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New Methods and Materials for Solid Phase Extraction and High Performance Liquid Chromatography

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GENERAL INTRODUCTION

Organization of Dissertation

This dissertation begins with a general introduction containing a literature review. This is followed by three research papers that are published or have been accepted for publication. The fourth paper will soon be submitted for publication. Permission from the publisher extending reproduction and distribution rights has been obtained. A general conclusion section follows the four papers. Each paper is similar to the published version, although additional figures and tables have been added. Figures and tables are contained in the text of the paper at the appropriate location. References cited within each paper are listed after the conclusions of each paper.

Solid Phase Extraction

The detection and quantification of organic compounds in various matrices is becoming more challenging every day. The number of controlled compounds and the demand for lower detection continue to rise. Most compounds cannot be determined directly without some sort of initial sample preparation step. This step is often the weakest link in the entire analytical determination. Sample preparation frequently introduces the major source of error and takes a significant amount of time[1]. Possibly the most important sample preparation technique is analyte pre-concentration. Most samples contain analytes in very low amounts and a concentration step is necessary to achieve the desired detection limit. Several sample preparation techniques that involve extraction, pre-concentration, or sample clean-up are available such as solid phase extraction (SPE), liquid-liquid extraction (LLE)[2], Soxhlet extraction[3], and supercritical fluid extraction[4]. For liquid samples, SPE is the most attractive choice. SPE may be used with solid samples if an initial liquid extraction step is used.

SPE is a relatively new technique that started in the early 1970's with a comprehensive study by Fritz, *et al.* on the use of XAD resins to extract organic molecules from water[5]. The use of SPE has continued to grow with advancements in methodology and materials. This growth has often been at the expense of LLE. SPE has many advantages over LLE, some of which are shown in Table I. One of the most important advantages is the lower cost of SPE. This cost savings stems from reduced analysis time, lower solvent usage, and lower solvent disposal costs. Several recent reviews on various aspects of SPE are available[6-15].

SPE is often done in small, plastic columns with a diameter of 0.5 to several centimeters. Fifty to several hundred milligrams of adsorbent are held in the column between two frits. Connecting the column to vacuum or positive air pressure completes the experimental setup for

Table I

Advantages of solid phase extraction over liquid extraction

Solid Phase Extraction	Liquid Liquid Extraction
on-site sampling possible	sample transported to lab
no emulsion formation	emulsion formation frequent
solvent purity not as important	must use high purity solvent
0.1-10 ml solvent	50-100 ml solvent
wide range of solvents to use	solvent must be immiscible with water
cleaner extracts due to adsorbent rinsing	no rinsing possible
easily automated	difficult to automate
low cost	high cost

SPE. The procedure usually consists of four steps. The first step involves rinsing the adsorbent with a suitable solvent. This step is necessary when hydrophobic adsorbents are used. These materials are non-polar and do not allow water to approach their surface easily. Rinsing with certain solvents modifies the resin surface and permits water to approach the adsorbent more easily and produces more efficient extractions. Two examples are methanol and acetone. These have both a polar end (-OH or -C=0) and a hydrophobic end ($-CH_3$). The hydrophobic portion coats the resin surface while the polar portion aids in attracting the aqueous sample. Although this is often a very important step, the research presented in the first chapter of this dissertation will show this may be disregarded with certain adsorbents.

The second step in SPE is the actual extraction. An aqueous sample is passed through an SPE column where the analytes encounter various attractive forces from the adsorbent. These forces may be dispersive, dipole-dipole, hydrogen bonding, ionic, or covalent[16,17]. These forces cause extraction of the analytes of the aqueous phase onto the stationary phase. Most SPE applications use hydrophobic interactions for the extraction. These interactions are simply dispersive forces between non-polar groups, and are weak and non-specific. Dipole and hydrogen bonding interactions are much stronger and more specific but not as common. This type of interaction occurs between electropositive and electronegative atoms, often hydrogen and oxygen, nitrogen, or fluorine. Extraction of charged molecules is due to the strong ionic interactions between ions of opposite charge. Covalent bonding is quite rare in SPE. This is the strongest type of interaction and the most specific. Examples of covalent interactions include the extraction of catecholamines on a boronic acid adsorbent[18-20] and the extraction of metal ions on a chelating adsorbent[21]. Other than the distinctive covalent bond, it is often a combination of forces that lead to extraction since many adsorbents contain both a carbon network and a functional group.

The third step is only necessary for complex samples. By choosing an appropriate rinsing solvent, compounds that interfere or are not of interest may be removed from the adsorbent. A pH change or organic/aqueous mixture may be used for this purpose.

The final SPE step is removing analytes from the adsorbent. Common solvents for this procedure are methanol, acetone, ethyl acetate, or a buffer of different pH. Elution may require only several hundred microliters of solvent. SPE typically results in a concentration factor of 10-1000 with values as high as 50,000 reported[22]. This concentration is imperative in achieving low detection limits.

Various sorbents are currently used for SPE. Much of the original work on the development of this technique used a polystyrene-divinylbenzene (PS-DVB) resin (Figure 1). This material consists of polystyrene copolymers cross-linked with divinylbenzene (DVB)[23] which imparts stability to the resin. PS-DVB may be classified as either microporous or macroporous. The synthesis procedure determines the resin type. Microporous resins are prepared by mixing a catalyst with the monomer in an aqueous solution. Heating causes polymerization to occur. These resins typically have low DVB cross-linking. If a water-immiscible organic solvent is added to the reaction mixture, a macroporous resins will form. The reaction solution dissolves the monomers, but not the polymers. During polymerization, the organic solvent fills the internal pores, resulting in a bead with larger pores and a high surface area. Most macroporous resins are cross-linked with higher percentages of DVB. This imparts rigidity and stability to the resin. The main physical difference between these two types of resin occurs when they are rinsed with solvents of different polarity. Microporous resins with low cross-liking will swell and shrink with

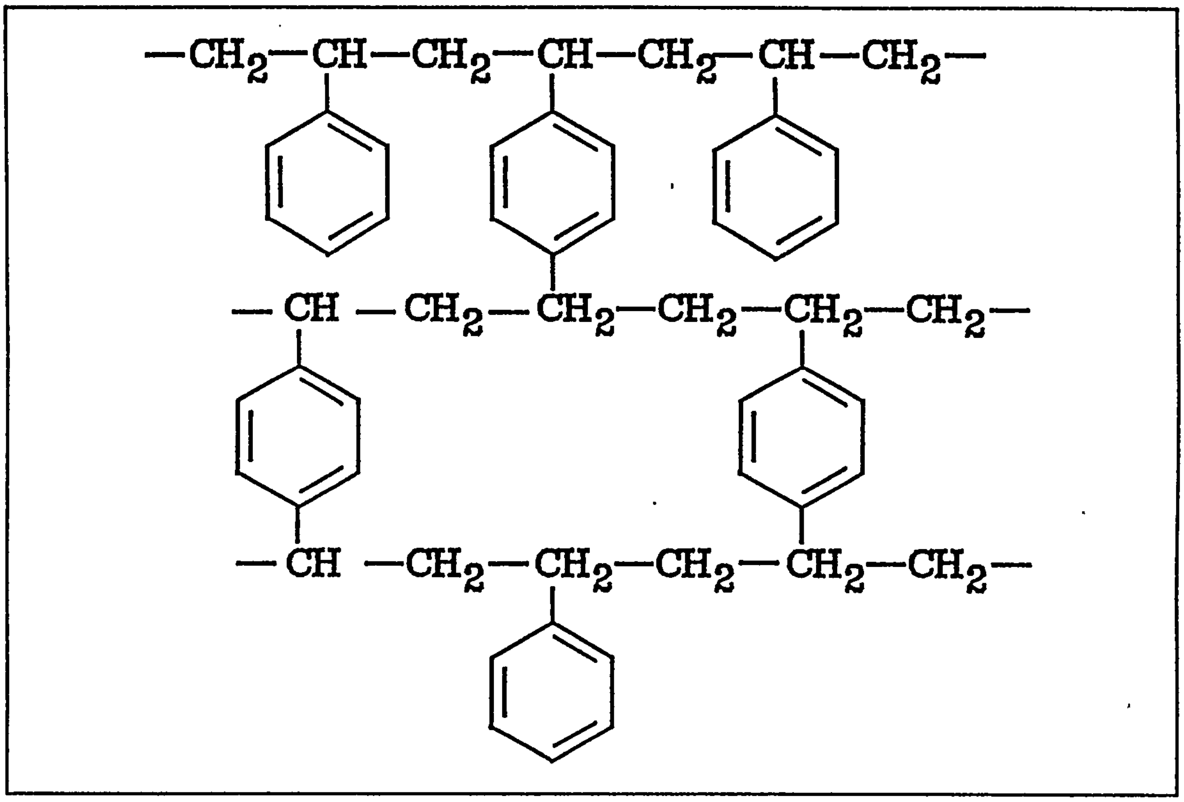

Fig. 1 Structure of polystyrene-divinylbenzene.

different solvents. Highly cross-linked macroporous resin will not swell or shrink. PS-DVB resins now used for SPE are almost exclusively macroporous. These particles are 10-50 µm in diameter and have surface areas in excess of 500 m^2/g. Currently, the most common SPE adsorbent is silica-C_{18}. This material consists of silica (-SiOH) modified with various alkyl chains[16]. The chain may contain functional groups such as CN, OH, NH_2, SO_3^-, or $N(CH_3)_3^+$. The functional groups alter the polarity of the silica and permit extraction of different classes of analytes.

A recent advance in SPE is the development of impregnated membranes[24-27]. These are comprised of silica or PS-DVB particles that are held in place with polytetrafluoroethylene or glass fibrils. Membranes have several advantages over loose resin SPE columns. The particles are held rigidly in place, therefore there is little chance of channeling, which arises when an adsorbent is not packed tightly into the column. Channeling occurs when the sample finds a path of lower resistance, and will tend to take this path instead of passing evenly through the adsorbent. The small particle size (5-10 µm) and uniform packing also lead to faster mass transfer kinetics. These characteristics allow for higher sample flow rates which decreases analysis time. Table II lists several types of adsorbents with their characteristic functional groups and typical applications.

SPE is applicable to almost any type of analyte provided a good choice of sorbent and solvent is made. Both solid and liquid samples may be analyzed although a prior extraction of a solid matrix is necessary. Recent applications for a variety of analytes are shown in Table III with references.

The effect of resin sulfonation on the retention of organic compounds is described in

Table II

Typical adsorbents used for solid phase extraction

	structure	references
PS-DVB	(figure 1)	28,29
anion-exchange	ϕ[1] - $N(CH_3)_3^+$	30
cation-exchange	ϕ - SO_3^-	31,32
Silica	Si - OH	33,34
C18	- Si -$C_{18}H_{37}$	35-40
C8	- Si -C_8H_{17}	41-43
phenyl	- Si -ϕ	44,45
cyanopropyl	- Si -C_3H_6CN	46-49
diol	- Si -$C_3H_6OCH_2(CHOH)_2$	50-53
aminopropyl	- Si -$C_3H_6NH_2$	54,55
phenylboronic acid	- Si -C_3H_6-NHϕ-$B(OH)_2$	56-58
cation-exchange	- Si -$C_2H_4\phi$-SO_3^-	59,60
anion-exchange	- Si C_3H_6-$N(CH_3)_3^+$	61,62
Membranes		
PS-DVB		63-65
silica - C18		66,67

[1] ϕ = phenyl group

Table III

Recent applications of solid phase extraction

Analyte Class	Matrix	References
Drugs/Biological compounds	Plasma	68-70
	Urine	71-73
Pesticides	Water	74-78
	Foods	79-81
Herbicides	Soil	82,83
	Water	84,85
	Blood	86
Organic Acids	Foods	87,88
	Urine	89,90
	Air particulates	91
Organic Amines	Water	92,93
	Foods	94,95
Carcinogens/Pollutants	Water	96-99
	Soil	100
	Tissue	101,102
	Urine	103
Metal Ions	Water	104-107
Inorganic Anions	Water	108,109

Chapter I of this dissertation. The degree of modification was found to be critical in determining the extraction efficiency of the resins. Breakthrough analysis was used as another method for determining extraction efficiency. Empore membranes embedded with sulfonated resin were used for the extraction of a variety of compounds.

Ion Chromatography

Ion chromatography (IC) is a common method used for the separation and determination of inorganic and organic ions. This technique was first described by Small, *et al.*[110] in 1975 and has since become a simple, reliable, and inexpensive method for the determination of ions in various mixtures.

The availability of a suitable detection technique hindered the initial development of IC. Conductivity was the obvious choice, but the high ionic strength of the eluent often swamped-out the analyte signal. Suppressors were necessary to reduce the background conductance and permit the ions to be detected. In 1979 Fritz, *et al.*[111] developed low-capacity resins for IC. These materials have capacities between 0.005 and 0.20 mequiv/g. The exchange sites are located on the outside of each polymer bead and provide efficient exchange characteristics. Low conductivity eluents were used which made eluent suppression unnecessary. The exchange groups on these resins are commonly $-SO_3^-H^+$ and $-N(CH_3)_3^+OH^-$ for cation and anion-exchange respectively.

A wide variety of eluents are used for IC. Salts of organic acids are often used for anion chromatography[111-113]. Aromatic acids such as benzoate and phthalate are common, and have the advantage of being a strong chromophore which permits indirect UV detection. The hydroxide ion has a high equivalent conductance and is often used as an eluent with indirect conductivity detection[114]. Inorganic mineral acids, such as nitric acid, are the most popular eluents for alkali metals and organic amines[115,116]. As with hydroxide, the hydrogen ion has a very high conductance, which is amiable with indirect conductivity detection. Organic bases, as with organic acids, may be used with conductivity or indirect UV detection[117,118].

Conductivity is the most common type of detector in use today[119]. Depending on the eluent composition, either increases or decreases in conductivity may be measured. Direct spectrophotometric detection is possible if the analyte ions absorb. Most simple ions do not absorb, but may be detected with indirect spectrophotometry if the exchange ion contains a chromaphore[120]. Pulsed electrochemical detections was developed by Johnson, *et al*[121]. in the early 1980's and is now commercially available. This is an especially powerful detection technique for saccarides[122], alcohols[123], alcoholamines[124], and sulfur compounds[125]. Mass spectrometry is becoming more popular as a detection device for IC[126-128]. In most cases, suppressed IC has been used to avoid salt accumulation in the ion source.

Several recent review articles on the methods and applications of IC are available. Fritz[129] discussed the fundamental principles and materials used for IC. In a later review, Dasgupta[130] discussed more recent advancements of IC. Walton published a chapter on IC, including the principles of ion-exclusion, ion-pairing, and ligand-exchange[131]. Haddad and Jackson published a very thorough book on the principles and applications of IC[132].

Although separation of the alkali metals has become routine, there is still interest in improving various aspects of these determinations. The simultaneous determination of +1 and +2 metals has been difficult to obtain because of the widely different ion-exchange selectivities[133].

Several methods have recently been proposed to accomplish this separation. Column switching techniques, in which two or more columns are used, have been described[134,135]. The sample passes through a low capacity column which retains +2 metals. The alkali metals are not retained on the first column and are passed through to a higher capacity column for separation. This technique is somewhat cumbersome, and simpler, single-column methods have been developed.

Hu, *et al.* have developed a silica-C18 modified stationary phase for the separation of alkali and alkaline earth cations[136-138]. An anionic bile salt micelle is hydrophobically attached to the C18 surface. This micelle possesses both ion-exchange and size-exclusion properties. Several applications for this stationary phase are given.

If ordinary stationary phases are used, eluents containing complexing agents are often needed. Yan and Schwedt studied the use of a variety of organic acids for the isocratic elution of alkali and alkaline earth metals[139]. A displacing counterion (Cu^{+2} or Ce^{+3}) was added to the eluent to permit indirect UV detection. Sato proposed two different eluents to accomplish this separation[140]. The eluents contained both an organic acid and organic base.

In the past few years, cation-exchange phases have been prepared by coating bonded silica with various resins. Polymers of styrene[141], glycidylmethacrylate[142], butadiene maleic acid[143], and acrylic acid[144] have been used. These stationary phases have low capacities and produce sharp peaks and short retention times. The low capacity allows for dilute eluents which makes suppression unnecessary. Alkali, alkaline earth, and heavy metals have been separated with these stationary phases.

Lowering detection limits for alkali metals, particularly in the presence of large excesses of other ions is always of interest. Dolgonosov and Krachak developed a highly selective method for the determination of the ammonium ion[145]. A suppressor column is used as a reaction chamber to selectively convert the ammonium ion to nitrogen gas and water. These neutral species generate large negative conductivity peaks. Ammonium was detected at the parts per billion level in the presence of 100-fold excess alkali metals.

Hashimoto and Okada developed a system for the trace determination of several alkali metals[146]. An eluent containing diaminopropionic acid was used with suppressed conductivity detection. Detection limits for NH_4^+, Li^+, Na^+, and K^+ were in the low parts per billion. This system is applicable to trace metal determinations in clean rooms.

Ivask and Pentchuk recently introduced the use of amino acids as modifiers for cation chromatography[147]. Glycine and ß-alanine were found to improve the separation of Na^+, K^+, and NH_4^+.

The simultaneous separation of cations and anions is currently of great interest. Several approaches have been taken to solve this problem. Initially, cation and anion-exchange columns were used in series[148,149]. Simpler methods that involve one column are now available. A simultaneous separation of metal ions and inorganic anions is possible if the metals form anionic complexes, as several do with EDTA[150,151]. Hu and Haraguchi developed a zwitterionic modified silica stationary phase[152]. Na^+ and K^+ were successfully separated from several inorganic anions.

Crown ethers and cryptands have been used to adjust retention and improve separation of cations and anions[153]. These compounds are used by permanently[154,155] or dynamically[156,157] coating a silica stationary phase. Metal ions interact differently with the ether cavity, which modifies their exchange behavior. Adsorbed crown ethers may be complexed with alkali metal cations to form an anion exchanger[158,159].

Several methods are available for the separation of organic amines. Alkyl amines are difficult to detect and a derivatization step is often necessary prior to a reversed phase separation[160-162]. Fewer procedures have been described for separating non-derivatized amines. Organic amines interact with ion-exchange stationary phases electrostatically through the nitrogen cation and hydrophobically through the carbon skeleton. This may lead to poor IC peak shapes. Organic solvents are often added to the mobile phase to decrease the hydrophobic interactions and improve peak shapes. Separating amines by IC is less common, even though no derivatization is necessary and conductivity detection is possible[163,164].

Organic modifiers such as methanol, acetonitrile, and acetone are sometimes added to IC eluents[165]. These solvents have a variety of effect on ions in solution[166]. Organic ions are retained by a combination of ion-exchange and hydrophobic interaction. Organic solvents in the eluent reduce the hydrophobic interaction and alter the retention mechanism. Organic solvents also modify the solvation of inorganic ions, which change retention characteristics. Although adding small amounts (10-20%) of organic solvent to ion chromatography eluents is not uncommon[167,168], little work has been done with predominately organic eluents. Hoffman and Liao have studied the retention process of aromatic amines in 100% organic solvents[169]. They assign numerical values to the ion-exchange and hydrophobic interaction contribution in the chromatographic retention of organic amines.

Chapters II and III of this dissertation discuss the effect of organic eluents on the separation of alkali metal ions and organic amines. A highly cross-linked PS-DVB resin was used for all research presented. Chapter II deals specifically with the alkali metals. The effect of organic solvents on retention and the addition of 18-crown-6 to the mobile phase are discussed.

Several unique separations are shown. In Chapter III, pure organic eluents were used for the separation of organic amines. These eluents effectively remove all hydrophobic interaction, leaving ion-exchange as the only retention mechanism. Different organic eluents were used and several separations presented.

High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) is probably the most widely used analytical technique today. This popularity is due to the necessity of a prior separation before almost any analytical determination. Unlike gas chromatography where the analytes must be volatile, HPLC is applicable to almost any compound. HPLC is a general term that encompasses many different forms of chromatography. This includes ion[129-132], reversed-phase[170-173], normal-phase[174-176], size-exclusion[177-179], and thin-layer[180-182].

Stationary phases for reversed phase HPLC are dominated by polymer and silica-based materials. Silica is generally more efficient than polymer phases, although it is not stable at extreme pH values. Stationary phases are the basis for considerable current research interest. Several recent investigations have examined the mechanism and theory behind stationary phase solvation and the retention process[183-186].

Many modes of detection are possible with HPLC. Currently, the most common detector is UV-Vis absorbance[187-189]. Unfortunately, this is not particularly selective or sensitive. Refractive index[190,191] is a near-universal detector but, like UV-Vis, is not very sensitive. Other detectors such as chemiluminescence[192,193], fluorescence[194,195], and electrochemical[196,197] are much more selective and sensitive. The detectors mentioned thus far do not provide significant qualitative information about the sample composition. Coupling HPLC to other instruments can provide this type of information. Mass spectrometers[198-200], FTIR instruments[201,202], or diode array detectors[203,204] provide more information and allow the determination of unknown analytes.

In chapter four of this dissertation, the use of Silicalite as an HPLC stationary phase is discussed. This material was first synthesized in 1978[205] and has since received very little attention as a possible chromatography adsorbent. Silicalite is a crystalline silica material that is hydrophilic but contains hydrophobic channels approximately 6 Å in diameter. Organic molecules in water are attracted to the channels while water, which associates into clusters 10-12 Å in diameter, is excluded. For small molecules size-exclusion is another possible selectivity mechanism. These facts make Silicalite a good choice for SPE or HPLC.

Much of the research involving Silicalite has related to adsorption of gasses. Hufton and Danner studied the adsorption of alkanes on Silicalite. They discussed both equilibrium properties and transport properties[206,207]. Various other studies of the sorption process have also been reported[208-213].

Shultz-Sibbel, *et al.* investigated the use of Silicalite for the adsorption of both gasses and liquids. Distribution coefficients were measured for many organic compounds in the gas phase. Capacities for pure organic liquids were also reported[214].

Fritz and Ogawa used Silicalite as an SPE adsorbent[215]. Good recoveries of aldehydes and ketones from aqueous samples were obtained.

Andronikashvili, *et al.* recently used Silicalite as a gas chromatography stationary phase[216]. A short column was packed with Silicalite and used for the separation of ortho, meta, and para isomers of aromatic compounds. Several separations are shown.

In Chapter IV, the use of Silicalite as an HPLC stationary phase is discussed. This material was found to be very useful for the separation of a variety of compounds. Isomers, which are difficult to separate of silica-C18 were easily resolved on Silicalite in only a few minutes. Several comparisons were made between Silicalite and silica-C18.

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EFFECT OF RESIN SULFONATION ON THE RETENTION OF POLAR ORGANIC COMPOUNDS IN SOLID PHASE EXTRACTION

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Abstract

The hydrophobic nature of polymeric resins used in solid-phase extraction (SPE) often limits their efficiency by preventing intimate surface contact with aqueous samples. A polymeric resin modified by a series of chemical derivatizations with sulfuric acid was found to display excellent surface hydrophilicity and improved extraction efficiencies. The degree of sulfonation was found to play a vital role in determining the SPE efficiency of such resins. By measuring the capacity factor (k′) of several polar organic solutes in pure water, an optimum sulfonation capacity of 0.6 mequiv/g was determined. Loose sulfonated resin and Empore® membranes embedded with sulfonated resin were used for SPE. Average recoveries were greater than 95% for both forms of sulfonated resin for a wide variety of organic compounds including phenols, alcohols, nitro molecules, aldehydes, esters and halogenated alkanes. Breakthrough curves for

p-cresol, ethyl acetoacetate, isophorone, and nitrobenzene were used to compare Empore membranes embedded with sulfonated and unsulfonated resins. The sulfonated membrane yielded sharper and more efficient breakthrough for all compounds tested.

Introduction

Sample preparation has been identified as often the slowest and therefore the most expensive step in the analytical process[1]. SPE has become the preferred technique for sample preconcentration. Being a multi-stage method, it is more efficient than simple liquid-liquid extraction. Analytes undergo multiple equilibrations with the resin and are therefore more strongly retained than in simple liquid extraction where only a single equilibration occurs. It is also more easily automated and much less polluting than liquid extraction techniques that use relatively large volumes of organic solvents[2]. An early comprehensive paper investigated thoroughly the technique, scope, and limitations of SPE with porous polymeric resins (Rohm & Haas, XAD)[3]. Several reviews on SPE have been published[4,5]. Bonded-phase silica particles (mostly C18 or C8) dominate the field, although porous polystyrene resins are finding increasing use because of their efficiency, ruggedness, and broader pH range.

One problem with extraction materials now used is the inability of aqueous solutions to adequately wet their surface, which is usually hydrophobic. This is true for both silica-C18 and underivatized PS-DVB. Pretreatment of the resin column or cartridge with methanol is usually necessary to obtain better surface contact with the aqueous solution[6,7].

Recently it has been shown that introduction of polar groups into a PS-DVB resin greatly increased the retention of polar organic compounds. Fritz and Sun[6] modified PS-DVB with alcohol and acetyl functional groups. These modified resins exhibited excellent hydrophilicity and a lesser dependence on wetting prior to extraction. They also yielded higher recoveries compared to their unmodified analogue. This was attributed to an increase in surface polarity allowing the aqueous sample to make better contact with the resin surface.

Fritz and Schmidt[8] used a sulfonated PS-DVB resin for the simultaneous extraction of bases and neutrals. Bases bind via ion-exchange and neutrals by hydrophobic interactions. A two-step elution was then used to elute the bases and neutrals sequentially. Excellent recovery of both bases and neutrals was obtained.

Mills, *et al.* recently compared sulfonated and unsulfonated silica-C18 for the extraction of triazine compounds[9]. Once again, higher recoveries were obtained with the sulfonated resin. This was not attributed to the increased resin hydrophilicity but to hydrogen bonding between the amine functionality of the analytes and the sulfonic acid group on the resin. They also determined distribution coefficients (K_D) for the atrazine compounds on both resins, and found much higher values on the sulfonated resin.

It is now shown that porous PS-DVB resins modified with surface sulfonic acid groups are superior to the unmodified PS-DVB for SPE of organic solutes from aqueous samples. The extent of the sulfonation was found to be critical in determining extraction efficiency of such resins. The modified resins can be used for SPE in either of two modes - resin packed into mini columns or disks of resin-loaded membranes.

Experimental

Reagents and Chemicals

Chemicals used for the sulfonation reactions were of reagent grade. All analytes studied were >99% pure and used as obtained from Aldrich and Fischer. Distilled water was further purified with a Barnstead Nanopure II System (Sybron Barnstead, Boston, MA, USA).

Several sulfonated resins were prepared from 8 μm PS-DVB obtained from Sarasep (Sarasep, Inc., Santa Clara, CA, USA). General reaction conditions are shown in Table I. Specifically, 2 g of resin was mixed with 5 ml glacial acetic acid to form a slurry. Concentrated sulfuric acid was added with stirring. After a given reaction time, the mixture was quickly added to ice water to quench the sulfonation reaction. The resin was then filtered through a medium glass frit, and rinsed with deionized (DI) water, methanol, 2-propanol, and finally acetone. After drying, the cation-exchange capacity was determined. One milliliter of 1 M HCl was passed through a known amount of resin to ensure all exchange sites were protonated. After rinsing with DI water to remove excess HCl, 5 ml of a standardized NaOH solution was slowly passed through the column and collected into a flask containing 10 ml of a standard HCl solution. Following another DI water rinse, this was titrated with NaOH to the phenolphthalein endpoint. Capacities were calculated as milliequivalents SO_3^- per gram of resin.

k' Determination

A small (20 x 2.1 mm I.D.) guard column (Supelco, Bellfonte, PA, USA) was filled with approximately 20 mg of resin. This guard column was contained within a guard column holder and connected to the HPLC system via two injection loops; a 10 μl loop from the injector (Rheodyne, Berkeley, CA, USA) and a 50 μl loop to the detector. The HPLC system consisted

Table I

General reaction conditions for resin sulfonation

Capacity (mequiv/g)	H_2SO_4 (ml)	Reaction time (min)	Temperature
0.0			
0.1	5	0.5	ice
0.4	50	2	ice
0.6	50	4	ice
1.0	50	10	room temp.
1.2	50	20	room temp.
1.5	50	90	room temp
2.1	50	90	50°C
2.7	50	90	85°C

of a Gilson Model 302B HPLC pump equipped with a Model 802B Gilson manometric module (Gilson, Middleton, WI, USA) and Model LP-21 Scientific Systems pulse dampener (Scientific Systems, State College, PA, USA) and a Kratos 783 UV-VIS detector (Applied Biosystems,Ramsey, NJ, USA). Retention times were measured with a Hitachi D-2000 Chromato-Integrator (EM Science, Cherry Hill, NJ, USA).

Fifty ppm samples were prepared by diluting stock solutions in DI water. Depending on the absorbance of the analyte, 10 - 50 μl were injected. Phenols were detected at 270 nm and the carbonyl compounds at 205 nm. DI water at 0.5 ml/min was used as the eluent. The column dead time, t_o, was determined using the retention time of bromide (sodium bromide). This value included the travel time through the tubing (60 μl total volume); therefore 0.12 min was subtracted from the measured time to calculate the true t_o. This value was also subtracted from all analyte retention times.

Capacity factors were determined for the unwetted and methanol wetted resin. Wetting was accomplished by injecting two successive portions of methanol from a 500 μl loop, followed by a 4 ml rinse with DI water. During the water rinse the 500 μl injection loop was replaced with the smaller loop for sample injection.

Procedure for SPE

The apparatus for SPE consisted of a 30 ml glass syringe barrel fitted with a luer tip. A 1.5 ml polypropylene SPE column (P.J. Corbert, St. Louis, MO, USA) was connected to the glass reservoir via a universal adapter. Loose 8 μm sulfonated resin and Empore (3M Co., St. Paul, MN, USA) membranes embedded with sulfonated resin were used as the SPE adsorbents. This was placed between two 20 μm polyethylene frits (P.J. Corbert, St. Louis, MO, USA) in the column. The bed height measured approximately 1 cm. Positive pressure was used to force liquids through the adsorbents. Prior to use, about 1 ml methanol and 2 ml water were used to rinse the column.

Samples were prepared by adding a 100 μl aliquot of a 150 ppm methanol solution containing 5-10 analytes to 15 ml of DI water. The final concentration of each compound in the sample was about 1 ppm. Air pressure was adjusted to provide a flow of 1-2 ml/min (30-60 psi). After loading, the glass reservoir was rinsed with 3-5 ml water and air was blown through the column to remove any remaining water. One milliliter ethyl acetate or methanol was used to elute the compounds into a GC vial. An internal standard (100 μl of a 150 ppm toluene solution in methanol) was added to the vial, which was then analyzed by gas chromatography. A Shimadzu GC 14A equipped with an AOC-14 autoinjector, flame ionization detector, and a C-R4A Chromatopac data analysis system (Shimadzu Corp., Kyoto, Japan) was used to separate and quantitate the analytes. The gas chromatographic column was a 15-meter SPB-5 (Supelco, Bellfonte, PA, USA). Recoveries were calculated as an average of 3 trials by comparing the relative peak areas with standards that were not subjected to SPE.

Breakthrough Curve Procedure

Breakthrough curves on a 46 mg sulfonated Empore membrane and a 44 mg unsulfonated Empore membrane were determined by passing a dilute solution (5-10 ppm) of analyte continually through the SPE column. Five or 10 ml fractions were collected in volumetric flasks. In order to concentrate the analyte, the collected sample was passed through another sulfonated Empore membrane, which was eluted with 1 ml of methanol into a GC vial. After addition of the internal standard, the analyte concentration was determined by gas chromatography. Fractions were collected until the concentration of analyte remained constant.

Results and Discussion

Sulfonation of Resins

Our goal was to make the resin surface more hydrophilic while keeping the interior surface hydrophobic enough to allow extraction of organic solutes. A fairly rapid sulfonation with sulfuric acid seemed appropriate to accomplish this because sulfonation of resins is known to proceed from the outside into the resin. Reacting for a short time will therefore only modify the surface of the resin bead. In order to achieve more even wetting of the resin with viscous concentrated sulfuric acid, the resin was first slurried with a little glacial acetic acid. Acetic acid has both a hydrophobic (-CH_3) and hydrophilic (-COOH) portion. This aids in contacting the polar acid and non-polar resin. Without the acetic acid the resin sits on surface of the sulfuric acid and does not react well.

Porous PS-DVB resin beads (8 μm) were reacted under a variety of conditions to produce sulfonated resins ranging in capacity from 0.1 to 2.7 mequiv/g. The sulfonation conditions and capacities are given in Table I.

Measurement of Capacity Factor

The efficiency of resins used for SPE is most commonly determined by measuring the percentage recovery of test solutes. However, this process depends on the efficiency of elution of the analytes from the SPE column as well as the efficiency of the initial extraction step. Laconto has pointed out that for 90% recovery, K_d for the extraction step may be anywhere between 10 and 100[5]. A better way to compare the behavior of different resins is to measure the capacity factor of the extraction step. Several methods have been used to measure k′ in aqueous solutions. One common method for measuring capacity factors consists of measuring k′ vs ϕ, the percentage of organic solvent in the eluent. After obtaining several points, an extrapolation to 0% organic solvent is possible. Although this is a common procedure[10], it may not be the most accurate for SPE where aqueous samples are used. Surface modifications are undoubtedly taking place due to adsorption of some of the organic portion of the eluent onto the resin surface. It is better to determine k′ in the same sample matrix that is common for SPE samples - 100% water. However, this can be difficult when k′ is very large. Mills . equilibrated a spiked aqueous sample with resin for 24 h. The analyte concentration remaining in the aqueous solution was used to calculate the equilibrium constant.

In the present work the k′ values of various analytes were determined by elution from a very small resin column using pure water as the eluent. The method is quick and convenient and requires no extrapolation since no organic modifier is used in the eluent. The capacity factor is determined from the recorded elution curve using the well-known relationship: $k' = (t_R - t_0)/t_0$. In order for this method to be feasible, the column must be small, otherwise many hours may be required for elution with water as the eluent. The values of k′ are also dependent on the measured t_0, although an error in t_0 will still give relative values of k′ that can be compared for different resins. Details of this method are given in the Experimental section.

Even with a small column non-polar compounds such as benzene would be retained for several hours and give flat elution "peaks". For this reason more polar, water-soluble compounds were chosen as test compounds: phenol, catechol, 2,3-butanedione, and ethyl pyruvate. These compounds are polar enough to elute in a reasonable time and are easily detected by a UV-Vis detector.

The k′ values of these four test compounds are given in Table II and are plotted against the sulfonic acid capacity of the resins in Figures 1-4. In each case the k′ increases with increasing sulfonation capacity, reaching a maximum at about 0.6 mequiv/g. Further increases in sulfonic acid capacity are marked by a rapid decrease in k′.

The increasing k′ values up to 0.6 mequiv/g can be attributed to the fact that a surface-sulfonated resin is more hydrophilic and therefore more easily wettable. The ability of water to come into intimate contact with the resin surface facilitates the transfer of analyte from the aqueous sample to the resin surface. The wettability of a resin may be quickly checked by adding a few milligrams of dry resin to water. Hydrophobic resins will remain on the surface of the water even if stirred. Hydrophilic resins will be dispersed throughout the solution due to the ability of polar surface to reduce the surface tension of the water, thus allowing water to closely approach the resin surface. In terms of capacity, 0.6 mequiv/g is the minimum sulfonation necessary to produce a hydrophilic resin surface. Underivatized PS-DVB and slightly sulfonated (up to 0.6 mequiv/g) are not wetted by water, while resins with a capacity of 0.6 mequiv/g or greater are wetted.

The lower k′ values above 0.6 mequiv/g may be attributed to lower overall hydrophobicity of the resin at higher concentrations of sulfonic acid groups. The hydrophobic resin matrix becomes increasingly shielded by the bulky, polar sulfonic acid groups. This shielding hinders the approach of the analytes to the resin surface. All of the compounds used in this experiment were quite polar, but a similar trend would be expected for more non-polar analytes, although the change may not be as dramatic. Obtaining curves for hydrophobic analytes would be difficult

Table II

Effect of sulfonation capacity on the retention of polar organic solutes in 100% water.

Values are an average of 2 trials. Bold numbers refer to methanol wetted resins.

sulfonation capacity (mequiv/g)	Capacity Factor							
	Phenol		Catechol		Ethyl pyruvate		2,3-Butanedione	
0.0	**49**	21	**10**	1	**0**	0	**1**	1
0.1	**124**	40	**32**	1	**4**	0	**2**	0
0.4	**350**	272	**45**	34	**49**	40	**4**	3
0.6	**457**	436	**90**	74	**79**	60	**14**	12
1.0	**381**	315	**70**	59	**55**	54	**8**	8
1.2	**324**	290	**78**	56	**57**	38	**7**	7
1.5	**209**	183	**45**	38	**34**	26	**6**	6
2.1	**127**	80	**25**	16	**16**	9	**4**	3
2.7	**55**	47	**12**	10	**6**	5	**2**	2

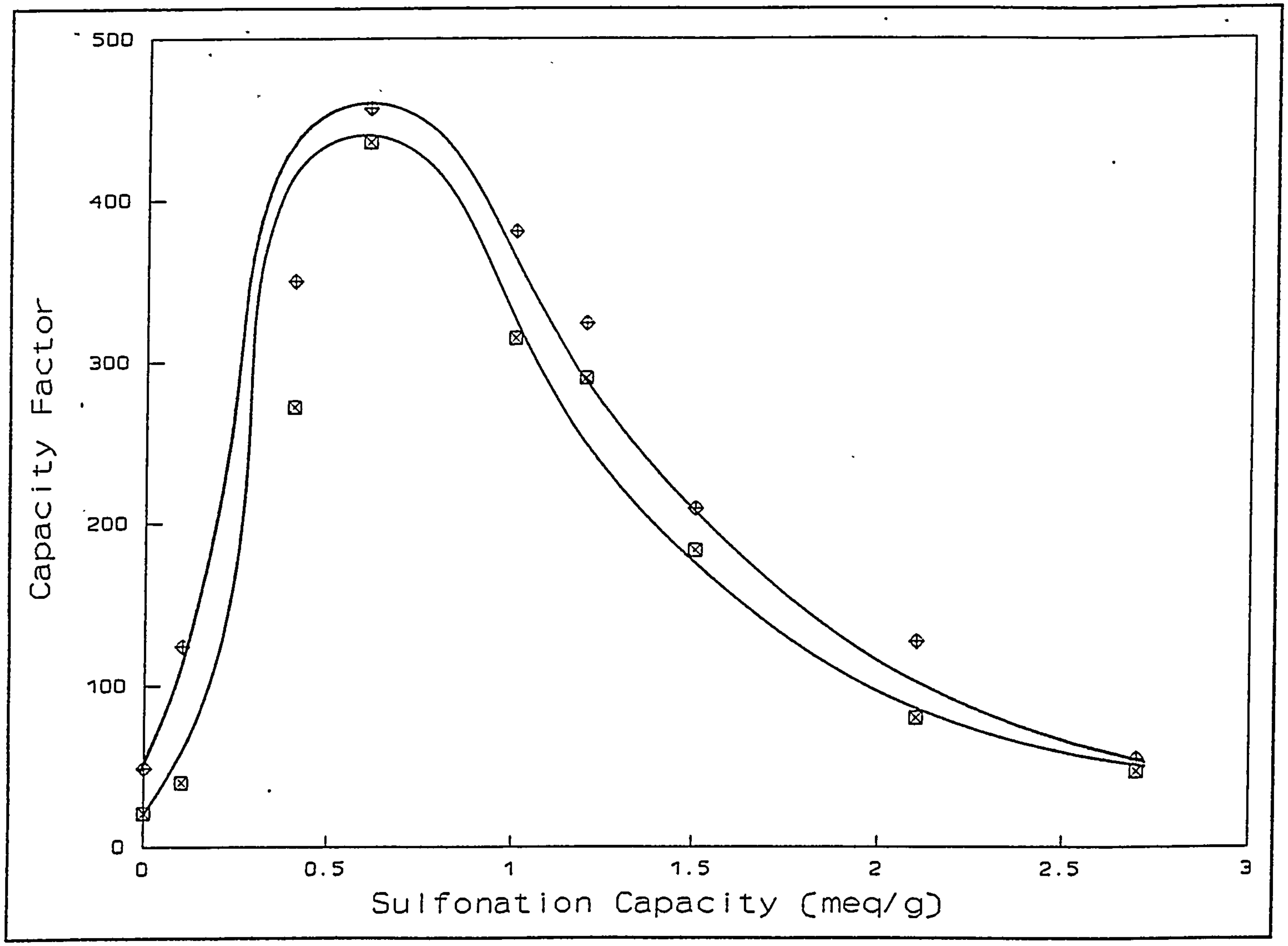

Fig. 1 Effect of PS-DVB sulfonation capacity on the retention of phenol in pure water for methanol wetted (top curve) and unwetted (bottom curve) resins.

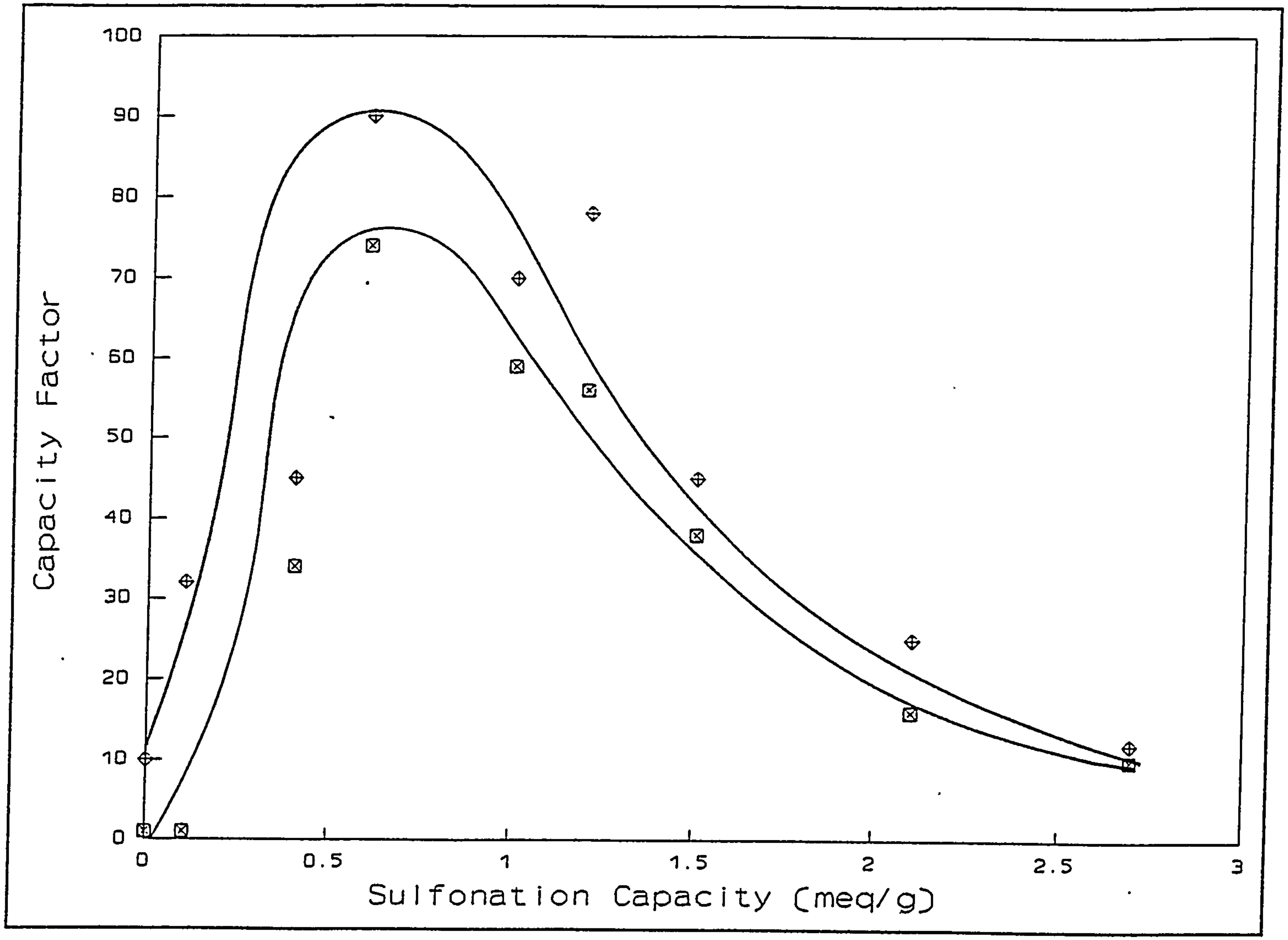

Fig. 2 Effect of PS-DVB sulfonation capacity on the retention of catechol in pure water for methanol wetted (top curve) and unwetted (bottom curve) resins.

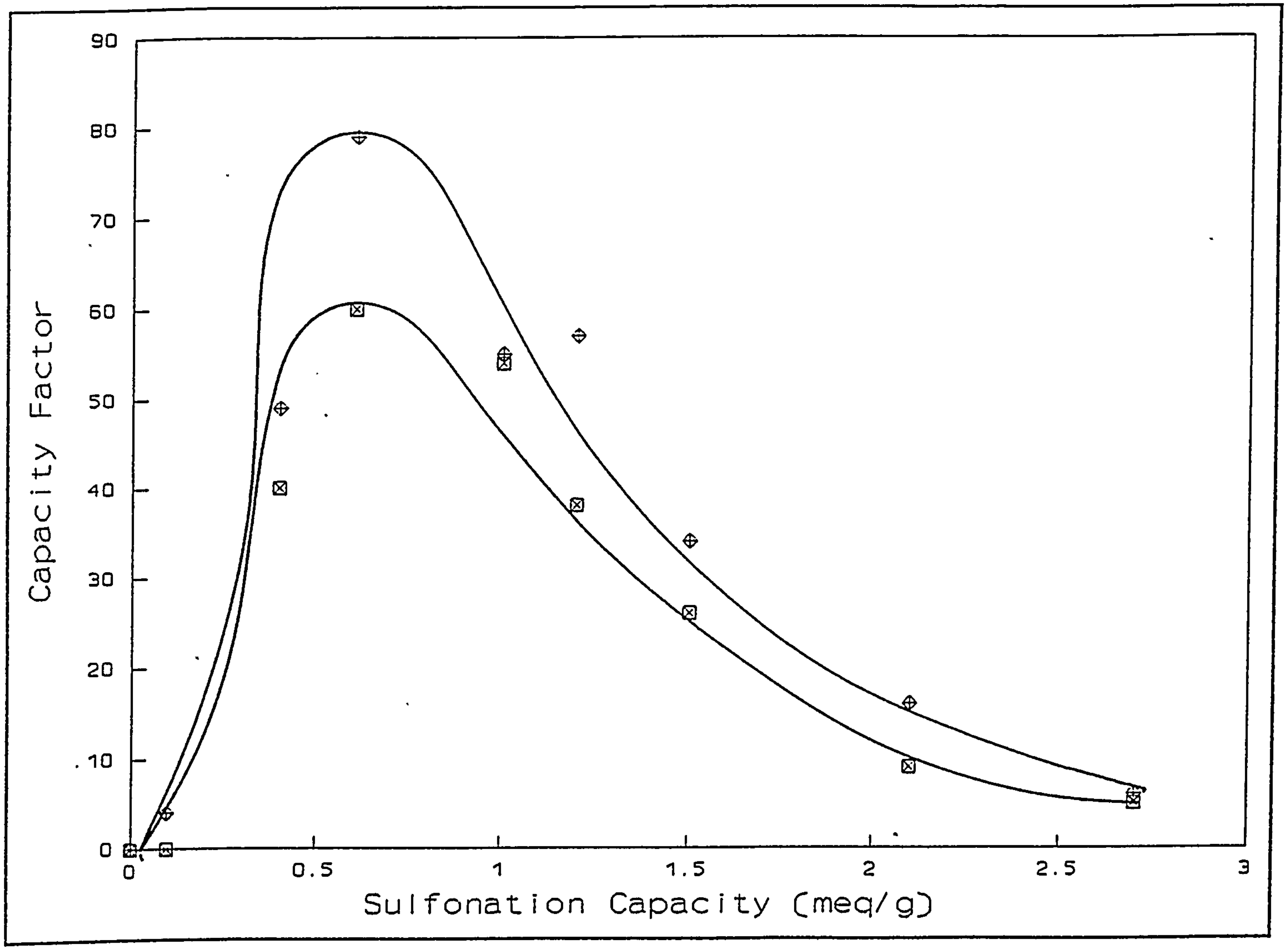

Fig. 3 Effect of PS-DVB sulfonation capacity on the retention of ethyl pyruvate in pure water for methanol wetted (top curve) and unwetted (bottom curve) resins.

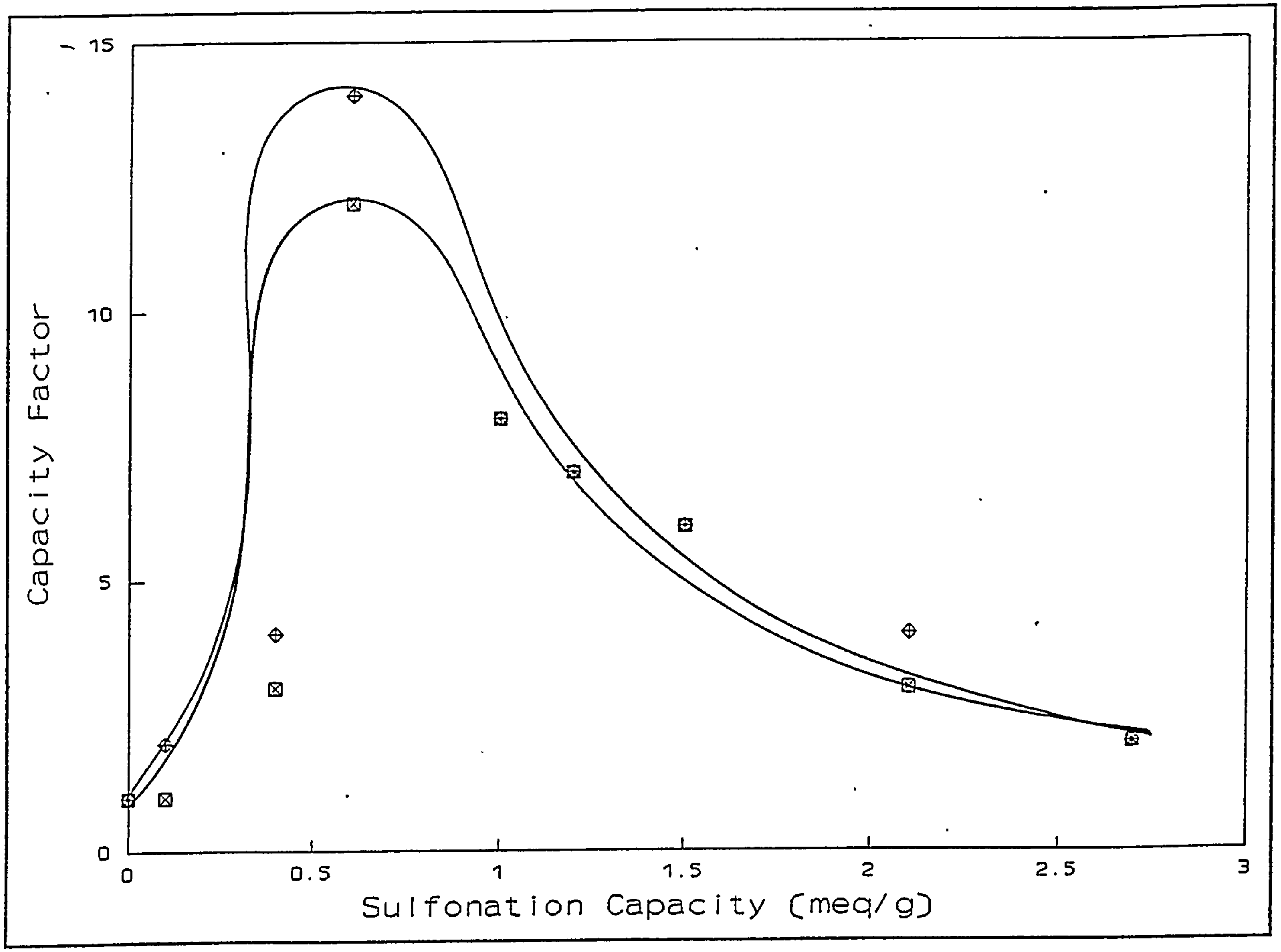

Fig. 4 Effect of PS-DVB sulfonation capacity on the retention of 2,3-butanedione in pure water for methanol wetted (top curve) and unwetted (bottom curve) resins.

and time consuming. The retention time for phenol, the most non-polar compound used for this study, was about 65 minutes. Judging by the peak shape, a maximum retention time of about 2 hours may still yield a detectable peak, which leads to an upper k′ limit for this technique of 1000.

SPE with Sulfonated Resins

The ability of sulfonated and unsulfonated resins to extract various organic test compounds from aqueous samples was compared using identical small columns packed with the resins. After the extraction step, the test compounds were eluted with 1.0 milliliter of ethyl acetate or methanol and determined by GC. The percent recoveries are given in Table III. The small resin size (8 µm) allows even the hydrophobic underivatized resin to extract the compounds, but the sulfonated resin, with a more polar surface is even more efficient for extracting these analytes. Note the sulfonation capacity for this table is 0.4 mequiv/g which is close to the optimum capacity of 0.6 mequiv/g. The effect of wetting the resin with methanol is also shown. As expected, this has a major effect on the underivatized resin, but is not as important with the sulfonated resin. The surface of the sulfonated resin is hydrophilic enough that methanol does not significantly modify it.

SPE with Resin-Loaded Membranes

Empore membranes embedded with sulfonated resin of approximately 0.6 mequiv/g were also used for SPE. Membranes embedded with other materials have been used and described previously[11-13]. They offer several advantages over loose resin including lower back pressure necessary to load samples, decreased channeling, and improved mass transfer[14-15]. In this study, sulfonated membranes were used to extract neutral organic compounds from water. Averaged

Table III

Comparison between sulfonated (0.4 mequiv/g) and unsulfonated resins for solid phase extraction

Values are an average of 3 trials. Wetting solvent is methanol.

Compound	Recovery(%)			
	sulfonated		unsulfonated	
	not wetted	wetted	not wetted	wetted
Anisole	94	93	83	89
Benzaldehyde	90	89	87	96
Nitrobenzene	96	95	88	96
Hexylacetate	94	94	84	82
Benzylalcohol	90	98	78	81
Phenol	98	95	77	89
Catechol	59	34	ND[1]	ND
m-Nitrophenol	98	99	89	95
Mesityl oxide	98	97	93	99
trans-2-Hexenyl acetate	93	90	79	89
Average ± R.S.D.(%) 2	95 ± 3.2%	94 ±3.4%	84 ± 5.5%	91 ± 6.3%

1 not detected
2 catechol not included

triplicate recoveries of 45 analytes are shown in Tables IV-VIII. Many classes of compounds arerepresented including phenols, alcohols, aldehydes, ketones, and esters. Polar analytes, like the phenols, and non-polar analytes such as the halogenated alkanes are all efficiently recovered. Recoveries are over 90% for all compounds with relative standard deviations commonly near 3%. These recoveries compare very favorably with data reported previously for alcohol and actyl derivatized PS-DVB resins and membranes[6,13].

Analysis of Breakthrough Curves

The adsorption capacity for several organic compounds was determined by passing a 5-10 ppm aqueous solution of the analyte through a resin-loaded membrane until breakthrough occurred. Since breakthrough is closely related to k'[4], the breakthrough volume (V_B) or retention volume (V_R) for a particular analyte is a good indication of the extraction ability of the resin. For this study, V_B is defined as the volume after extrapolating the middle portion of the curve to the x-axis, and V_R as the volume at $C/C_o = 0.5$. C/C_o is the ratio of analyte effluent concentration to influent concentration. The resin load capacity may also be determined from a breakthrough curve. This is the total number of moles of analyte adsorbed by a resin, and is calculated by multiplying V_R by the influent concentration[16].

Breakthrough curves of several compounds were compared for two Empore membranes - one embedded with underivatized PS-DVB and the other with sulfonated PS-DVB (0.6 mequiv/g capacity). Breakthrough curves for four compounds are shown in Figures 5-8. The sulfonated resin membrane, being more hydrophilic, produced a sharper breakthrough than the underivatized membrane, which allowed breakthrough almost immediately.

The parameters calculated from the breakthrough curves are shown in Table IX.

Table IV

Recoveries of phenols (1 ppm) with a sulfonated Empore membrane (0.6 mequiv/g)

	Recoveries (%)
phenol	98
p-cresol	102
2,5-dimethylphenol	98
p-chlorophenol	97
o-chlorophenol	95
m-nitrophenol	97
p-isopropylphenol	96
4-secbutylphenol	98
4-tertbutylphenol	100
2-methylresorcinol	91
4-hexylresorcinol	101
catechol	53
Average (w/o catechol)	97 %

Table V

Recoveries of alcohols (1 ppm) with a sulfonated Empore membrane (0.6 mequiv/g)

	Recoveries (%)
1-hexanol	94
cyclohexanol	93
2-ethyl-1-hexanol	97
benzyl alcohol	94
1-octanol	96
phenethyl alcohol	96
2-phenyl-1-propanol	98
dodecyl alcohol	94
Average	95 %

Table VI

Recoveries of aldehydes/ketones (1 ppm) with a sulfonated Empore membrane (0.6 mequiv/g)

	Recoveries (%)
isophorone	100
hydroquinone	96
acetophenone	102
hexyl aldehyde	95
octyl aldehyde	96
nonyl aldehyde	93
9-anthraldehyde	98
salicylaldehyde	100
benzaldehyde	104
Average	98 %

Table VII

Recoveries of esters/ethers (1 ppm) with a sulfonated Empore membrane (0.6 mequiv/g)

	Recoveries (%)
anisole	96
phenetole	91
ethyl acetoacetate	97
methyl benzoate	96
isopentyl benzoate	92
ethyl cinnamate	95
hexyl acetate	93
trans-2-hexenyl acetate	91
Average	94 %

Table VIII

Recoveries of nitrogen compounds (1 ppm) with a sulfonated Empore membrane (0.6 mequiv/g)

	Recoveries (%)
nitrobenzene	100
3-nitroacetophenone	98
m-nitrophenol	97
benzonitrile	99
benzothiazole	97
o-nitrotoluene	97
Average	98 %

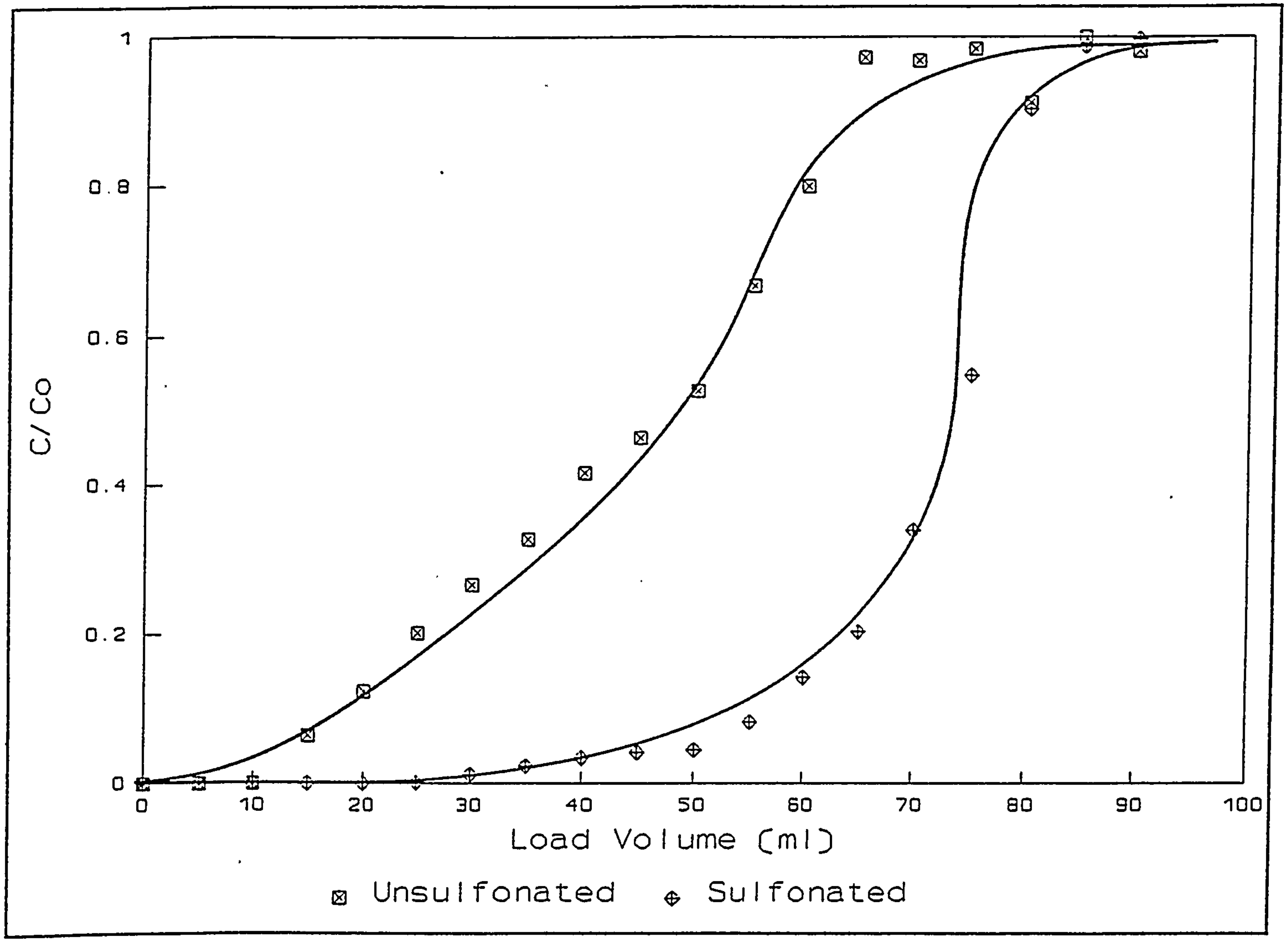

Fig. 5 Breakthrough curve for *p*-cresol on a sulfonated and unsulfonated Empore membrane.

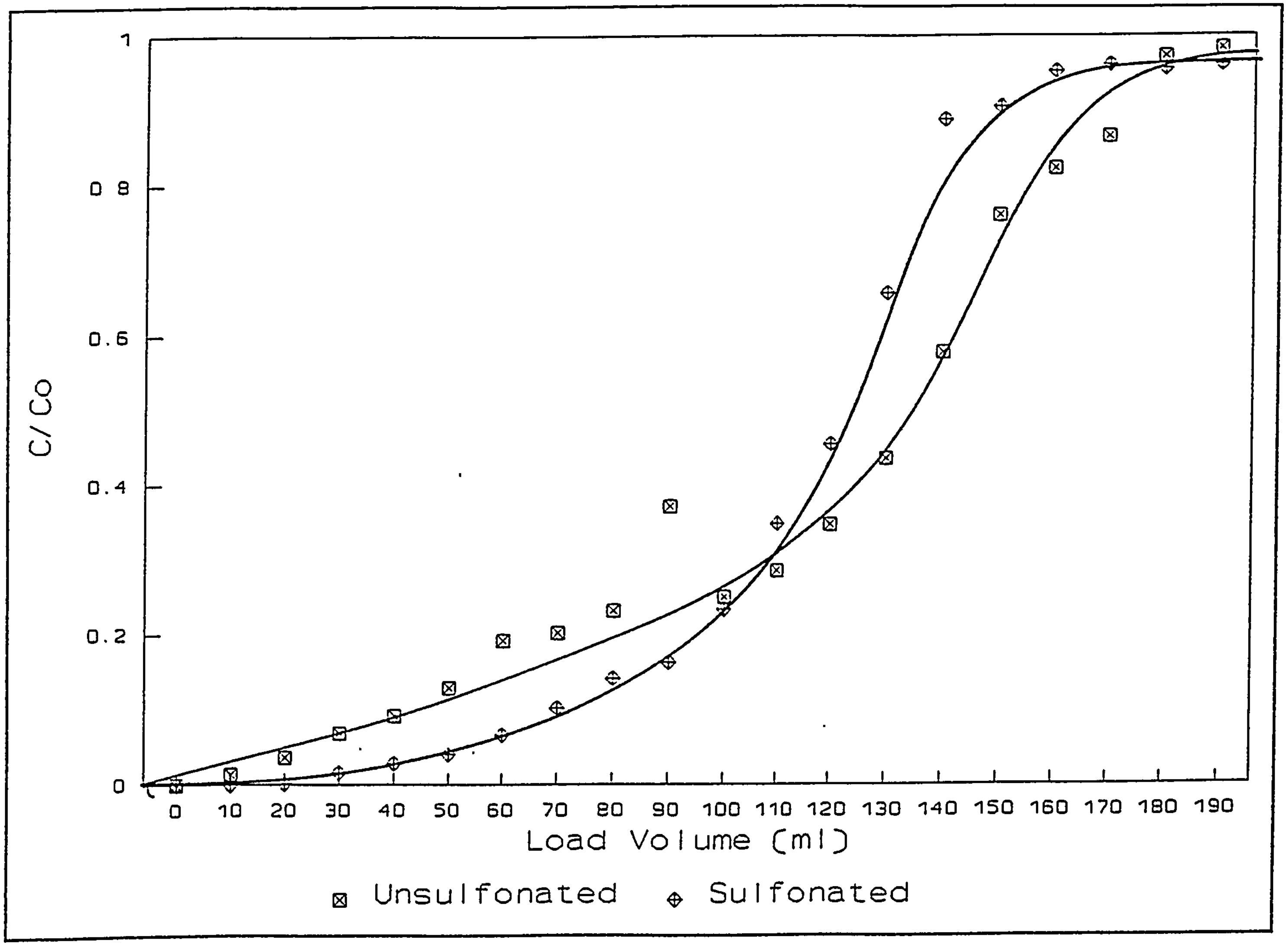

Fig. 6 Breakthrough curve for nitrobenzene on a sulfonated and unsulfonated Empore membrane.

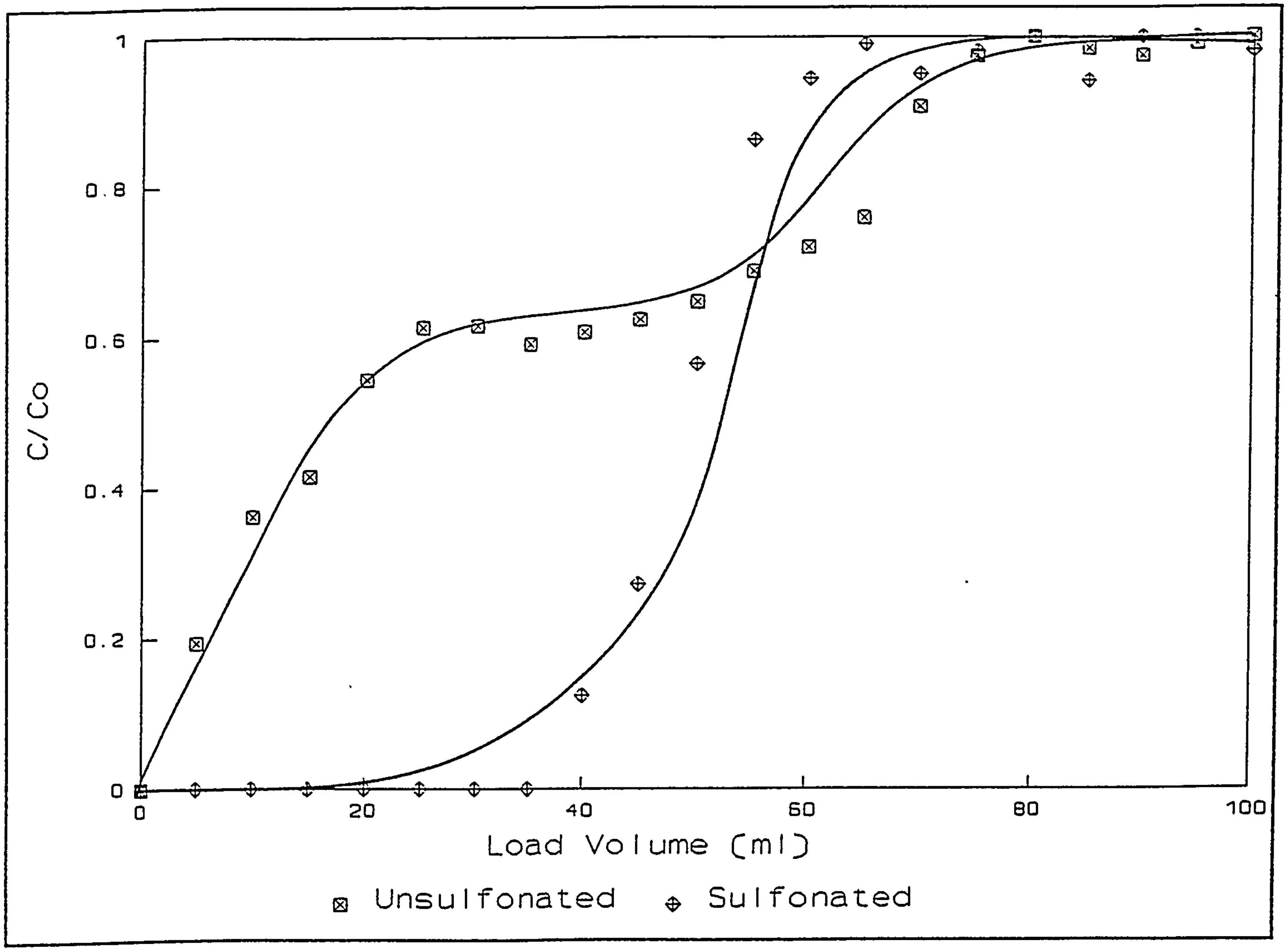

Fig. 7 Breakthrough curve for ethyl acetoacetate on a sulfonated and unsulfonated Empore membrane.

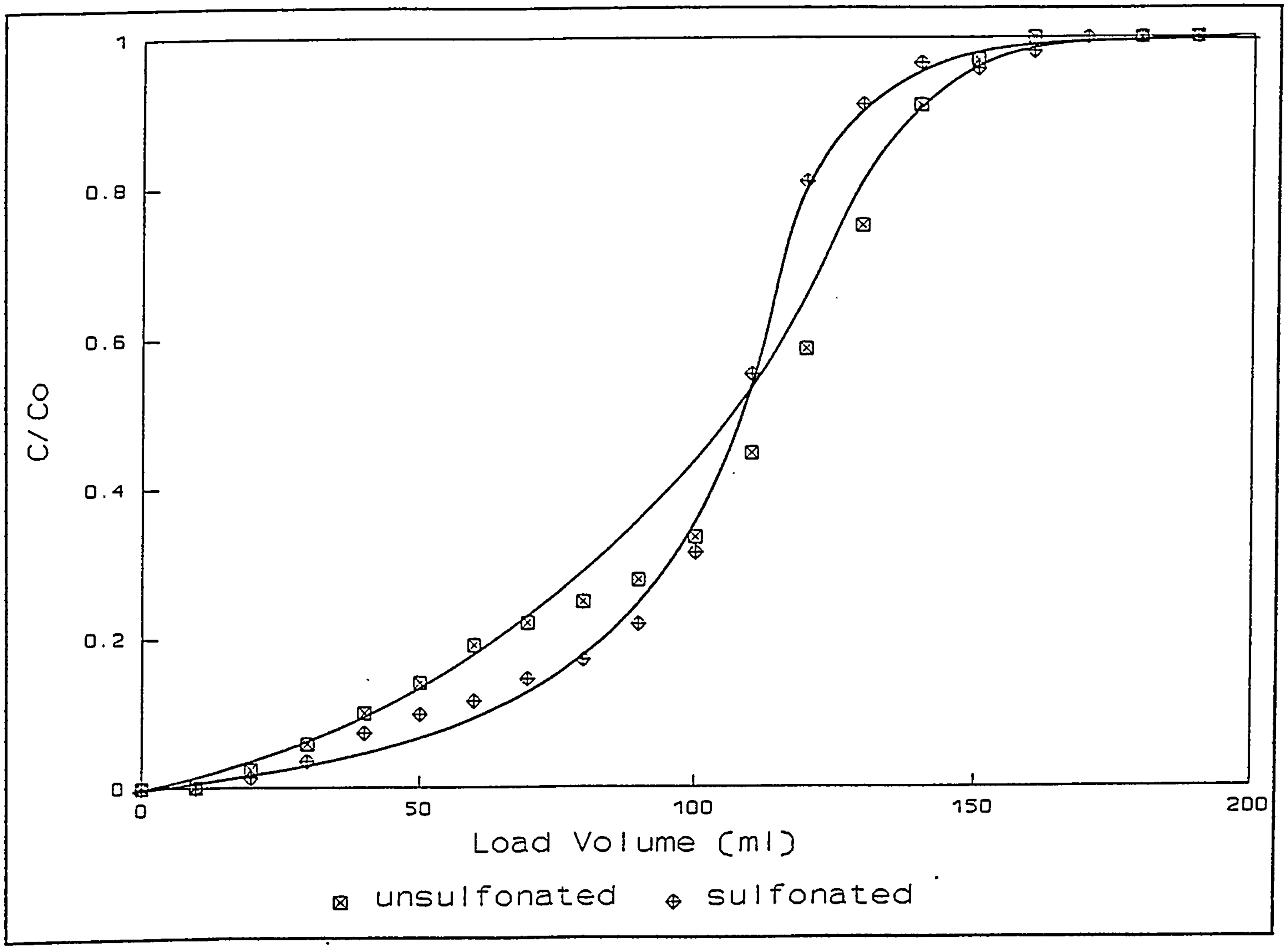

Fig. 8 Breakthrough curve for isophorone on a sulfonated and unsulfonated Empore membrane.

Table IX.

Comparison of breakthrough data for Empore membranes embedded with unsulfonated PS-DVB and sulfonated (0.6 mequiv/g) PS-DVB

Compound	Load Capacity (10^{-6} moles/g)		V_R (ml)		V_B (ml)	
	Sulf.	Unsulf.	Sulf.	Unsulf.	Sulf.	Unsulf.
p-Cresol	74.2	47.4	74	46	34	12
Ethyl acetoacetate	39.3	13.1	46	15	38	0
Nitrobenzene	223	241	126	132	76	28
Isophorone	173	185	109	114	45	27

Extraction efficiency of the more polar compounds, p-cresol and ethyl acetoacetate, was dramatically increased with the sulfonated membrane. Load capacity and V_R were similar on both membranes, but V_B was significantly larger for all compounds on the sulfonated membrane.It may be expected that less polar compounds would be more easily extracted with an underivatized resin, but this is not necessarily the case. The less polar compounds, nitrobenzene and isophorone, do have slightly higher load capacities and V_R on the underivatized membrane, but V_B is still much lower. This is caused by the poor curve shape and early breakthrough on the underivatized membrane. V_B is the parameter of most concern for SPE because dilute samples are usually used and the load capacity of a resin is seldom approached. A sulfonated membrane would therefore be the better adsorbent to use for SPE of these types of compounds.

Conclusions

Introduction of hydrophilic functional groups to PS-DVB increased the extraction efficiency of polar organic molecules from water. In this work, the effect of resin sulfonation capacity has been determined. Sulfonated resins lead to better recoveries of many compounds from aqueous solutions. A capacity of 0.6 mequiv/g was determined to be the optimum degree of sulfonation. Empore membranes embedded with sulfonated resin are simple to use and provide efficient extraction of many compounds. Breakthrough analysis was used to compare sulfonated and unsulfonated membranes. Breakthrough on the sulfonated membrane was much sharper, indicating a much more efficient extraction process.

Acknowledgements

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ION-CHROMATOGRAPHIC SEPARATION OF ALKALI METALS IN NON-AQUEOUS SOLVENTS

A paper accepted for publication in the *Journal of Chromatography A*

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Abstract

Ion-exchange chromatography is a common method for the separation and determination of metal cations. Although much research has been done on improving various aspects of this technique, the use of non-aqueous eluents has received little attention. In this work, the effect of organic solvents on the retention of alkali metal cations on a macroporous polystyrene-divinylbenzene resin was studied. Retention of alkali cations generally increased as the organic content in the eluent increased for most organic solvents, although a maximum does occur at 75% organic for some ions. Since organic solvents do not solvate these cations in the same manner as water, increases in the separation factors and changes in elution order were observed. Several separations that are not possible with aqueous eluents are shown.

The effect of crown ethers in the mobile phase was also investigated. 18-crown-6 (18C6)

altered the retention of most alkali metal cations. In some cases 18C6 changed elution order or improved peak shape. Separations with an organic eluent and 18C6 modifier are also shown.

Introduction

Factors that influence the aqueous selectivity of cation-exchange resins for various +1 metal cations have been studied extensively over many years. Diamond and coworkers[1,2] proposed a theory of water-enforced ion pairing to explain selectivity toward various cations. Electrostatic attraction between the sulfonate groups within the ion-exchange resin and the alkali metal cations suggests that cations with the smallest ionic radii would be the most strongly retained. The Pauling radii in Table I[3] would predict a chromatographic elution order of Cs^+, Rb^+, K^+, Na^+, Li^+, which is exactly the opposite of that observed in ion-exchange chromatography. However, hydrated ionic radii and approximate hydration number (Table I) are in the opposite order to the Pauling radii, with Li^+ being the most highly hydrated. The cation-exchange resins

Table I

Ionic radii of alkali metal cations

	Li^+	Na^+	K^+	Rb^+	Cs^+
Pauling radii, Å	0.60	0.96	1.33	1.48	1.69
Hydrated radii, Å	3.40	2.76	2.32	2.28	2.28
Approximate hydration number	25.3	16.6	10.5	10.0	9.90

used in classical ion-exchange chromatography are highly sulfonated and take up a great deal of water inside the microporous resin. The hydration of the alkali metal ions thus would remain much the same inside the resin as in the aqueous mobile phase.

The effects of performing ion-exchange separations in aqueous-organic or in organic solvents have been studied by a number of investigators. A recent review concludes that ion-exchange in non-aqueous solvents is very complicated from a theoretical point of view[4]. Organic solvents are obviously going to affect the solvation of alkali cations compared to the situation in water alone. The dielectric constant, viscosity, and other effects will also be different in organic solvents. In the present work the ion-chromatographic separation of alkali metals and the ammonium ion was studied in pure organic and aqueous-organic eluents. Some major changes in selectivity were observed and several practical separations obtained.

Experimental

Chromatographic System

The chromatographic system consisted of several components. An Alltech 425 HPLC pump (Alltech Associates, Inc., Deerfield, IL, USA) was used to deliver a flow of 1 ml/min. A 7125 Rheodyne injector (Rheodyne, Berkeley, CA, USA) delivered a 10 μl sample which was detected with an Alltech 320 conductivity detector (Alltech Associates, Inc., Deerfield, IL, USA). A Hitachi D-2000 integrator (Em Science, Cherry Hill, NJ, USA) was used to measure retention times. Separations were recorded by a Servogor 120 chart recorder (Abb Goerz Instruments, Vienna, Austria), and a Keithley Chrom 1-AT data acquisition board (Keithley MetraByte Corp., Taunton, MA, USA) with Labtech Notebook software (Laboratory Technologies Corp., Wilmington, MA, USA). Columns were packed with a Shandon HPLC packing pump (Shandon Southern, Sewichley, PA, USA) at 3000 psi.

Reagents and Chemicals

The cation-exchange resin was prepared in our lab from 5 µm macroporous polystyrene-divinylbenzene (Sarasep Inc., Santa Clara, CA USA). Two grams of resin were slurried with a few milliliters of glacial acetic acid and placed in an ice bath. Five milliliters of concentrated sulfuric acid was added to the resin with stirring. The resin mixture was reacted for 30 seconds then poured into ice water to quench the reaction. This procedure produced a sulfonic acid cation-exchange resin with a capacity of approximately 0.15 mequiv/g. Absolute ethanol was punctilious grade and used as obtained from Quantum (Quantum Chemicals, Newark, NJ, USA). All other organic eluents used were of HPLC grade and used as obtained from Fisher (Fisher Scientific, Pittsburgh, PA, USA) and Sigma (Sigma Chemical Company, St. Louis, MO, USA). The salts and methanesulfonic acid eluent were all of reagent grade and used as obtained from Aldrich (Aldrich Chemical Company, Inc., Milwaukee, WI, USA) and Fisher. Either halide or acetate salts were dissolved in organic solvents to prepare 1000 ppm stock solutions which were then diluted with eluent to produce samples of desired concentrations.

Results and Discussion

Type of Resin

Ion-chromatographic separations of alkali metal cations are generally performed with sulfonated microporous polymeric resins[5,6] or with resins coated with a sulfonated latex. A lightly sulfonated macroporous resin with a very high degree of cross-linking was selected for the present study. Such a resin would be less likely to undergo volume changes due to swelling and should be more compatible with organic solvents.

A separation of alkali metal ions was first attempted in water alone using the lightly sulfonated macroporous cation-exchanger with aqueous 3 mM methanesulfonic acid as the eluent. Under these conditions the sample cations exhibited very similar retention times (Figure 1). A much better separation would be obtained with a microporous cation-exchanger[5]. The selectivity of the macroporous resin for alkali metal ions was improved considerably by chemically introducing hydroxymethyl groups[7] prior to sulfonation.

These results seem to indicate that solvation of the resin plays a role in imparting selectivity for the various sample ions. Microporous cation-exchange resins form a gel and are highly hydrated within. With sulfonated macroporous resins, the hydrated alkali metal ions may be repelled somewhat by the hydrophobic resin matrix. The presence of hydroxymethyl groups on the macroporous resin makes it less hydrophobic and was found to improve selectivity for the hydrated alkali ions.

When the macroporous resin column in Figure 1 was used with the same acidic eluent in 100% methanol, the chromatographic separation was improved considerably (Figure 2). Now the alkali metal ions are solvated with methanol and the resin matrix is probably coated with a thin layer of methanol, which makes the ions and the resin surface more compatible with one another.

Ion Chromatography in Organic Solvents

Ion-exchange selectivity in organic solvents and in mixed solvents involves a complex series of effects. The dielectric effect and ionic solvation seem to play the major roles, but interactions involving the solvent, resin exchange sites, analyte ion, and eluting ion also affect

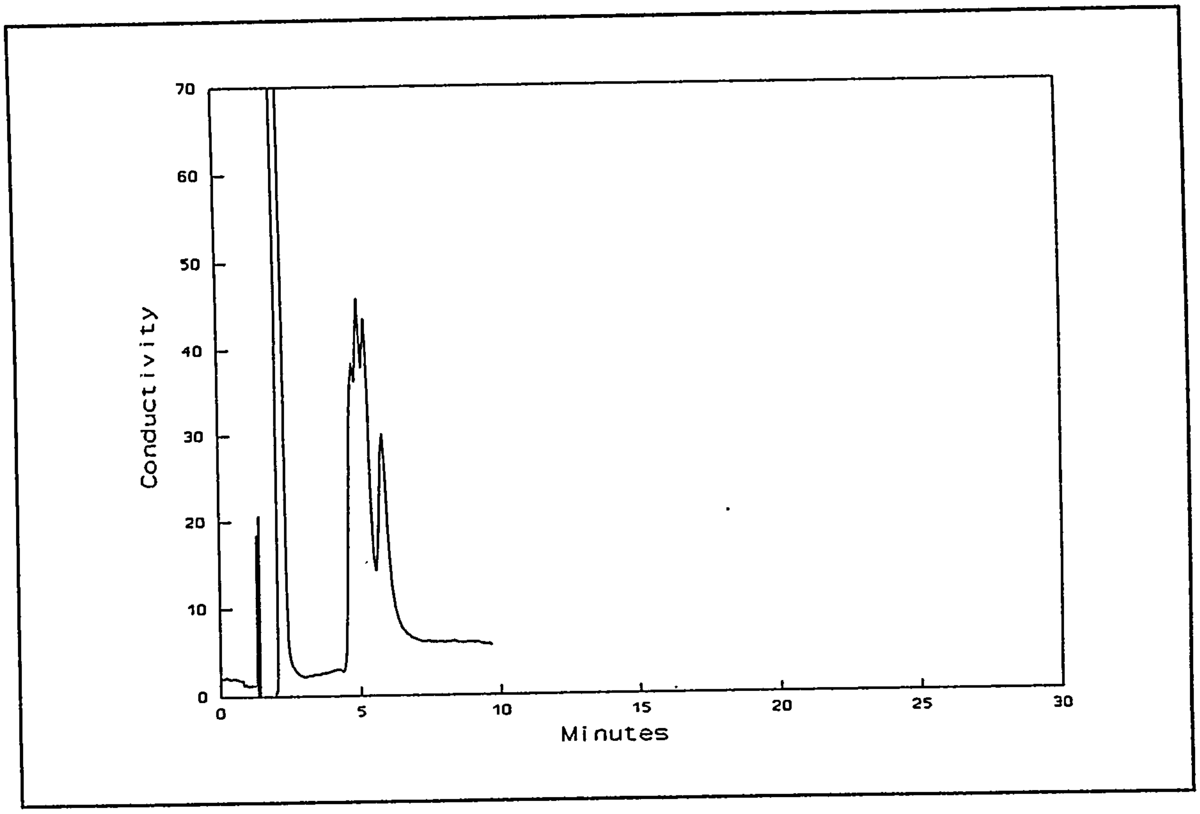

Fig. 1 Separation of 2-48 ppm Li^+ (1), Na^+ (2), K^+ (3), Rb^+ (4), and Cs^+ (5) on a 15 cm cation-exchange column with 3 mM methanesulfonic acid in water as the eluent.

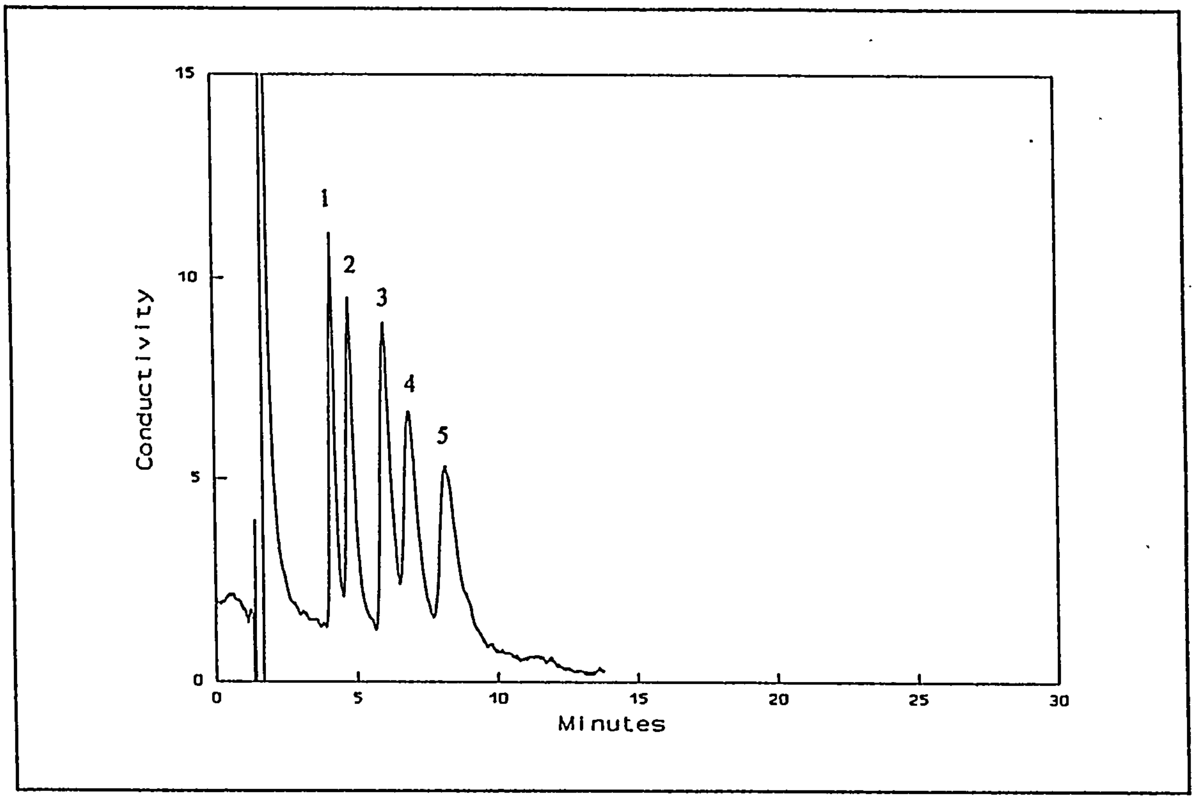

Fig. 2 Same separation as Figure 1 with 3 mM methanesulfonic acid in methanol as the eluent.

retention[8].

Capacity factors for the alkali metal cations were measured in eluents containing 0-100% organic solvent (Table II). The capacity factors, k′, of the cations generally increase with greater organic content of the solvent. This trend was observed for all eluents up to a composition of 75% organic/25% water. Retention decreased when the organic content was increased to 100% for all cations in methanol and several cations in acetonitrile and ethanol. Increased retention of sample cations may be explained at least partly by the lower dielectric of organic solvents. This favors ion-pair formation with the result that analyte cations will be electrostatically attracted more strongly to the resin sulfonate anion. Solvation also appears to be a major force. As the organic content is increased toward 100%, analyte cations must become less solvated by water and more solvated by the organic solvent molecules which are all larger than water molecules[8]. The larger radii should inhibit the approach of the cation to the resin and therefore cause a decrease in the retention times. At about 75% methanol this effect becomes more important than the continued decrease in dielectric. The data for ethanol also support this. Maxima are observed for Li^+ and Na^+ which are the most highly solvated ions. Replacing water with ethanol in the hydration sphere should have a larger effect on the solvated radii of these ions than the other less solvated alkali ions. The change in solvated radii is therefore more important for Li^+ and Na^+ than the decrease in dielectric. No maxima is observed for 2-propanol, indicating that the decrease in dielectric is more important than the change in solvation radii. The maxima in methanol are represented graphically in Figure 3. A similar maximum has been observed by others[9-12].

Linear plots were obtained in 100% methanol for log k′ vs. log H^+ (methanesulfonic acid),

Table II

Capacity factors in organic and mixed solvents with 0.5 mM methanesulfonic acid as the eluent

Solvent	Li^+	Na^+	K^+	Rb^+	Cs^+	NH_4^+
100% Water	2.27	2.27	2.71	2.74	2.96	3.04
Methanol						
25%	2.66	2.47	2.77	2.80	2.98	3.13
50%	3.10	3.07	3.31	3.39	3.80	3.70
75%	4.43	5.07	6.32	7.30	8.33	5.82
100%	2.08	2.82	3.73	4.33	5.15	3.09
Ethanol						
25%	2.75	2.57	2.78	2.78	2.96	3.13
50%	3.25	3.09	3.37	3.48	3.76	3.78
75%	4.61	5.14	6.90	7.62	8.76	5.98
100%	1.86	3.84	7.24	8.84	9.87	2.12
2-Propanol						
25%	2.20	1.98	2.11	2.05	2.17	2.45
50%	2.26	2.14	2.35	2.41	2.62	2.88
75%	3.41	3.52	4.42	4.81	5.66	4.69
100%	8.84	12.3	19.5	>20	>20	4.54
Acetonitrile						
25%	2.10	2.10	2.37	2.38	2.54	2.51
50%	2.50	2.38	2.89	2.98	3.35	3.10
75%	3.00	3.12	3.79	3.95	4.45	3.98
100%	4.46	2.11	1.75	1.59	1.54	2.40

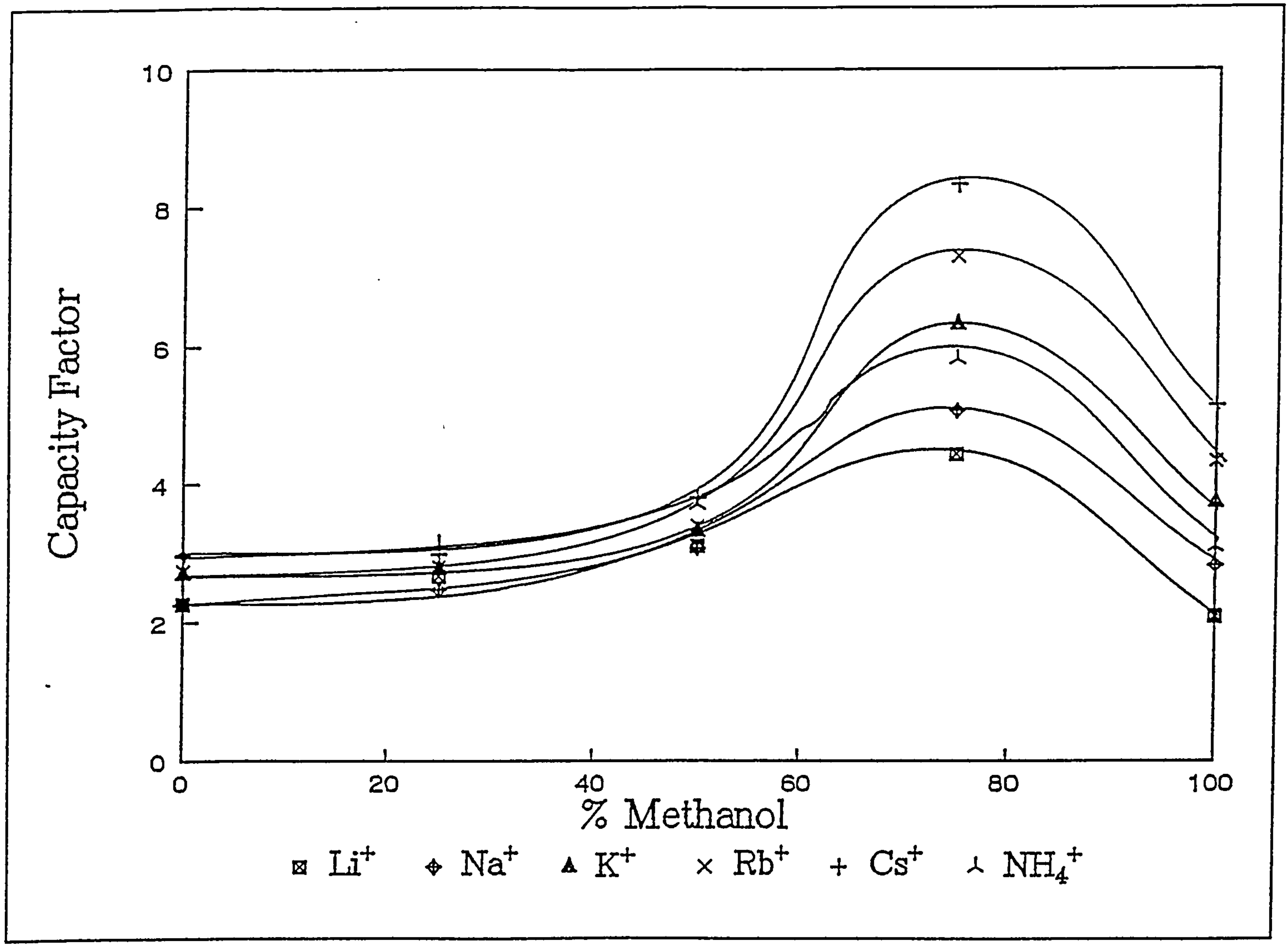

Fig. 3 Effect of methanol on the retention of alkali metals. The eluent contained 0.5 mM methanesulfonic acid.

as shown in Figure 4. Slopes of all the cations were very close to the theoretical slope of -1.0 (± 0.02). This indicates the ion-exchange mechanism is well-behaved.

The separation factor for potassium/ammonium on most chromatographic systems is usually fairly small. However, the ratio of retention times is quite large in 100% ethanol: $t_{K^+}/t_{NH_4^+} = 7.24/2.12 = 3.4$. The chromatogram in Figure 5 shows that an ammonium peak of only 10 ppm can easily be separated from a 1,000 ppm K^+ peak. An even larger ratio of $t_{K^+}/t_{NH_4^+}$ is found in 2-propanol. However, the peaks in 2-propanol were much broader than in methanol and ethanol, and the sensitivity of the conductivity detector was appreciably lower.

Retention in acetonitrile followed the same general trend as with the alcohol eluents. In 100% acetonitrile a unique elution order was observed; Cs^+ eluted first and Li^+ eluted last. This is the reverse of the normal elution order for the alkali metals. The reason for the reversal is not clear, but the inability of acetonitrile to hydrogen bond and the lack of a lone electron pair on an oxygen atom may play a role. Unfortunately, the chromatographic peaks obtained in 100% acetonitrile were generally quite broad.

Effect of 18-crown-6

Crown ethers have been known for many years to complex alkali metal cations[13]. In recent years they have been used to manipulate selectivity in ion chromatography[14-16]. Often they are used in a water-organic mobile phase to dynamically coat a silica-C18 or polymeric resin column. The retention mechanism involves ligand-exchange rather than ion-exchange. In this research a crown ether was added with methanesulfonic acid to the organic eluent. The crown ether was sufficiently soluble that no dynamic coating of the stationary phase took place[14]. This was verified by plotting log k′ vs. log H^+ for Li^+ and K^+ in organic eluents containing 10 mM

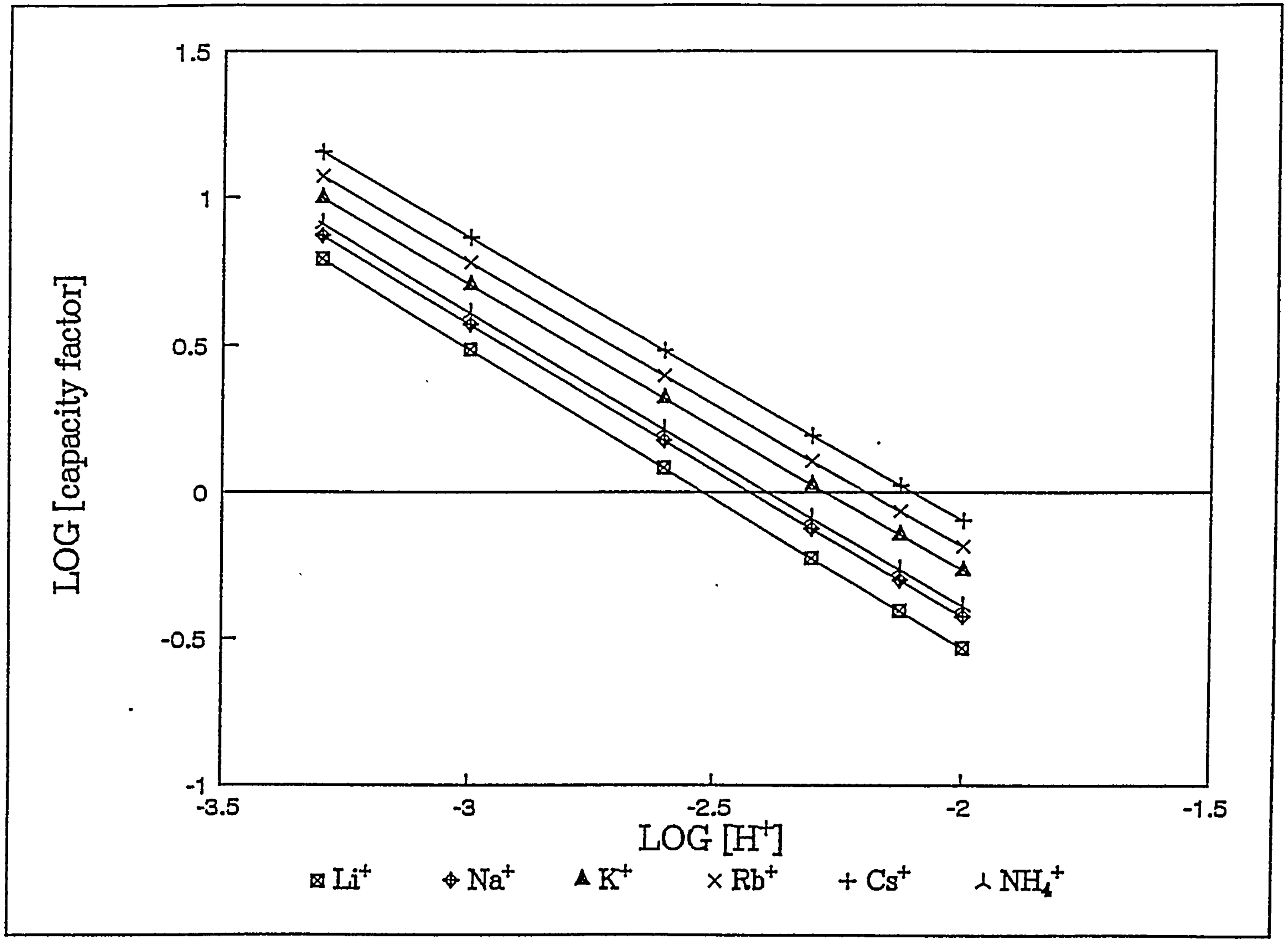

Fig. 4 Effect of methanesulfonic acid concentration on the retention of alkali metals in 100% methanol.

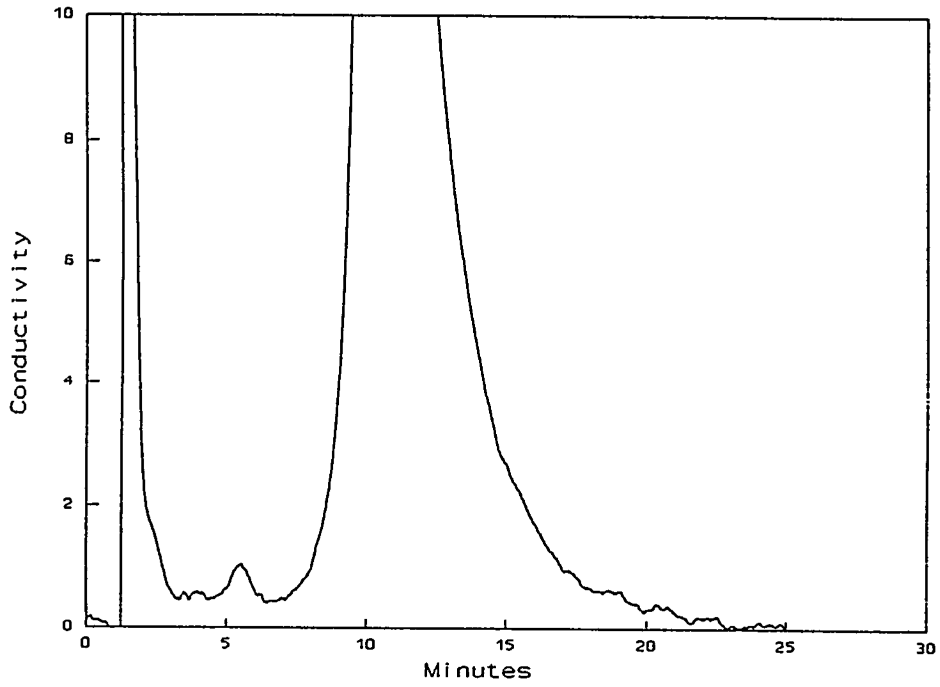

Fig. 5 Separation of 10 ppm NH_4^+ and 1000 ppm K^+ on a 10 cm column with 1 mM methanesulfonic acid in 100% ethanol as the eluent.

18C6. 100% acetonitrile and 75% methanol were tested. In both eluents,slopes of the Li^+ and K^+ lines were very close to -1.0. If 18C6 were coating the resin, a second retention mechanism of ligand-exchange would affect the K^+ ion significantly more than the Li^+ ion and a difference in slopes would be expected. The slopes were nearly identical in both eluents, therefore it was concluded that no coating was taking place and the retention mechanism was purely ion-exchange.

The alkali metal ions have larger formation constants with 18C6 in organic solvents than in water[17]. It was expected that 18C6 would increase retention due to formation of a larger cationic complex. This was found to be generally true but not necessarily because of the formation of larger cations.

The effect of 18C6 on the ion-exchange behavior of alkali metals in non-aqueous solvents is shown in Table III. Although increasing concentrations of 18C6 in acetonitrile increase the capacity factors of all of the ions studied, the large increase in the k′ of lithium was the most striking. The separation factor (k'_{Li^+} / k'_{Na^+}) was 3.8 compared with 2.6 with no crown ether. The presence of the crown ether also sharpens the Li^+ peak in the separation of Na^+ and Li^+ (Figure 6). This permitted the separation of 1 ppm Li^+ from 500 ppm Na^+ as shown in Figure 7. Data in Table III suggest small amounts of Li^+ can be separated from much larger amounts of all other alkali metal ions as well.

H^+ does not complex 18C6 in water or methanol, but it does have a large formation constant in acetonitrile (K_f=$10^{6.5}$)[17]. The longer retention times in acetonitrile may be a consequence of the strong complex between H^+ and the crown ether. This complex is stronger than any complex with alkali metal ions. A large H^+ complex would be a weaker eluting species

Table III

Effect of 18C6 on k′ of alkali metal ions in organic solvents

10 cm column with 2 mM methanesulfonic acid in methanol as the eluent

	mM 18C6			
	0	0.1	1.0	10
Li$^+$	1.45	1.31	1.32	1.34
Na$^+$	1.80	2.06	2.21	2.20
K$^+$	2.55	2.67	2.63	2.59
Rb$^+$	3.05	4.08	4.05	4.01
Cs$^+$	3.77	4.17	4.21	4.23
NH$_4^+$	1.93	2.71	3.34	3.36

10 cm column with 1 mM methanesulfonic acid in acetonitrile as the eluent

	0	0.1	1.0	10
Li$^+$	3.57	4.59	7.17	8.97
Na$^+$	1.39	1.33	1.64	2.34
K$^+$	1.34	1.26	1.65	2.48
Rb$^+$	1.26	1.26	1.73	2.45
Cs$^+$	1.24	1.27	1.71	2.74
NH$_4^+$	1.50	1.39	2.18	3.35

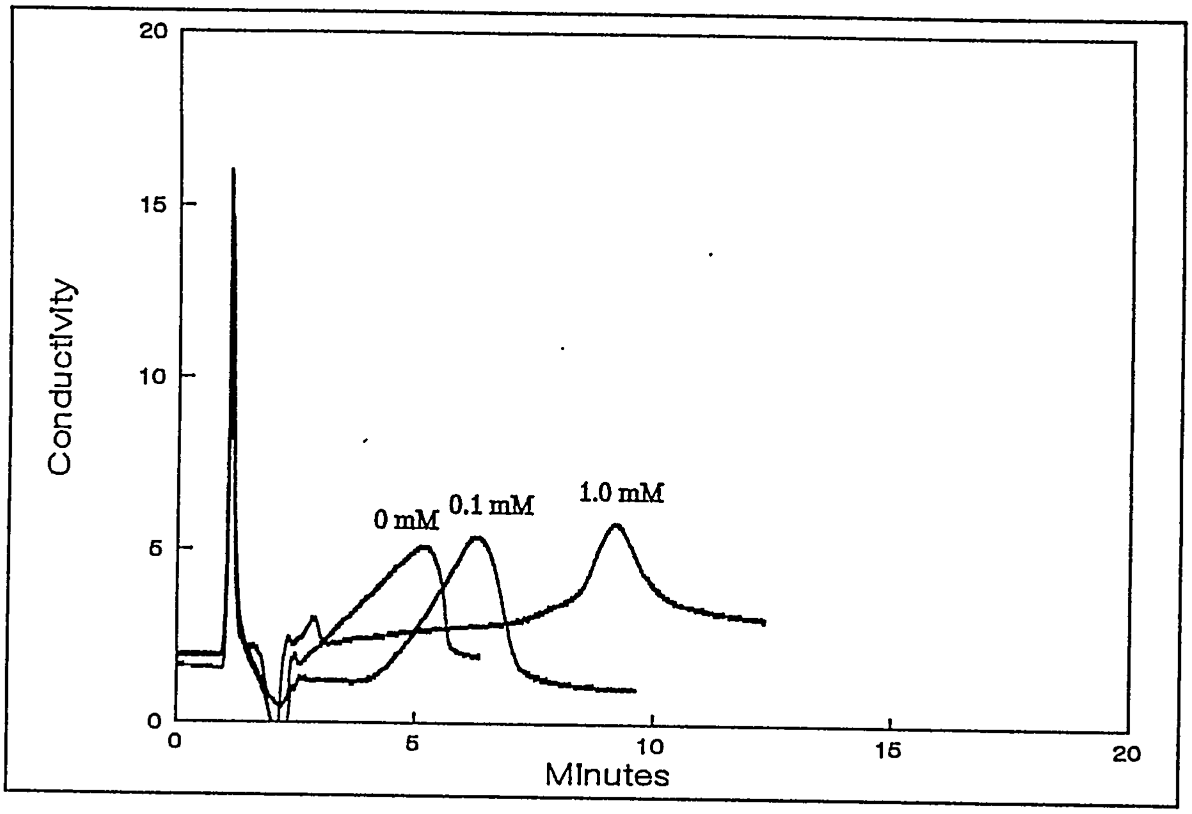

Fig. 6 Effect of 18-crown-6 on the peak shape and retention of Li^+(8 ppm) with 1 mM methanesulfonic acid in acetonitrile as the eluent.

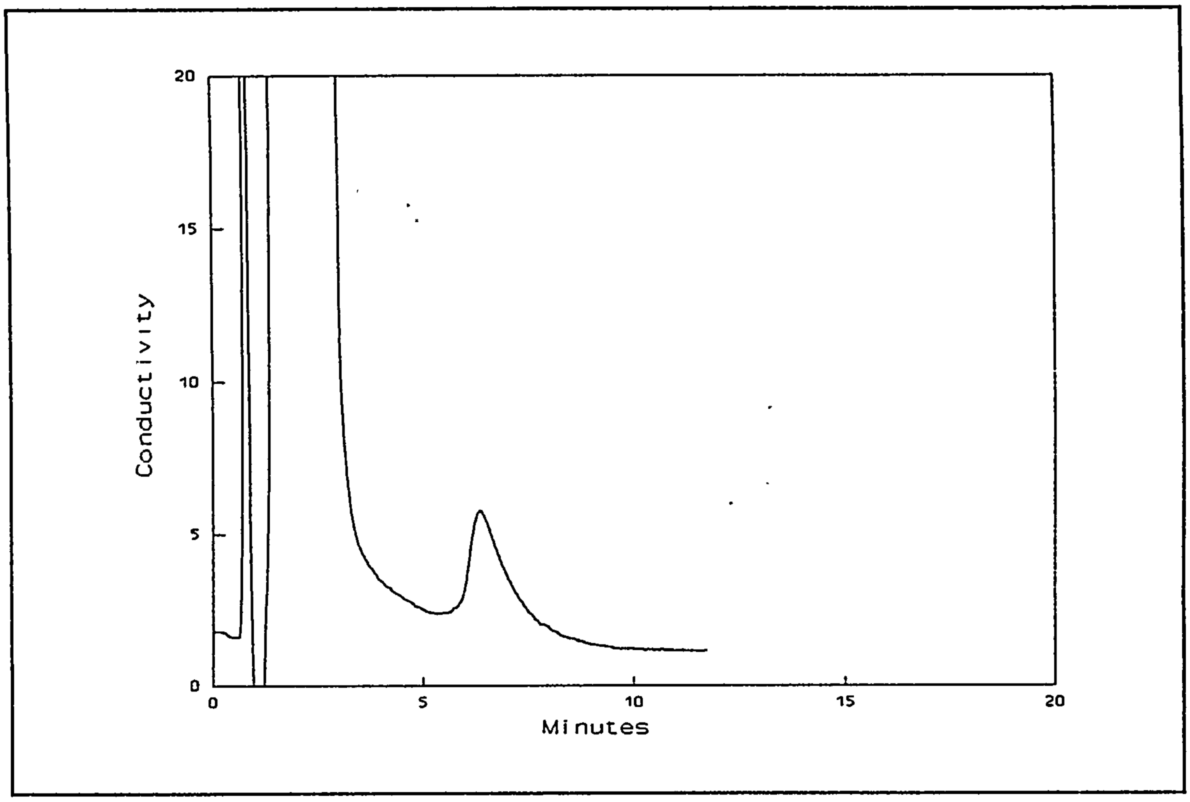

Fig. 7 Separation of 500 ppm Na^+ and 1 ppm Li^+ on a 10 cm column with 1 mM methanesulfonic acid and 1 mM 18C6 in 100% acetonitrile as the eluent.

than H^+ alone and the retention times of the alkali metals would increase. The alkali metal formation constants with 18C6 are generally higher in methanol than in acetonitrile except for Li^+, which is not complexed. Addition of 0.1 mM 18C6 increases k′, but increasing the concentration further has little effect. H^+ is scarcely complexed in methanol, so the changes in retention are due to the interaction of the crown ether with the metal cations. It is unclear why K^+ is affected so little since it has the largest K_f with 18C6. The other cations generally elute in order of decreasing K_f. The metals with higher K_f values spend more time as a larger, complexed ion. The larger ions have the largest k′ values in methanol.

Including NH_4^+ in a separation with the alkali metals was not possible in 100% methanol. It eluted very closely to Na^+ and could not be separated. The addition of 18C6 did adjust the retention times but not in a manner to allow a separation. An eluent of 75% methanol was found to increase separation factors of several cations. Adding 18C6 to this eluent further increased the resolution and improved peak shapes. A separation of four ions in 75% methanol, 1.5 mM methanesulfonic acid, and 1 mM 18C6 is shown in Figure 8. The effect of 18C6 is easily seen when comparing this to a similar separation without 18C6 (Figure 9).

Conclusions

The use of non-aqueous solvents with macroporous cation-exchange resin permits several separations that are very difficult with aqueous eluents. Methanol was found to be the most favorable solvent due to the best combination of resolution and peak shape. Acetonitrile and ethanol, although producing broader peaks, were useful for separating ions that usually elute close

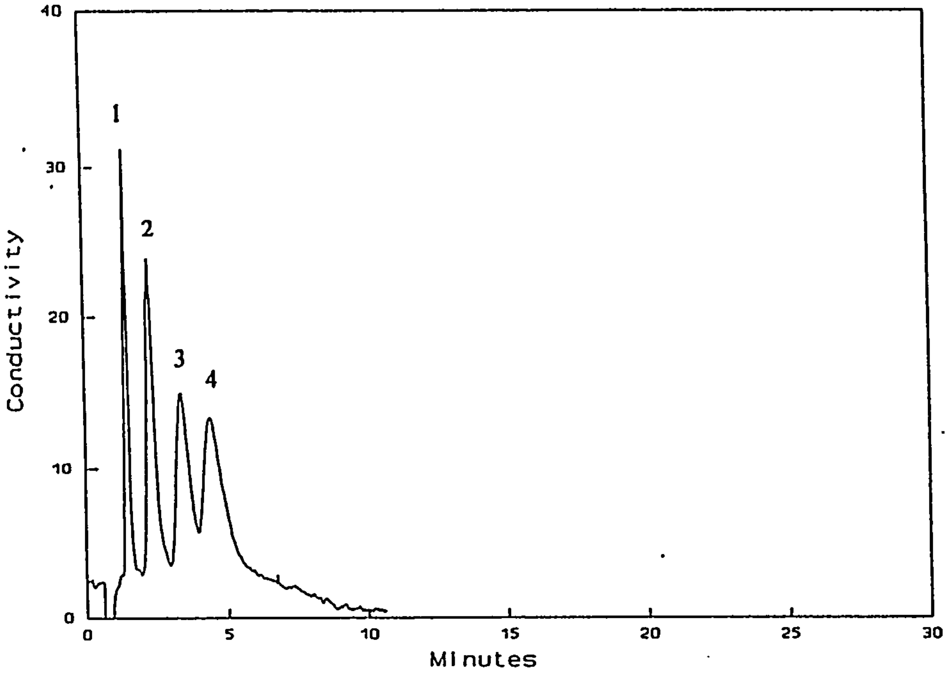

Fig. 8 Separation of 1 ppm Li^+ (1), 3 ppm Na^+ (2), 3 ppm NH_4^+ (3), and 9 ppm K^+ (4) on a 5 cm column with 1.5 mM methanesulfonic acid and 1 mM 18C6 in 75% methanol as the eluent.

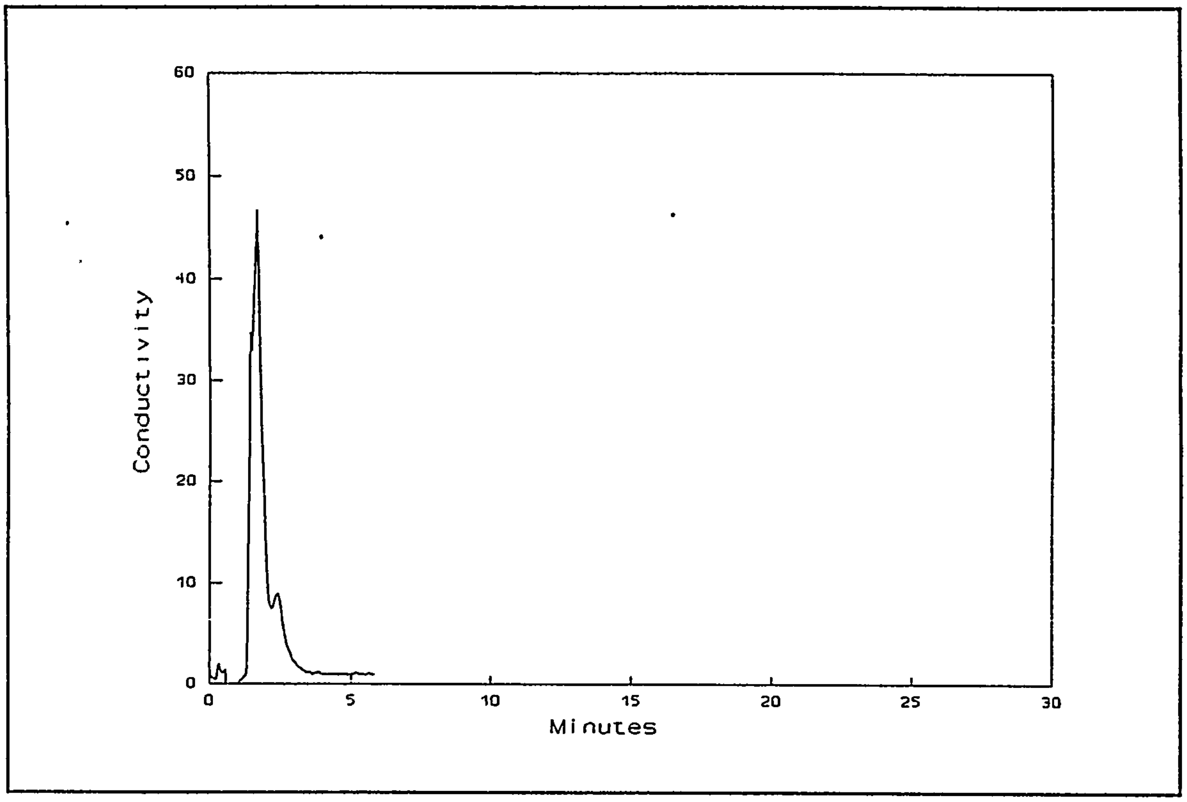

Fig. 9 Same separation as Figure 8 but without 18C6.

together, Li^+/Na^+ and K^+/NH_4^+ respectively. Elution order in acetonitrile is reversed from that found with aqueous eluents: $Cs^+<Rb^+<K^+<Na^+<Li^+$. Addition of 18C6 to the mobile phase improves both peak shape the resolution of several ions.

Acknowledgements

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CATION-EXCHANGE CHROMATOGRAPHY IN NON-AQUEOUS SOLVENTS

A paper accepted for publication in the *Journal of Chromatography A*

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Abstract

Much of the selectivity of organic ions in conventional ion chromatography comes from hydrophobic interaction between the carbon chain of the analyte ions and the polymer matrix of the ion-exchanger. By operating in organic solvents containing little, if any, water, the true ion-exchange selectivity of various organic ions can be measured. Capacity factors, k′ for a series of protonated amine cations were measured in methanol, ethanol, 2-propanol and acetonitrile using a polymeric cation-exchanger resin, conductivity detection, and eluents of methanesulfonic acid in the same organic solvent. Plots of log k′ vs. log methanesulfonic acid concentration were linear with slopes close to the theoretical slope of -1.0 except for acetonitrile where the slopes averaged -0.82. The capacity factors showed little change with increasing carbon chain length in n-alkylamine cations, but increased substantially at fixed eluent concentration in going from

methanol, to ethanol, to 2-propanol and especially to acetonitrile.

Several practical separations of organic amine cations were demonstrated in non-aqueous media. One advantage of this technique is that neutral organic compounds elute very quickly in non-aqueous media and thus do not interfere with the chromatographic separations of ions.

Introduction

Organic solvents have been used in classical ion-exchange chromatography to aid in the separation of metal cations[1-3]. Formation of metal complexes with chloride and other anions was found to occur much more readily in organic solvents than in water alone. Organic solvents have also been added to aqueous eluents in modern ion chromatography to modify the behavior of various ionic analytes. However, the percentage of organic solvent in the eluent has generally been < 20%. Rabin and Stillian recently discussed practical aspects on the use of organic solvents in ion chromatography[4]. The solvents were used primarily for selectivity mediation of the ion-exchange process for separation of various anions.

In the ion-chromatographic separation of organic cations, it has long been known, or at least suspected, that the mechanism involved more than simple ion-exchange. Lee and Hoffman[5,6] have shown that two mechanisms occur in such cases - ion-exchange and hydrophobic interaction between the sample cations and the resin matrix. For example, these authors showed that the slopes of the linear plot of log k′ vs. carbon number for protonated amine cations were less in an eluent of 70% acetonitrile (30% water) than with an eluent of 30% acetonitrile. This was attributed to the greater hydrophobic interaction present with the 30% acetonitrile eluent.

The purpose of the present investigation was to study ion chromatography in organic solvents containing little if any water. Under these conditions, solvation of the lipophilic part of the cation should be sufficient to virtually eliminate the hydrophobic interaction between the sample cations and the resin. In this way the true ion-exchange selectivity can be measured. This paper deals with ion-exchange chromatography of organic cations in non-aqueous solvents. A companion paper is concerned with alkali-metal cations in organic solvents[7].

Experimental

Chromatographic System

The chromatographic system consisted of several components. An Alltech 425 HPLC pump (Alltech Associates, Inc., Deerfield, IL, USA) was used to deliver a flow of 1 ml/min. A 7125 Rheodyne injector (Rheodyne, Berkeley, CA, USA) delivered a 10 µl sample which was detected with a Alltech 320 conductivity detector. For all separations, decreases in conductivity were measured. A Hitachi D-2000 integrator (EM Science, Cherry Hill, NJ, USA) was used to measure retention times. Separations were recorded by a Servogor 120 chart recorder (Abb Goerz Instruments, Vienna, Austria), and a Keithley Chrom 1-AT data acquisition board (Keithley MetraByte Corp., Taunton, MA, USA) with Labtech Notebook software (Laboratory Technologies Corp., Wilmington, MA, USA). Columns were packed with a Shandon HPLC packing pump (Shandon Southern, Sewickley, PA, USA) at 3000 psi.

Reagents and Chemicals

The cation-exchange resin was prepared in our lab from 5 µm macroporous polystyrene-divinylbenzene (Sarasep Inc., Santa Clara, CA, USA). Two grams of resin were slurried with a few milliliters glacial acetic acid. An excess of concentrated sulfuric acid was then added to the resin slurry and placed in an ice bath. This reaction mixture was stirred for 0.5 to 4 min to produce the desired exchange capacity of 0.15 or 0.65 mequiv/g. Methanol, acetonitrile and 2-propanol were of HPLC grade and used as obtained from Fisher (Fisher Scientific, Pittsburgh, PA, USA). Punctilious absolute ethanol was used as obtained from Quantum (Quantum Chemicals, Newark, NJ, USA). All eluents were prepared daily. The analytes and methanesulfonic acid eluent were all of reagent grade and used as obtained from Lancaster (Lancaster Synthesis, Inc., Windham, NH, USA), Aldrich (Aldrich Chemical company, Inc., Milwaukee, WI, USA), and Fisher. Stock solutions of 1000 ppm were used to prepare all samples.

Results and Discussion

Types of Resin

One common resin used for separation of cations in modern ion chromatography is a sulfonated microporous resin of low exchange capacity[8,9] or a resin coated with a latex of low cross-linking. These materials tend to swell somewhat in water to form a gel. In the present study, a sulfonated macroporous resin of high cross-linking was used. This resin is compatible with a wide variety of organic solvents and appears to undergo little if any swelling in going from one solvent to another.

Effect of Eluent Cation Concentration

After trying several different inorganic acids, methanesulfonic acid was selected as the eluting acid for the separation of protonated amine cations. In ion chromatography of cations with H^+ as the eluting cation, k′ should vary according to the following equation:

$$\log k' = -m \log H^+ + b$$

where m is the slope of a linear plot. Linear plots were obtained for the C_1-C_{10} n-alkylamines in methanol, ethanol, 2-propanol and acetonitrile. The slopes were very close to the theoretical slope of -1 in the three alcohols and only a little less than -1 in acetonitrile (Table I). Figures 1 and 2 graphically display the ion-exchange behavior of three alkyl amines and 3 aniline compounds. These eluents effectively removed the hydrophobic interaction as was evident by the closeness of the measured slope to the theoretical slope of -1.0.

Effect of Solvent on k'

A plot of log k' vs. the number of carbon atoms is linear for members of a homologous series in HPLC[10]. The slope of such a plot is an indication of the extent of the hydrophobic effect of the carbon chain on the capacity factor, k'.

The capacity factors of C_1-C_{10} n-alkylamine cations were measured under identical chromatographic conditions in each of four organic solvents. The results are shown graphically in Figure 3. The values of k' were very similar for the various amines in any given solvent although slight variations were present at the extremes. This could be interpreted as some residual hydrophobic attraction for the larger amines, or poor ionic solvation for the smaller amines in certain solvents. The k' values for the homologous series increase in the order methanol < ethanol < propanol << acetonitrile. These results might be explained by a lower degree of solvation of the RNH_3^+ in acetonitrile than in the alcohols where hydrogen bonding through the -OH and interaction with a lone electron pair on oxygen were possible. A higher degree of solvation would attract the RNH_3^+ more strongly to the liquid mobile phase and

Table I

Slopes of Log t_R' - Log $[H^+]$ lines in organic solvents. The resin contained 0.65 mequiv/g of $-SO_3^-H^+$. Eluents contained 75, 50, 25, and 10 mM methanesulfonic acid.

	Methanol	Ethanol	2-Propanol	Acetonitrile
Methylamine	-0.95	-1.02	-0.92	-0.85
Ethylamine	-0.94	-1.00	-0.92	-0.85
Propylamine	-0.93	-1.01	-0.93	-0.84
Butylamine	-0.93	-1.01	-0.92	-0.83
Pentylamine	-0.92	-1.00	-0.93	-0.81
Hexylamine	-0.90	-1.00	-0.93	-0.80
Heptylamine	-0.92	-1.00	-0.93	-0.78
Octylamine	-0.92	-1.01	-0.92	-0.79
Nonylamine	-0.90	-1.00	-0.92	-0.82
Decylamine	-0.89	-1.01	-0.92	-0.81

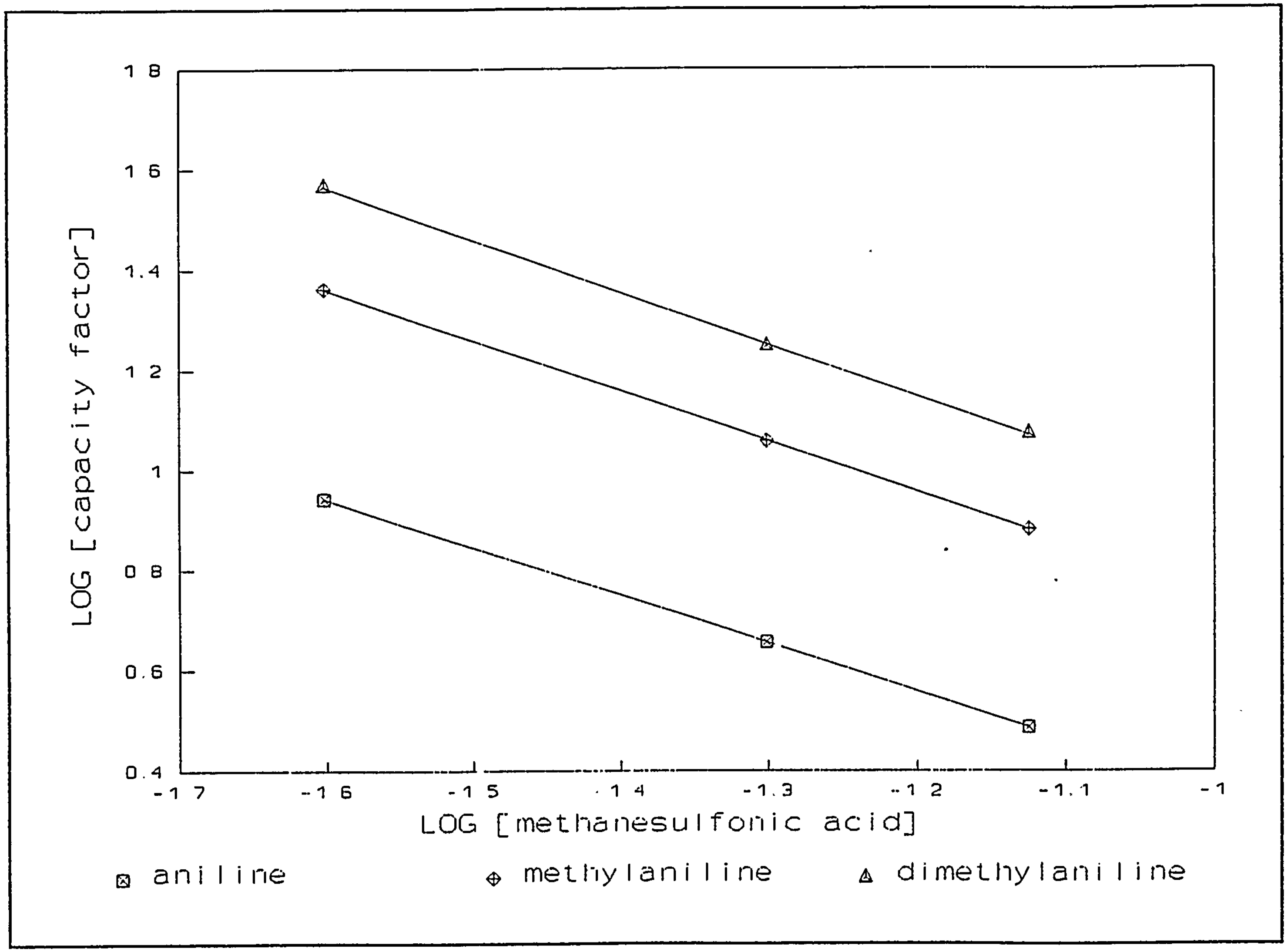

Fig. 1 Effect of [H⁺] on the retention of aniline cations in 100% ethanol.

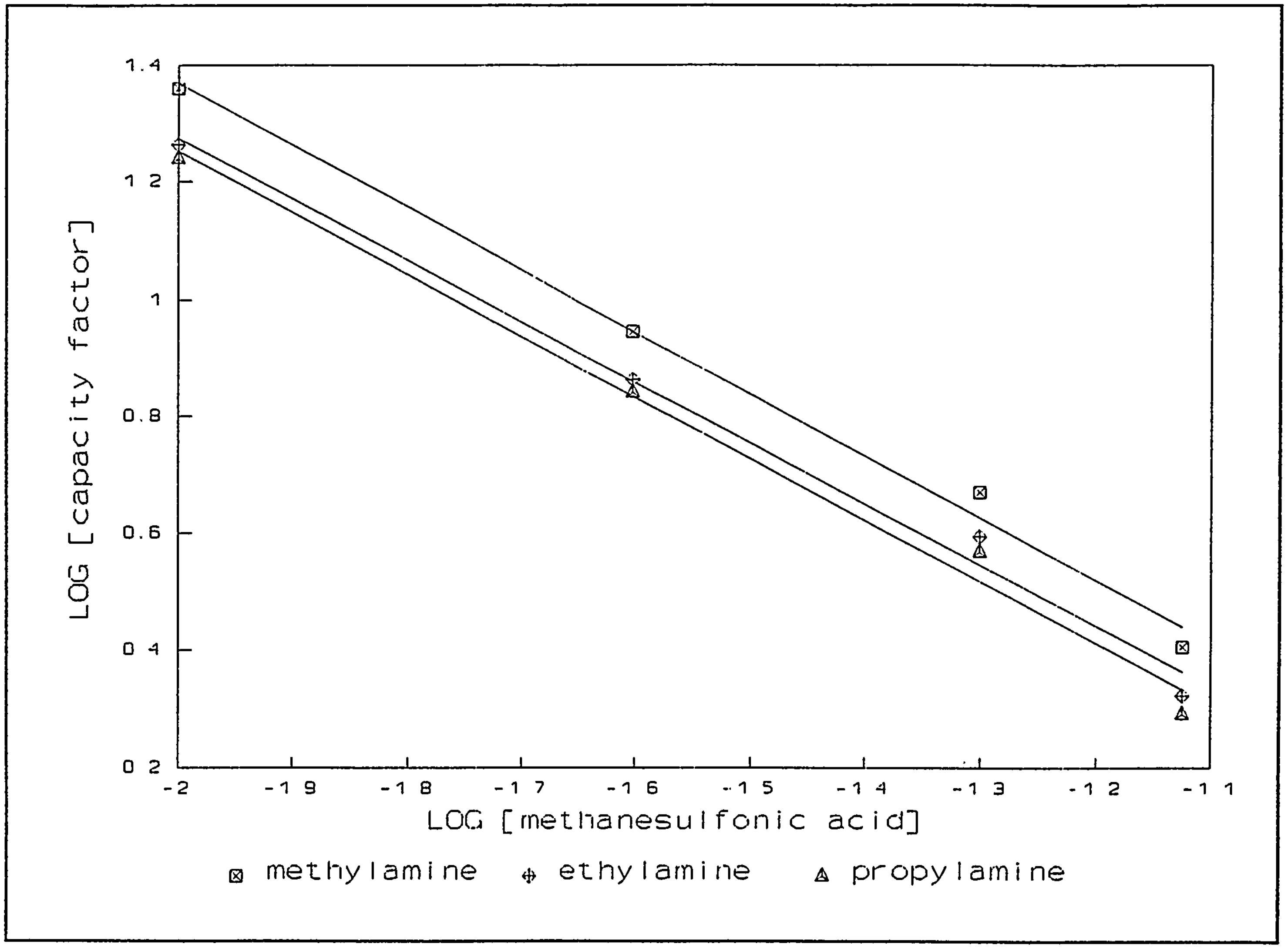

Fig. 2 Effect of [H$^+$] on the retention of n-alkyl amine cations in 100% ethanol.

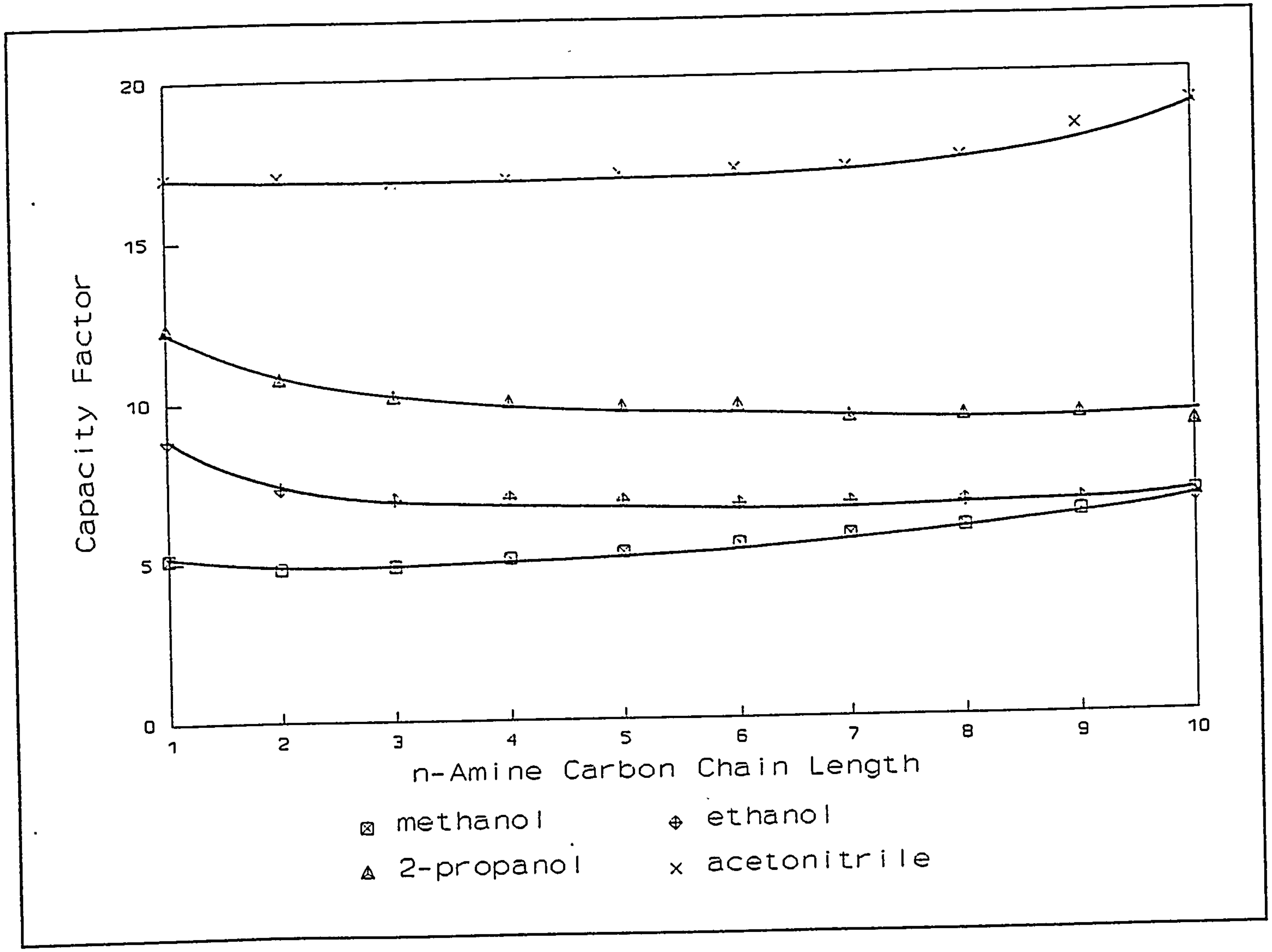

Fig. 3 Effect of solvent on the retention of n-alkylamines. Eluent contained 25 mM methanesulfonic acid.

reduce retention.

The capacity factors of a number of amine cations are given in Table II. Elution is strongly dependent on solvation of the amine cation and the dielectric of the eluent. The effect of dielectric is seen by the 2-propanol k′ values in Table II. Although 2-propanol should be a stronger eluent for the amines, retention times are longer. This is due to the low dielectric of 2-propanol which favors the amine cation remaining paired to the resin exchange sites. Methanol and ethanol have more moderate dielectric constants but have different efficiencies for solvating straight-chain and aromatic amines as can be seen by the capacity factors of the octylamine and aniline cations.

Separation of Protonated Amine Cations

The data in Figure 3 indicate that none of the solvents studied would be a suitable choice for separation of the n-alkylamine ions. However, an excellent separation was obtained for the protonated cations of aniline, N-methylaniline, and N, N-dimethylaniline as shown in Figure 4. These ions differ only by one or two methyl groups. Good separations were also obtained in methanol for a sample containing n-octylamine, di-n-octylamine and tri-n-octylamine (Figure 5) and for another sample containing ethylamine, diethylamine, and triethylamine (Figure 6).

Conductivity detection can be used in any of the solvents studied but the sensitivity is better in eluents with higher dielectric constants (methanol and acetonitrile). Elution of an amine cation reduces the concentration of the more mobile H^+ and thereby gives a peak of decreased conductance for the alcohol solvents. Conversely, H^+ has a lower conductivity than the amine cations in acetonitrile and a positive signal was measured. For convenience, the chromatographic peaks are displayed graphically on a conventional y-axis of increasing signal.

Table II

Capacity factors of protonated amine cations in alcohol solvents on a 5 cm lightly sulfonated column. Eluent contained 1mM methanesulfonic acid.

AMINE	Methanol	Ethanol	2-Propanol
aniline	1.98	1.62	5.78
N-methylaniline	3.18	4.10	13.64
N,N-dimethylaniline	4.37	7.68	27.6
n-octylamine	2.39	1.82	4.83
di-n-octylamine	4.57	3.26	9.43
tri-n-octylamine	7.54	4.61	17.6

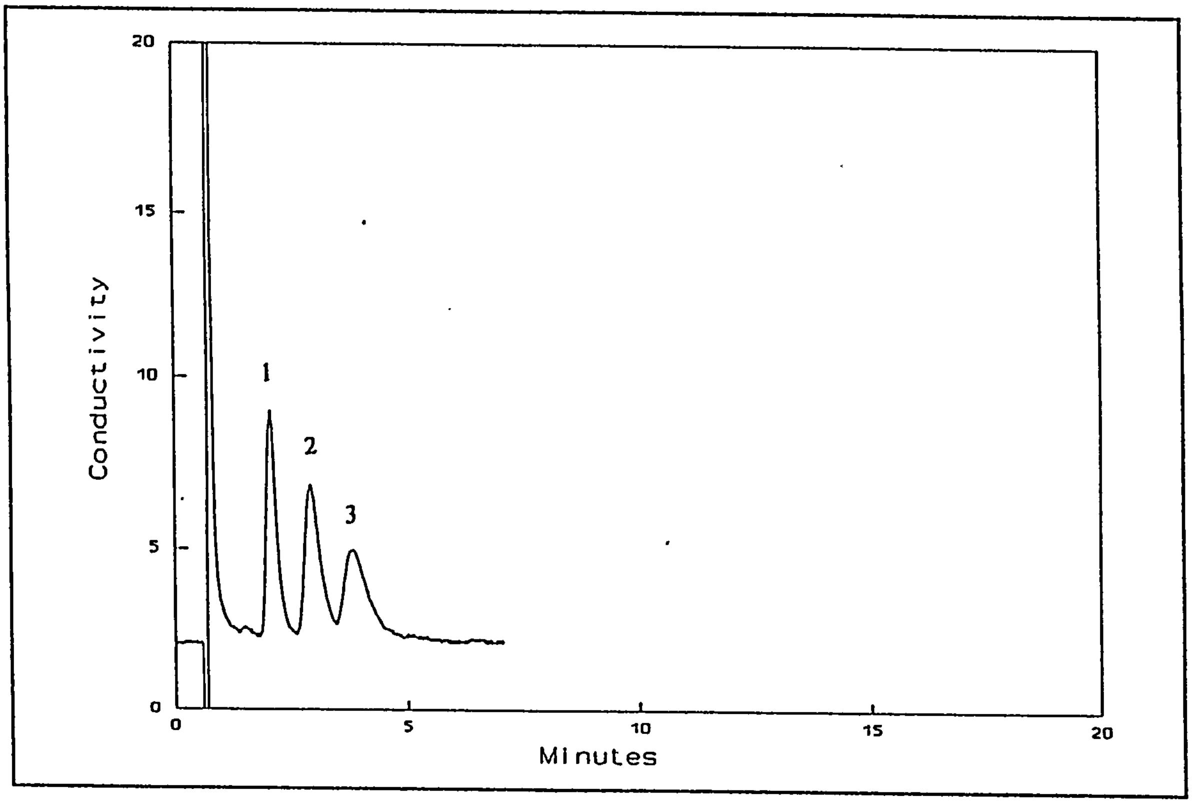

Fig.4 Separation of 12.5 ppm aniline (1), N-methylaniline (2), and N,N-dimethylaniline (3) on a 5 cm sulfonated resin column (0.15 mequiv/g) with 1 mM methanesulfonic acid in methanol as the eluent.

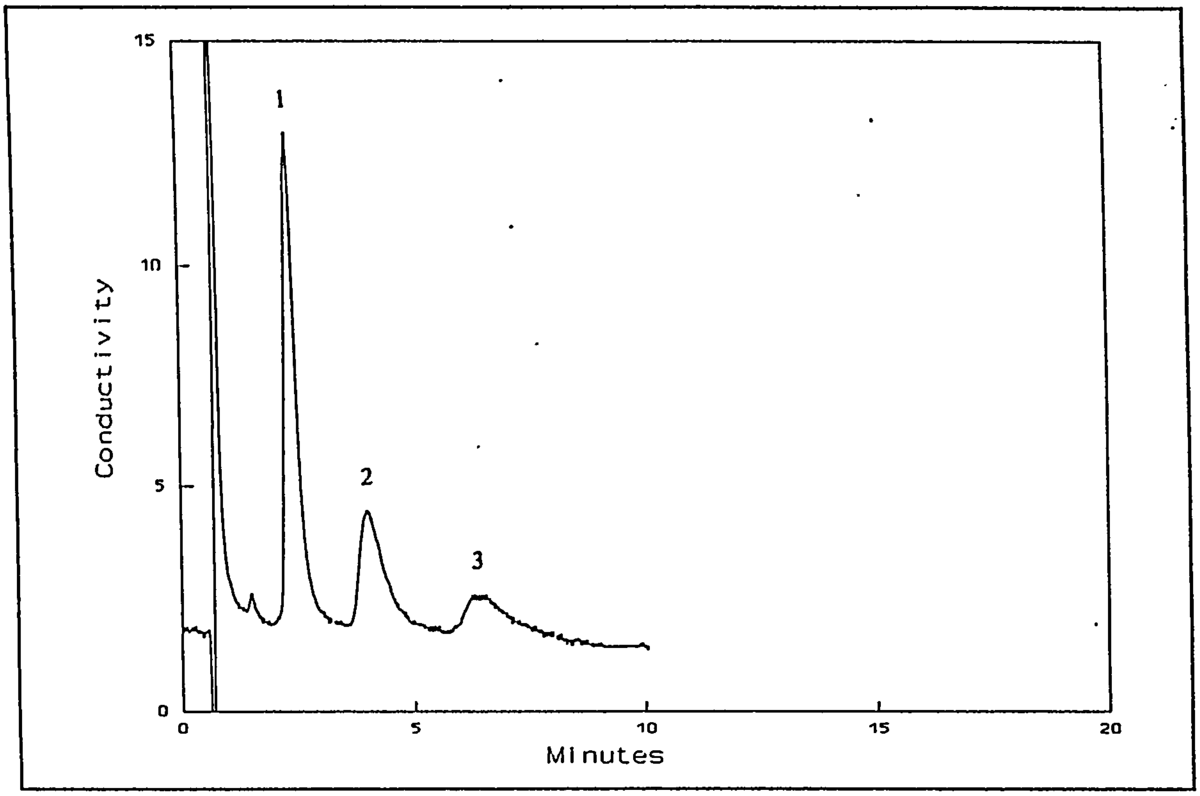

Fig. 5 Separation of 50 ppm n-octylamine (1), di-n-octylamine (2), and tri-n-octylamine (3) on a 5 cm lightly sulfonated column with 1 mM methanesulfonic acid in methanol as the eluent.

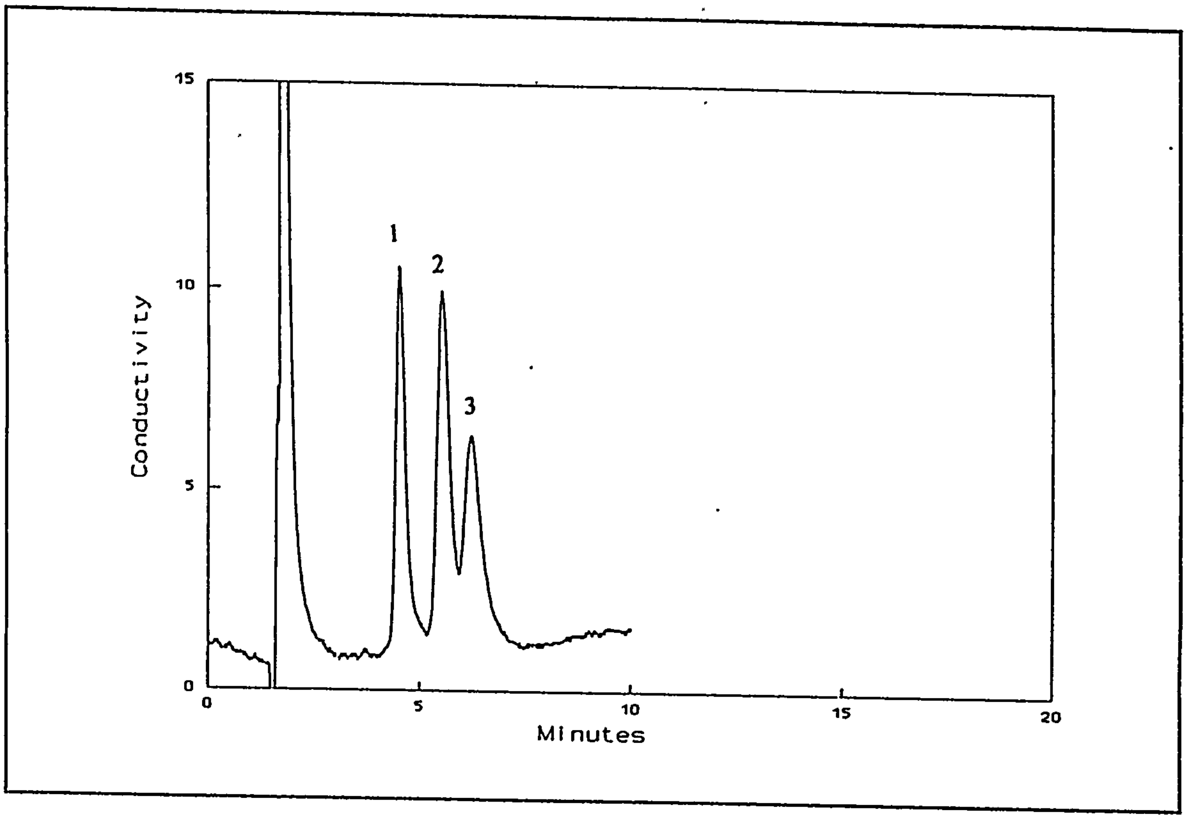

Fig. 6 Separation of 50 ppm ethylamine (1), diethylamine (2), and triethylamine (3) on a 15 cm lightly sulfonated column with 5 mM methanesulfonic acid in methanol as the eluent.

The capacity factors of most neutral organic compounds are very low in the solvents studied. This means that neutral organic compounds will be eluted quickly and not interfere with the chromatographic separation of the amine cations. A separation of 50 ppm aniline, N-methylaniline, and N,N-dimethylaniline in a sample spiked with 10,000 ppm of toluene is shown in Figure 7. The toluene elutes in a very compact, early peak.

Determination of Trace Amines in Organic Solvents

With the use of organic eluents, it should be possible to detect very small amounts of organic bases in solvents. A large volume of organic solvent may be injected. The amine will be retained by electrostatic attraction while the solvent passes through the column with the injection peak. In Figure 8, 50 ppb aniline in toluene (with 0.75 mM methanesulfonic acid) is determined. The detection limit of this system was determined by analyzing the baseline noise after elution of the aniline peak. Using three times the standard deviation of the noise as the criteria, a detection limit of 5 ppb was determined. This type of trace analysis should be applicable to many bases in organic solvents.

Conclusions

A plot of log k′ vs. log H^+ (from methane sulfonic acid) was linear with a slope close to -1 in each of the four solvents studied. This is an indication that the separation mechanism is pure ion-exchange and not based on hydrophobic attraction. The capacity factors of C_1-C_{10} alkylamine cations showed very little change with regard to the number of carbon atoms. However, amine cations such as aniline, N-methylaniline, and N, N-dimethylaniline, were easily separated. These results indicate that the size and shape of the cation, particularly near the

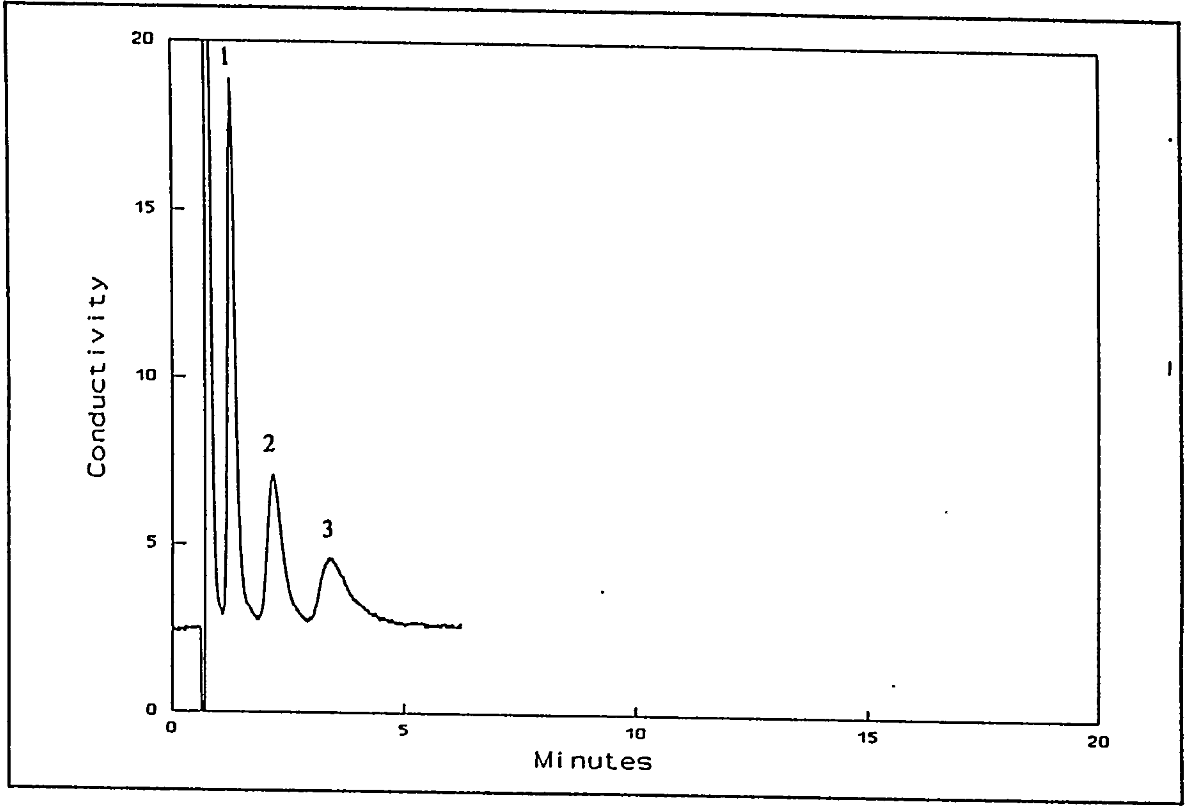

Fig. 7 Separation of 50 ppm aniline (1), N-methylaniline (2), and N,N-dimethylaniline (3) in a sample spiked with 10,000 ppm toluene on a 5 cm lightly sulfonated column with 2.5 mM methanesulfonic acid in ethanol as the eluent.

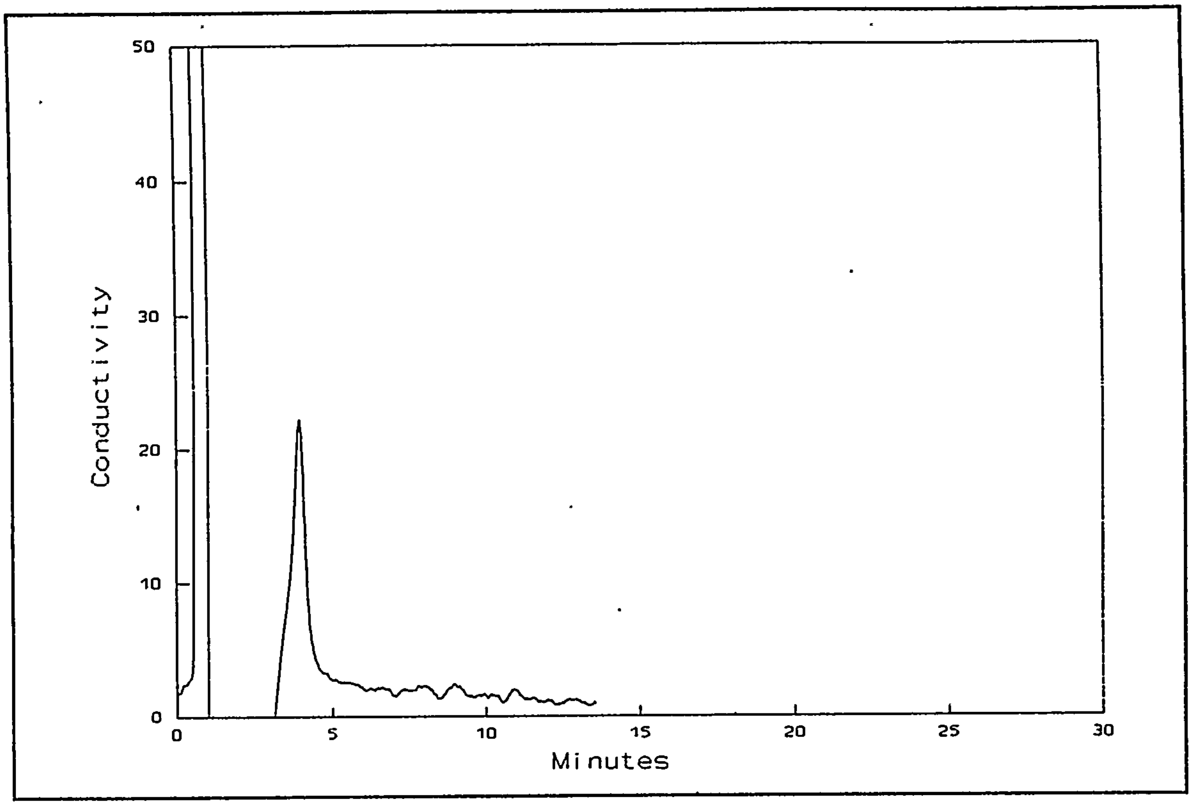

Fig. 8 Injection of 200 µl toluene with 50 parts per billion aniline and 0.75 mM methanesulfonic acid. The eluent is 0.75 mM methanesulfonic acid in methanol at 1.5 ml/min.

nitrogen, affect the ion-exchange selectivity.

Practical separations of amine cations can be obtained in methanol or ethanol with indirect conductivity detection. Large concentrations of neutral organic compounds in the sample do not interfere with the chromatographic separation and determination of amine cations.

Acknowledgements

We wish to thank Alltech Associates, Inc., Deerfield, IL, U.S.A. for a gift of the chromatographic pump and #320 conductivity detector used in this work. We also thank Doug Gjerde of Sarasep Inc. (Santa Clara, CA, USA) for the resin used in this work. This research was supported by a grant from the 3M Co., St. Paul, MN, U.S.A. The work was performed in the Ames Laboratory of Iowa State University. Ames Laboratory is operated for the U.S. Department of Energy under contract No. W-7405-Eng-82.

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SILICALITE AS A STATIONARY PHASE FOR HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

A paper to be submitted for publication in the *Journal of Chromatography A*

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Abstract

A silica-based molecular sieve was used as a stationary phase for reversed phase high performance liquid chromatography (HPLC). This material is very hydrophilic but possesses hydrophobic channels which are approximately 6Å in diameter. Small molecules may partition into and out of the channels. Larger molecules have more difficulty interacting with this stationary phase. This size-exclusion effect is a second selectivity mechanism that may be used to obtain unique separations. Comparisons between Silicalite and silica-C18 are shown along with several separations that demonstrate the capabilities and limitations of this material as an HPLC stationary phase. Larger-scale separations of *cis/trans* isomeric pairs using displacement chromatography was also performed on Silicalite. Milligram quantities of pure *cis* or *trans* isomer were collected using this technique.

Introduction

Silicalite is one particular polymorph of SiOH. It contains 5-membered rings of alternating silica-oxygen arranged in a tetrahedral pattern. This material is hydrophilic but contains hydrophobic channels approximately 6 Å in diameter. Silicalite was first synthesized in 1978[1], but has received very little attention as a possible chromatography stationary phase. Organic molecules are attracted to the hydrophobic channels while water, which associates into clusters 10-12 Å in diameter, is excluded. For smaller molecules, size-exclusion and hydrophobic interaction are possible retention mechanisms. These characteristics make Silicalite an interesting choice as an HPLC stationary phase.

Much of the research involving Silicalite has related to the adsorption and kinetic mechanisms of gas adsorption[2-10]. Fritz and Ogawa used Silicalite as an SPE adsorbent. Good recoveries of aldehydes and ketones from aqueous samples were obtained[11].

Andronikashvili, *et al.* recently used Silicalite as a gas chromatography stationary phase. A short column was packed with Silicalite and used for the separation of ortho, meta, and para isomers of aromatic compounds. Several separations are shown[12].

For this work, Silicalite was packed into HPLC columns and used for the separation of a variety of neutral organic compounds.

Experimental

Chromatographic System

The chromatographic system consisted of several components. A SpectraPhysics P2000 (Spectra-Physics Analytical, Freemont, CA, USA) HPLC pump was used for mobile phase delivery. A 7125 Rheodyne injector (Rheodyne, Berkeley, CA, USA) delivered a 10 μl sample to the column and was then detected with a Kratos 783 UV-VIS detector (Applied Biosystems, Ramsey, NJ, USA). Separations were recorded by a Servogor 120 chart recorder (Abb Goerz Instruments, Vienna, Austria), and a Keithley Chrom 1-AT data acquisition board (Keithley MetraByte Corp., Taunton, MA, USA) with Labtech Notebook software (Laboratory Technologies Corp., Wilmington, MA, USA).

The setup for the displacement chromatography experiment consisted of two HPLC systems, shown in Figure 1. A 10-25 cm displacement separation column was connected directly between pump #1 and injector #1. After leaving the displacement column, the eluent passed through a 5 μl loop on injection valve #1. A second, smaller column was used to obtain the analytical separation of the effluent from the displacement column. An Alltech 425 HPLC pump (Alltech Associates, Inc., Deerfield, IL, USA) was used to deliver the mobile phase to the analytical column. Turning injection valve #1 allowed a 5 μl portion of the displacement column effluent to be directed to the analytical column and on to the UV-Vis detector for quantitation. The analytical separation took only two minutes or less, therefore fractions could be analyzed at least every two minutes.

Reagents and Chemicals

All analytes and solvents used were of reagent grade, and used as obtained from Aldrich (Aldrich Chemical Company, Inc., Milwaukee, WI, USA) and Fischer (Fisher Scientific, Pittsburgh, PA, USA). Distilled water was further purified with a Barnstead Nanopure II System (Sybron Barnstead, Boston, MA, USA).

The Silicalite particles were between 5 and 10 μm and used as obtained from the 3M

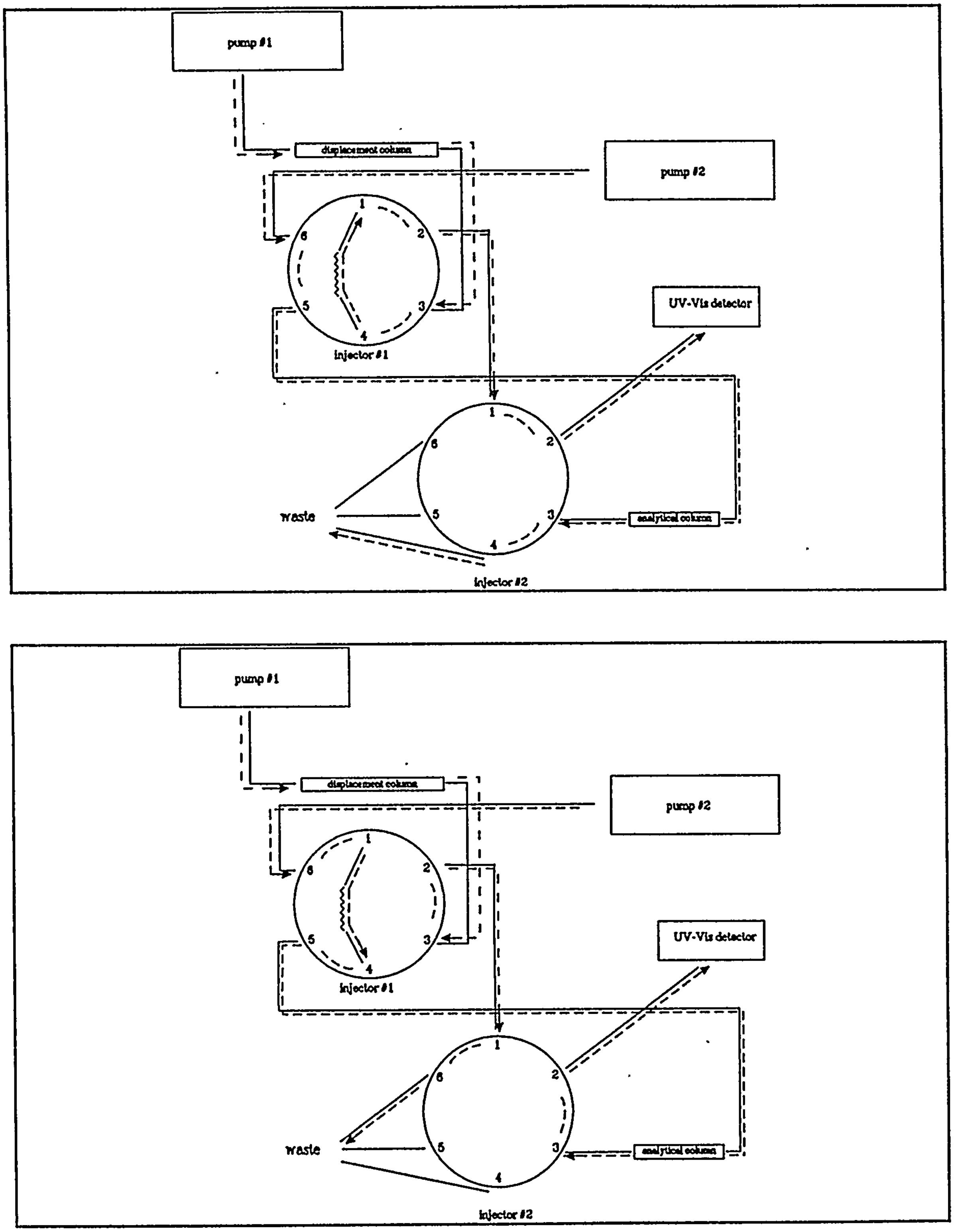


Fig. 1 Experimental setup for displacement chromatography. Load (TOP) and inject (BOTTOM) positions are shown.

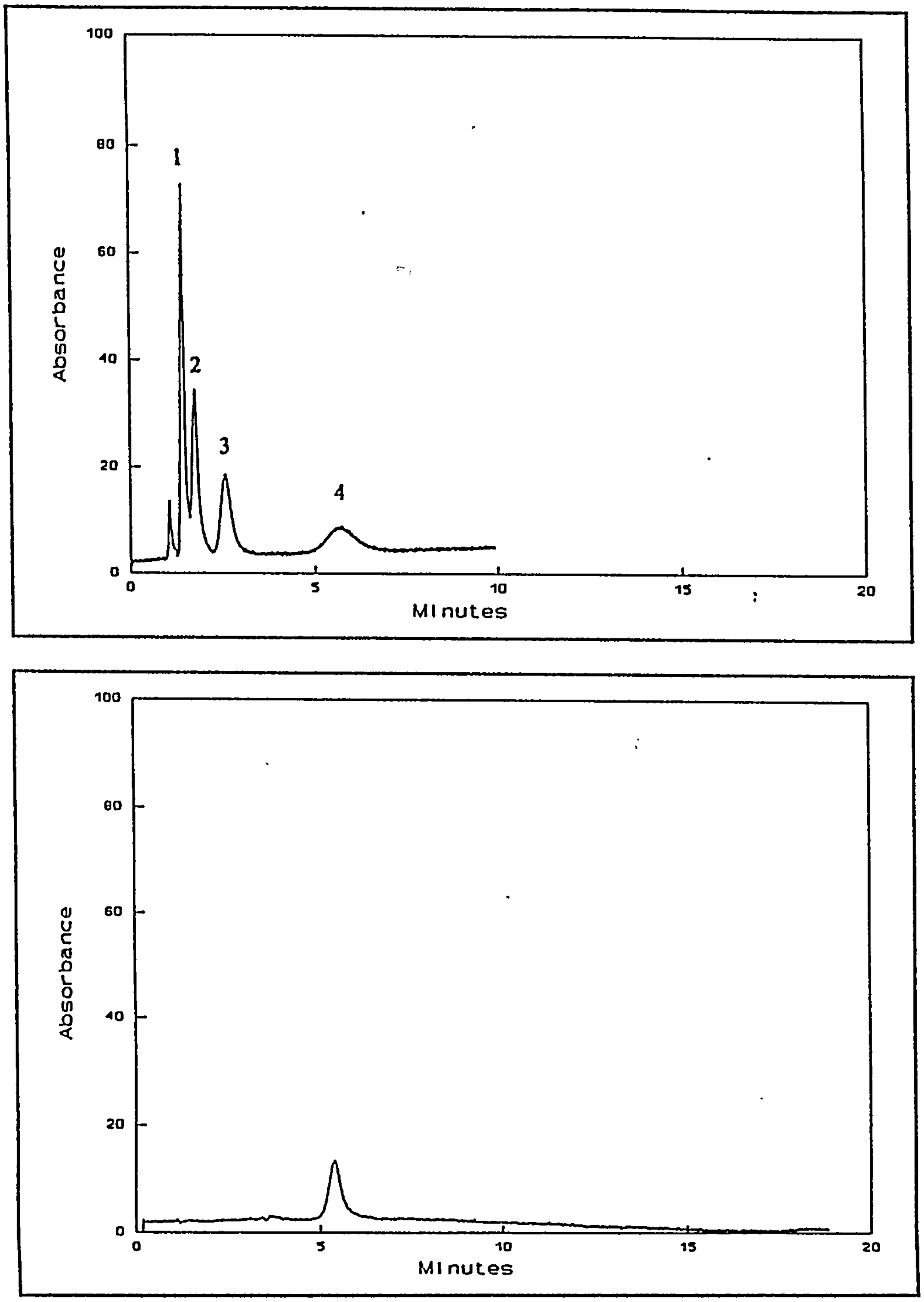
company (3M Company, St. Paul, MN, USA). The following packing procedure was followed. Silicalite was added to 2-propanol and sonicated under vacuum for 15 minutes or more. This removed any trapped air bubbles in the Silicalite and produced a slurry which was then packed into 4.6 mm i.d. HPLC columns. A Shandon HPLC packing pump (Shandon Southern, Sewichley, PA, USA) was used at a pressure of 4000 psi. Nucleosil silica-C18 particles of 5 µm diameter (Alltech Associates, Inc., Deerfield, IL, USA) were slurried in methanol and pack at a pressure of 3000 psi. Samples were prepared by diluting 1000 ppm stock solutions with the eluent to the final desired concentration.

Results and Discussion

Analytical Separations

The additional mechanism of size-exclusion should permit unique separations to be obtained with a Silicalite stationary phase. The characteristics of Silicalite were investigated by separating a variety of compounds. A 10 cm Silicalite column packed with 5-10 µm particles was used for all separations unless otherwise noted. Several comparisons were made between a 10 cm Silicalite column and a 10 cm silica-C18 column packed with 5 µm particles. In most comparisons, the flow rate or percentage acetonitrile in the eluent has been modified to yield similar retention times.

A separation of several ketones is shown in Figures 2 and 3. A 50% acetonitrile eluent permits a nice separation, however methanol eluted these compounds very slowly. As shown in Figure 3, after nearly 20 minutes only one peak (acetone) has eluted. Methanol is known to be a somewhat weaker eluent for HPLC, but is particularly poor for Silicalite. This may be due to



Figs. 2,3 Separation of acetone (1), 2-butanone (2), 2-pentanone (3), and 2-hexanone (4) on a 10 cm Silicalite column with 50% acetonitrile (Top) and 50% methanol (Bottom) as the eluent.

methanol self-associating via hydrogen bonding much like water.

Phenols were chosen as one group of compounds to study. The benzene ring diameter is approximately 5-6 Å in diameter, and should be able to interact with the hydrophobic channels of Silicalite. Numerous phenols are available with many different substituents. These substituted phenols should permit the investigation of the size-exclusion effect of Silicalite. A gradient elution of several *p*-alklyphenols is shown in Figure 4. The background absorbance rises due to the increased acetonitrile concentration, but has been subtracted from the phenol separation to produce the flat baseline. These phenols are substituted in the *para* positions with methyl through heptyl groups. Heptylphenol is obviously larger than cresol, although is it not excluded from the Silicalite channels. The alkyl groups are opposite the -OH group, which should allow the molecules to "slide" into the channels. They can fit in one direction, but not the other. This is the same elution order expected for a silica-C18 stationary phase.

Figures 5-11 are separations of various phenol mixtures. Figure 5 and the comparison made in Figures 6 and 7 are shown to demonstrate the size-exclusion effect. The most bulky substituted phenol in Figure 5, 2,6-di-*tert*-butyl-4-methylphenol, elutes very near the injection peak, even earlier than phenol. This molecule is too bulky to fit into the channels and was therefore not retained. Also note the nice separation of the trimethylphenol isomers (this separation is compared with silica-C18 in Figures 8 and 9). The size-exclusion effect is also shown in Figures 6 and 7 with several chlorophenols. The largest molecule, pentachlorophenol, elutes first on the Silicalite column, but comes out much later on silica-C18. As before, this molecule is excluded from the channels due to the large size. The elution order of the mono substituted chlorophenols is 2-, 3- and finally 4-chlorophenol. This order might be expected

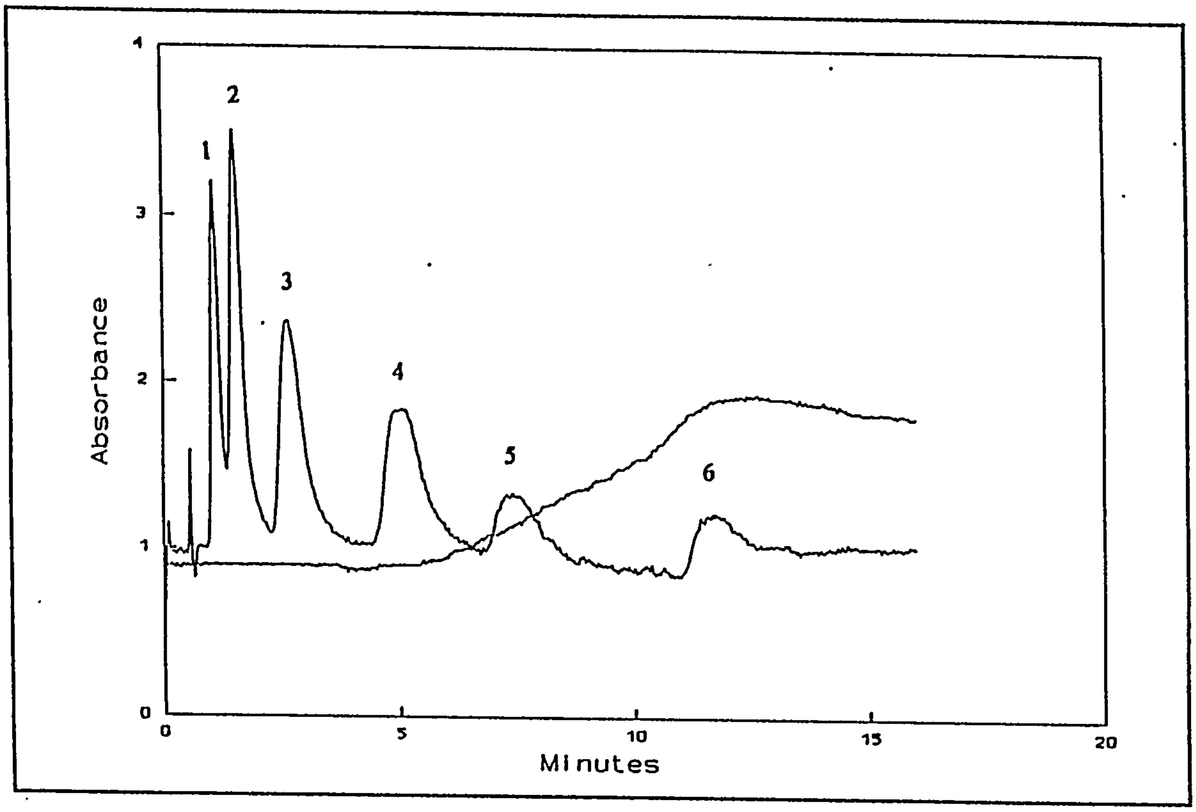


Fig 4. Separation of n-alkylphenols on a 5 cm Silicalite column with background subtraction.

Gradient: 30 % acetonitrile for 2 minutes.
30-50 % acetonitrile from 2-10 minutes.
50 % acetonitrile for 5 min at 1 ml/min.

Peaks: 4-cresol (1), 4-ethylphenol (2), 4-propylphenol (3), 4-n-butylphenol (4), 4-n-amylphenol (5), 4-n-heptylphenol (6)

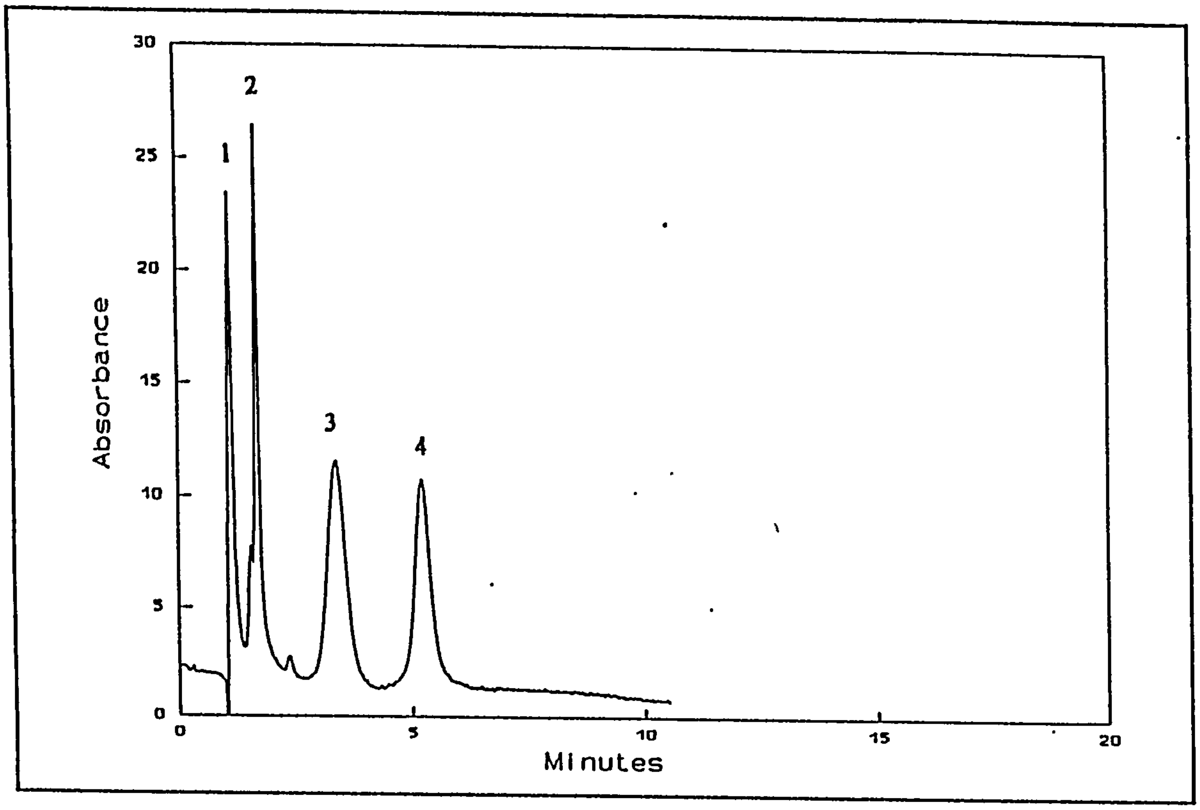
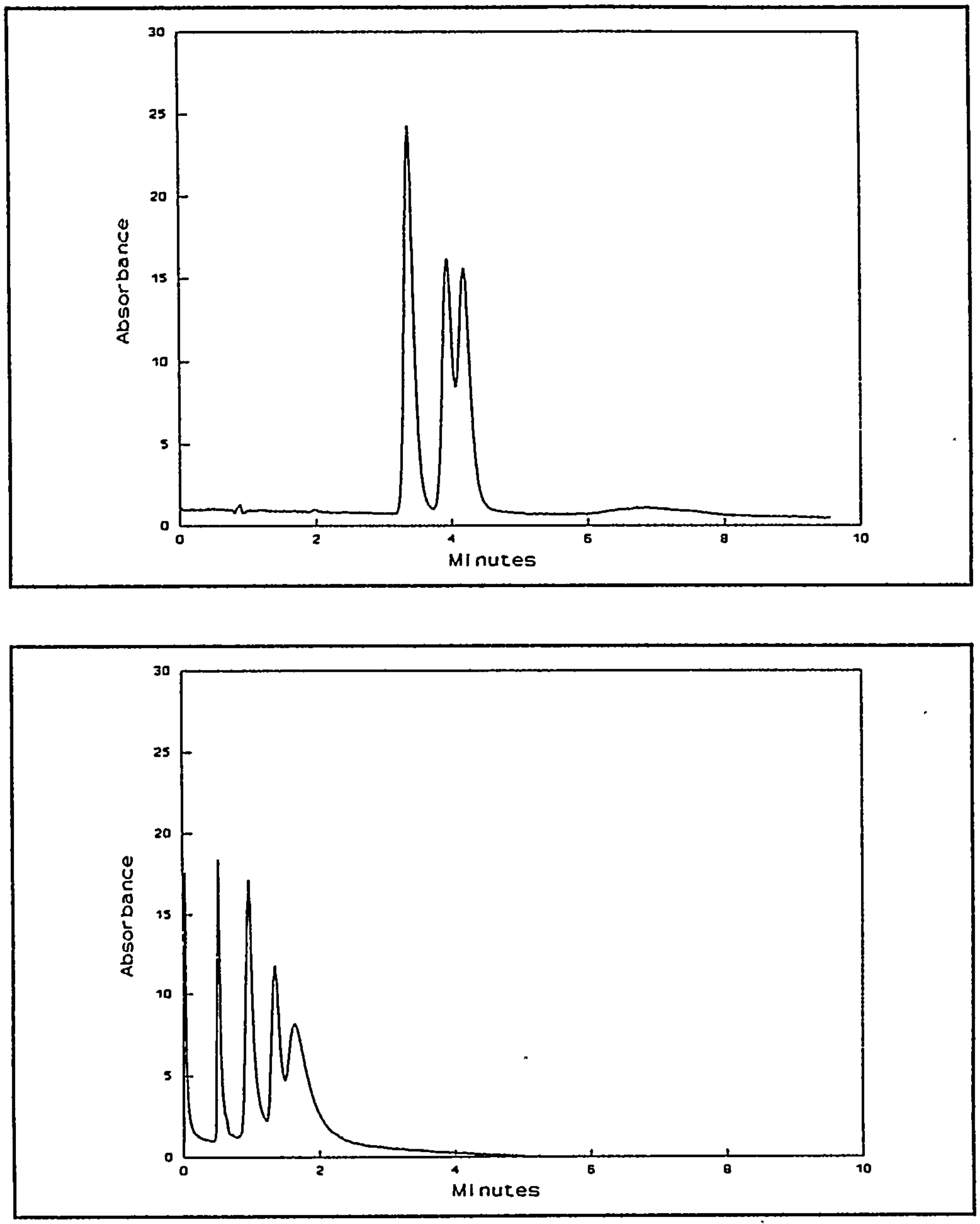


Fig. 5 Separation of 1-5 ppm 2,6-di-*tert*-butyl-4-methylphenol (1), phenol (2), 2,4,6-trimethylphenol (3), and 2,3,6-trimethylphenol (4) on a 10 cm Silicalite column with 30% acetonitrile at 1 ml/min as the mobile phase.



Figs. 6,7 Separation of 10 ppm 2-, 3-, 4-chlorophenol, and pentachlorophenol on 10 cm columns with 35% acetonitrile at 1.25 ml/min as the mobile phase.

TOP: silica-C18

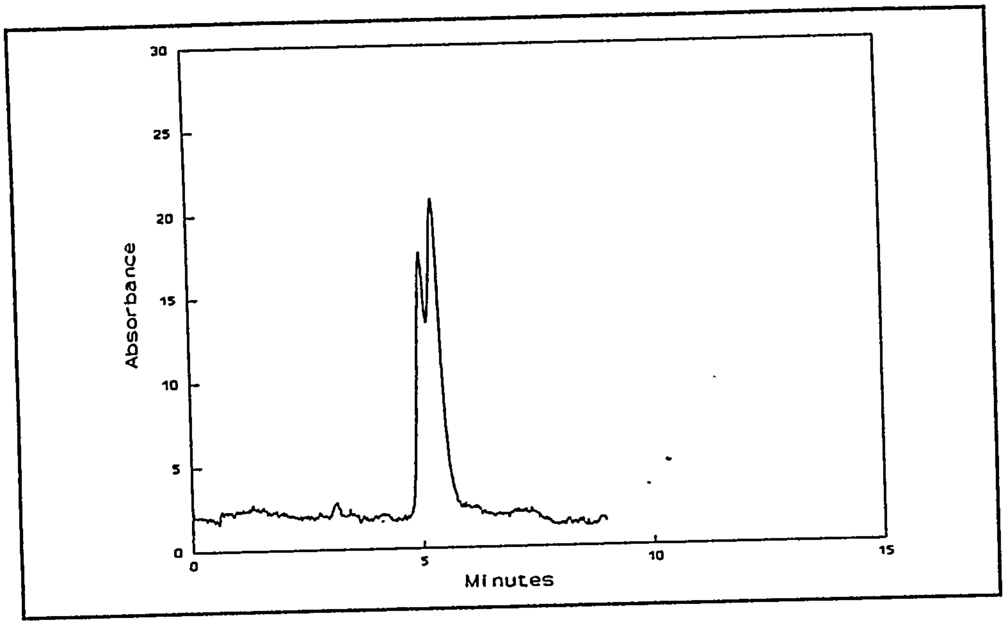
Elution order is 2-, 4-, 3-, pentachlorophenol.

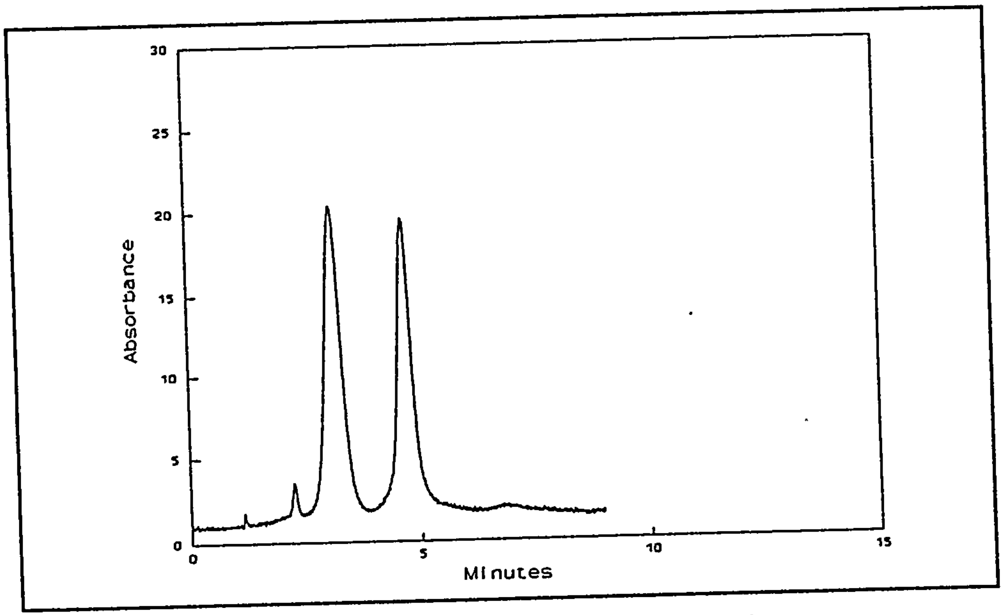
BOTTOM: Silicalite

Elution order is pentachlorophenol, 2-, 3-, 4-.

based on the molecular shape. 4-chlorophenol is able to fit more easily into the channels and therefore retained the longest. The other isomers are more bulky and elute earlier. A separation of two trimethylphenols is shown in Figures 8 and 9. The 2,4,6- isomer, which is more bulky than 2,3,6-trimethylphenol, elutes first on Silicalite. The elution order is reversed on the silica-C18. The separation of three *tert*-butylphenols is compared in Figures 10 and 11. Note the improved resolution and different elution order on the Silicalite. The elution order is somewhat difficult to explain. The *para* compound elutes first, although based on molecular shape, it should partition into the channels more easily than the *meta* or *ortho* compounds.

Many phenol positional isomers were separated nicely with Silicalite. A more challenging separation would be *cis/trans* stereoisomers, which have the same connectivity. Figures 12-21 compare the separation of several *cis/trans* isomeric pairs. The first comparison in Figures 12 and 13 is *cis* and *trans* 1,2-dichloroethylene. These are fairly small molecules, but the different position of the chloro group was enough to alter the interaction with the Silicalite channels. The silica-C18 column does provide slight separation, but would require longer analysis time for complete separation. Both isomers of 1,2-dichloroethylene may be purchased separately, and elution order was therefore confirmed. The other isomers used for comparisons cannot be purchases separately, but are sold as "predominately *trans*". It was assumed that the smaller peak is due to the *cis* isomer. The next three comparisons for crotonaldehyde, crotyl alcohol, and 4-hexene-3-one were all very similar. A near-baseline separation was obtained with Silicalite in under four or five minutes. No separation, or very slight separation was obtained with the silica-C18. In each case, the cis isomer eluted first. The shape of the *trans* isomer must permit a better fit into the Silicalite channels. The separation of *cis/trans* crotononitrile is shown in

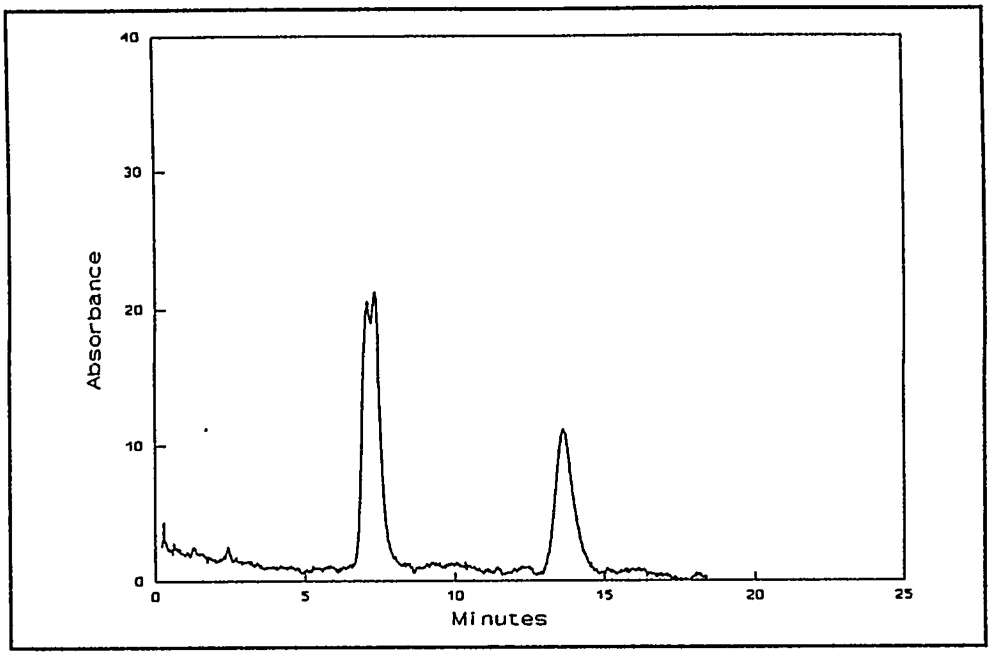


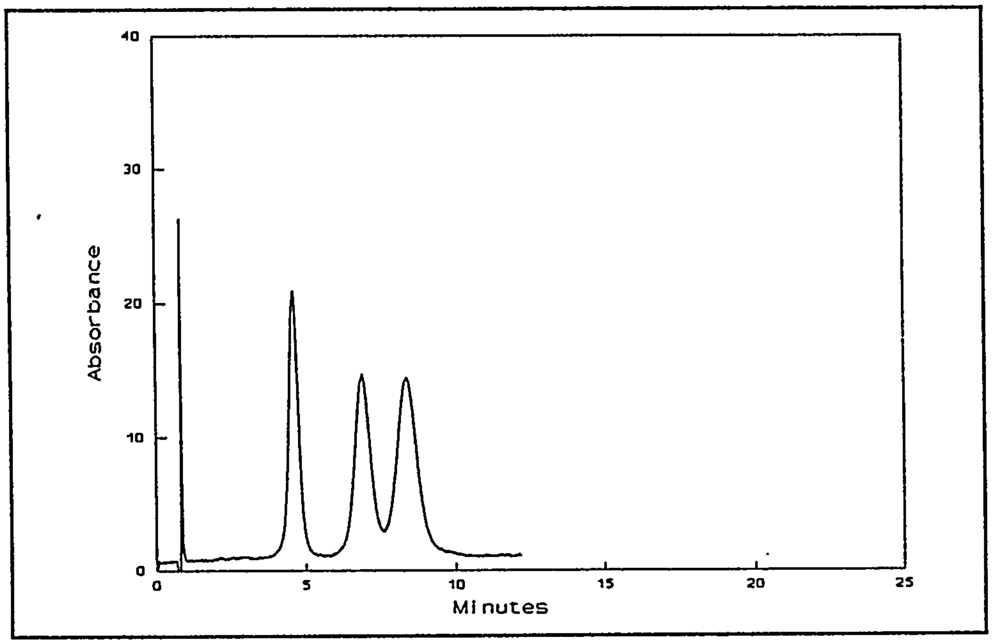


Figs. 8,9 Separation of 25 ppm 2,3,6-trimethylphenol and 2,4,6-trimethylphenol on 10 cm columns with 32% acetonitrile as the eluent.

TOP: silica-C18 with flow of 2 ml/min. Elution order is 2,3,6-, 2,4,6-trimethylphenol.

BOTTOM: Silicalite with flow of 1 ml/min. Elution order is 2,4,6-, 2,3,6-trimethylphenol.

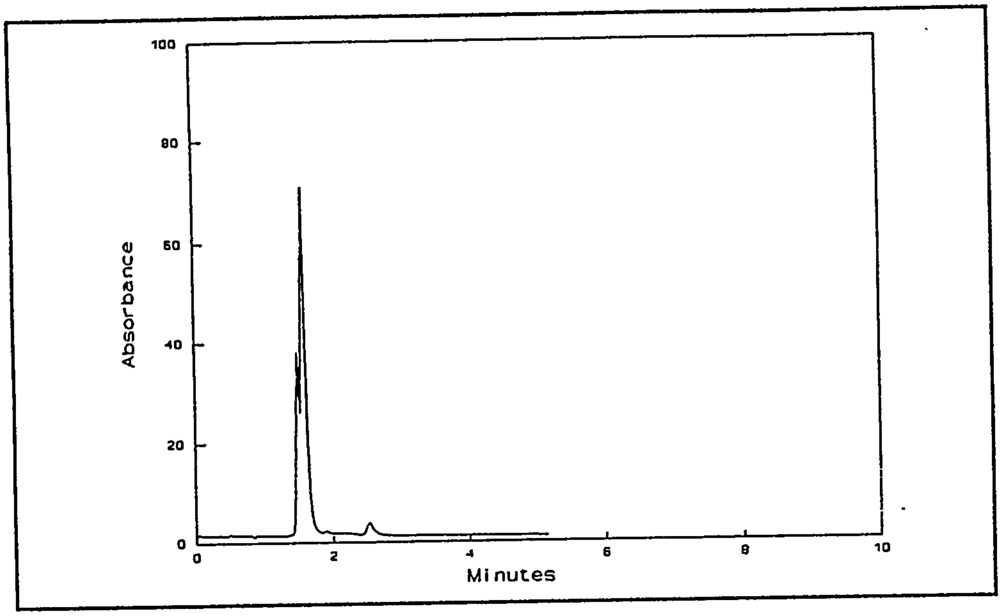


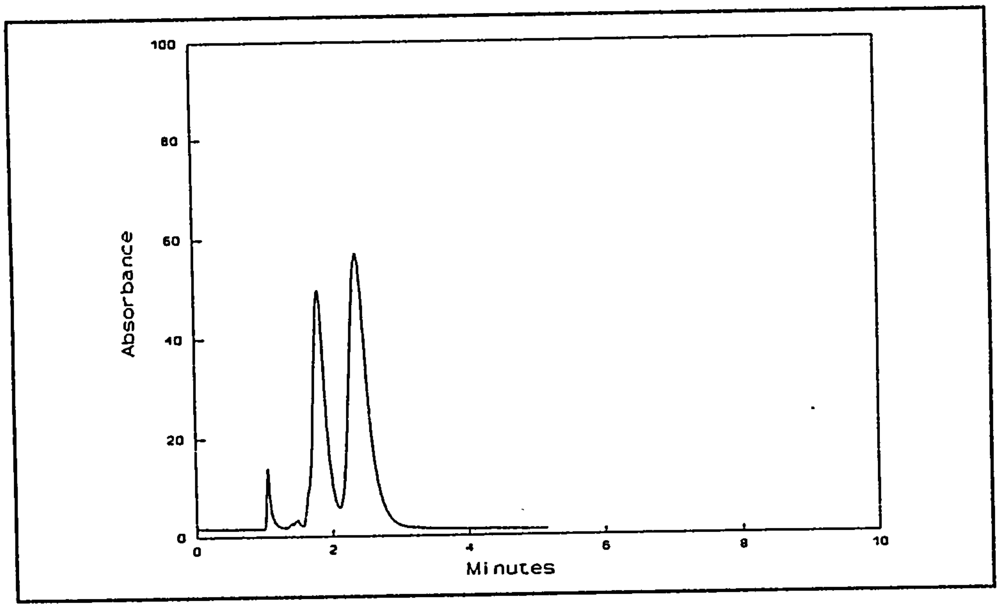


Figs. 10,11 Separation of 25 ppm 2-, 3-, and 4-*tert*butylphenol on 10 cm columns with 32% acetonitrile as the eluent.

TOP: silica-C18 with flow of 2 ml/min. Elution order is 3-, 4-, 2-*tert*butylphenol.

BOTTOM: Silicalite with flow of 1.5 ml/min.

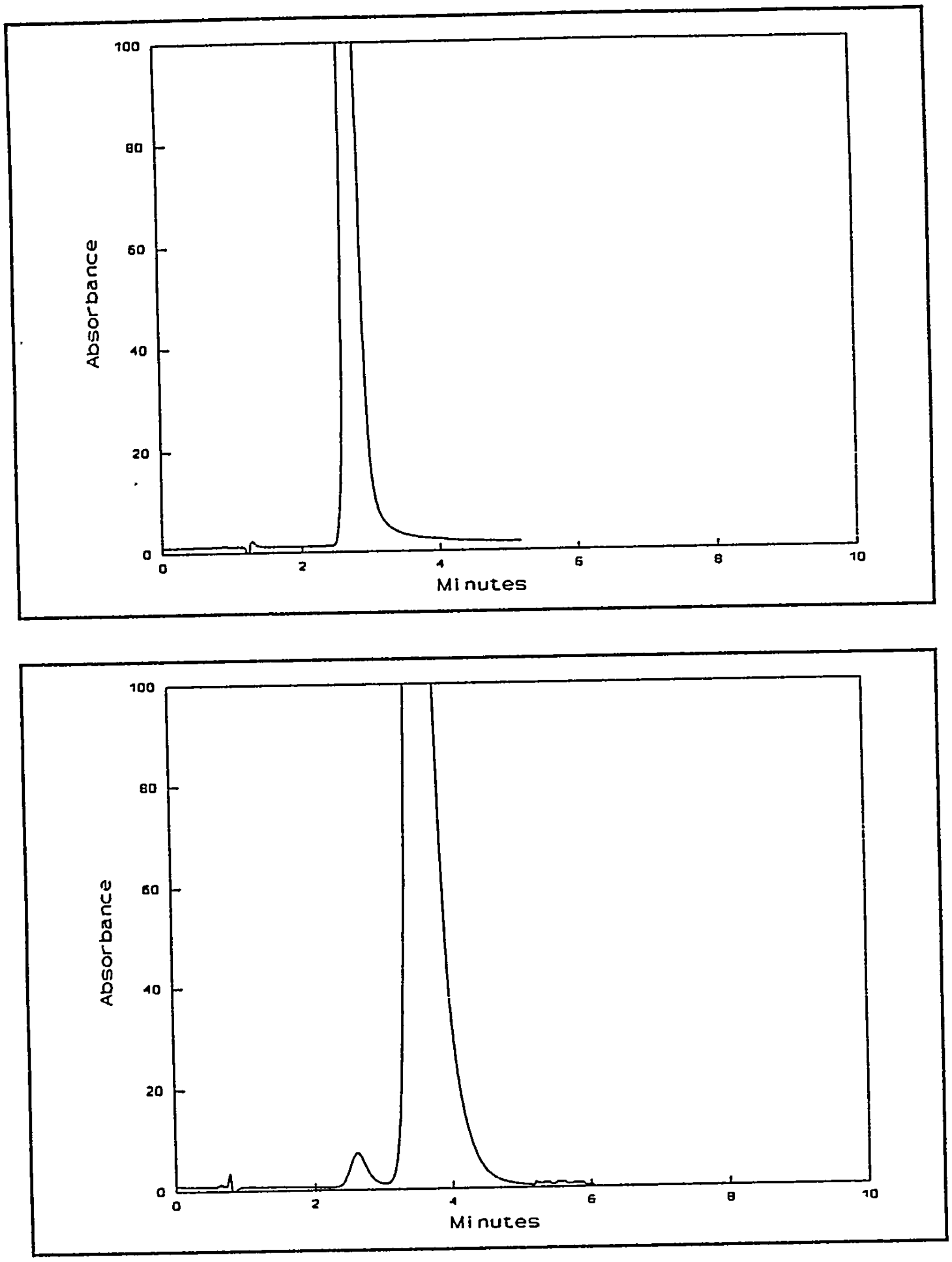




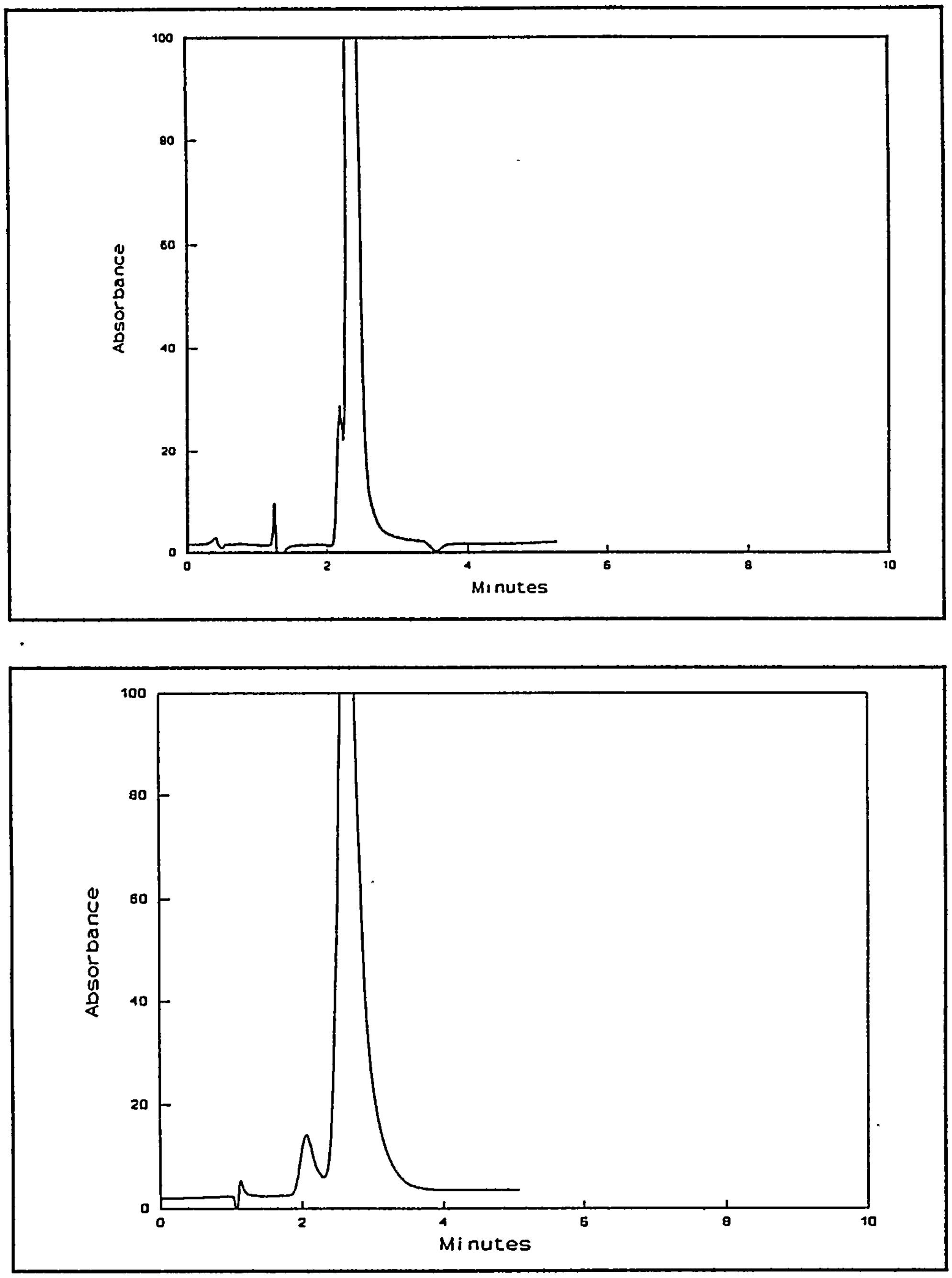
Figs. 12,13 Separation of 25 ppm *cis/trans* 1,2-dichloroethylene on a 5 cm column with acetonitrile at 1 ml/min as the eluent.

TOP: silica-C18 with 60% acetonitrile.
Elution order is *cis, trans.*

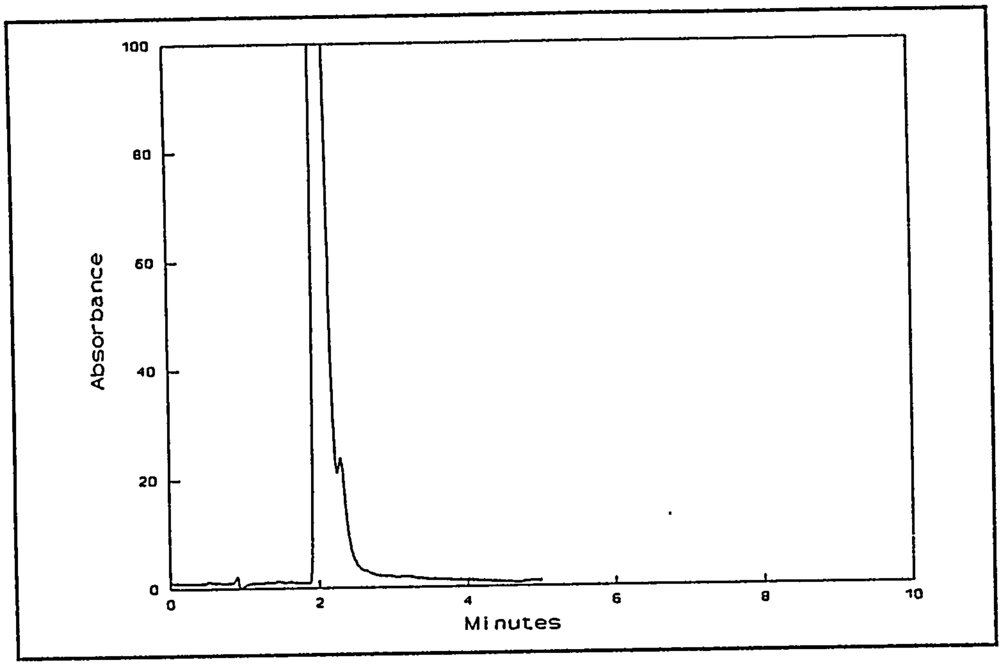
BOTTOM: Silicalite with 75% acetonitrile.
Elution order is *cis, trans.*

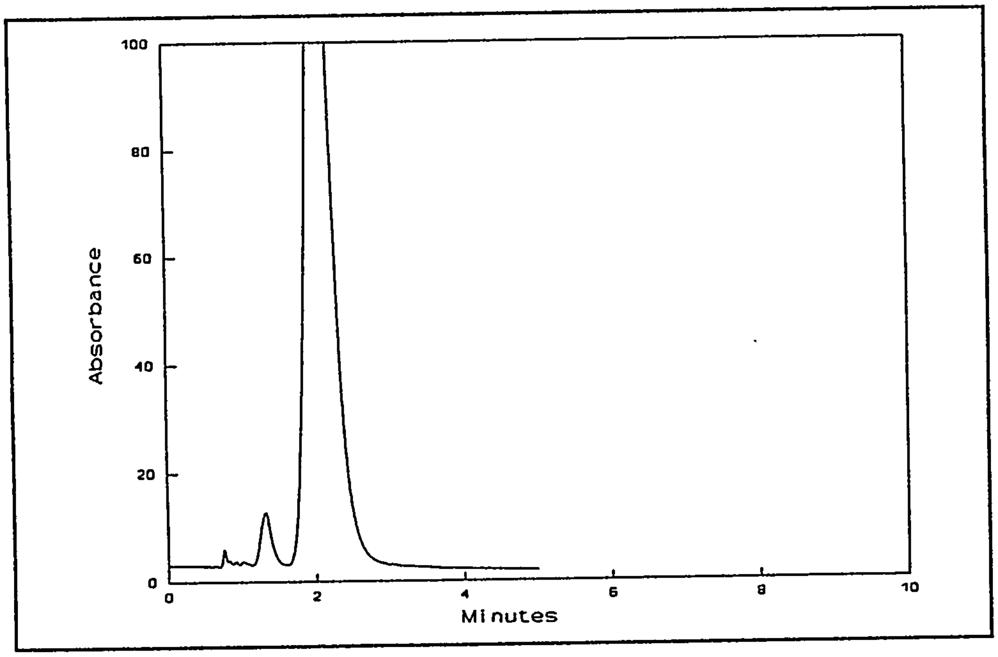


Figs. 14,15 Separation of 10 ppm *cis/trans* crotonaldehyde on a 10 cm column with acetonitrile at 1 ml/min as the eluent.
TOP: silica-C18 with 12% acetonitrile.
BOTTOM: Silicalite with 30% acetonitrile.



Figs. 16,17 Separation of 50 ppm *cis/trans* crotyl alcohol on a 10 cm column with acetonitrile a 1 ml/min as the eluent.
TOP: silica-C18 with 12% acetonitrile.
BOTTOM: Silicalite with 20% acetonitrile.





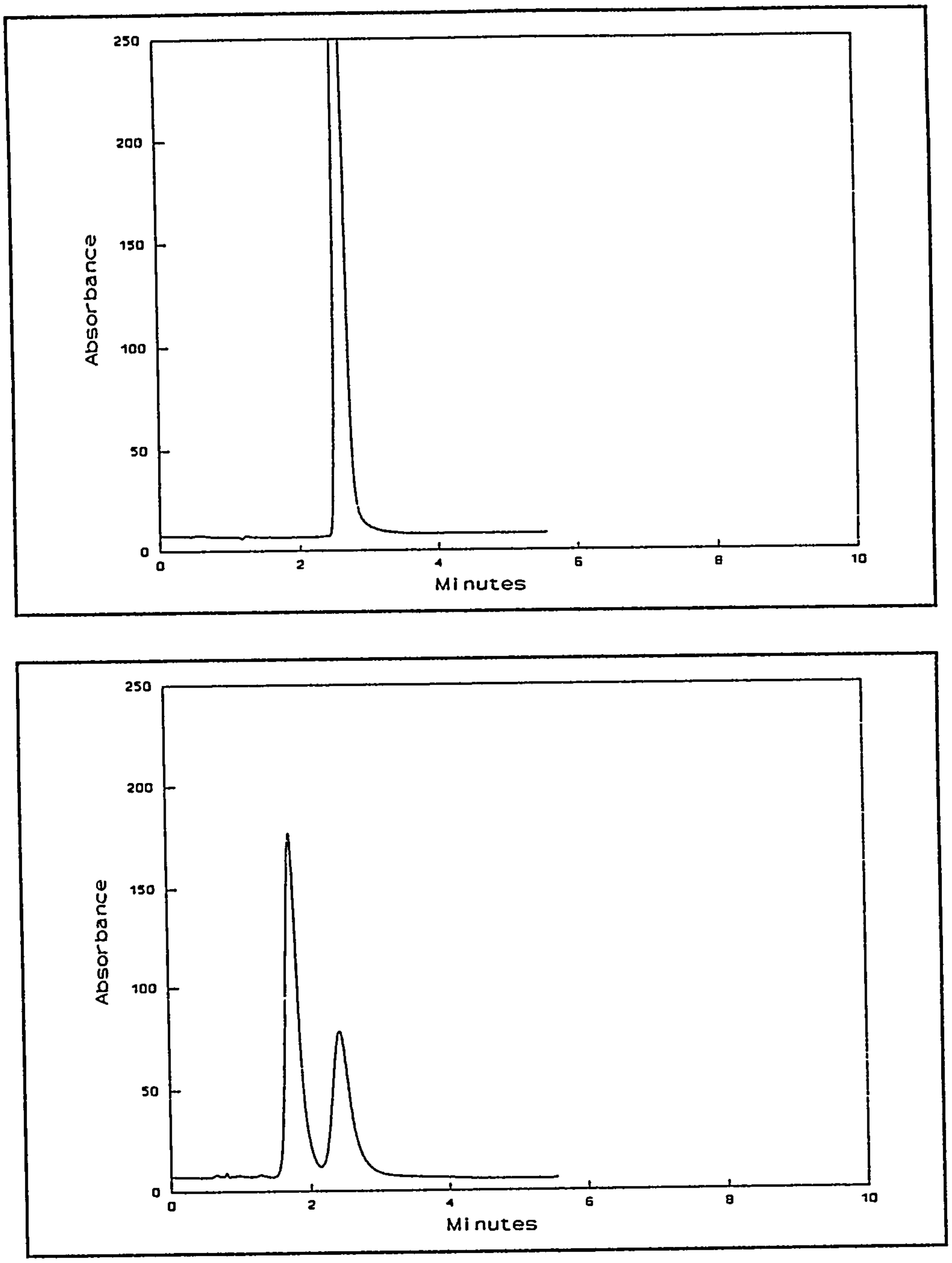
Figs. 18,19 Separation of 15 ppm *cis/trans* 4-hexene-3-one on 10 cm columns with 80% acetonitrile at 1 ml/min as the eluent.
TOP: silica-C18 with 50% acetonitrile.
BOTTOM: Silicalite with 80% acetonitrile.

Figures 20 and 21. This compound contains similar amounts of each isomer. These isomers were resolved in three minutes with Silicalite, while no separation was obtained with silica-C18. It is interesting to note the first peak is larger. Crotononitrile may be slightly enriched with the *cis* isomer, or the elution order was opposite from the previous chromatograms. It is difficult to determine which is true without the pure isomers to confirm retention times. These separations were reproducible. Very similar separations were obtained on different columns several months apart.

Displacement Chromatography

The ability of Silicalite to efficiently separate *cis/trans* isomers was taken a step further to a prep scale. Displacement chromatography was used to attempt a separation of 25-50 mg crotononitrile on a 10 or 15 cm Silicalite columns. A saturated solution of crotononitrile in water (≈ 30,000 ppm) was loaded on the Silicalite, after which the eluent containing the displacing molecule was pumped through the column. The displacement column effluent was sent through a 5 µl injection loop on the way to the detector. Turning an injection loop sent a 5 µl portion of the effluent to a second column that performed the *cis/trans* isomer separation and quantitation. This allowed the effluent to be monitored every 1-2 minutes, the time necessary for the analytical separation (Figure 1).

n-Butanol is a common molecule for displacement chromatography, and was one of the first eluents used. Various concentrations up to about 6% butanol in water were used but with little success. The best separation with n-butanol is shown in Figure 22. Crotononitrile was loaded at 0.25 ml/min and eluted with 0.73 M n-butanol at 0.25 ml/min. Both isomers tail significantly, indicating that butanol is not a strong displacing molecule on Silicalite. Other



Figs. 20,21 Separation of 10 ppm *cis/trans* crotononitrile on 10 cm columns with acetonitrile at 1 ml/min as the eluent. **TOP:** silica-C18 with 25% acetonitrile. **BOTTOM:** Silicalite with 50% acetonitrile.

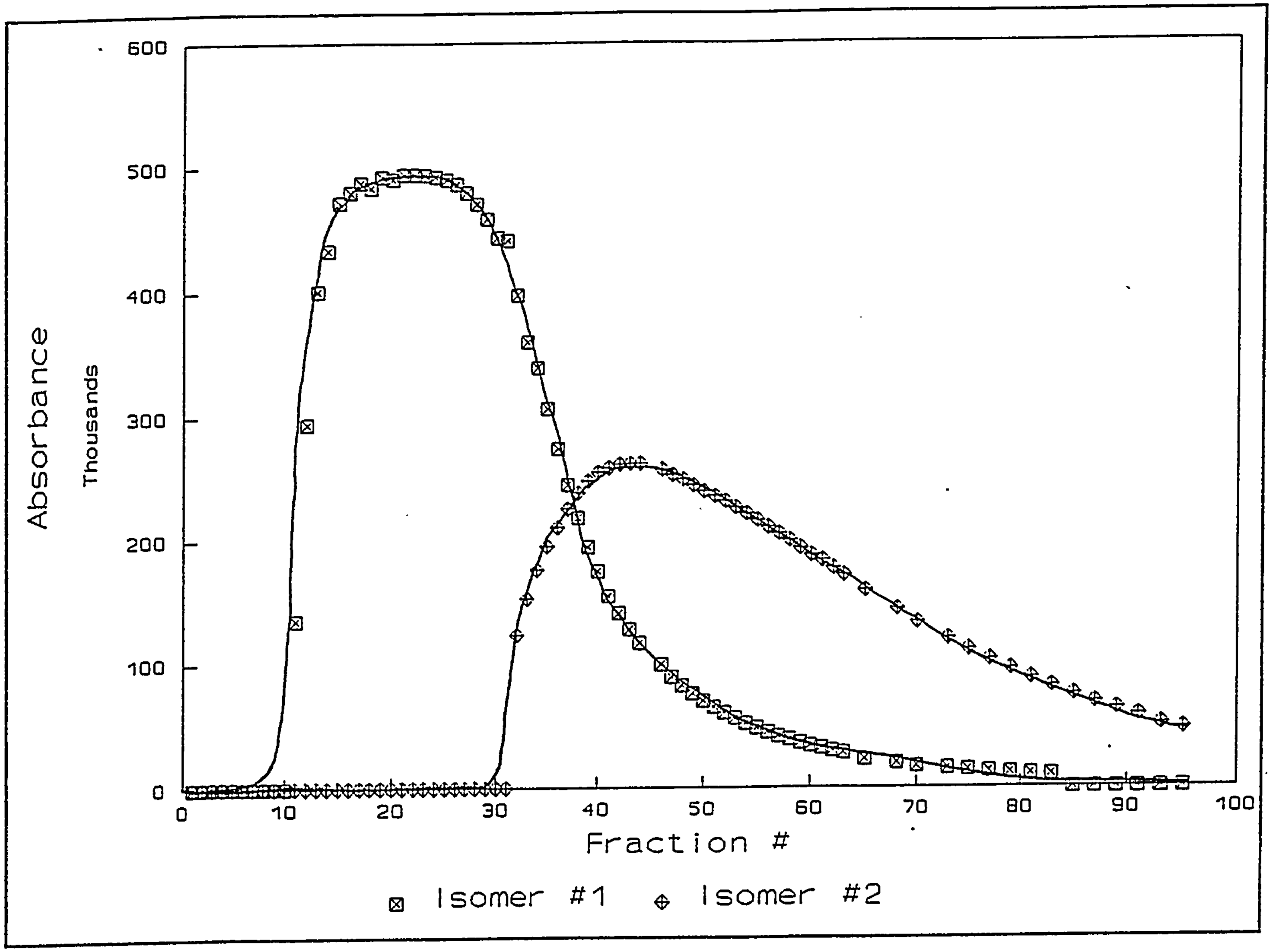


Fig. 22 Displacement chromatogram of 25 mg crotononitrile on a 10 cm Silicalite column. First fraction collected 75 minutes after elution.

alcohols, ketones, esters, and ethers were tested as displacing molecules. Several flow rates, columns, and concentrations were also tested. The optimum conditions were found to be a load solution of 30,000 ppm crotononitrile at 0.2 ml/min and an eluent of 2.5% ethyl propionate at 0.2 ml/min. The chromatogram obtained under these conditions is shown in Figure 23. The separation was much improved, although slight overlap still occurred as did tailing of the second peak. The composition of crotononitrile is approximately 70% *trans* / 30% *cis*, based on the peak area of an analytical separation. Injection of 50 mg is therefore about 35 mg *trans*. Based on the curve in Figure 23, 28-30 mg of pure *trans* crotononitrile could have been recovered. Considering the inability of silica-C18 to separate *cis/trans* mixtures, this larger-scale separation is quite good. This type of separation is one exciting possible application of a Silicalite stationary phase.

Conclusions

Silicalite has been shown to be a viable HPLC stationary phase. The size-exclusion effect provides another mechanism to provide increased selectivity. Molecules that are too large elute very quickly, while smaller molecules may partition into and out of the channels. Differences in selectivity and efficiency were observed between Silicalite and silica-C18 for many compounds. *Cis/trans* stereoisomers, which are difficult to separate on silica C18 were well separated on Silicalite in only a few minutes. A larger scale separation using displacement chromatography was possible with Silicalite. With this technique, milligram quantities of pure isomer may be collected.

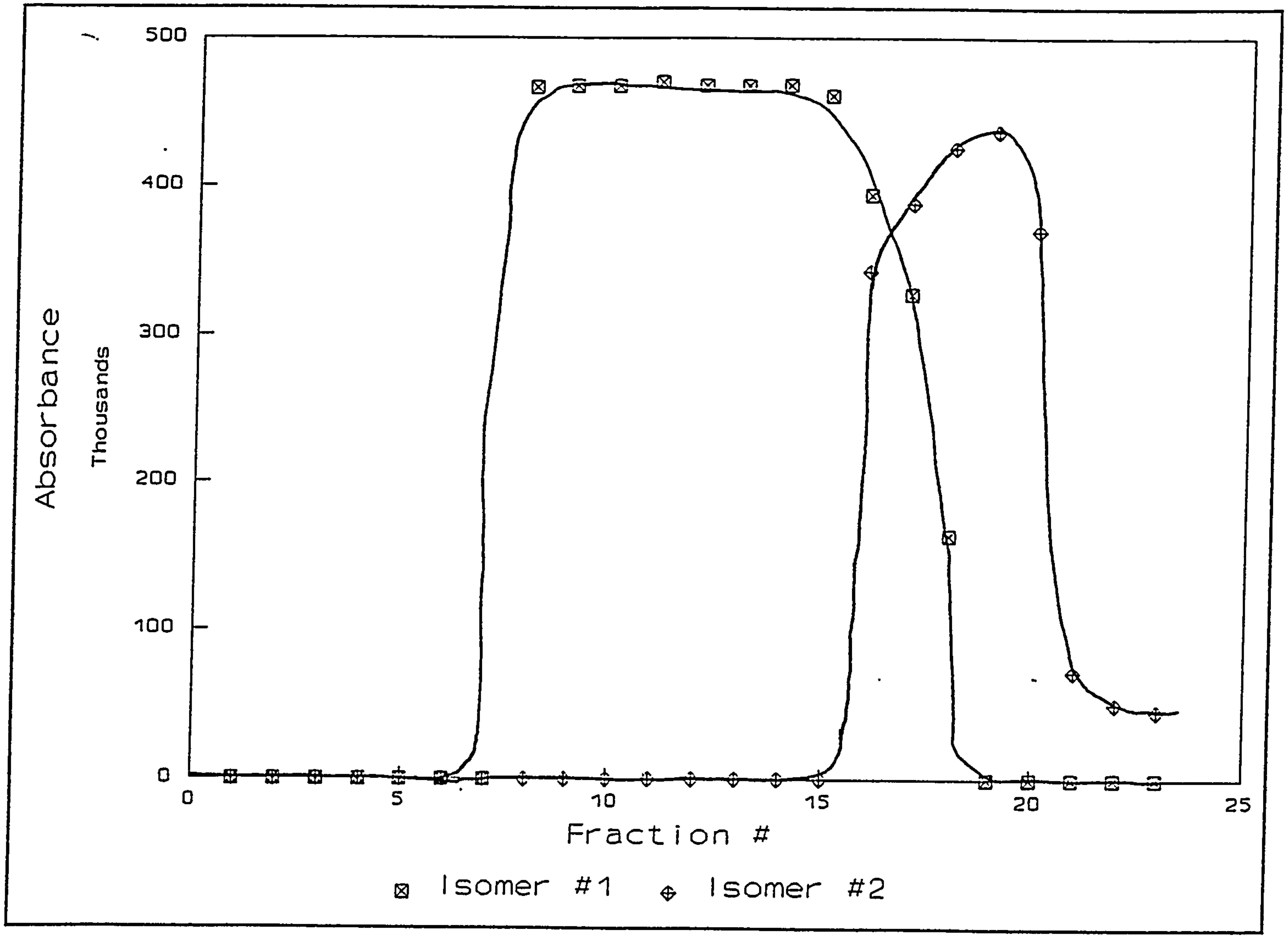


Fig. 23 Displacement chromatogram of 50 mg crotononitrile on a 15 cm Silicalite column. First fraction collected 75 minutes after start of elution.

Acknowledgements

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GENERAL CONCLUSION

Introduction of hydrophilic functional groups to PS-DVB resins increases the extraction efficiency of polar organic molecules from water. In this work, the effect of resin sulfonation has been determined. Sulfonated resins yield better recoveries of many compounds from aqueous solutions. Capacity factors of several organic compounds were measured in pure water, which permitted the optimum sulfonation capacity to be determined. A capacity of 0.6 mequiv/g was found to retain polar organic compounds most efficiently. Empore membranes embedded with sulfonated resin are simple to use and provide efficient extraction of many compounds. Breakthrough analysis was used to compare sulfonated and unsulfonated Empore membranes. Breakthrough on the sulfonated membrane was much sharper, indicating a more efficient extraction process.

Non-aqueous eluents used with macroporous cation-exchange resins permit several separations that are very difficult to obtain with aqueous eluents. Methanol was found to be the most favorable solvent due to the best combination of resolution and peak shape. Acetonitrile and ethanol, although producing broader peaks, were useful for separating ions that usually elute close together, Li^+/Na^+ and K^+/NH_4^+ respectively. Elution order in acetonitrile is reversed from that found with aqueous eluents: $Cs^+<Rb^+<K^+<Na^+<Li^+$. Addition of 18C6 to the mobile phase improved both peak shape resolution of several ions.

Ion-exchange of organic amines may be studied if organic eluents are used. These eluents will effectively remove all hydrophobic interaction. A plot of log k′ vs. log H^+ (from methanesulfonic acid) is linear with a slope close to -1 in each of the four solvents studied. This is an indication that the separation mechanism is pure ion-exchange and not based on hydrophobic attraction. The capacity factors of C_1-C_{10} alkylamine cations showed very little change with regard to the number of carbon atoms. However, amine cations such as aniline, N-methylaniline, and N,N-dimethylaniline, are easily separated. These results indicate that the size and shape of the cation, particularly near the nitrogen, affect the ion-exchange selectivity. Practical separations of amine cations were obtained in methanol and ethanol with indirect conductivity detection. Large concentrations of neutral organic compounds in the sample did not interfere with the chromatographic separation and determination of the amine cations.

Silicalite has been shown to be a viable HPLC stationary phase. The size-exclusion effect provides another mechanism that improves selectivity. Molecules that are too large to fit into the channels elute very quickly, while smaller molecules enter into the channels. Phenol positional isomers were separated on Silicalite. Improved resolution and changes in elution order were observed when compared to silica-C18. *Cis/trans* stereoisomers, which are difficult to separate on silica-C18 are efficiently separated on Silicalite. A larger-scale separation using displacement chromatography is possible on Silicalite. Using this technique milligram quantities of pure isomer could be collected.