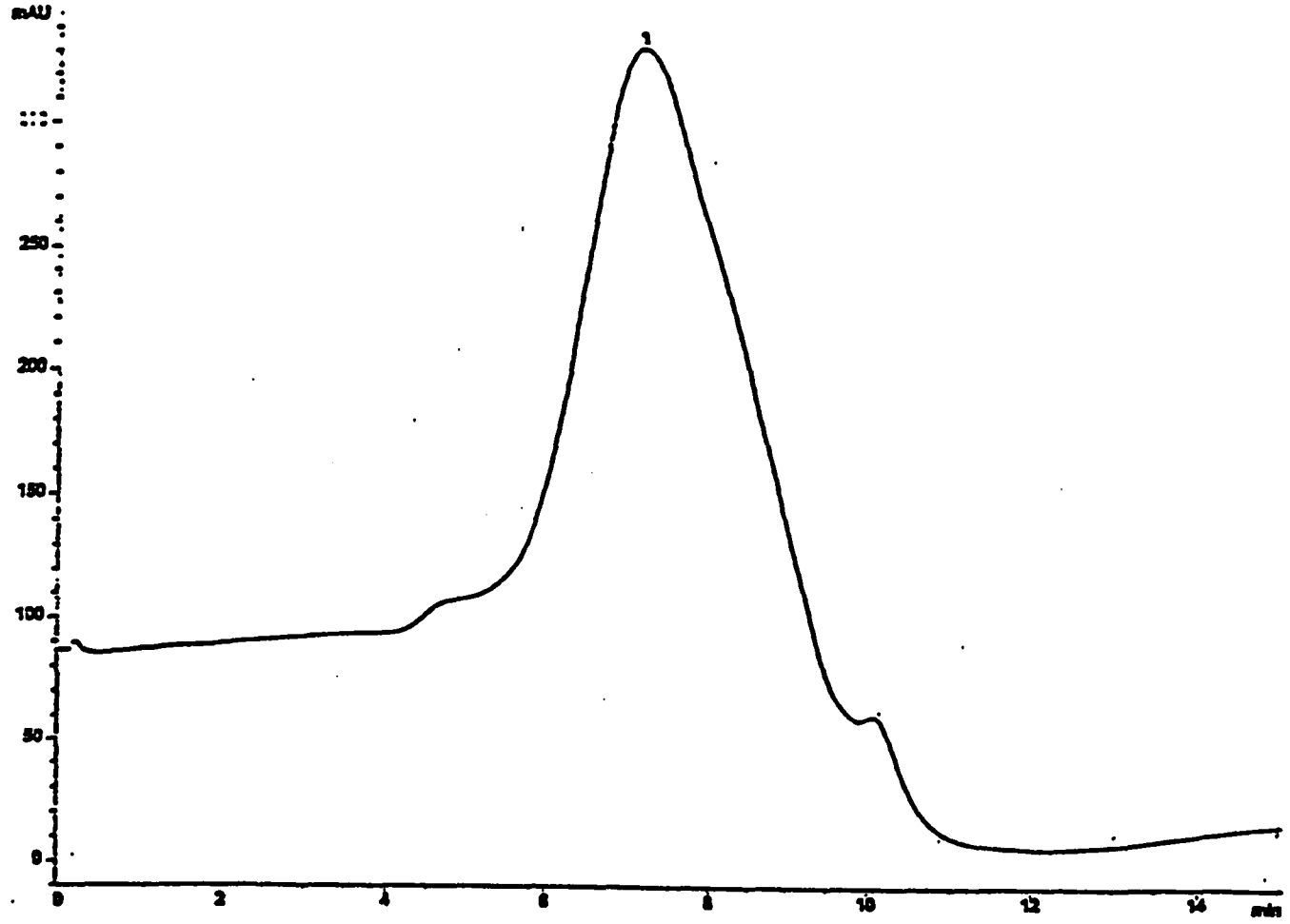

Figure 16. Blank run with acetonitrile gradient on a Supelcosil LC-18 (150X4.6 mm I.D) column. Eluent, acetonitrile-water (10:90) for four minutes, followed by a constant ramp to acetonitrile-water (40:60) at ten minutes, and then a constant level of acetonitrile-water (40:60) for the remainder of the run. The mobile phase was buffered at pH 9.2 with a 0.03 M phosphate buffer and containing 50 mM Brij-S; flow-rate, 1 ml/min; detection, UV at 254 nm. Peak: 1=Brij-S.

Mechanism

Initially it was assumed that the Brij-S surfactant in solution enveloped the serum proteins in a protective sheath and thus prevented them from being adsorbed on the silica C18. However, observations made in the gradient elution experiment pointed toward a mechanism in which the silica C18 surface was coated with a thin layer of Brij-S. The negative charge of this adsorbed layer of Brij-S would repel proteins, which also have a negative charge at pH 9.2, and thereby prevent protein adsorption. Support for this mechanism was provided by experiments in which the silica C18 was precoated with Brij-S by a dynamic equilibrium. After a wash period, a serum sample containing no Brij-S passed quickly and completely through the HPLC column. Drug analytes had long retention times in this system when compared to those obtained with the Brij-S containing mobile phase separations (Figure 17). This showed that while the coated resin prevented adsorption of the proteins in the sample solution, due to the lack of surfactant in solution, longer retention times for drugs resulted.

Incorporation of Brij-S in the mobile phase used for direct-injection HPLC has an additional function besides coating the silica C18 stationary phase. The Brij-S seems to form complexes with analytes in solution and reduce their retention times. This mechanism is suggested by the fact that increasing concentrations of Brij-S resulted in faster elution of the analytes with sharper peaks, while no effect is observed on the protein peak.

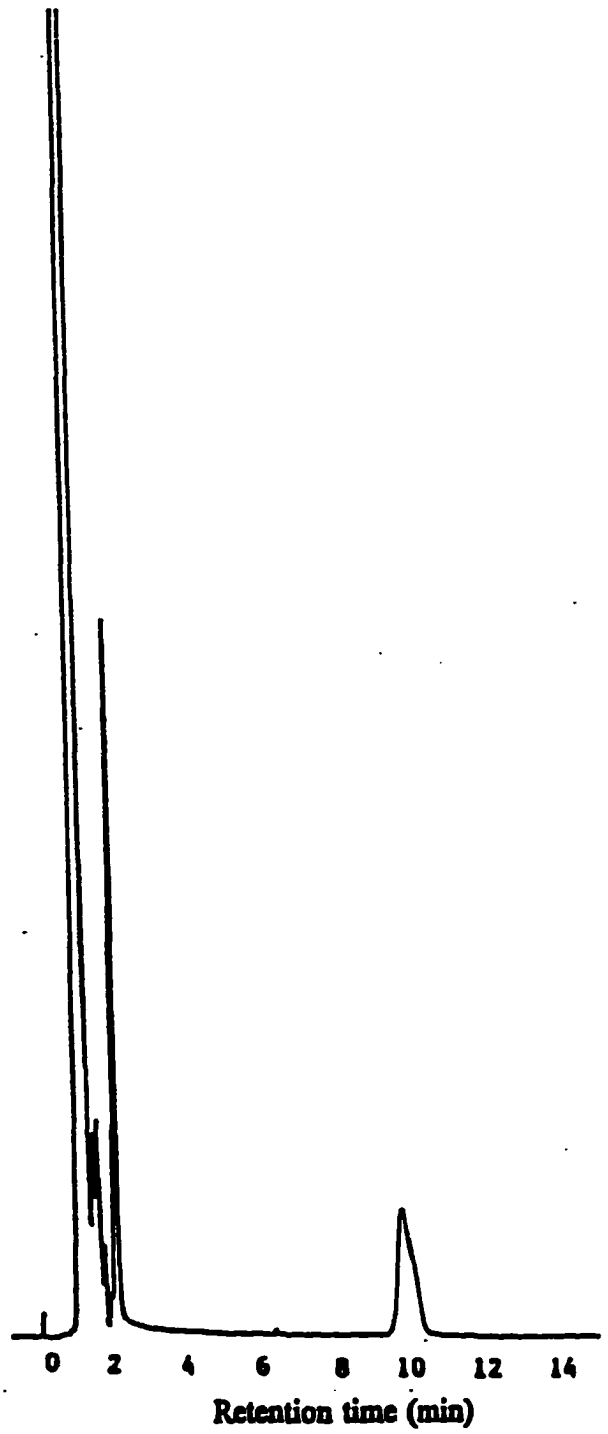

Figure 17. Elution of undiluted serum from a Supelcosil LC-18 (150 X4.6 mm I.D.) Column. Eluent, acetonitrile-water (10:90) buffered at pH 9.2 with a 0.03 M phosphate buffer with no surfactant. The column was coated for 30 minutes with a 100 mM Brij-S mobile phase and then allowed to equilibrate for 2 hours with the acetonitrile-water mobile phase. Flow rate, 1 mL/min; detection, UV at 254 nm.

Conclusions

In the chromatographic determination of drugs in serum by direct-injection HPLC, the surfactant in the mobile phase accomplishes two purposes. First, it prevents adsorption of large bio-molecules and causes them to be eluted rapidly and cleanly. Second, it permits elution of the drugs at a much lower organic solvent concentration than would otherwise be necessary (13, 14). Sulfonated Brij-30 is shown to be much more efficient than the commonly used SDS. By using optimum separation conditions, separations of a wide range of drugs in serum were achieved. By introducing a gradient, analysis times were cut in half with no additional instrumentation required. The use of an appropriate surfactant is an easy way to simplify and speed up the chromatographic analysis of biological fluids.

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MEMBRANE-BASED MICRO SOLID-PHASE EXTRACTION OF ORGANIC COMPOUNDS

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Abstract

A technique is described for performing membrane-based micro solid-phase extraction (MMSPE) directly in a syringe. The same syringe used for the extraction was then used for the elution and subsequent injection of the eluted volume directly into either a gas chromatograph or liquid chromatograph system. A membrane 1 mm thick and 0.7 mm in diameter was placed in a removable ferrule between the plunger and needle of a syringe. Membranes containing sulfonated, C18, and anion exchange resins were used in the study. The technique employed exhaustive extraction of the analytes of interest, with extractions >90% for most compounds tested. With the very small elution volumes which were required with this technique, concentration factors of up to 200 were achieved with just 1.0 ml of sample solution.

Introduction

Solid-phase extraction (SPE) has many advantages over liquid-liquid extraction (LLE). SPE has higher concentration factors, better extraction efficiencies, is more easily automated, is less labor-intensive, and above all uses much less solvent than LLE (1-3). As hazardous waste production faces tighter controls, the use of organic solvents in the laboratory must be minimized. This is a primary reason why many methods are being converted from LLE to SPE. Conventional SPE uses just a few milliliters of solvent compared to the liters that can be consumed in a single LLE extraction.

SPE uses a column packed with resin through which an aqueous sample is passed. As the sample passes through the bed of resin, the analytes are both extracted and concentrated on the resin. After extraction, the analytes of interest are eluted from the resin with a suitable solvent. Once eluted, the analytes are generally analyzed by either gas chromatography (GC) or high performance liquid chromatography (HPLC). While resin beds are traditionally packed with 40 μm particles and are 1 cm thick (4), many papers have been published which utilize smaller resin particles and thinner resin beds (5-7).

Recently resin-loaded membranes have been introduced which enmesh 5 μm resin particles in a network of teflon fibrils (8-11). These membranes provide fast flow rates with excellent recoveries. Ambrose and Fritz (12) introduced a system which utilized very thin membranes held in the hub of a needle. A membrane 4 mm in diameter was held in place with a specially manufactured screen system. With this system, elution volumes from 20-50 μl were used and very good recoveries were reported. While this method minimized the amount of required eluting solvent, working with such small elution

volumes in the open laboratory can be very difficult and introduce large errors into the analysis.

Solid-phase micro extraction (SPME) has also received much interest as an alternative to conventional SPE. With SPME, a thin polymer coating is applied to a fiber (13-17). The coating is then exposed to organic analytes contained either in solution or in the headspace above a solution. After equilibration, the fiber is removed from the solution and inserted into a specially designed GC interface where the analytes which were adsorbed onto the polymer coating are thermally desorbed from the fiber. This system is ideal in that it needs no organic solvent for elution. The primary drawback to SPME is that it is an equilibration technique. Uptake is based on the partitioning of the analytes between the fiber and solution, with complete extraction of analytes seldom achieved. The system is also utilized almost entirely for GC and a specially designed interface for injection onto the GC chromatograph is required. An interface for using SPME with HPLC has been introduced, but again a specially designed interface is required (18).

The present work describes a system that retains all of the advantages of column-based SPE, while miniaturizing the entire process. The membrane-based micro-solid-phase extraction (MMSPE) system is easily automated, lends itself to being both a portable and compact system, and elution of analytes is achieved with just 1-10 μl of solvent. Finally, due to the very small elution volumes, after extraction the analytes are eluted off of the membrane directly into either an HPLC or GC system. This setup eliminates the problems encountered when trying to transfer such small volumes of solution. The entire system required no special instrument modifications for either the GC or HPLC injections.

Experimental

Reagents and chemicals

All analytes used in the study were >99% pure and obtained as received from Fisher Scientific (Pittsburgh, PA, USA), Aldrich Chemical Co. (Milwaukee, WI, USA), and Sigma Chemical Co. (St. Louis, MO, USA). Stock solutions were used to prepare all sample solutions. Acetonitrile was of HPLC grade and used as obtained from Fisher. Serum (Sigma) was reconstituted with distilled deionized water that had passed through a Barnstead Nanopure II system (Sybron Barnstead, Boston, MA, USA). Sheets of experimental sulfonated polystyrene-divinyl benzene (PS-DVB), C18, and amine-based anion exchange resin were obtained from the 3M Co. (St. Paul, MN, USA).

HPLC system

The HPLC system for the isocratic separations consisted of several components. A Dionex SXP pump (Dionex, Sunnydale, CA, USA) was used to provide a constant flow rate of 1 ml/min. A Rheodyne injector (Rheodyne, Berkeley, CA, USA) was used to make 5 µl and 20 µl injection. A Kratos Spectra Flow 783 UV absorbance detector (Kratos Analytical Instruments, Ramsey, NJ, USA) was used for detection. Finally a Servago 123 chart recorder was used for plotting chromatograms. All separations were performed on a Supelcosil LC-18 column (150 mm x 4.6 mm i.d.).

GC system

Samples were injected manually from the syringe into a Shimadzu (Kyoto, Japan) GC 14A equipped with a flame ionization detector and a C-R4A Chromatopac data

analysis system. The GC column used was a Supelco SPB-5 column, 30 m x 0.32 mm i.d. with a stationary phase thickness of 1 μm. Recoveries were calculated as an average of three trials by comparing the relative peak areas between the analyte of interest and a standard that had not undergone the SPE technique.

Procedure for MMSPE

The apparatus used for MMSPE is shown in Figure 1. The apparatus utilized a 3.0 ml Whatman filtration tube for the sample reservoir. This was interfaced to a 50 μl Hamilton series 1705 Gastight glass syringe using a 22 gauge stainless steel needle with a bevelled rubber septum. An immobilizing clip held the two pieces together and provided a pressurized seal to eliminate leakage. The schematic of the Hamilton syringe is shown in Figure 2. Approximately 1 mm of the influent end of the Hamilton needle was trimmed off by the Iowa State Chemistry Machine Shop. The membrane to be utilized for analysis was then packed into the ferrule, providing a resin bed 1 mm in depth and 0.7 mm in diameter.

Sample loading and elution

Before extraction, all resin membranes were first rinsed with acetonitrile and water. For sample volumes less than 100 μl, the sample was injected directly into the barrel of the syringe as four 25 μl aliquots. The sample was then forced through the membrane with the plunger. For larger sample volumes, the entire apparatus was assembled as shown in Figure 1. Once the apparatus was assembled, it was attached to a positive pressure source. By applying 30 psi to the reservoir, a flow rate of 0.05 - 0.2 ml/min was

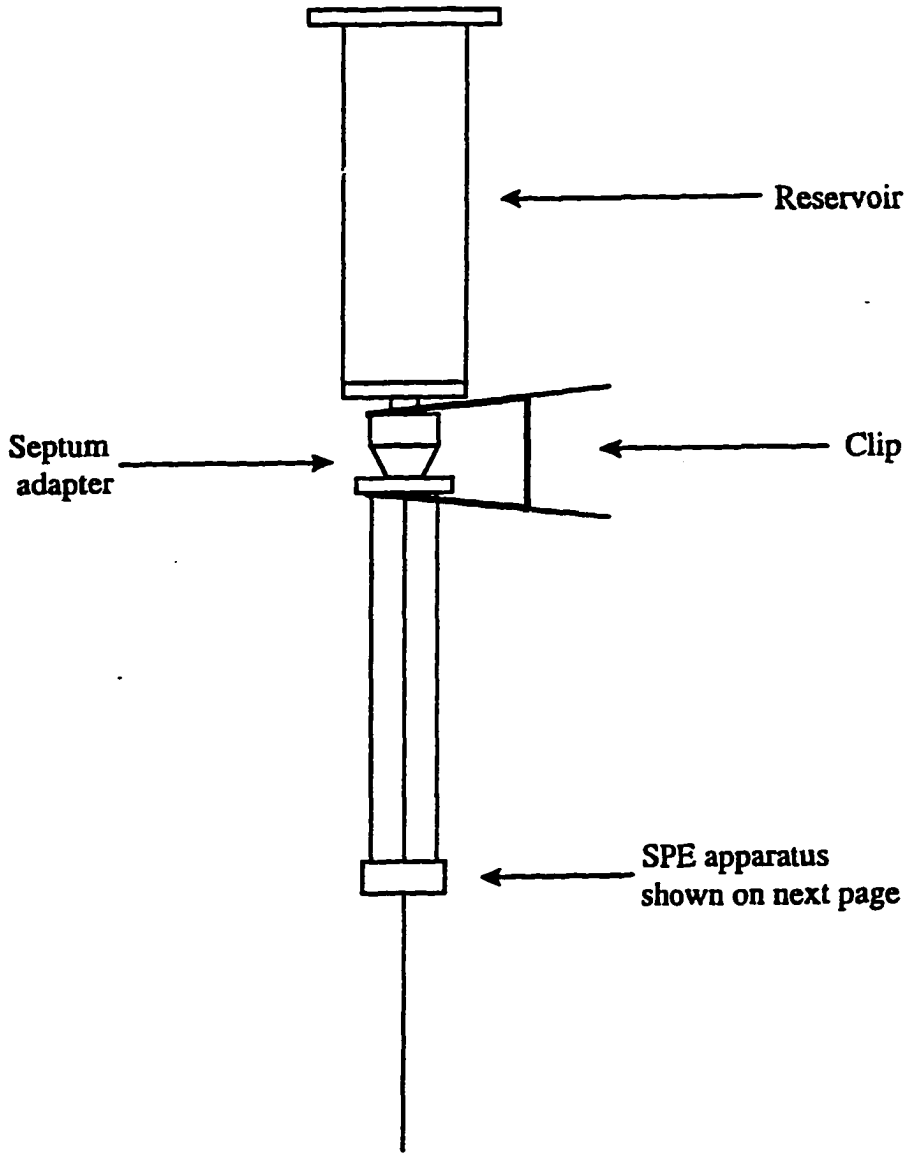

Figure 1. SPE in a syringe apparatus.

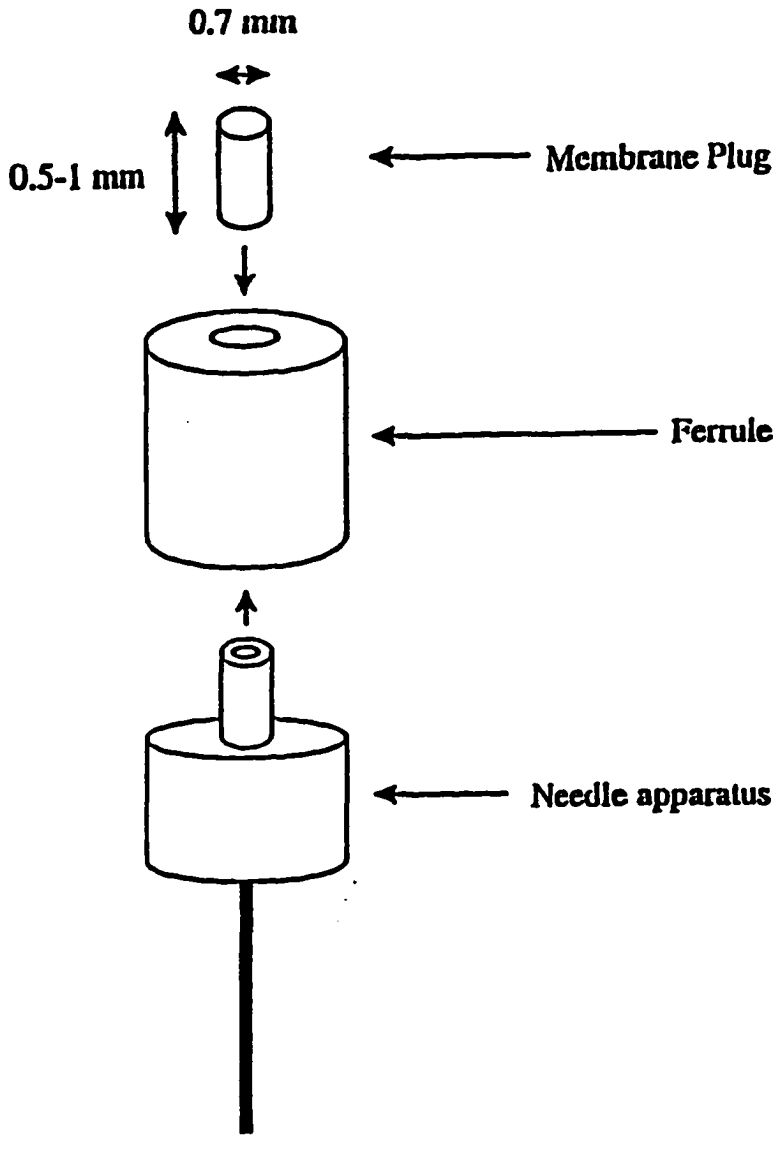

Figure 2. Expanded view of SPE apparatus.

achieved. After the sample had passed through the syringe, the apparatus was taken apart and a plunger was inserted into the Hamilton syringe to push any remaining solution through the membrane plug. For elution of the analytes from the resin, acetonitrile or another eluting solvent was drawn into the syringe through the needle. The solvent was allowed to stay in contact with the resin for 60 seconds before being forced back through the membrane by applying positive pressure with the plunger. For GC the sample was injected directly onto the GC column for analysis. For HPLC the sample was either first eluted into a vial and then injected into the chromatographic system, or injected directly into the liquid chromatographic system via a 5 µl injection loop.

RESULTS AND DISCUSSION

MMSPE assembly

The assembly for MMSPE (Figs. 1 and 2) has been carefully developed to give both efficient extraction and elution of organic compounds. The membrane plug (0.7 mm x 1 mm) weighed 0.3 mg based on the average weight of ten membranes. The calculated volume of the membrane was 0.385 µl. Due to the small diameter of the membrane plug, no support above or below the membrane was required. The only modification required to the syringe was to cut approximately 1 mm from the top of the needle. The void area created was filled with the membrane plug. The commercially available syringes were then used with no further modifications.

With the setup described, the membrane was first treated with acetonitrile and water by manually pushing a small volume of each solution through the syringe. After pretreatment, the sample solution was passed through the syringe. For small volumes, the

sample was added in 25 µl aliquots directly into the syringe barrel. The sample was then forced through the membrane with the syringe plunger. For larger volumes, 0.1-3 ml of the sample solution was placed in the 3 ml reservoir and the entire apparatus was attached to a positive pressure source. This setup eliminated the need to manually force the larger volumes through the syringe.

Breakthrough curves

To determine the practicality of using such a small amount of resin-loaded membrane for extraction of organic compounds, total capacity was determined for a membrane plug. The breakthrough curves for anisole and benzophenone on a sulfonated resin membrane were constructed, and the information used to calculate loading capacity for the membrane. A 1 ppm anisole solution was passed through the resin in 100 µl aliquots. The anisole peak was monitored with HPLC using UV detection at 254 nm. A similar procedure was used for benzophenone, using a 5 ppm solution of benzophenone and passing the solution through in 50 µl aliquots. The breakthrough curve is the ratio of analyte concentration to influent concentration (C/C_0) plotted against the load volume (V_L). Breakthrough is considered to be at $C/C_0 = 0.5$, but since quantitative extraction is desired with SPE, $C/C_0 = 0.1$ was also calculated. The resin load capacity was determined from the breakthrough curve. The resin load capacity is the total number of moles of analyte that can be adsorbed by a resin. This number is calculated by multiplying the breakthrough volume at $C/C_0 = 0.5$ by the influent concentration used (19).

Figures 3 and 4 show the breakthrough curves for anisole and benzophenone, respectively. It was calculated from these figures that breakthrough of $C/C_0 = 0.5$ occurred

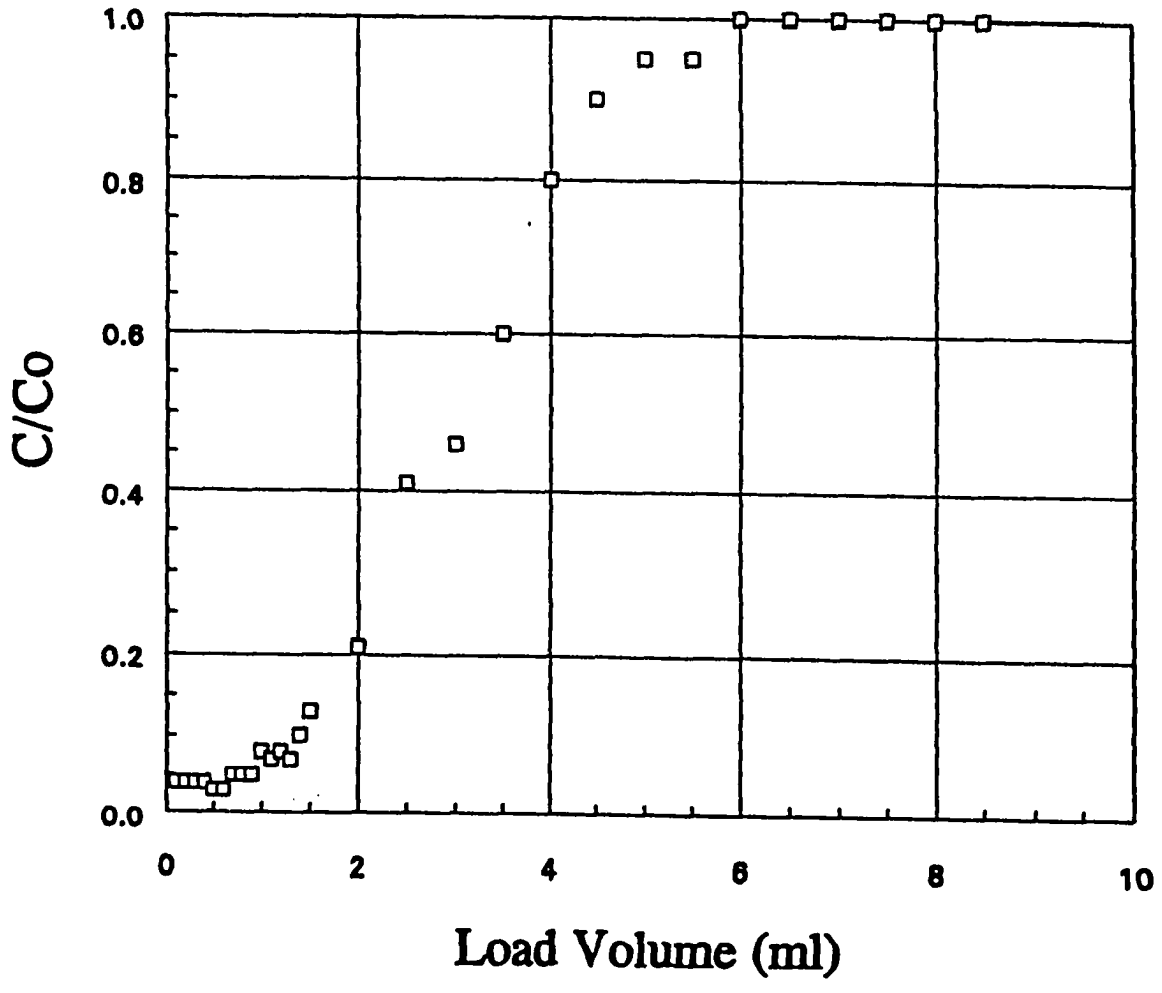

Figure 3. Breakthrough curve for anisole on a sulfonated Empore membrane using the SPE in a syringe setup. Load volume is based on a 1 ppm solution.

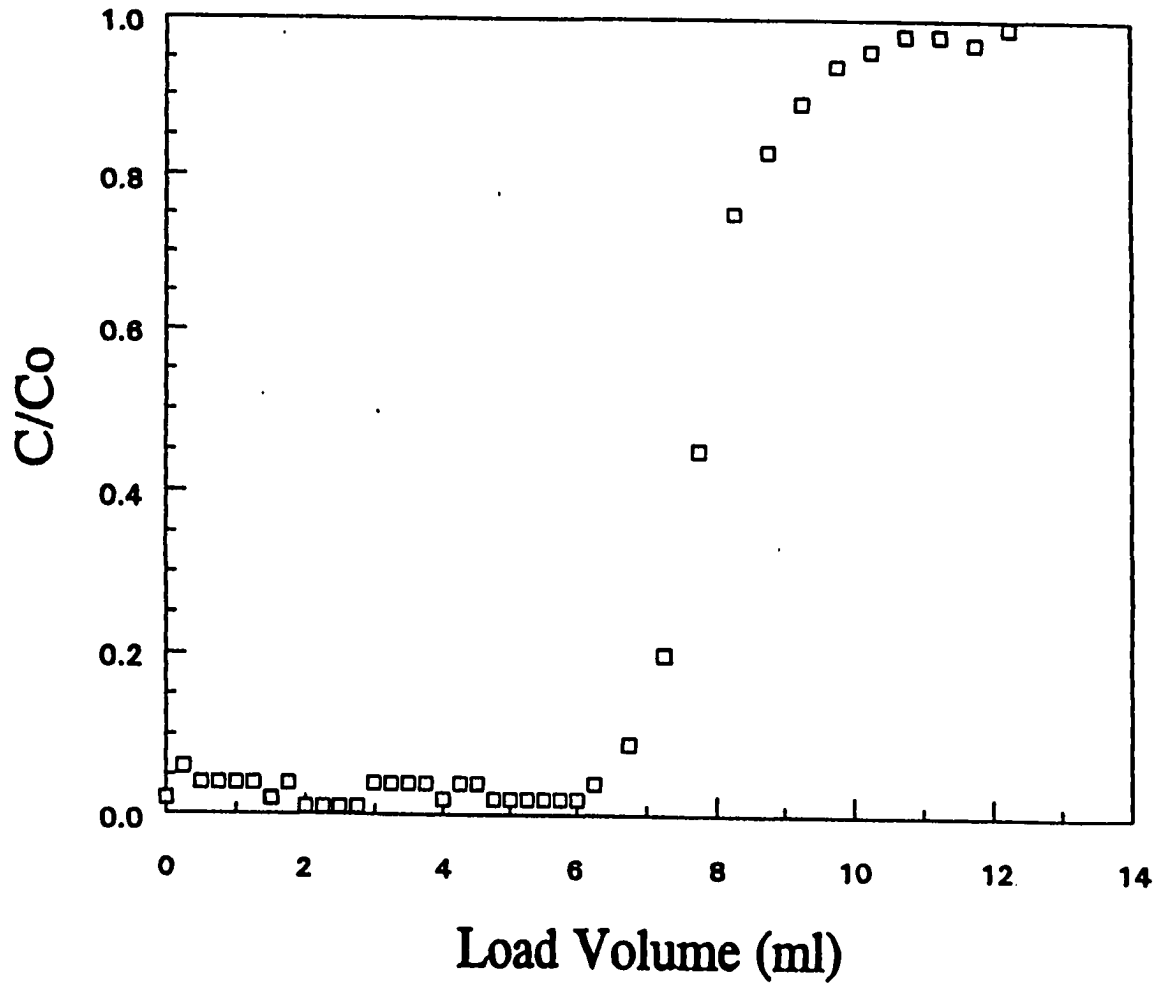

Figure 4. Breakthrough curve for Benzophenone on a sulfonated Empore membrane using the SPE in a syringe setup. Load volume is based on 1 ppm solution.

occurred at 2.9 ml for anisole and 8.0 ml for benzophenone. The loading capacities based on the breakthrough curves were calculated to be 29.6 X 10^{-6} moles for anisole and 43.4 X 10^{-6} moles benzophenone. For anisole, 29.6 X 10^{-6} moles is equal to 30 μg anisole. Based on the average membrane weight of 0.3 mg found previously, this would be equivalent to a 1.0% loading of the resin membrane. For benzophenone, 43.4 X 10^{-6} moles were taken up or 7.8 mg of benophenone for a 2.6% loading of the membrane. From these experiments, it was determined that MMSPE would provide the capacity required for extraction of trace amounts of organic compounds.

MMSPE of phenols

Three different membranes were tested for the extraction of phenols from aqueous solutions including a sulfonated PS-DVB resin, a C18 resin, and an anion exchange resin. For the extraction tests, a 50 μl aliquot of 5 ppm phenol solution was passed through the membrane plug. The sample solution was buffered at pH 9 with a phosphate buffer. The effluent from the extraction step was then analyzed by HPLC and compared to a standard solution to determine the percentage extraction. Table 1 compares the three membranes for extraction efficiency of seven phenols. Many of the phenols were poorly extracted with both the C18 and the sulfonated membrane. The anion exchange resin took up the anionic phenols very strongly and recoveries increased to >95% for all phenols tested.

Using the sulfonated resin membrane, a 200-fold concentration of phenols was performed with direct injection of the concentrated phenols into a GC chromatograph. The experiment consisted of passing 1.0 ml of a 5 ppb sample solution through the SPE apparatus. After extraction the phenols were eluted with 5 μl of 0.1 M HCl in acetonitrile,

Table 1. Percentage extraction of phenols from aqueous solutions using an Empore membrane containing sulfonated polystyrene-divinylbenzene resin contained in the SPE in a syringe apparatus.

Compound	Sulfonated PS/DVB	C18	Anion Exchange
Phenol	39	47	96
m-cresol	63	69	99
4-ethylphenol	83	93	98
4-propylphenol	95	96	99
4-butylphenol	97	93	100
4-amylphenol	97	93	100
4-heptylphenol	99	89	100

* All extraction performed on aqueous samples buffered at pH 9
* Results based on the average of three trials
* The average relative standard deviation for each set of three trials was 2.73%

with the eluate injected directly from the MMSPE apparatus into the GC. For extraction the acetonitrile was drawn up through the membrane and allowed to equilibrate for 60 seconds. The acetonitrile was then pushed quickly into the GC. The conditions for GC analysis were a 60 °C initial temperature with a ramp to 180 °C at 8 °C/min. A split ratio of 1:25 was used for the injection of the 5 µl elution volume directly onto the GC column. The initial 5 ppb sample solution is shown in figure 5, with the standard and concentrated phenols solution shown in figures 6 and 7. As expected, the recoveries of the earliest eluting phenols are low, but all phenol peaks are easily identified after the preconcentration with MMSPE.

A similar experiment was performed with an amine-based anion exchange resin and chromatography of the phenols by HPLC with UV adsorption. Before extractions were performed, the anion exchange resin was first rinsed with acetonitrile, water and NaOH to ensure the resin was in the basic form. Following the NaOH treatment, the resin was again rinsed with deionized water. For this work, 3.0 ml of a 5 ppb solution of phenols was passed through the MMSPE apparatus. After extraction, the phenols were eluted with 15 µl of 0.01 M HCl in acetonitrile. The eluent was collected in a vial and then drawn into an HPLC syringe for injected into the HPLC system. The phenols were separated with a 60% acetonitrile/40% water mobile phase buffered at pH 9.0 with a 10 mM phosphate buffer. The initial 5 ppb solution before preconcentration is shown in figure 8. A 5 ppm standard solution and preconcentrated phenol solution are shown in figure 9. With the anion exchange resin, recovery of phenol jumped to 73% compared to 22% achieved with the sulfonated resin.

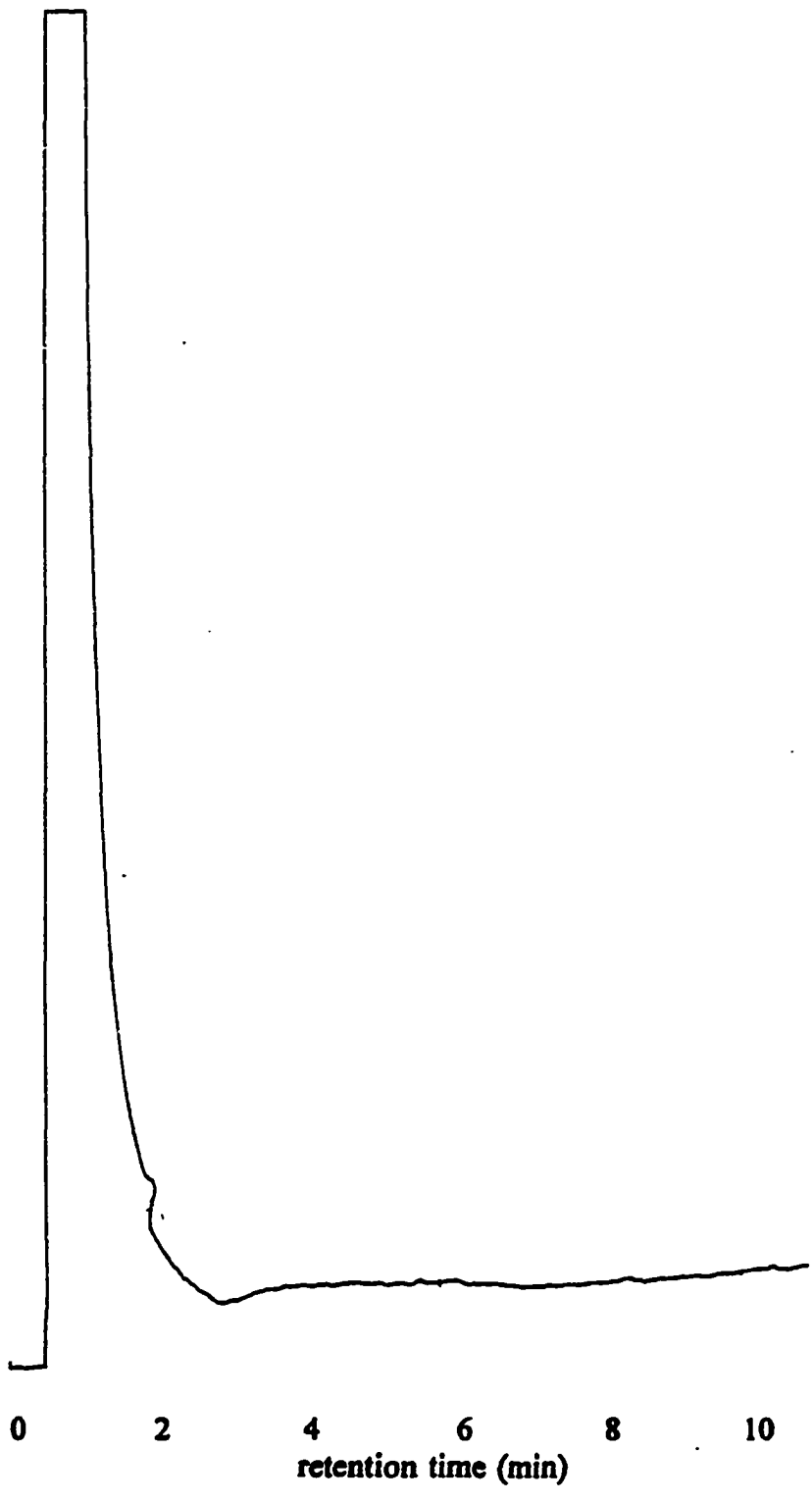

Figure 5. GC of 5 ppb phenol standard solution. Separation performed on a 15 m Supelco SPB-5 column. Initial temperature = 60 °C, Ramp to 180 °C at 8 °C/min.

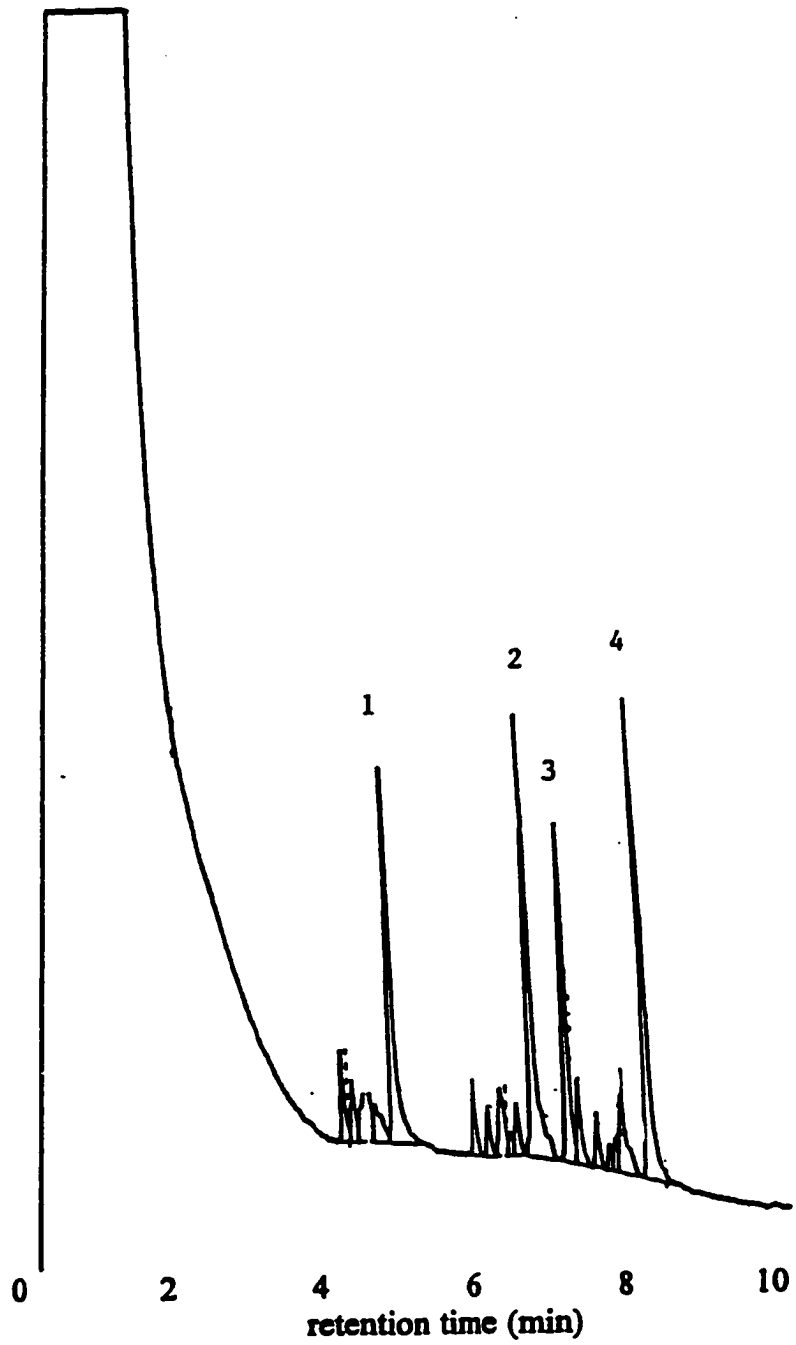

Figure 6. GC of a 1 ppm phenol standard solution. Separation performed on a 15 m Supelco SPB-5 column. Initial temperature = 60 °C, Ramp to 180 °C at 8 °C/min. Peak identification: 1 = phenol, 2 = p-cresol, 3 = nitrophenol, 4 = 2,4-dimethylphenol.

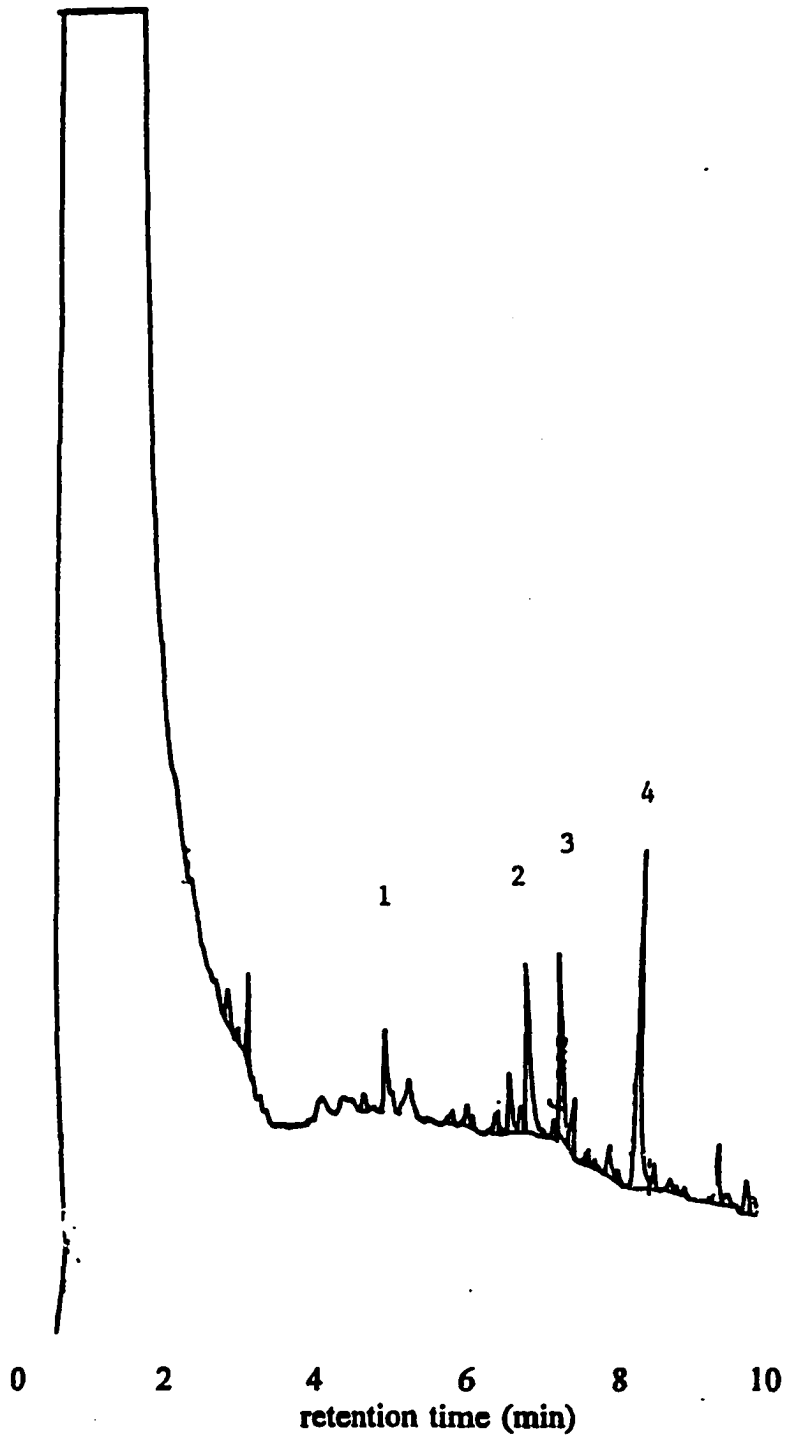

Figure 7. GC of a 5 ppb phenol solution after a 200-fold preconcentration performed on the MMSPE apparatus. Conditions and peak identifications same as Figure 5.

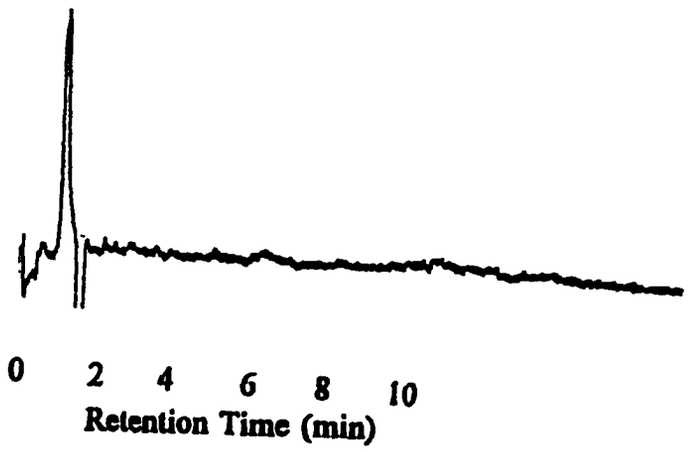

Figure 8. HPLC of 5 ppb phenol standard. HPLC performed with 60% Acetonitrile/40% water on a Supelcosil LC-18 column. Flow rate 1 ml/min. Detection performed with UV at 254 nm.

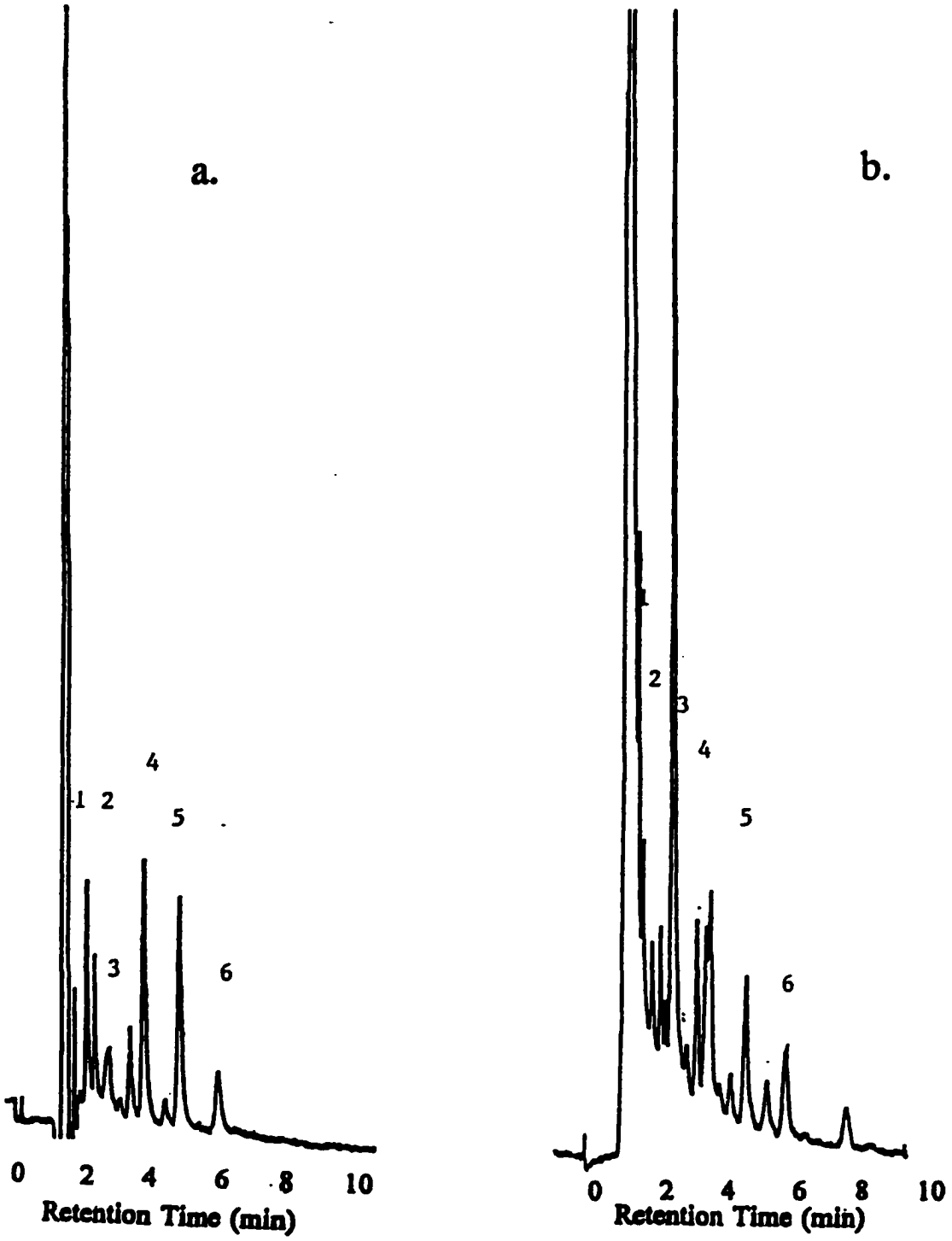

Figure 9. Recovery and preconcentration of phenols from aqueous solutions. MMSPE performed on an anion exchange resin-loaded membrane. Elution with 0.1 M HCl in ACN. HPLC performed with 60% ACN/40% water on a Supelcosil LC-18 column. Flow rate 1 ml/min. (a) 1 ppm standard (b) 5 ppb after 200-fold concentration. Peaks identification: 1=phenol, 2=m-cresol, 3=4-ethylphenol, 4=4-propylphenol, 5=4-butylphenol, 6=4=amylphenol.

MMSPE of benzenes

For the extraction of benzene-based compounds, a membrane containing sulfonated PS-DVB was compared to one impregnated with C18 resin. The sulfonated PS-DVB membrane proved to be better for many of the more weakly retained compounds, while both membranes proved to take up most of the tested compounds at levels near >90%. The analysis of eight aromatic compounds by GC after preconcentration is shown in Figures 10 and 11. In this experiment a simple 2-fold concentration step was performed. A 50 µl sample containing 5 ppm of eight substituted benzenes was passed through the MMSPE apparatus followed by elution with 25 µl of acetonitrile. For better accuracy 10 ppm propylbenzene was spiked into the acetonitrile used to elute the compounds and was used as an internal standard.

A sulfonated PS-DVB membrane was again compared to a C18 membrane for the uptake of benzene-based compounds. With the MMSPE apparatus it was found that while both membranes give similar results for the uptake of small polar compounds, the sulfonated membrane is clearly better for the uptake of more hydrophobic compounds. Table 2 is the comparison of extractions of eleven benzenes with sulfonated PS/DVB and C18 loaded membranes. Both membranes provided recoveries >90% for all but benzene, with the sulfonated PS/DVB membrane giving slightly higher recoveries for most compounds.

Conclusions

While miniaturization of chromatographic techniques is a current trend in analytical chemistry, the problems with these techniques are often overlooked. MMSPE is a

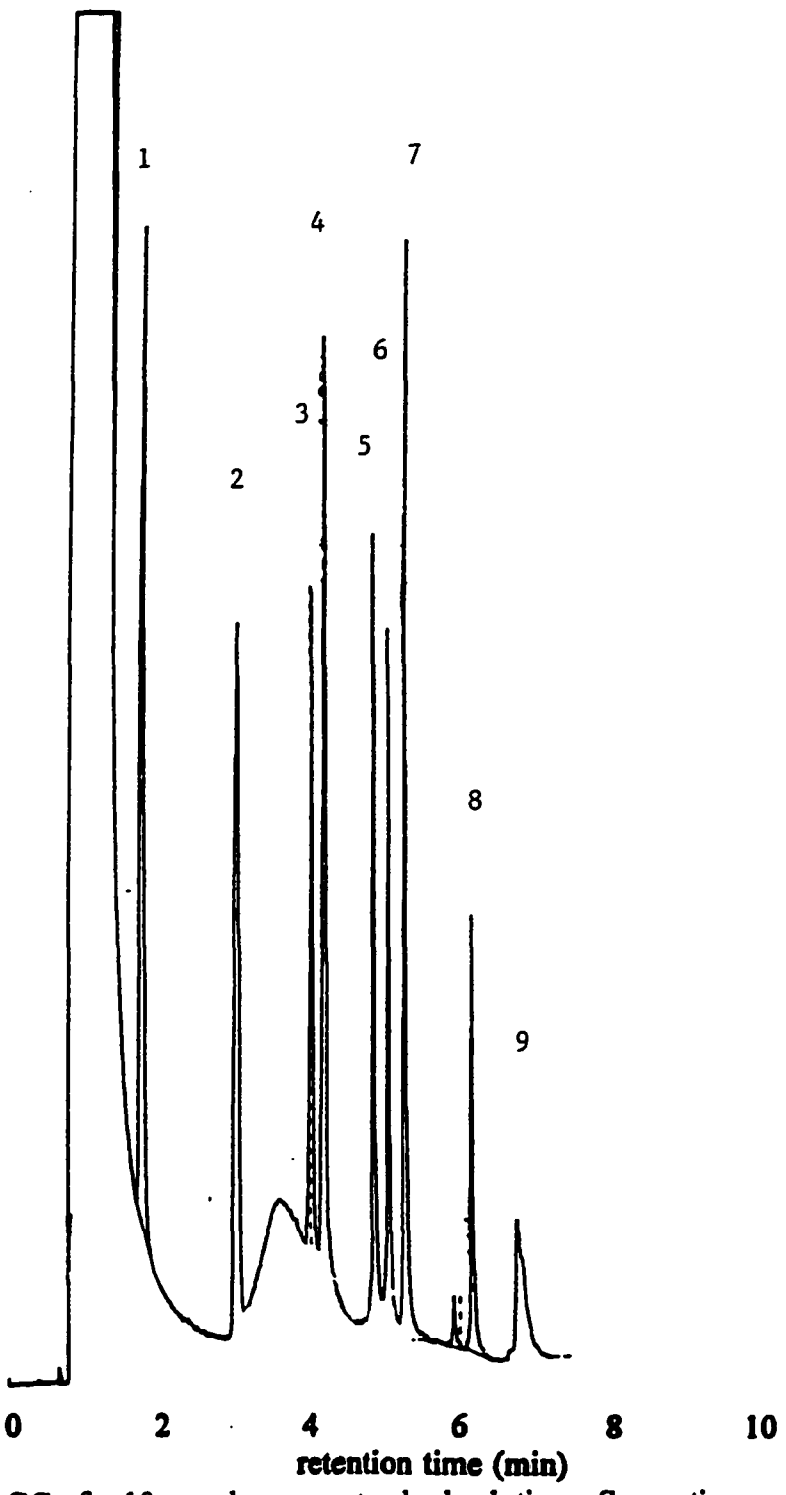

Figure 10. GC of a 10 ppm benzene standard solution. Separation performed on a 15 m Supelco SPB-5 column. Initial temperature = 60 °C, Ramp to 180 °C at 8 °C/min. Peak identification: 1 = benzene, 2 = toluene, 3 = chlorobenzene, 4 = bromobenzene, 5 = ethylbenzene, 6 = anisole, 7 = propylbenzene, 8 = o-dichlorobenzene, 9 = methylbenzoate.

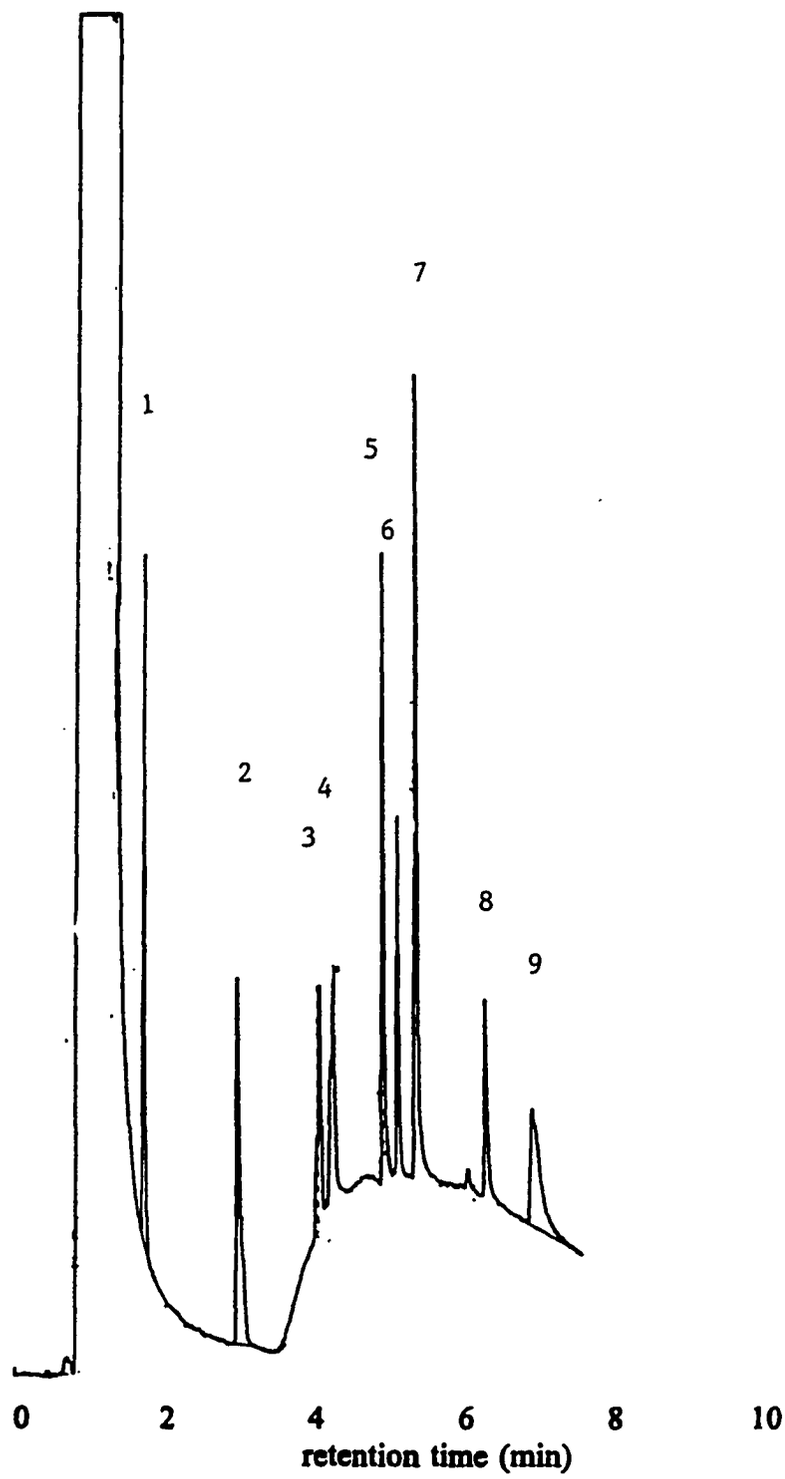

Figure 11. GC of a 5 ppm benzene standard solution after a 2-fold concentration performed on the MMSPE apparatus. Conditions and peak identifications same as Figure 10.

Table 2. Percentage extraction of substituted benzenes with sulfonated PS-DVB and C18 resin-impregnated membranes.

Compound	Sulfonated PS/DVB	C18
benzene	87	79
toluene	93	93
ethylbenzene	100	99
propylbenzene	100	100
butylbenzene	100	100
benzylbromide	97	92
bromobenzene	97	92
chlorobenzene	96	94
Benzylchloride	94	94
benzyl acetate	96	76
benzonitrile	99	97

technique which provides all of the advantages of traditional SPE, while eliminating many of the problems encountered with some of the current miniaturized techniques. SPE in a syringe allows high extraction efficiencies while eliminating the need to handle small volumes of eluting solvents. Excellent uptake and elution of a wide range of organic compounds was demonstrated with this technique. By switching between currently available resin-impregnated membranes, the scope of MMSPE is easily enlarged. Not only was MMSPE used for GC, it was also used for HPLC with no additional apparatus. With additional refinements to the apparatus, MMSPE will provide a miniaturized technique that is both dependable and simple to use.

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GENERAL CONCLUSIONS

By using resins that are much smaller than conventional solid phase extraction, and carefully optimizing reaction condition, resins can be produced that give very good recoveries in the batch mode. These resins provided high capacities and excellent recoveries in both the column and the batch mode. Many of the problems usually encountered when using chelating resins are avoided in the batch mode. Equilibration times, elution off of the resin, and memory effects when reusing the resin are avoided in the batch mode. Due to the very strong complexation achieved with these resins, recoveries of metals in the batch mode are also higher than when simple ion exchange resins are used.

The DSX-100 instrument from Cetac Technologies showed excellent reproducibility and recovery of metal ions with a fully automated system. Concentration factors between 10-100 were demonstrated, along with excellent elimination of interfering ions in the matrix. Concentrated salt brines were even used as representative samples, with recoveries of uranium near 80% with a ten-fold concentration effect.

The use of a new surfactant, Brij-S proved to be an excellent choice when compared to SDS. The surfactant serves two purposes in the separation of drugs from biological fluids in direct-injection HPLC. First, it prevents adsorption of the large biomolecules and causes them to be eluted rapidly and cleanly. Second, it permits elution of the drugs at a much lower organic solvent concentration than would otherwise be necessary. Brij-S is found to elute the large biomolecules almost with the identical strength at SDS, while the elution of drugs with Brij-S is a great improvement over SDS.

Peak shapes are sharper, drugs are eluted faster, and different selectivities for particular drugs are found. By using a solvent gradient in the sulfonated Brij-30 system, analysis times were cut in half and late eluting peaks were are sharpened, with no additional instrumentation required. The choice of an appropriate surfactant for a separation is an easy way to achieve excellent separations in the minimum amount of time when drug separations in biological fluids are required.

The technique of SPE in a syringe has many advantages over convention SPE and SPME. SPE in a syringe miniaturizes traditional SPE, requiring just 5-20 μl of solvent to elute compounds from the small membrane bed. Since the technique requires such small elution volumes, the eluted compounds can be eluted from the syringe, directly into a GB or HPLC system, with no handling of the eluant required. This eliminates the risk of error due to evaporation or mishandling. SPE in a syringe is also an exhaustive extraction technique, providing the lowest limits of detection and the easiest quantification.

SPE in a syringe is shown to be an effective form of SPE. Recoveries of phenols on an anion exchange resin were found to be >90%, with limits of detection pushed down to the parts per billion range. This technique was also effective in removing drugs from serum and in the extraction of polyaromatic hydrocarbons before analysis with HPLC.

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of your kids with level heads that helped us all go far (no matter what some high school guidance counselor predicted). Thanks for your support and always telling me I could accomplish whatever I set my mind to, no matter how discouraged I may of been at the time.

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