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Improved Resins and Novel Materials and Methods for Solid Phase Extraction and High Performance Liquid Chromatography

by

Freeze, Ronald

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Ames Laboratory, U.S. DOE
Iowa State University
Ames, Iowa 50011

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DISCLAIMER

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

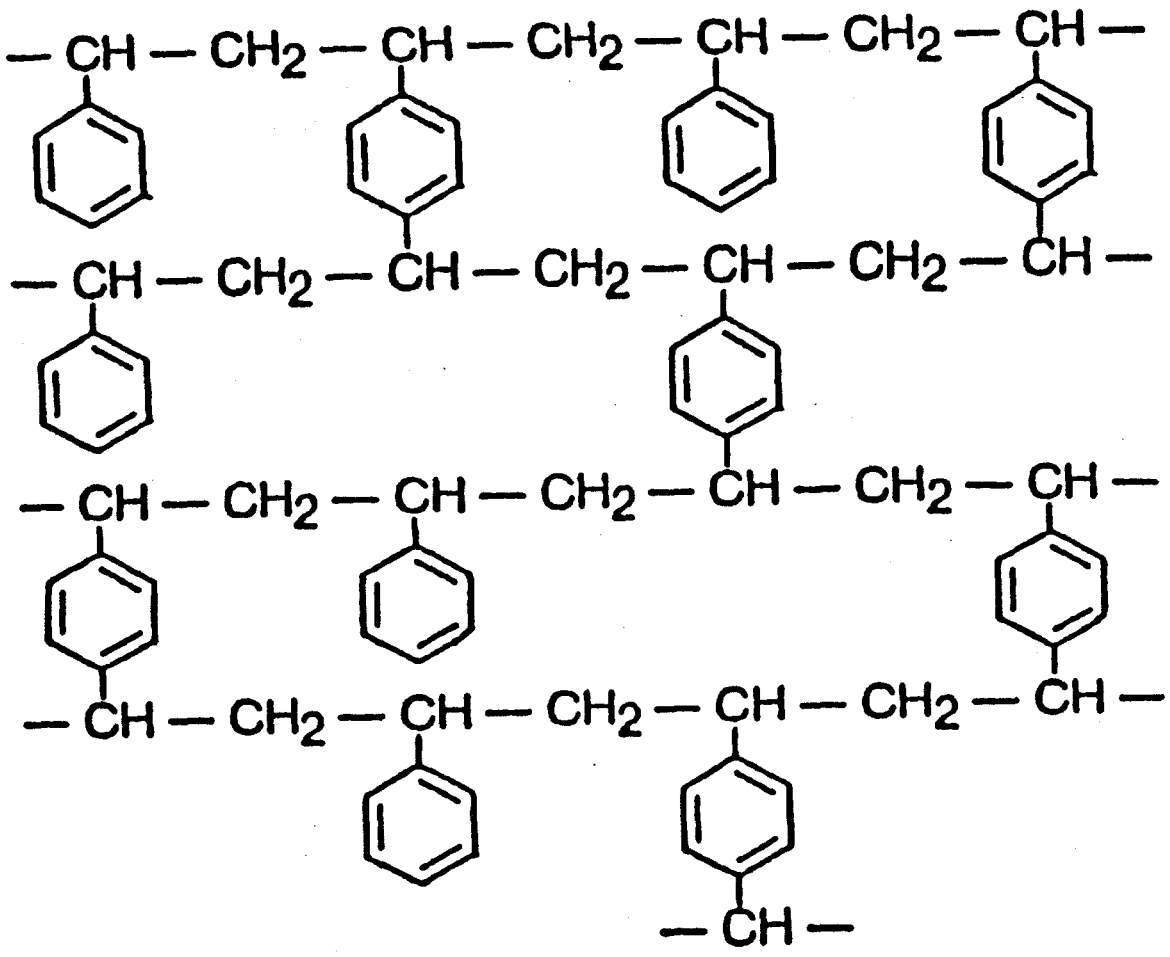

Figure 1. Chemical Structure of Polystyrene divinylbenzene (PS/DVB).

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	$R\text{-}N(CH_2COO^-Na^+)_2$	16-19
dithiocarbamate	$R\text{-}NHCS_2^-$	20-24
8-hydroxyquinoline	C_9H_6NOH	25-27
thiouronium	$R\text{-}SC(NH_2)NH$	28,29
hydroxamic acid	R-CONHOH	30-35
cation exchange	$R\text{-}SO_3^-$	36,37
anion exchange	$R\text{-}N(CH_3)_3^+$	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

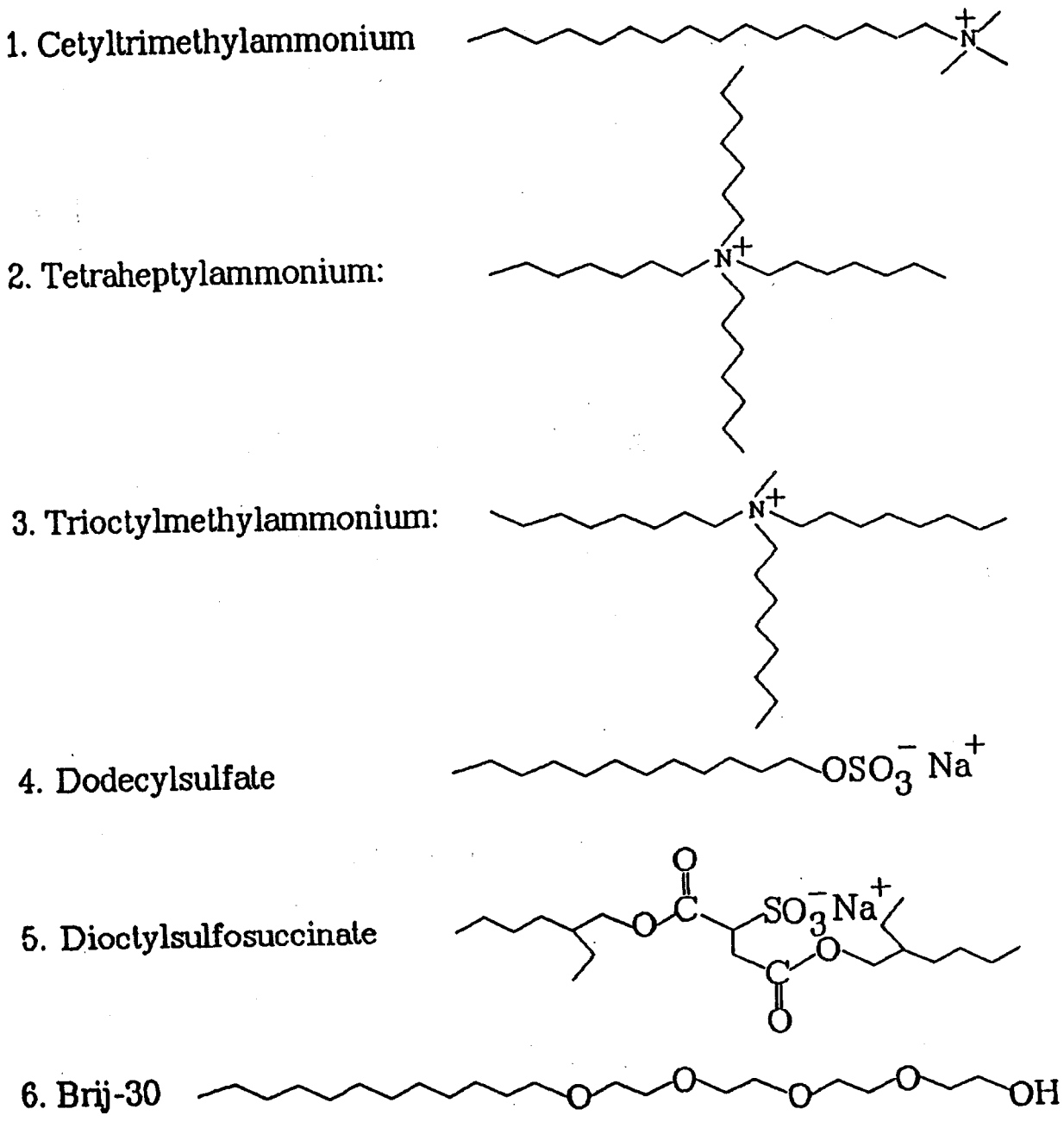

Figure 2. Structures of common mobile phase additives.

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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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 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. 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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