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Method development for high performance liquid chromatography: novel organic modifiers and column packing conditions

Xue Li
Iowa State University

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Method development for high performance liquid chromatography: Novel organic modifiers and column packing conditions

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Xue Li

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This work is dedicated to my parents.

I will forever be indebted to them

for the sacrifices they made for me,

for their everlasting love and support,

and for the values they instilled in me.

TABLE OF CONTENTS

GENERAL INTRODUCTION

Dissertation Organization

This dissertation begins with a general introduction containing a literature review. References cited in the literature review are compiled in the reference section within the general introduction. This is then followed by four chapters all of which are in the format of research papers. They have been accepted, have been submitted or will soon be submitted for publication. A general conclusion section follows the four chapters. The second paper is similar to the publication 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.

High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) emerged in the late 1960's. This emergence was driven by the need for an instrumental technique that could separate water soluble, thermally labile, non-volatile compounds with speed, precision and high resolution, stimulated by the discovery of RNA and DNA [1]. Since then, HPLC has grown tremendously and has become probably the most widely used analytical technique in analytical, biochemical, pharmaceutical, medical and many other industrial laboratories. This popularity is due to the versatility, selectivity and sensitivity of HPLC as well as the necessity of a prior separation before almost any analytical determination. Unlike gas chromatography where the analytes must be volatile and the mobile phase can only be a few gases, HPLC is

applicable to almost any compound and a vast variety of mobile phases are available. Unlike capillary electrophoresis where the sample loading capacity is too small for many applications and the reproducibility is still questionable despite its increasing popularity, HPLC enjoys micro to preparative and production scale and it has become a very robust and rugged technique.

Today, HPLC is a general term that encompasses many different forms of chromatography. This includes adsorption [2], reversed-phase , normal-phase, ion-exchange, ion-pair [3-5], ion-exclusion [3-6], size-exclusion [7, 8], displacement [9], affinity [10, 11], immunoaffinity [12-15], thin-layer [16], chiral [17], liquid-liquid partition [18], hydrophobic interaction [19, 20], hydrophilic interaction [21-23] and multimode [24, 25] chromatography as well as several newly emerged modes such as perfusion [26-30], optical [31], temperature-responsive [32, 33], electrochemically modulated chromatography [34-44] and capillary electrochromatography [45, 46].

Many modes of detection are possible with HPLC. UV-Vis absorbance is routinely used for the detection of compounds with UV chromophore. Refractive index detection is a near-universal detector but, like UV-Vis, does not provide very sensitive detection. However, laser-based light scattering mass detector overcomes the problems with refractive index detectors [47]. Excellent progress has been made in the area of specific detectors. Chemiluminescence [48, 49], fluorescence, indirect florescence and laser induced fluorescence [50-53], radioactivity [54], and electrochemical [55, 56] detectors have all been developed.

Coupling HPLC to other instruments has increased the power of HPLC greatly. For example, HPLC coupled with mass spectrometers [30, 57-59], FTIR instruments [60, 61], diode array detectors [62, 63] or NMR instruments [64, 65] provides more information and allows the determination of unknown analytes. The coupling of HPLC with other separation methods such as LC-GC, LC-CE, and the coupling between different retention mechanisms of HPLC have provided powerful separation tools for chromatographers when dealing with complex matrices that require high resolution power. This is so-called multidimensional chromatography [66-69].

The applications of HPLC are simply too many to cover inclusively in this brief literature review. A quick survey of the 1995 Application Reviews issue of *Analytical Chemistry* reveals that HPLC is employed in almost every area covered in this issue including pesticides, coatings, process analytical chemistry, geological and inorganic materials, synthetic polymers and rubbers, food, peptides and proteins, air pollution, environmental analysis, forensic science, pharmaceuticals and drugs, petroleum and coal, industrial hygiene, water analysis and every aspect of clinical chemistry [70]. Indeed, it is amazing just to think about the popularity of HPLC from major components to trace analysis, from food, soil and water to poison, drug and trace evidence, from organic to inorganic analysis, from natural to synthetic products, from peptides and proteins to RNA and DNA, from industry to clinics, from separation and purification to structure elucidation. The future of HPLC seems to be even brighter with gas chromatography, supercritical fluid chromatography, field flow fractionation and capillary electrophoresis to complement it.

Although HPLC has become a well established technique, advances are still being made in the fundamental understanding of various separation mechanisms as well as improvements in equipment capabilities.

In this dissertation, HPLC methods were developed for the separations of various organic compounds, including acids, bases and neutral organic compounds on silica-based as well as polymer-based columns. Several types of mobile phase additives were explored. An improved method to pack HPLC columns is proposed.

Mobile Phase Modifiers for HPLC Separations

Retention behavior in liquid chromatography is a function of the strength of the solute-mobile phase and solute-stationary phase interactions. In LC, it is the mobile phase that enjoys a lot more variety than the stationary phase. Once a stationary phase is chosen, selectivity is adjusted by modifying the mobile phase.

The solvent properties of the greatest chromatographic interest are solvent strength and selectivity. Strength, in the sense of chromatography, is synonymous with its polarity. For the purpose of adjusting the solvent strength of normal phase chromatography, a polar solvent (methanol, acetonitrile, tetrahydrofuran, etc) is incorporated into a non-polar solvent (hexane, benzene, etc). To adjust the solvent strength of reverse phase chromatography, a non-polar solvent (methanol, acetonitrile, tetrahydrofuran, etc) is incorporated into a polar solvent (water). The solvent strength is controlled by the percentage of the modifying solvent. Selectivity has been described as "the ability of a given solvent to selectively dissolve one

compound as opposed to another, where the polarities of the two compounds are not obviously different" [71]. Snyder [71, 72] has described a useful scheme for classifying common solvents according to their polarity or chromatographic strength and their selectivity based on the principal intermolecular forces responsible for their properties. A selectivity triangle for solvents was constructed and offered a structured methodology for solvent selection.

Most frequently, mixtures of solvents instead of a single pure solvent are used as the mobile phase. Binary solvent mixtures provide a simple way to control solvent strength but are limited in controlling selectivity. Choosing a solvent of similar strength but with different selectivity can improve peak resolution. Use of ternary or quaternary solvent mixtures often allows fine tuning of solvent selectivity while maintaining a constant solvent strength [73-75]. According to Lehrer, the use of four solvents is required to accomplish optimization of resolution in HPLC with one of the four being the strength adjusting solvent (water for RPLC and n-hexane for NPLC) and the rest being three selectivity adjusting solvents, each of which comes from one apex of the Snyder selectivity triangle (methanol, THF and acetonitrile for RPLC, and ether, methylene chloride and chloroform for NPLC) [76].

Influence of the nature of organic modifiers in mobile phase of non-aqueous RPLC was studied by Heron and Tchapla following the common provision of solvophobic and partition theories [77]. It was found that for the mixtures of acetonitrile or nitromethane in methanol, the retention of solutes vs. the percentage of organic modifier can be correlated with the variation of mobile phase surface tension vs. the percentage of organic modifier in

non-aqueous RPLC. This is not true when THF, acetone, methylenechloride or chloroform was used as the modifier. In this case, it is the dielectric constant of the mobile phase that affects the retention. Solvents of the latter type result in a conformational modification of the alkyl chain of the silica C-18 stationary phase.

Besides using mixed solvents as the mobile phases, mobile phase additives with different functionalities have also been investigated. Various inorganic salts such as KCl, $CaCl_2$ and $ZnCl_2$ have been used as mobile phase modifiers for the separation and stability indicating assay of atenolol and the presence of $CaCl_2$ and $ZnCl_2$ was found to enhance the UV response of atenolol [78]. In another application, NaCl was added to a mobile phase of acetonitrile/water to adjust the selectivity of mono- and disaccharides. The presence of NaCl in the mobile phase allows the resolution of sugars from each other and the separation of sugars from the chloride interference peaks in food matrixes [79].

Diethylamine was used in acetonitrile/water or ethanol/hexane eluent and allowed the resolution of the four stereoisomers of nadolol using the same chiral column by RPLC and NPLC respectively [80].

0.1% trifluoroacetic acid added into 40% acetonitrile/water allows the successful diastereomeric separation of synthetic neuropeptides which are not separable without the presence of TFA [81].

The separation of several closely related nerve agent degradation products, which are organic acids with extremely similar ion exchange capacity was not possible with typical eluents used for anion exchange chromatography (NaOH/water). Addition of acetonitrile to

the mobile phase provided additional selectivity and allowed successful separation of them. The presence of the acetonitrile results in mixed ion exchange and reverse phase separation mechanism. This is due to the ability of acetonitrile to swell the stationary phase particles, which reduces the ion-exchange sites but exposes more hydrophobic surface leading to a increased reverse phase effect. Competition of the two mechanisms maximizes the resolution when the solvent strength is just low enough for the analytes to remain on the column long enough to be separated [82].

A neutral, coordinatively-unsaturated metal complex was introduced as a mobile phase modifier for HPLC of amino alcohols [83]. Interaction with the metal complex and the subsequent increase in solute retention was shown to be dependent on steric, dipole and solvation effects.

Different carboxylic acids were tested by Naleway and Hoffman [84] as mobile phase modifiers. The effect of acid chain length, pH, and eluent composition on retention was studied. The retention of both neutral and positively charged compounds was influenced by the dissociation equilibrium of the carboxylic acid in the mobile phase. The inflection in the retention of neutral and charged solutes as pH was changed occurred at the pK_a of the acid in the mobile phase.

Heldin *et al.* [84] studied the separation of enantiomers of acids, esters, and amino alcohols of moderate hydrophobicity using (2R,3R)-dicyclohexyl tartrate (DCHT) in phosphate buffer as a mobile phase and porous graphitic carbon as a stationary phase. DCHT was found to have a high affinity for the graphitic carbon phase with this mobile phase. It

seemed to be adsorbed on the support as a monolayer. Changes in the concentration of the mobile phase had only a minor influence on the amount adsorbed and addition of acetonitrile to the mobile phase decreased the amount of DCHT adsorbed on the carbon support.

Short- and long-chain alcohols are commonly used as mobile phase modifiers. They were used in nonpolar mobile phases, such as hexane or heptane, for the chromatographic separation of several bi- and trifunctional solutes [86] and fat-soluble vitamins in vegetable oil compounds [87].

The diastereomeric and enantiomeric separation of cyclopent-2-ene-1,4-diol and bicyclo[3.1.0]hexane-1,4-diol derivatives was carried out by HPLC on chiral stationary phase [88]. The separation was influenced greatly by the kind of alcohol modifiers in the mobile phase. The retention times and separation factor increase on changing 2-propanol to ethanol and to a mixture of methanol and ethanol. This was attributed to the polarity difference between methanol, ethanol and propanol.

Michels and Dorsey [89] measured the retention behavior of a variety of solutes using a homologous series of normal alcohols as organic modifiers in hydro-organic mobile phases. The results imply that a systematic change in the extent of solvation of the stationary phase occurred with respect to the size of the organic modifier.

Lau and Simpson [90] proposed a way to control variation in separation characteristics of different columns. The effect of chain length of a homologous series of alcohol modifiers in different solvent mixtures of methanol/water was studied on commercially available columns. Columns could be modified to present the same surface area/volume for interaction

with solute molecules and hence to obtain equivalent separations.

Morris and Fritz [91] found that small straight-chain alcohols added to aqueous solvent had a dramatic effect on the chromatographic behavior of small polar compounds such as alkane carboxylic acids. The alcohols were believed to coat the surface of the polymeric resin due to a dynamic equilibrium in which the alcohol additives were distributed between the mobile and stationary phases.

Study by Scott and Simpson [92] showed that short-chain aliphatic modifiers such as alcohols, carboxylic acids and aldehydes can be adsorbed onto the surface of ODS reverse phase from aqueous solution and their adsorption follows Langmuir-type adsorption isotherm. This coating modifies the properties of the C-18 material and reduces the hydrophobic attraction of the acid analytes for the C-18 silica.

By spectroscopic method, Montgomery and Wirth [93, 94] found that for chromatographic surfaces in contact with water, C-18 chains lie flat on the surface when the mobile phase is water. At low concentrations, short-chain alcohols primarily wet the interface, and at high concentrations, methanol and 1-propanol solvate the C-18 chains. Long-chain alcohols can wet the interface as well as short-chain alcohols, but only a minute amount of long-chain alcohols in the mobile phase are required. The wetting of the interface is accomplished by adsorbing of the alcohols in a sub-monolayer through interpenetrating with the chains of the C-18 chains on the stationary phase with -OH groups sticking out, which makes the surface more hydrophilic.

Kirkland [95] used aprotic modifiers such as acetonitrile, methyl-*tertyl*-butyl ether

(MTBE), THF, methylene chloride and ethyl acetate to replace normally used alcohol modifiers in non-polar solvent for the optimization of the selectivity and resolution of racemic drugs. Superior separations were obtained on derivatized cellulose carbamate HPLC columns. It was proposed that the improvement in enantiomer resolution when using aprotic solvents largely occurs when hydrogen bonding is the major source of chiral interaction.

Cyclodextrins and their derivatives have become the most popular mobile phase modifiers in HPLC separations of optical, geometrical and structural isomers [96-100]. The formation of inclusion complexes driven by several intermolecular interactions between cyclodextrins and analyte molecules results in the selectivity in separations. The fixed cavity sizes of cyclodextrins govern the mechanism of solubilization/retention of the analyte by forming species of defined stoichiometry.

Warner *et al.* demonstrated that ß-cyclodextrin (CD) can be used as mobile phase modifiers for the separation of polycyclic aromatic hydrocarbons [101-103]. Alcohols added to the mobile phase promoted the formation of cyclodextrin inclusion complexes with the polynuclear species by forming ternary complexes. The same authors also measured the formation constants for ß-CD complexes of anthracene and pyrene using RPLC [104]. They found that pyrene complexation was not observed until tertyl-butyl alcohol or cyclopentanol was added to the mobile phase, and that the analyte:CD stoichiometry was 1:1 and 1:2 for anthracene and pyrene, respectively.

Many chiral separations were performed using mobile phases containing alcohols as the additives [105-108]. In most cases, it was found that the steric structure of the alcohol

modifier had an effect on the capacity factor and stereoselectivity. Results indicated that the alcohols would compete with the solutes for chiral and achiral binding sites and that the steric bulk around the hydroxyl moiety of the modifiers played a role in this competition. In a study by Enquist and Hermansson [109], the adsorption of propanol and acetonitrile on the chiral column was measured and monolayers were found at modifier concentrations of 10% and 15% respectively.

A mathematical model was developed by Armstrong *et al.* [110] using basic equilibrium-driven approach to explain the effect of organic modifier and pH on the retention and selectivity in RPLC separation of alkaloids on CD-bonded phases. It was proposed that organic modifiers such as acetonitrile and methanol compete with the solutes in binding with the adsorption sites on the stationary phase.

Macrocyclic antibiotics are among the newest chiral selectors used for the enantioseparations in LC and CE, as mobile phase additives as well as chiral selectors bonded to the stationary phase [111, 112]. Typical examples are rifamycin B, vancomycin, ristocetin A, teicoplanin and thiostreptin. They are enantioselective via several different mechanisms including π-π complexation, hydrogen bonding, inclusion in a hydrophobic pocket, dipole stacking, steric interactions, or combinations thereof. They have been utilized successfully in hundreds of enantiomeric separations.

Surfactants are another type of important mobile phase modifiers used in HPLC separations. They have been used for ion-pair chromatography (IPC) and micellar liquid chromatography (MLC). In ion-pair chromatography [113-116], surfactants are used as mobile

phase modifiers at concentrations below their critical micelle concentrations. They are adsorbed onto the stationary phase from the mobile phase and act as dynamic ion exchangers for ionic analyte species. Charged analytes such as rare earth metals [114] and inorganic anions [115, 116] can be retained and separated. IPC greatly extended the capabilities of HPLC for the separations of charged species despite its several shortcomings [113].

In micellar liquid chromatography, surfactants are used in aqueous solvent at concentrations above their critical micelle concentrations [117-120]. Some of the surfactants are adsorbed onto the stationary phase with the majority forming micelles in the mobile phase. The retention of analytes is determined by their partition between the micelle pseudophase, the bulk mobile phase and the stationary phase. Solvent strength and selectivity can be controlled by the choice of surfactant (head group type and/or hydrophobic chain length), the concentration of surfactant [121-124] and pH of the eluent [125, 126]. Selectivity in MLC not only depends on the eluent composition but also the solute type and its location on/in the micelles [127]. MLC has been used for the quantitative structure-activity relationship study [128, 129], in direct body fluid injection for therapeutic drug monitoring [130-134], for the analysis of nucleotides and bases [135] and for the quantitative determination of bilirubin species [136], in protein separations [137], in the determination of inorganic cations and anions [138, 139], and for isomer separations [140].

Despite of the merits of MLC, its applications seem to be hindered by its inferior efficiency to conventional hydro-organic HPLC [141, 142]. Various approaches have been attempted to overcome this. The most common and effective one has been using small

percentage of organic solvent such as a short-chain alcohol as the eluent modifier and 3% 1-propanol is the best so far [143] although the breakdown of micelles limits the amount of alcohol which can be incorporated into the mobile phase [144-146]. It is believed that the presence of this small amount of alcohol in the mobile phase facilitates the mass transfer of the solute between the micelle, bulk eluent and stationary phase. Besides, the alcohol modifier also provides greater selectivity since it has a greater effect on the retention of the hydrophobic solutes than the hydrophilic ones. A model describing the effect on retention upon the addition of alcohols to the mobile phase of MLC has recently proposed by Jemenez *et al.*[147]. In a study by Garcia and coworkers, alcohols (methanol, n-propanol and n-butanol) and NaCl were used as mobile phase modifiers for the separation of benzene and naphthalene derivatives by MLC [148]. The dependence of selectivity on the nature and concentration of surfactants in the mobile phase, on the nature of the additives (alcohols and salt) and on the percentage of the alcohols was studied. Optimized selectivity was found to correspond to the use of SDS at low concentration and the addition of an alcohol at medium chain length.

Chapter I of this dissertation discusses the use of straight-chain alcohols, diols and amino alcohols as mobile phase modifiers for HPLC separation of carboxylic acids and organic bases. The analytical columns are silica C-18, or polystyrene/divinylbenzene with or without sulfonate groups. The improvement in separations as a result of the use of additives are shown and the mechanisms are proposed.

Chapters II and III deal with the separations of various organic compounds using

single and mixed surfactants as the mobile phase modifiers on silica C-18 columns. The systems attempted are of a bridge between conventional and micelle LC in that surfactants are used as the mobile phase modifiers but micelle formation is not the prerequisite of the improved separations obtained. The tremendous improvement in the separations of many compounds is shown and the probable separation mechanism is studied.

HPLC Column Packing Conditions

An efficiently packed chromatographic column is critical in order to obtain superior separations. However, column packing and evaluation has been somewhat neglected [149]. Most reports on HPLC employ commercial columns which are much more expensive and less flexible than purchasing packing materials and packing columns on-site. Only those who have to pack their own columns have done the column packing themselves, such as those people working on column packing techniques, experimenting with new phases or working with packed capillary and open tubular columns where no commercial resources are dependably available in market.

There are generally three types of column packing methods: dry packing [150-152], high pressure slurry packing [150-153] and compression slurry packing [154, 155]. In dry packing, the dry packing material is slowly poured into the column and the packing bed is formed under the action of vibration, rotation and/or tamping. Dry packing is suitable for large particles with particle diameter (d_p) > 20 μm. For particles with d_p < 20 μm, this technique is poor due to the fact that the mechanical action applied in packing can not

disperse the particles well to prevent the agglomeration of the small particles. Even for large particles, the column efficiency and its reproducibility often are not very good [156].

Compression slurry packing is another way of packing HPLC columns and it can be axial or radial compression or both. Radial compression is accomplished by applying gas or liquid pressure on a flexible-walled cartridge which is filled with packing materials [157]. Axial compression is accomplished by moving a piston in the column, which is filled with slurry, to force the solvent out of the end frits and make a filtration bed in the column [155]. This technique fits the packing of large columns because of the low packing pressure required and excellent column efficiency obtained. Annular expansion, *i.e.*, compression in both axial and radial directions, is available by insertion of a plunger in the center of a column. The major drawback of the compression slurry packing is the requirement of special equipment, which can be expensive.

The first high pressure slurry packing method was reported by Kirkland [150]. In this technique, the suspension of packing materials is stored in a reservoir which is connected to the column. A packing solvent is then introduced under high pressure and at high flow-rate into the reservoir to push the slurry into the column to form the resin bed by filtration. This packing method is considered to be the most successful and most popular method for particles with $d_p < 20$ μm and columns with 20 mm I.D. or smaller. The packing of large scale columns or with particles of 1 μm or smaller would impose extreme technical requirements for the instrumentation.

Compared to dry packing methods, slurry packing results in more favorable packing

conditions. This is because the attraction between the particles is drastically reduced as a result of the dispersion of the stationary phase particles in a solvent [158, 159].

It used to be important to choose slurry solvents with similar density as the particles when the particle sizes of commonly used packing materials were large, *i.e.*, balanced-density slurry packing. For particle sizes smaller than 5 μm, which are a commonplace in today's practice, solvent density is not critical as before. However the selection of solvent is still very important since the small particles of the packing materials tend to form aggregates. The co-existence of aggregates and discrete particles is analogous to using a packing with a wide particle size range. Packing agglomeration causes nonuniform compaction during the packing process resulting in a wide variation of flow velocity through the column and thus very poor column efficiency [151, 160, 161]. So, it is important to have a solvent which can disperse the particles well and prevent the formation of aggregates. For this purpose, dilute ammonium hydroxide solution was used to slurry silica microspheres successfully by Kirkland [162]. Chemically, the microspheres became all negatively charged in the presence of base, resulting in repulsion of the particles and stabilization of the slurry with minimal aggregation. Organic acids such as n-heptanoic and dichloroacetic acid have also been used to slurry silica-based HPLC packing materials [163]. This approach minimized the particle agglomeration by decreasing the amount of dissociated silanols. Sedimentation packing technique was recently introduced by Wang, Hartwick, Miller and Shelly [164]. With this approach, a slurry consisting of the packing and a deflocculating solvent, acetone, was poured into the column and the bed was formed by sedimentation. Then, the bed was solidified by a flocculating

solvent, methanol/water (1:1). The function of the deflocculating solvent is to disperse the particles and prevent the agglomeration of them and the function of the flocculating solvent is to solidify the packing bed. Vissers, *et al.* [165] reported that the performance of slurry-packed microcolumns was predominantly determined by the selection of the packing solvent, which preferably has to be coagulating solvent, and the selection of the slurry solvent was of minor importance.

Packing pressure is another important parameter. Hu *et al* [166] studied the effect of packing technique in slurry packing on column efficiency under various packing pressure and pressurizing conditions with CCl_4 as the slurry medium. In the case of packing 10 cm x 4.6 mm with 5 μm microparticular Si, 220 kg/cm^2 packing pressure gave similar effect to that with 350 kg/cm^2. One-step (sudden) pressuring technique seems to be preferable to the gradual pressuring one. Using gigaporous polymeric packings (4000 Å mean pore size), Freitag, Frey and Horvath [167] studied the behavior of slurry-packed columns in protein separations. They found that the conditions employed during the packing process have dramatic effect on the properties of such columns and this can be attributed in part to the deformability of the particles. An increase in packing pressure up to 6000 psi resulted in a high mass-transfer efficiency and lower column permeability due to the decrease in interstitial porosity. Further increase in the packing pressure beyond 6000 psi will cause lower column efficiency due to the formation of a low-porosity layer of highly compressed particles at the downstream end of the column during high pressure packing losing the packing uniformity along the column axis.

Slurry packing technique cannot be freely applied to pack large scale columns or

columns with small particles since the back pressure will become dangerously high as the column length increases or the particle sizes decreases. Recently, electrokinetic packing was invented by Yan [168] to complement with the existing packing techniques. Columns are packed using electroosmotic flow induced by an electrical double layer on the internal wall of a fused silica capillary and the electrophoretic mobility of positively- or negatively-charged particles of the packing materials. This method is particularly applicable for particles sizes smaller than 1 μm and column internal diameter between 10 and 500 μm and several columns can be packed at the same time. Columns packed using this method are useful for the separations by micro-HPLC and electrochromatography.

Chapters IV of this dissertation is a study of the slurry packing method. In this study, effect of column packing conditions such as packing pressure, and slurry/packing solvent on the performance of the resulting column was evaluated using macroporous polymer resin and silica C-18 as the packing materials. A practical method to generate high efficiency HPLC columns is proposed.

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28

ORGANIC MODIFIERS FOR THE SEPARATIONS OF ORGANIC ACIDS AND BASES BY HPLC

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

Xue Li and James S. Fritz
Ames Laboratory, U.S. Department of Energy and
Department of Chemistry, Iowa State University
Ames, Iowa 50011, U.S.A.

ABSTRACT

Neutral eluents were proved to be applicable for the separations of organic acids and bases on reversed phase high performance liquid chromatographic columns when a straight chain alcohol, diol or amino alcohol is used as the organic modifiers in the mobile phase. Upon incorporating 2% 1-butanol, 1% 1,2-hexanediol, or 0.25% 1,2-octanediol into the aqueous eluent, the separation of alkane carboxylic acids on a silica C-18 column was improved greatly in terms of both separation time and peak shape. When the same silica C-18 column was pre-coated with 1,2-decanediol, good separations of these acids were obtained with either water alone or 1% 1-butanol as the eluent. When 1.5% 1-hexanol, 0.09% 1-

decanol or 0.01% 1-dodecanol was added into 30% acetonitrile/water, much sharper peaks and shorter retention times resulted for a group of seven to eight aromatic bases on an underivatized polystyrene/divinylbenzene column. Columns packed with lightly sulfonated polystyrene/divinylbenzene pre-treated with amino alcohols such as ethanolamine, 3-amino-1-propanol or 4-amino-1-butanol allows much improved separations of alkane carboxylic acids when water or methanol/water mixture was used as the eluent. The retention times of analytes on the treated column were affected by the ion exchange capacity of the stationary phase and the hydrocarbon-chain length of amino alcohol used to treat the column. The effect of these alcohols, diols and amino alcohols on the separations was believed to be that these molecules coat onto the surface of the stationary phase by hydrophobic interactions or electrostatic attractions resulting in a more hydrophilic surface of the stationary phase and hence less hydrophobic interactions between analytes and stationary phase, and reduced retention times and improved separations.

INTRODUCTION

Ion exclusion chromatography is often used for the separation of hydrophilic molecules such as weak acids or weak bases. Such separations are usually performed on gel type ion exchange columns of fairly large dimensions with primarily aqueous solution as the eluent [1, 2]. The separation mechanism was long considered to be the partitioning of the solute acids or bases between the eluent and the water inside the resin gel. Hydrophobic interactions between the analyte molecules and polymeric resin matrix play a role in these separations [3-

5]. Separations of weak acids or bases generally require eluents containing dilute acid or base to suppress the ionization of the analytes in order to obtain sharp peaks. However, Tanaka *et al.* were able to obtain good separations of alkane carboxylic acids using eluents containing no acid but only a neutral sugar-alcohol additive [6]. Separations of carboxylic acids and small polar compounds have been reported by Morris and Fritz [7] on smaller, lightly sulfonated non-gel polymeric resins. The same authors have also found that small straight-chain alcohols added to aqueous solvent had a dramatic effect on the chromatographic behavior of small polar compounds such as alkane carboxylic acids. The alcohols were believed to coat the surface of the polymeric resin due to a dynamic equilibrium in which the alcohol additives were distributed between the mobile and stationary phases [8].

The present work was performed in parallel with and in continuation of the work by Morris and Fritz. Here, typical reversed phase HPLC columns, such as silica C18 and underivatized polystyrene/divinylbenzene columns were used for the separation of carboxylic acids and organic bases, respectively. Low percentage of 1-butanol or 1,2-diol present in aqueous eluent allows dramatic reduction in retention time of carboxylic acids and excellent separations of these compounds on a silica C-18 column. The same column pre-coated with 1,2-decanediol permits much improved separation of theses acids when water alone or 1% butanol in water was used as the eluent. Separation of organic bases on underivatized polystyrene/divinylbenzene resin column was also improved greatly when a straight-chain alcohol, such as 1-hexanol and 1-dodecanol, was incorporated in eluent containing 30% acetonitrile.

Coating of organic modifiers onto the stationary phase can be accomplished not only by hydrophobic interactions but also electrostatic attractions. This idea was explored in the final part of this paper. Good separations of alkane carboxylic acids were obtained using a lightly sulfonated polystyrene/divinylbenzene column coated with amino alcohols. The coating was accomplished by running acidified amino alcohol solution through columns packed with lightly sulfonated polymeric resin.

EXPERIMENTAL

Chromatographic System

The chromatographic system consists of several components, including a LKB 2156 solvent conditioner (LKB, Bromma, Sweden). A Dionex DXP pump (Dionex, Sunnydale, CA, U.S.A.) was used to deliver a flow of 1 ml/min. A 7010 Rheodyne injector (Rheodyne, Berkeley, CA, U.S.A.) delivered 10 μl sample. The bases were detected by a Kratos Spectra flow 783 UV absorbance detector (Kratos Analytical Instrument, Ramsey, NJ, U.S.A.), and the carboxylic acids were detected by a Dionex CDM-3 conductivity detector (Dionex, Sunnydale, CA, U.S.A.). Separations were recorded by a Servogor 120 chart recorder (Abb Goerz Instrument, Vienna, Austria). The column used for the separation of the bases was prepared in our lab using 5-μm underivatized macroporous PS/DVB resin (Sarasep Inc., Santa Clara, CA). The same resin was functionalized with a sulfonic acid group at various exchange capacities and used for the separation of carboxylic acids using amino alcohol as the modifier. A Supelcosil LC-18 column (150 mm x 4.6 mm I.D.) (Supelco, Inc., Bellefonte, PA, U.S.A.) was used for the separation of carboxylic acids using straight-chain alcohols and diols as the

modifiers. A Shandon HPLC packing pump (Shandon Southern, Sewickley, PA) was used for column packing.

Reagents and Chemicals

Reagents used for the sulfonation reactions were of reagent grade, and methanol and acetonitrile were of HPLC grade. They were used as obtained from Fisher (Fisher Scientific, Pittsburgh, PA, U.S.A.) and Allied Chemical (Allied Corporation, Morristown, NJ, U.S.A.). The mobile phase additives and analyte chemicals were of reagent grade and were all used as obtained from Aldrich (Aldrich Chemical Company, Inc., Milwaukee, WI, U.S.A.). All eluents were prepared daily. Stock solutions were used to prepare all sample solutions by diluting with mobile phase. A Barnstead Nanopure II system (Sybron Barnstead, Boston, MA, U.S.A.) was used to further deionize distilled water for all eluents and sample mixtures.

Chromatographic Procedure

A flow rate of 1 ml/min was selected for all the chromatographic separations. The separation column was equilibrated with mobile phase until the baseline was stabilized. Sample injections were made at this point. The eluted bases were detected by a UV-vis detector at 262 nm with an output range of 0.010 AUFS, and the eluted acids were detected by a conductivity detector with the output range of 3 μS, 10 μS or 30 μS.

Capacity factor, k', was calculated according to expression: $k' = (t_r - t_o)/t_o$. The system dead time, t_o, used to calculate capacity factor k', was measured by injecting nitrate solution into the system. An average of at least three replicates was used to do all the calculations.

RESULTS AND DISCUSSIONS

Separations of Carboxylic Acids on a Silica C-18 Column

Fig. 1 is a separation of five carboxylic acids, formic through valeric acid, on a silica C-18 column with water alone as the eluent. Because of the strong hydrophobic interactions between butyric acid, valeric acid and the surface of the C-18 stationary phase, these two compounds were retained strongly on the stationary phase so that butyric acid had a long retention time, and valeric acid failed to elute. When 2% butanol was added to the otherwise aqueous eluent, the retention times of the analytes were all reduced but to different degrees and excellent separations of these acids within 6 minutes resulted (Fig. 2). To study the effect of butanol concentration on the retention times of these acids, capacity factors of the analytes were plotted against the concentration of butanol in the eluent (Fig. 3). The presence of butanol in the eluent reduces the retention of the analytes dramatically. However, this effect is much more dramatic when the concentration of butanol in eluent is below 1.5%. When further increasing the concentration of butanol above 1.5%, the reduction in the retention times was not as much. This effect cannot be explained by increased solvating ability of the eluent because even at 2% butanol, there still is 98% water in the eluent. Moreover, if it were because of the solvating ability change, the further increase in the concentration of butanol beyond 1.5% would result in at least as much effect as when it is below 1.5%. On the other hand, the effect of butanol on the retention of the carboxylic acids can easily be explained by considering the physical adsorption of butanol onto the surface of the stationary phase. A study by Scott and Simpson [9] showed that short-chain aliphatic moderators such as alcohols,

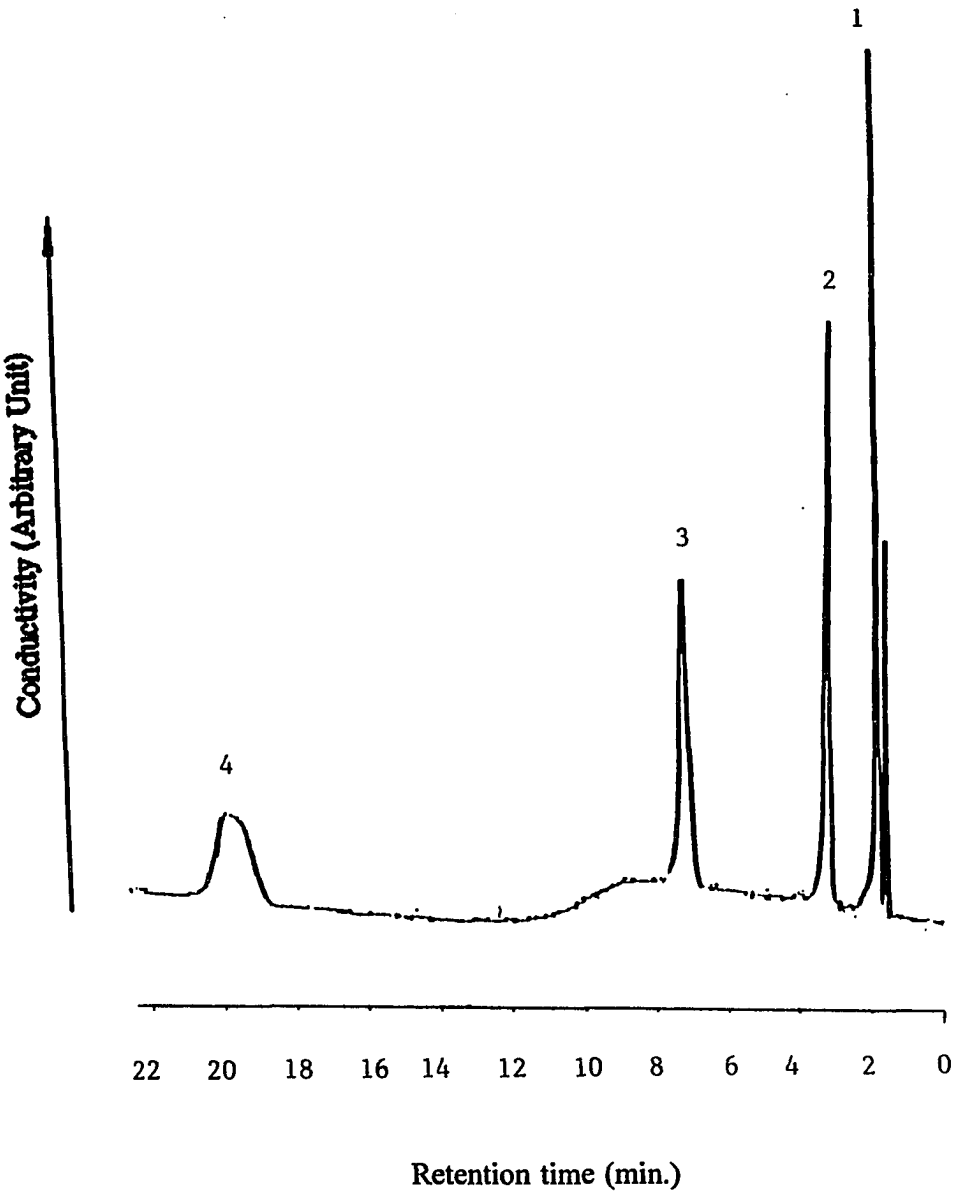


Fig. 1. Chromatographic separation on Supelcosil LC-18 (150 mm x 4.6 mm I. D.) column. Eluent: water. Flow rate: 1 ml/min. Detection: conductivity. Peak identification: 1 = formic acid, 2 = acetic acid, 3 = propionic acid, 4 = butyric acid. Valeric acid failed to elute.

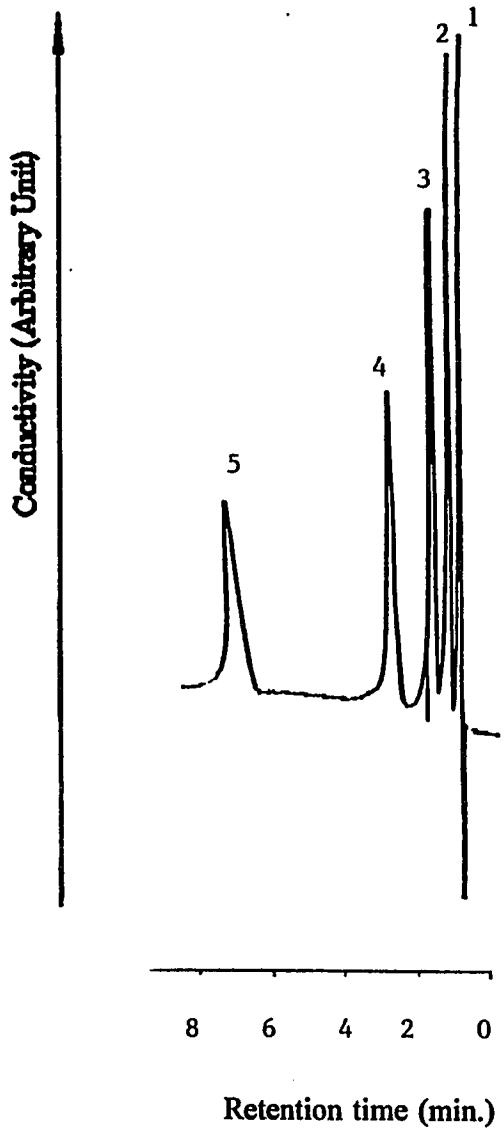


Fig. 2. Chromatographic separation on Supelcosil LC-18 (150 mm x 4.6 mm I. D.) column. Eluent: 2% 1-butanol/water. Flow rate: 1 ml/min. Detection: conductivity. Peak identification: 1 = formic acid, 2 = acetic acid, 3 = propionic acid, 4 = butyric acid, 5 = valeric acid.

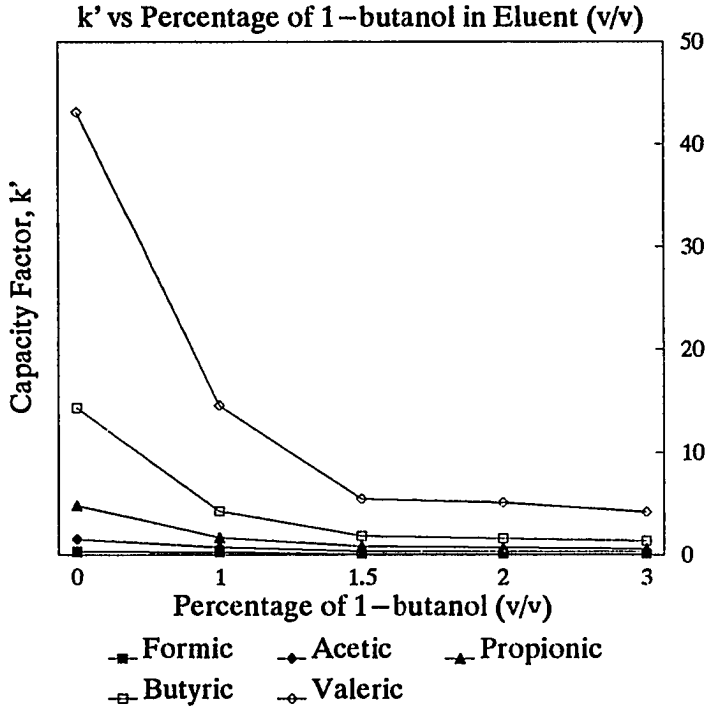


Fig. 3. Effect of 1-butanol on the retention of carboxylic acids. Experimental conditions are the same as Fig. 1.

carboxylic acids and aldehydes can be adsorbed onto the surface of ODS reverse phase from aqueous solution. Their adsorption follows a Langmuir-type adsorption isotherm. The coverage of the ODS surface by alcohol monolayer increases with the concentration of alcohol in the solution phase rapidly until the concentration reaches about 1% for butanol and 2-3% for propanol. Then, the increase in percent coverage slows down and finally reaches a plateau. When butanol concentration reaches *ca.* 1.5%, about 98% of the ODS surface is covered by butanol. This coating modifies the properties of the C-18 material and reduces the hydrophobic attraction of the acid analytes for the C-18 silica. Our experiments seem to be reminiscent of the adsorption isotherm of 1- butanol on ODS surface reported by Scott and Simpson. Other authors have also studied the adsorption of straight-chain alcohols on the surface of silica C-18. By spectroscopic method, Montgomery and Wirth [10] found that for chromatographic surfaces in contact with water, long-chain alcohols can wet the interface as well as short-chain alcohols, but only a minute amount of long-chain alcohols in the mobile phase is required. The wetting of the interface is accomplished by adsorption of the alcohols in a sub-monolayer through interpenetrating with the chains of the C-18 chains on the stationary phase with -OH groups sticking out, which makes the surface more hydrophilic.

It was felt that 1,2-diols would also coat the resin through a dynamic equilibrium and provide a hydrophilic surface. The separation of alkane carboxylic acids was next investigated with straight-chain 1,2-diols as organic modifiers. A Supelcosil C-18 silica column was again used along with primarily aqueous eluents. Fig. 4 shows a good separation of all five carboxylic acids with an aqueous eluent containing 1% (v/v) 1,2-hexanediol. This is a much

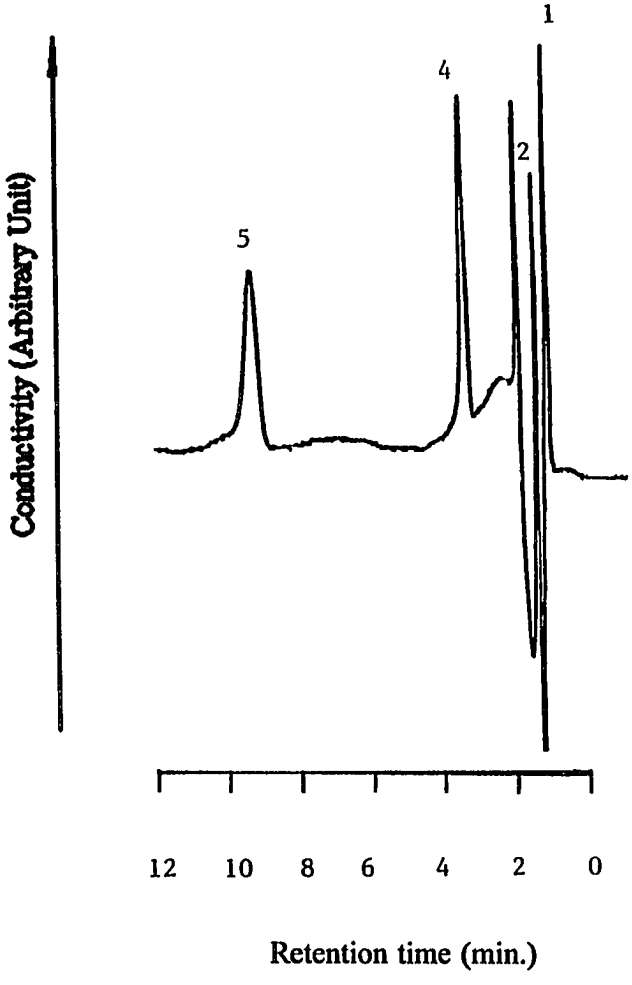


Fig. 4. All the experimental conditions are the same as Fig. 2, except that the eluent is 1% 1,2-hexanediol/water (v/v).

improved separation compared with that of Fig. 1 except there are some negative dips and an unknown broad peak early in the separation. A much better separation was obtained with an aqueous eluent containing only 0.25% 1,2-octanediol (v/v) (Fig. 5). The longer hydrocarbon chain apparently results in effective surface coating of the C-18 silica at a lower concentration than 1,2-hexanediol. 1,2-decanediol was too insoluble in water alone to include in the aqueous eluent. In this case, a 1% (w/v) solution of the diol in 60% methanol-40% water was run through the column at a very slow flow rate for several hours. Then the column was equilibrated with deionized water. This treatment should result in a stable, even coating of 1,2-decanediol on the C-18 silica surface. A fairly good separation of the carboxylic acids was obtained except for rather severe tailing of the last two peaks (Fig. 6A). An improved separation was obtained with this treated column by incorporating 1% 1-butanol in the eluent (Fig. 6B). For comparison, the same separation (1% butanol) on an untreated column is shown in Fig. 6C. The small amount of 1-butanol present in the eluent probably coated the surface which is not covered by 1,2-decanediol so that a more hydrophilic surface of the stationary phase was resulted from the combination of the two modifiers. The idea of using one additive in the eluent and using another one to pre-treat the column provides more possibilities in improving the separations of carboxylic acids.

Separations of Organic Bases on Underivatized Polymer Columns

The use of straight-chain alcohols and diols as mobile phase modifiers is not limited to the separation of acids nor to predominantly aqueous eluents. Fig. 7A shows a separation of seven basic compounds using 30% acetonitrile-70% water as the mobile phase. The

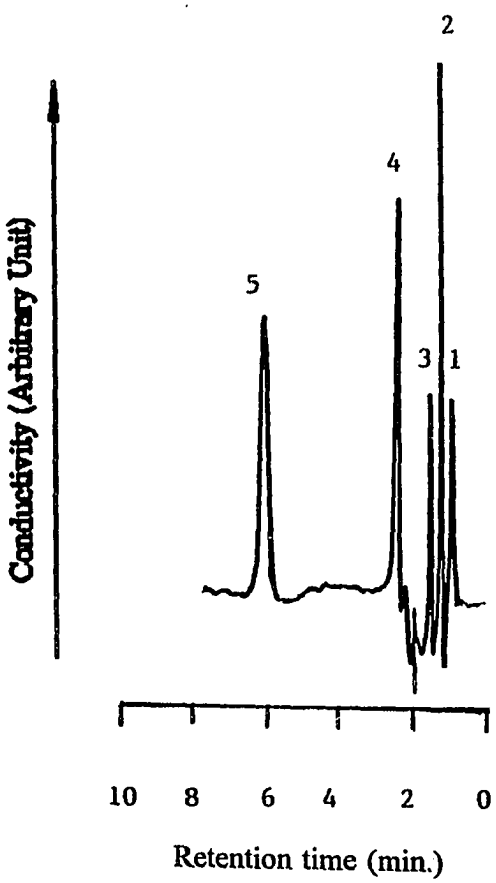


Fig. 5. All the experimental conditions are the same as Fig. 2, except that the eluent is 0.25% 1,2-octanediol/water (v/v).

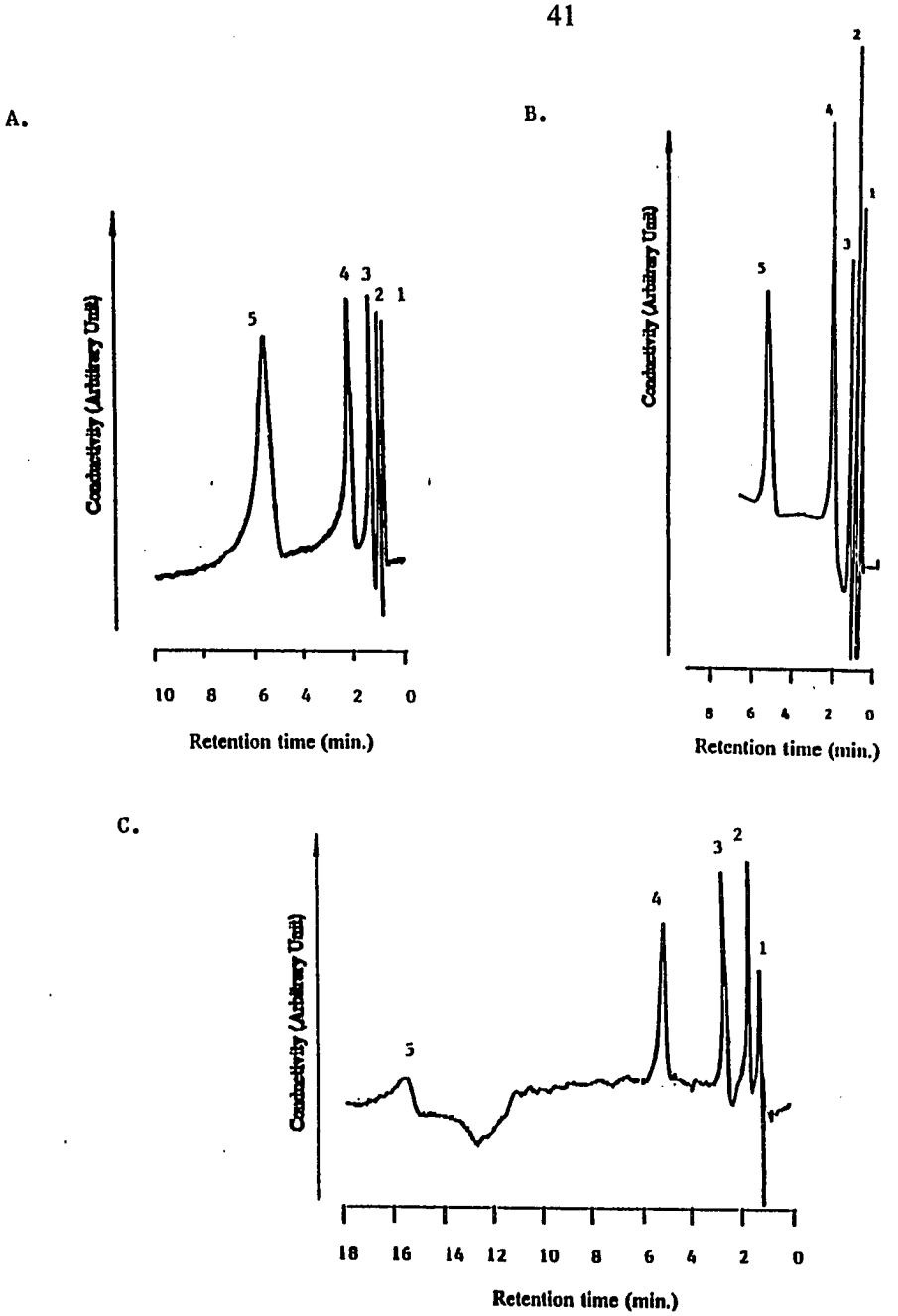
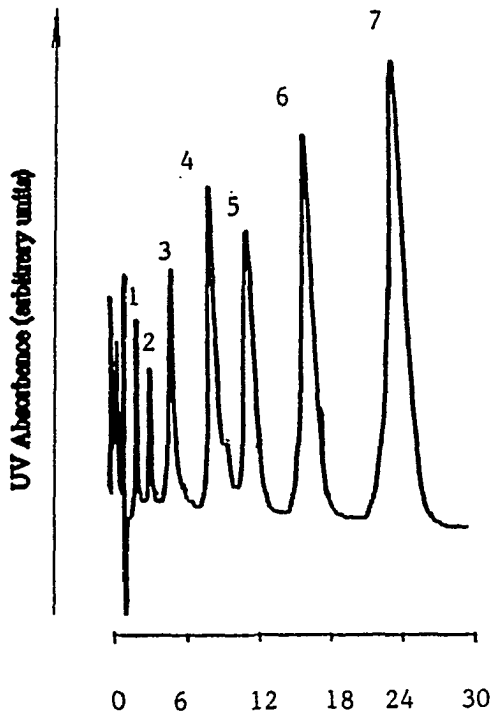


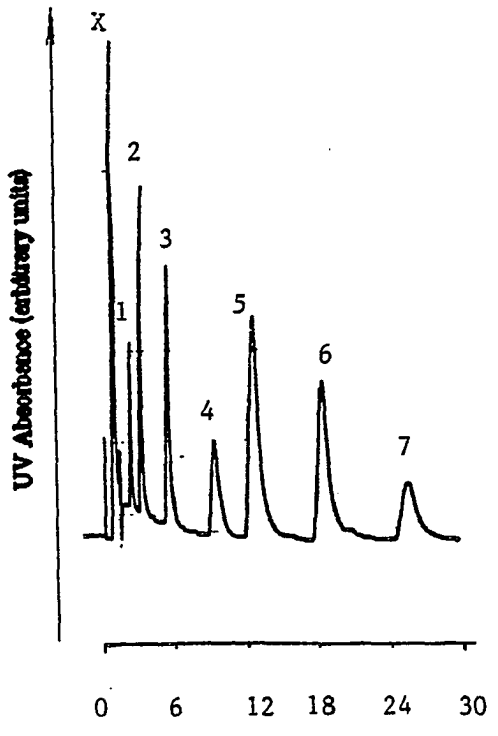
Fig. 6. All the conditions are the same as Fig. 2, except that: A: Column was precoated with 1,2-decanediol, Eluent: water. B: Column was pretreated 1,2-decanediol, Eluent: 1% 1-butanol. C: Non-treated column, Eluent: 1% 1-butanol.

Fig. 7. Chromatographic separations on underivatized polystyrene/divinylbenzene column (100 mm x 4.6 mm). Eluent: A. 30% acetonitrile/water, B. 30% acetonitrile/water containing 0.1 M methylamine. Flow rate: 1 ml/min. Detection: UV @ 262 nm. Peak identification: 1 = pyridine, 2 = 4-picoline, 3 = aniline, 4 = quinoline, 5 = quinaldine, 6 = N-methylaniline, 7 = 4-benzylpyridine.

A


UV Absorbance (arbitrary units)
1
2
3
4
5
6
7
0
6
12
18
24
30
Retention time (min.)

B

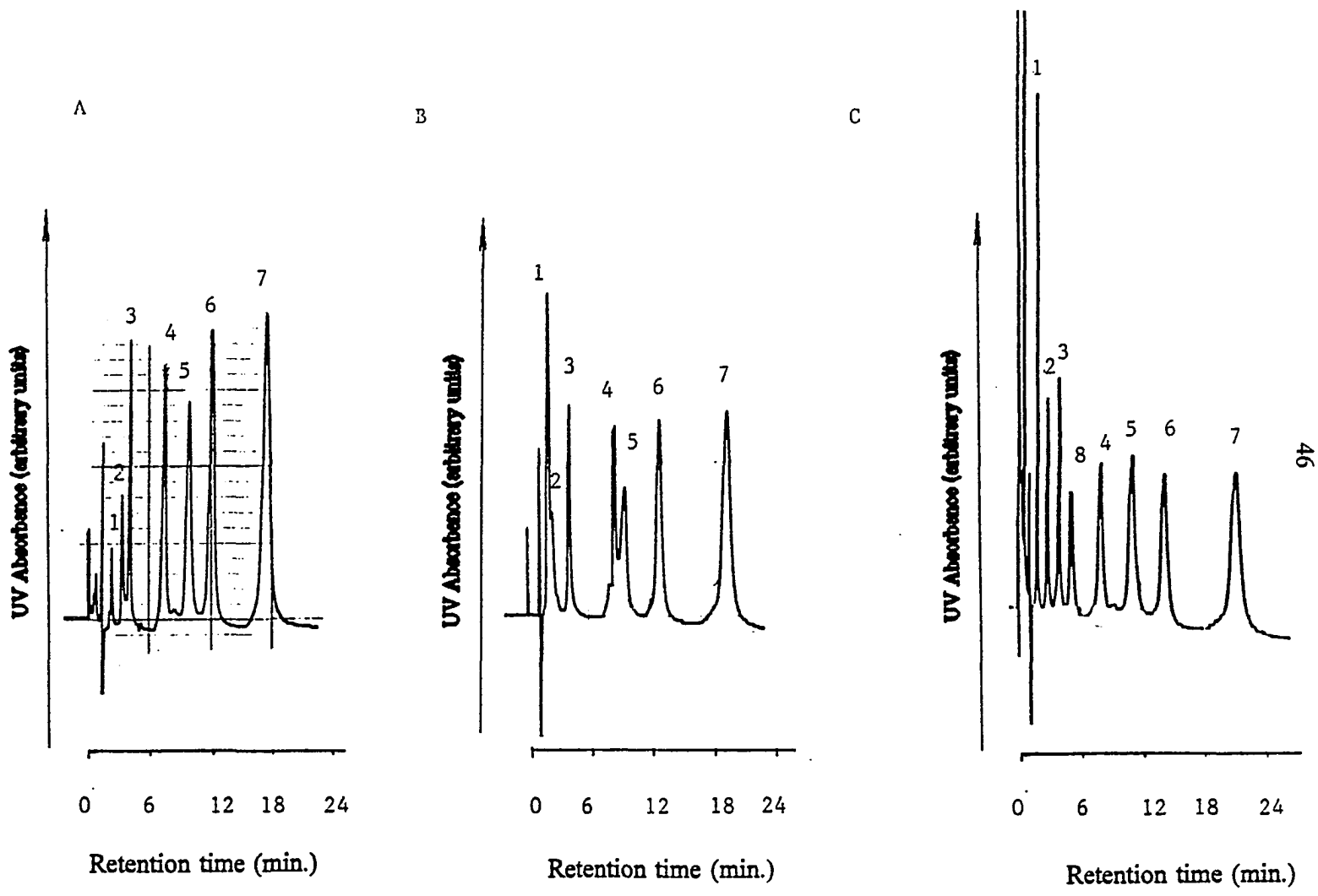

UV Absorbance (arbitrary units)
X
1
2
3
4
5
6
7
0
6
12
18
24
30
Retention time (min.)

separation is complete except peak 4 and 5. However, the peaks are quite broad. This is probably due to the different polarity between the protonated and deprotonated forms of the bases so that their interactions with the stationary phase would be different resulting in different retentions and hence broad peaks. In hope of sharpening the sample peaks, 0.1 M methylamine was added to the eluent to suppress the protonation of the analytes. As shown in Fig. 7B, the presence of methylamine in the eluent reduced the widths of the sample peaks. However, all of them are severely tailed. On the other hand, when 1.5% 1-hexanol (v/v) was added into the eluent, the peaks were all sharp and symmetrical, and their retention times were reduced with the total separation time being 18 minutes now (Fig. 8A) although the peaks of picoline and aniline are overlapped. When 0.09% 1-decanol (v/v) was used as the mobile phase modifier, sharper and symmetrical sample peaks were again obtained with the elution time being about 18.5 minutes (Fig. 8B) although there was peak overlapping between pyridine and picoline, and between quinoline and quinaldine. When 0.01% 1-dodecanol (w/v) was used, sharper and symmetrical peaks were obtained with the separation time being about 23.5 min. Because of the sharpness of the peaks and also the selectivity change compared with no modifiers, a separation of eight compounds was allowed (Fig. 8C).

Separations of Carboxylic Acids on Sulfonated Polymer Columns

The dynamic equilibrium between the mobile and the stationary phase allows the coating of straight-chain alcohols and diols onto the surface of the stationary phase. This coating strongly affects the separation of alkane carboxylic acids and organic bases. Thus the effect of attaching an amino alcohol group to the polymeric resin was investigated. This was

Fig. 8. Chromatographic separations on underivatized polystyrene/divinylbenzene column (100 mm x 4.6 mm). Eluent: 30% acetonitrile/water containing A. 1.5% 1-hexanol, B. 0.09% 1-decanol and C. 0.01% 1-dodecanol. Flow rate: 1 ml/min. Detection: UV @ 262 nm. Peak identification: 1 = pyridine, 2 = 4-picoline, 3 = aniline, 4 = quinoline, 5 = quinaldine, 6 = N-methylaniline, 7 = 4-benzylpyridine, 8 = 4-ethylpyridine.


A
B
C
UV Absorbance (arbitrary units)
1
2
3
4
5
6
7
8
0
6
12
18
24
Retention time (min.)

made possible by lightly sulfonating the resin to produce surface SO_3^- groups and pumping an acidic aqueous solution of amino alcohol through the resin column. The protonated amino alcohol ($\overset{+}{N}H_3ROH$) would attach to the sulfonate group ($-SO_3^-$) on the resin surface by electrostatic attraction. The hydroxyl group points outward from the resin surface and should give an effect similar to alcohols and diols on the resin surface.

The first additive employed was ethanolamine. Fig. 9 shows the attempted separation of five carboxylic acids on lightly sulfonated polymeric resin with water alone as the eluent. The hydrophobic attraction of the alkyl chain of the analytes for the resin is too great and the separation is incomplete. After treating the column with an acidic (pH = 3) ethanolamine solution, the same separation was tried with pure water as the eluent and the chromatogram in Fig. 10A was obtained. Retention times of the acids were reduced dramatically. Valeric acid was eluted at about 28 min. Further improvement in separation with the ethanolamine coated column was obtained when an alcohol was added to the aqueous eluent. A much improved separation of all five analytes was obtained with 40% methanol (Fig. 10B). A very fast separation was obtained with an aqueous eluent containing only 0.2% 1-butanol, in which valeric acid was eluted within 2 minutes (Fig. 10C). Formic acid eluted at the time of the system peak. With lightly sulfonated resin columns not treated with ethanolamine, 60% methanol or 5% butanol is generally used in the eluent.

The second additive of this type tried is 3-amino-1-propanol. The column was pretreated by the same procedure as with ethanolamine. Fig. 11 shows the separation of the five acids on the column treated with 3-amino-1-propanol using 20% methanol/water as the

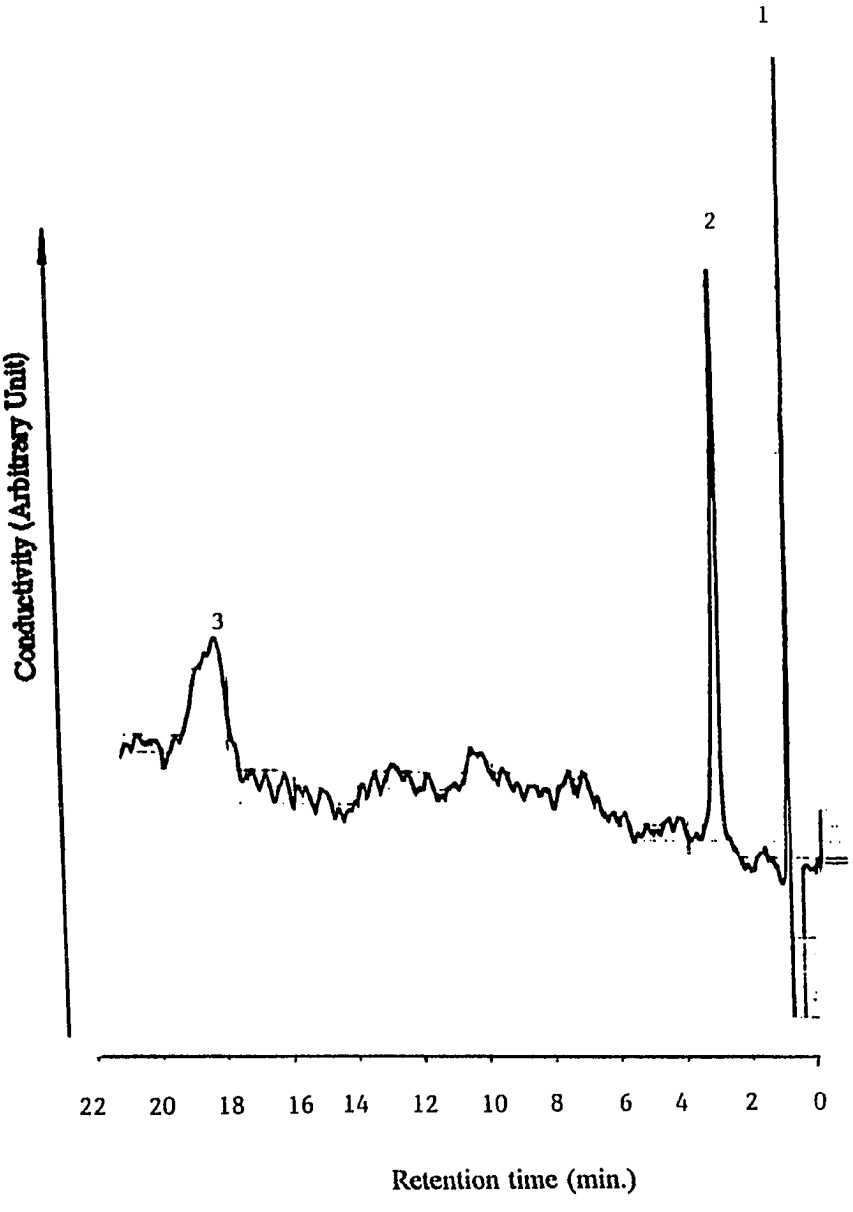
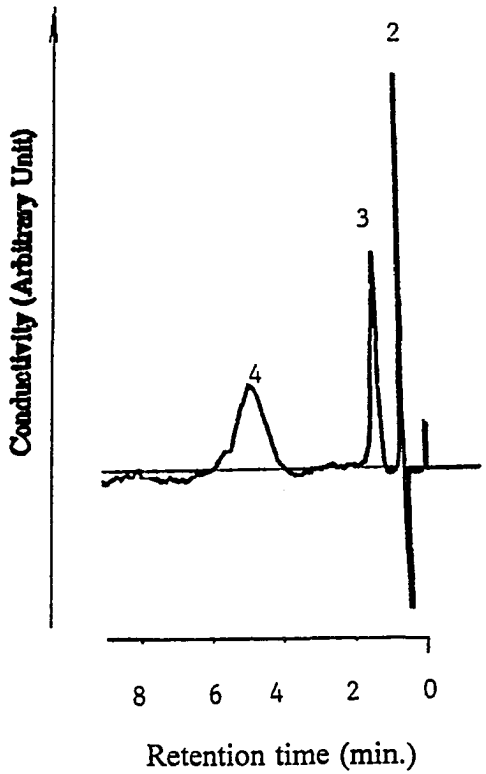


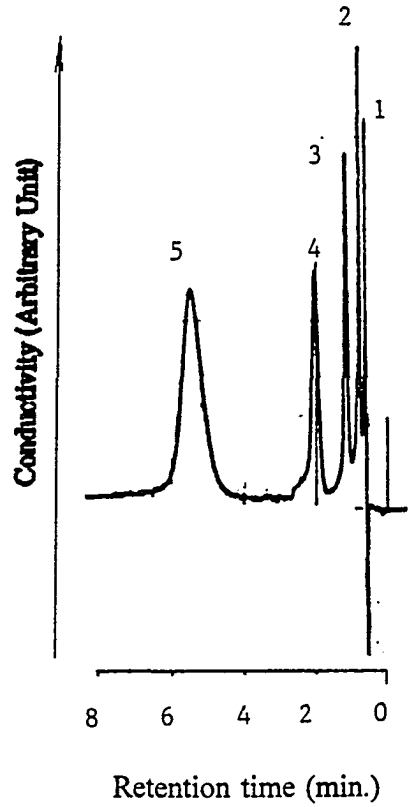
Fig. 9. Chromatographic separation on column packed with lightly sulfonated 5 μm PS/DVB (100 mm x 4.6 mm I. D., sulfonation capacity: 0.30 meq/g). Eluent: water. Flow rate: 1 ml/min. Detection: conductivity. Peak identification: 1 = formic acid, 2 = acetic acid, 3 = propionic acid. Butyric acid and valeric acid failed to elute.

Fig. 10. Chromatographic separation on column packed with lightly sulfonated 5 μm PS/DVB pre-treated with ethanolamine (100 mm x 4.6 mm I. D., sulfonation capacity: 0.30 meq/g). Eluent: A: water, B: 40% methanol/water and C: 0.2% 1-butanol/water. Flow rate: 1 ml/min. Detection: conductivity. Peak identification: 1 = formic acid, 2 = acetic acid, 3 = propionic acid, 4 = butyric acid, 5 = valeric acid.

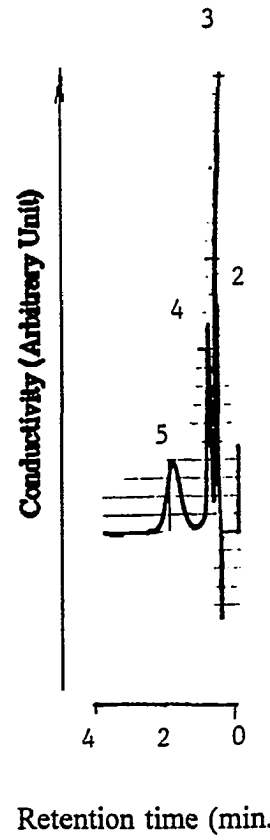
A.


Conductivity (Arbitrary Unit)
2
3
4
8
6
4
2
0
Retention time (min.)

B.


Conductivity (Arbitrary Unit)
2
1
3
5
4
8
6
4
2
0
Retention time (min.)

C.


Conductivity (Arbitrary Unit)
3
2
4
5
4
2
0
Retention time (min.)

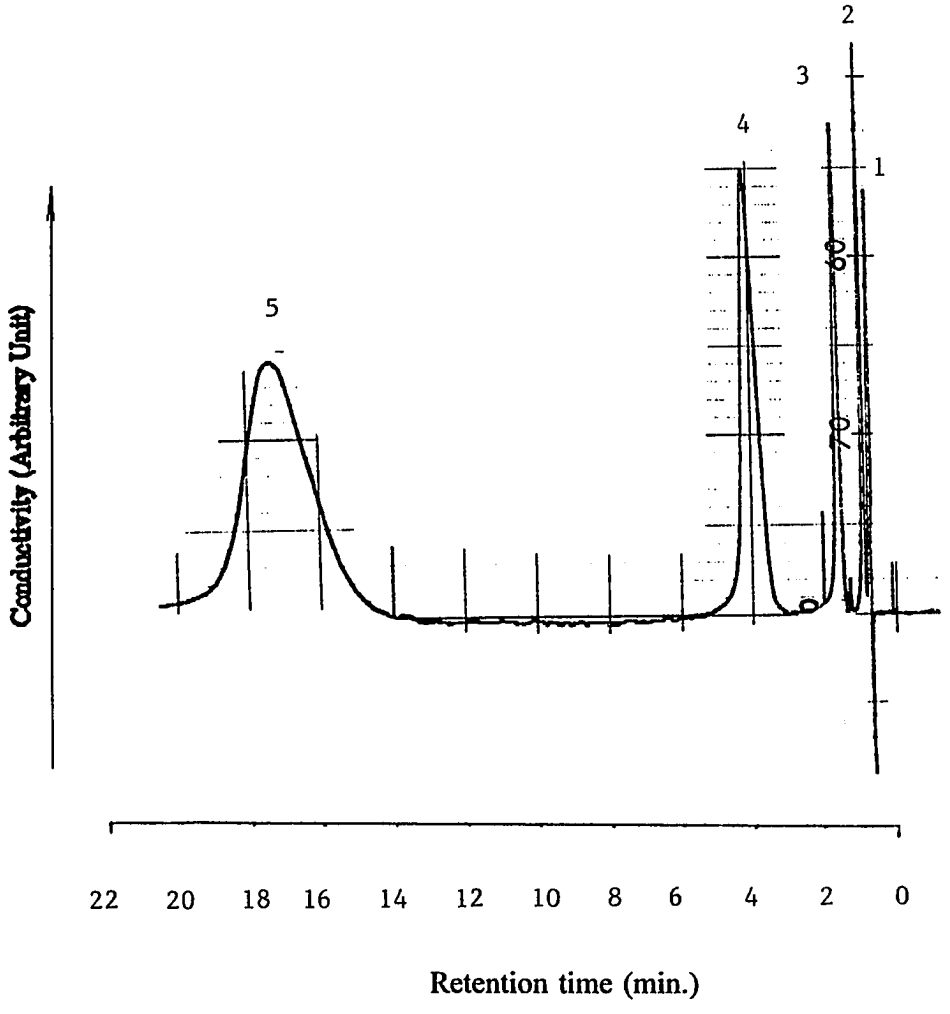


Fig. 11. Chromatographic separation on column packed with lightly sulfonated 5 μm PS/DVB pre-treated with 3-amino-1-propanol (100 mm x 4.6 mm I. D., sulfonation capacity: 0.30 meq/g). Eluent: 20% methanol/water. Flow rate: 1 ml/min. Detection: conductivity. Peak identification: 1 = formic acid, 2 = acetic acid, 3 = propionic acid, 4 = butyric acid, 5 = valeric acid.

eluent.

4-amino-1-butanol was also used to coat the stationary phase. Table I shows the retention times of formic to valeric acids on columns packed with PS/DVB resin of the same sulfonation capacity and coated with ethanolamine, 3-amino-1-propanol or 4-amino-1-butanol under the same experimental conditions. Retention times of the analytes obviously increase with the length of the carbon chain of the amino alcohol for each analyte. This is reminiscent of the effect of varying brush lengths in bonded-phase silica columns, i.e., C2, C8, C18, etc [11].

Table II shows the retention times of these five acids on 3-amino-propanol coated columns of different sulfonation capacities under the same experimental conditions. The reaction conditions for the sulfonation reactions, and the sulfonation capacities and wettability of the resins by water and 20% methanol/water are as indicated in Table III. It was found that the retention of carboxylic acids increases with increasing sulfonation capacity until the resin becomes wettable by water. When passing this point, a decrease in retention resulted from further increase in the sulfonation capacity. Table IV shows the retention times obtained on 3-amino-1-propanol coated columns at different capacities with 20% methanol as the eluent. The resins are all wettable by 20% methanol. It is apparent that the increase in sulfonation capacity results in continuous decrease in the retention of the analytes. Dumont and Fritz studied the effect of sulfonation capacity on the retention of polar organic compounds in water [12]. They found that the retention times of the test compounds increase with increasing sulfonation capacity, reaching maximum at about 0.6 meq/g. Further increases in sulfonation

Table I. Effect of hydrocarbon-chain length of amino alcohols on the retention of carboxylic acids. Chromatographic column: 100 mm x 4.6 mm I. D., packed with lightly sulfonated 5 μm PS/DVB with sulfonation capacity: 0.002 meq/g. Other conditions are the same as Fig. 9.

Compounds	Retention time on columns coated with		
	Ethanolamine	3-amino-1-propanol	4-amino-1-butanol
Formic acid	na[1]	0.22	0.27
Acetic acid	0.31	0.40	0.57
Propionic acid	0.66	1.50	2.65
Butyric acid	2.39	7.40	14.51
Valeric acid	28.00	na[2]	na[3]

[1]Peak is at injection dip.

[2]Valeric acid failed to elute.

[3]Valeric acid failed to elute.

Table II. Resin sulfonation reaction conditions, sulfonation capacities and their wettability by water and 20% methanol-80% water[1]

Resin	Reaction Conditions		Capacity (meq/g)	Wettability	
	Reaction time (min.)	Temperature		Water	20% methanol
A	120	90°C	1.30	Yes	Yes
B	10	Room Temp.	0.30	Yes	Yes
C	5	Room Temp.	0.046	Partial	Yes
D	1.7	Room Temp.	0.025	Partial	Yes
E	0.5	Ice	0.002	No	Yes

[1] Other sulfonation reaction conditions are (1) 25 ml concentrated sulfuric acid was added into 2 g underivatized PS/DVB resin stirred in 5 ml glacial acetic acid, and (2) the reaction was stopped by pouring the reaction mixture into ice-water bath.

Table III. Effect of the sulfonation capacity on the retention of carboxylic acids. Chromatographic columns are precoated with 3-amino-1-propanol. Sulfonation capacities are as shown in Table II. Eluent is water. Other conditions are the same as Fig. 9.[2]

Compounds	Retention time on column				
	A	B	C	D	E
Formic acid	0.25	0.32	0.31	0.29	0.22
Acetic acid	0.28	0.54	0.49	0.42	0.40
Propionic acid	0.61	2.25	2.07	1.61	1.50
Butyric acid	1.61	13.50	11.72	8.82	7.40
Valeric acid	7.16	na	na	na	na

[2]na: Valeric acid failed to elute.

Table IV. Effect of the sulfonation capacity on the retention of carboxylic acids. Eluent: 20% methanol/water. Other conditions are the same as in Table III.

Compounds	Retention time on column		
	A	B	E
Formic acid	0.32	0.29	0.26
Acetic acid	0.43	0.41	0.40
Propionic acid	0.62	0.76	1.00
Butyric acid	1.08	2.01	3.20
Valeric acid	3.25	8.59	14.63

capacity result in a rapid decrease in the retention of the test analytes. The increasing retention up to 0.6 meq/g can be attributed to the fact that the higher the sulfonation, the more hydrophilic and wettable by water the resin is. Because the ability of water to come into contact with the resin surface facilitates the transfer of analyte from aqueous solution to the resin surface, longer retention time is obtained on columns with higher sulfonation capacities. Once the sulfonation capacity is high enough, wettability is no longer a factor. The overall hydrophilicity of the resin will determine the hydrophobic interaction of the analyte with the resin surface. The higher the sulfonation capacity, the more hydrophilic the resin is and hence the less hydrophobic interaction and shorter retention times. The present study mirrored the findings by Dumont and Fritz. In the current system, the wettability of the sulfonated resin could affect (a) the contact of the amino alcohol solution with the resin surface during the pre-treatment step as well as that of the eluent with the resin surface during the separation

step and (b) the percentage coverage of the hydrophobic resin matrix by the hydrophilic amino alcohol group.

CONCLUSIONS

Straight-chain alcohols, diols and animo alcohols were successfully used as organic modifiers for the separations of organic acids and bases. These were achieved by incorporating the modifiers into the eluent or using a solution to pretreat the separation column.

The function of these mobile additives is to coat the surface of the stationary phase and make it less hydrophobic. Those modifiers with longer hydrocarbon chains are more effective than those with shorter chains because they have higher tendency to be adsorbed onto the surface of the stationary phase. Lower concentrations in the eluent are therefore needed to produce an effective coating.

This concept (dynamic coating) should have far reaching possibilities for HPLC. Use of surface modifiers has clear advantages over chemically derivatized stationary phases in that the properties and thus selectivity of the stationary phase can be altered and fine tuned by carefully choosing the chemical nature of the modifier.

ACKNOWLEDGEMENTS

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NOVEL ADDITIVES FOR THE SEPARATIONS OF ORGANIC COMPOUNDS BY HPLC

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Xue Li and James S. Fritz
Ames Laboratory, U.S. Department of Energy and
Department of Chemistry, Iowa State University
Ames, Iowa 50011, U.S.A.

ABSTRACT

This paper proposes the use of novel surfactant additives at concentrations generally above their critical micelle concentrations in aqueous solution but without micelle formation for the separations of various organic compounds. The presence of these additives in organic-water mixtures greatly improves the separations of alkylbenzenes, polycyclic aromatic hydrocarbons, alkylphenols, and some other aromatic compounds. Compared with separations obtained without additives, shorter retention times and sharper peaks are obtained. The reason for the improvement appears to be a stronger interaction between analyte molecules and mobile phase due to the presence of long hydrocarbon chain(s) or polyoxypropylene segments

in the additive molecules. The retention times of late-eluting compounds are reduced by a larger percentage than the retention times of earlier peaks. This effect is similar to gradient elution but is obtained using only isocratic elution with an organic-water eluent containing an appropriate additive. Solvent strength and selectivity can be varied by controlling the type and concentration of the additive. Binding constants between solute and surfactant additives were calculated by relating capacity factor to surfactant concentration.

INTRODUCTION

The idea of adding an organic modifier to the mobile phase to improve HPLC separations has been studied. Addition of a suitable organic modifier results in additional interactions either between analytes and the stationary phase , as when a long chain alcohol is used to coat the surface of the stationary phase [1], or between analytes and the mobile phase, as occurs when cyclodextrins are used for chiral separations [2]. Jorgenson *et al.* postulated a dynamic association equilibrium between the tetrahexylammonium ion and neutral organic solutes in acetonitrile-water [3]. They used this association to separate several neutral organic compounds by CZE. However, no one has ever taken advantage of this kind of equilibrium for HPLC separations.

Surfactants, such as sodium dodecylsulfate (SDS) [4] and cetyltrimethylammonium bromide (CTAB) [5], have been used in ion-pair chromatography, generally at concentrations below their critical micelle concentrations. They coat the stationary phase and act as dynamic ion exchangers for ionic analyte species. Charged analytes such as rare earth metals [4] and

inorganic anions [5] can be retained and separated using stationary phases such as silica C-18. Surfactants have also been used in aqueous solvent at concentrations above their critical micelle concentration to replace the organic solvent component used in conventional hydro-organic chromatography. This is so-called micellar liquid chromatography [6, 7]. Micellar liquid chromatography has several advantages over conventional HPLC, but it is often associated with broader peaks and hence lower efficiency compared to conventional HPLC due to slow mass transfer from micelle to stationary phase [6, 7].

In this paper, the effect of surfactants is studied in mobile phases containing 39-70% organic solvent. Under these conditions, the formation of micelles is unlikely. However, a dramatic decrease in retention time was observed with several of the additives studied and sharp peaks were obtained. This is like a "bridge" step between micellar liquid chromatography and conventional HPLC because surfactants are used at concentrations above their critical concentrations in aqueous solution but no micelle formation is required.

EXPERIMENTAL

Chromatographic System

The chromatographic system consists of several components. A Dionex DXP pump (Dionex, Sunnydale, CA, U.S.A.) was used to deliver a flow of 1 ml/min. A 7010 Rheodyne injector (Rheodyne, Berkeley, CA, U.S.A.) delivered 10 μl sample which was detected with a Kratos Spectra flow 783 UV absorbance detector (Kratos Analytical Instrument, Ramsey, NJ, U.S.A.). Separations were recorded by a Servogor 120 chart recorder (Abb Goerz

Instrument, Vienna, Austria). Supelcosil LC-18 columns (150 mm x 4.6 mm I.D.), or in several cases, an Alltech Nucleosil C18 column (150 mm x 4.6 mm I.D.) were used as separation columns.

Reagents and Chemicals

Methanol and acetonitrile were of HPLC grade and used as obtained from Fisher (Fisher Scientific, Pittsburgh, PA, U.S.A.). The mobile phase additives and analyte chemicals were reagent grade except phenylacetaldehyde, which was 90%, and were all used as obtained from Aldrich (Aldrich Chemical Company, Inc., Milwaukee, WI, U.S.A.), J.T.Baker (J.T. Baker Chemical Co., Phillipsburg, NJ, U.S.A.), BASF (BASF Corporation, Parsippany, NJ, U.S.A.) and Sigma (Sigma Chemical Company, St. Louis, MO, U.S.A.). All eluents were prepared daily. Stock solutions were used to prepare all sample solutions by diluting with mobile phase. A Barnstead Nanopure II system (Sybron Barnstead, Boston, MA, U.S.A.) was used to further deionize distilled water for all eluents and sample mixtures.

Chromatographic Procedure

A flow rate of 1 ml/min was selected for all the chromatographic separations. The separation column was equilibrated with mobile phase containing no additives, i.e., 60%, 50% or 39% acetonitrile, or 70% methanol, until the baseline was stabilized. Then the desired eluent was used. The baseline was stable after about 0.5 h. Sample injections were made at this point. The eluted species were detected by a UV-vis detector at 254 nm with an output range of 0.010 AUFS.

Capacity factor, k', was calculated according to expression: $k' = (t_r - t_o)/t_o$. The system

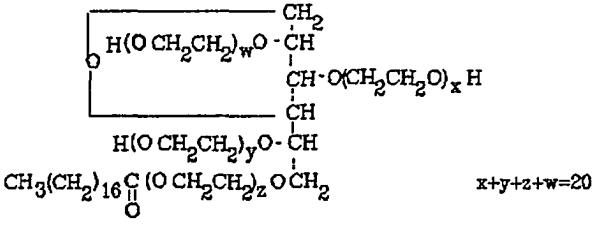
dead time, t_o, used to calculate capacity factor k', was measured by injecting nitrate solution into the system. An average of at least three replicates was used to do all the calculations.

RESULTS AND DISCUSSIONS

Effect of Additives

Table I is a list of additives that have been used for the separations of the analytes. Pluronic L-31 contains alternating hydrophobic polyoxypropylene and hydrophilic polyoxyethylene segments. The others are all amphophilic compounds with one or more long alkyl hydrophobic chains and a hydrophilic head group varying in chemical nature. These additives are either ionic or nonionic. The hydrophilic part helps to solubilize them in aqueous-organic solvent while the hydrophobic part helps them to fulfill their function as mobile phase modifiers to improve the separations of various organics. Figures 1-5 show separations with and without additives in the mobile phase. In Figure 1 a separation of alkylphenols is shown with 60% acetonitrile-water as the mobile phase. The separation took about 22 min to finish. When 50 mM Pluronic L-31 was added to the mobile phase, a baseline separation was still obtained in only 14 min (Figure 2). For the separation of benzene through perylene, the additive effect is even more evident. As shown in Figure 3, these six compounds were completely separated with 60% acetonitrile/water as the mobile phase. However, it took more than 54 min to elute all the analytes from the column because of the strong interactions between stationary phase and these very hydrophobic analytes. When 40 mM Tween-60, which contains one saturated C-17 hydrocarbon chain in each molecule, was

Table I. Mobile phase additives utilized for the separations of organic compounds.

Abbreviation	Name	Structure
1. THPA	Tetraheptylammonium	$(CH_3CH_2CH_2CH_2CH_2CH_2CH_2)_4\overset{+}{N}$
2. CTAB	Cetyltrimethylammonium	$CH_3(CH_2)_{15}\overset{+}{N}(CH_3)_3$
3. SDS	Sodium dodecylsulfate	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2OSO_3^-$
4. DOSS	Dioctylsulfosuccinate	$CH_3CH_2CH_2CH_2CH(CH_3CH_2)CH_2O_2C\text{-}CH_2$ $CH_3CH_2CH_2CH_2CH(CH_3CH_2)CH_2O_2C\text{-}CH\ SO_3^-$
5. Brij-30	Plolyoxyethylene(4) dodecyl ether	$CH_3(CH_2)_{11}(OCH_2CH_2)_4OH$
6. Tween-60	Polyoxyethylene(20) sorbitan monostearate	 x+y+z+w=20
7. Pluronic L-31	Polyoxyethylene-polyoxypropylene copolymer	$H(OCH_2CH_2)_a(OCH_2\underset{CH_3}{CH})_b(OCH_2CH_2)_aOH$ M.W=1900 19% EO, w/w
8. 1-dodecanol		$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2\ OH$
9. 1,2-decanediol		$CH_3(CH_2)_7\underset{OH}{CH}\,CH_2OH$

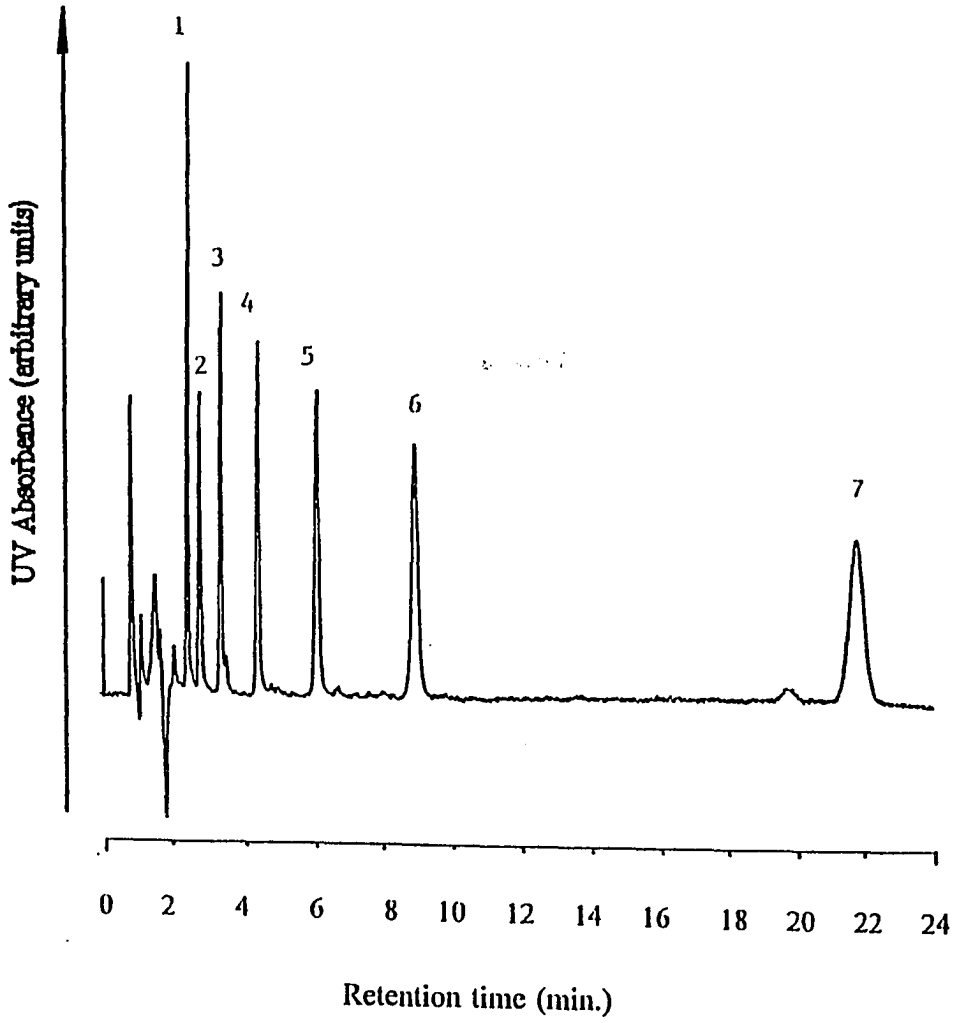


Fig. 1. Chromatographic separation on Supelcosil LC-18 (150 mm x 4.6 mm I.D.) column. Eluent: 60% acetonitrile/water. Flow rate: 1 ml/min. Detection: UV @ 254 nm. Peak identification: 1 = phenol, 2 = p-cresol, 3 = 4-ethylphenol, 4 = 4-n-propylphenol, 5 = 4-n-butylphenol, 6 = 4-n-amylphenol, 7 = 4-n-heptylphenol.

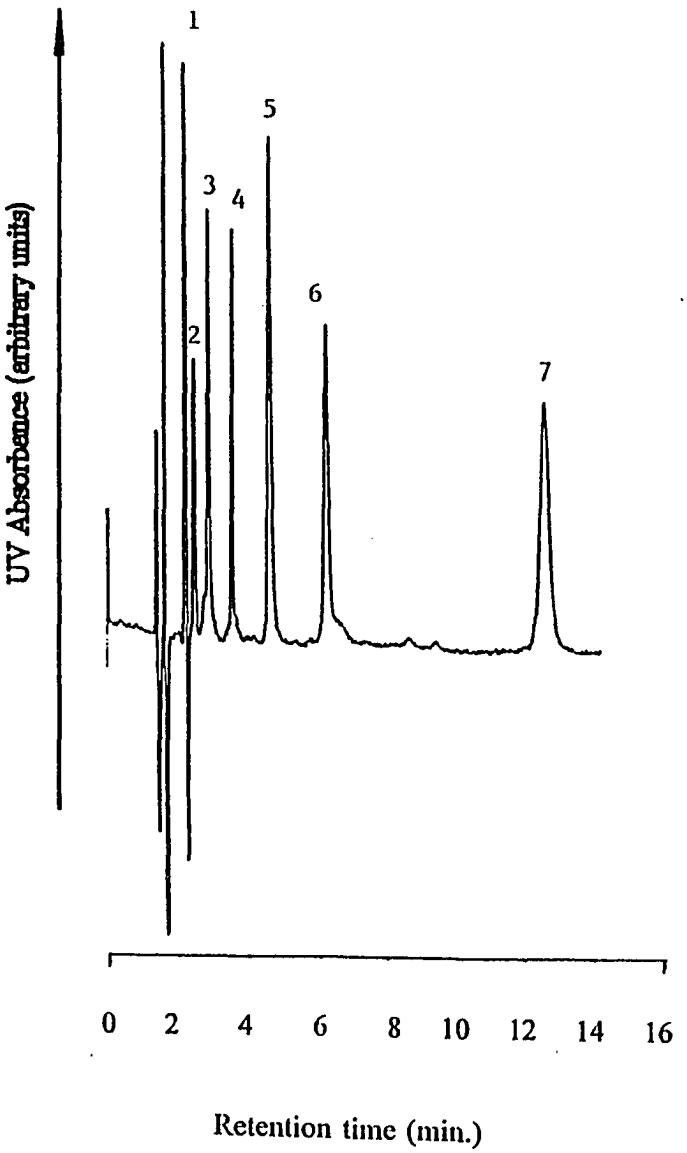


Fig. 2. All conditions are the same as in Fig. 1, except that the eluent is 60% acetonitrile/water containing 50 mM Pluronic L-31.

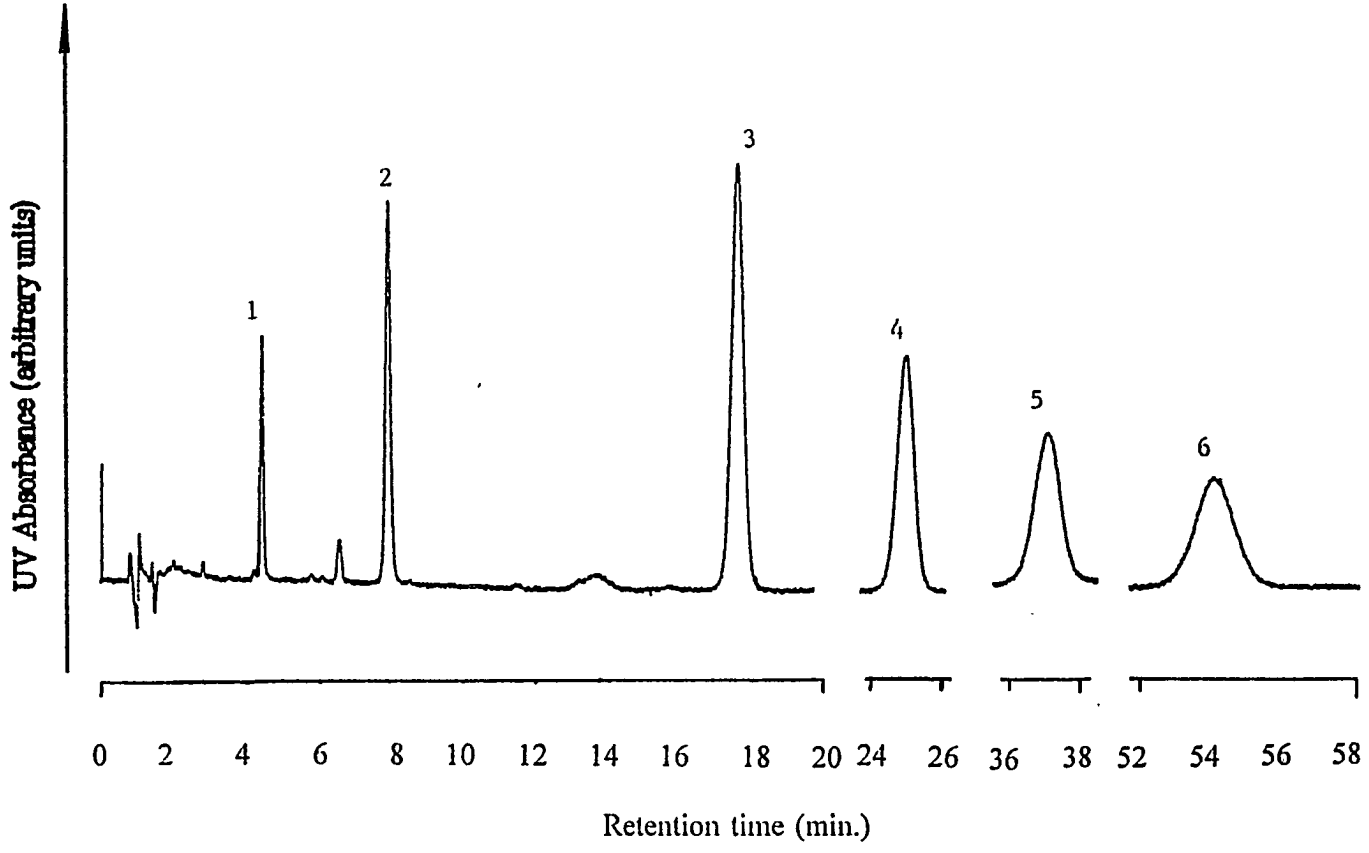


Fig. 3. Chromatographic separation on Supelcosil LC-18 (150 mm x 4.6 mm I.D.) column. Eluent: 60% acetonitrile/water. Flow rate: 1 ml/min. Detection: UV @ 254 nm. Peak identification: 1 = benzene, 2 = naphthalene, 3 = anthracene, 4 = pyrene, 5 = chrysene, 6 = perylene.

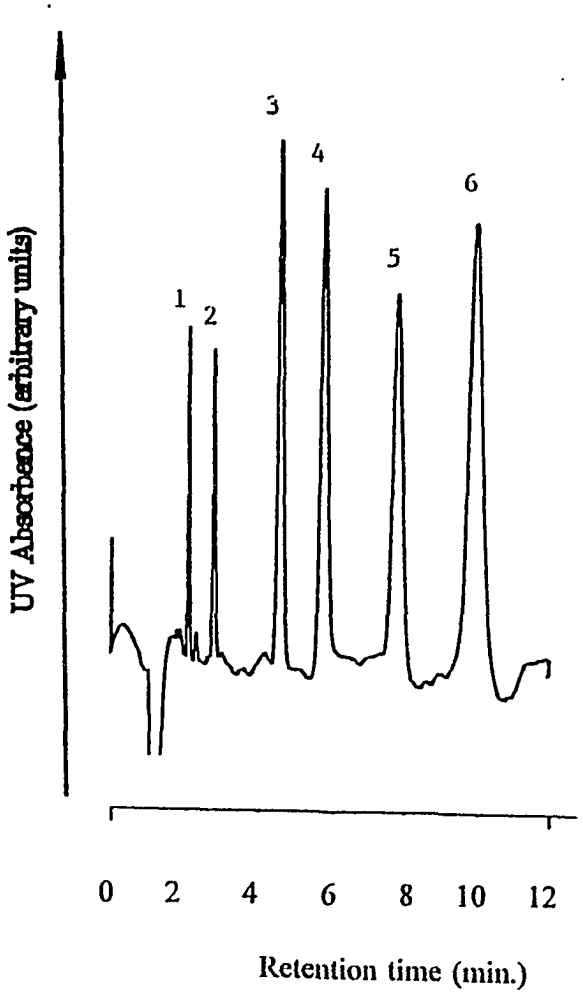


Fig. 4. All conditions are the same as in Fig. 3, except that the eluent is 60% acetonitrile/water containing 40 mM Tween-60.

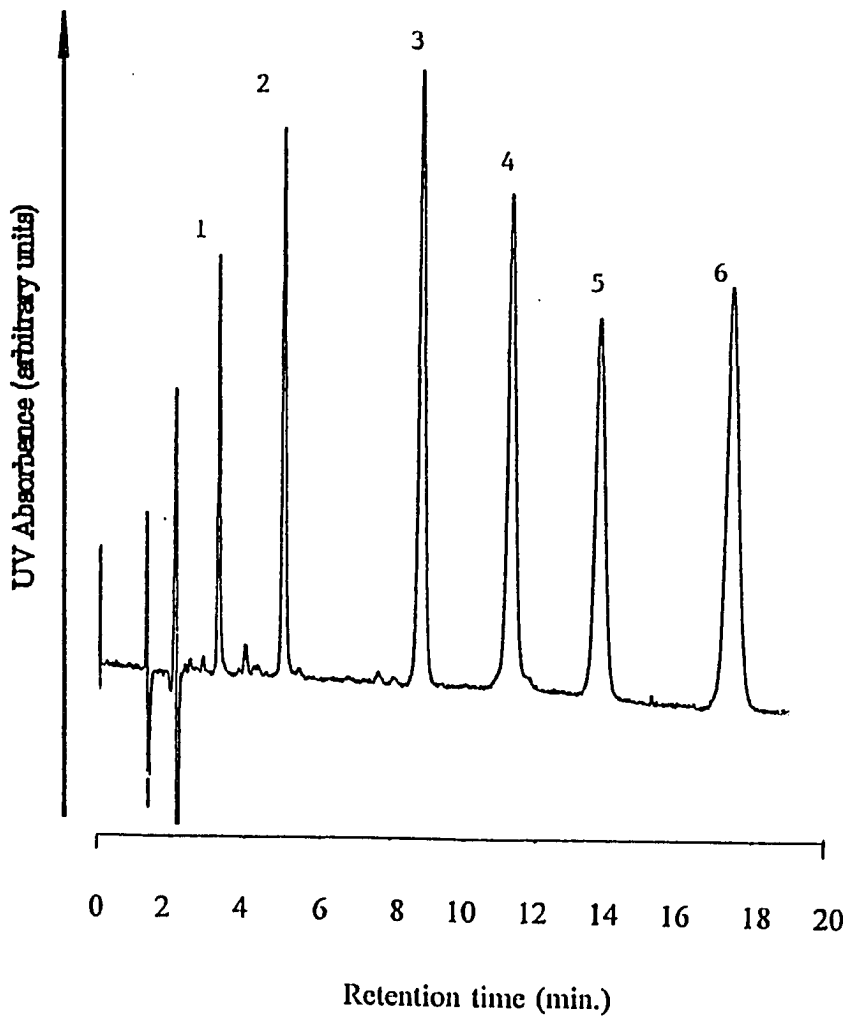


Fig. 5. All conditions are the same as in Fig. 3, except that the eluent is 60% acetonitrile/water containing 50 mM tetraheptylammonium bromide.

added to the mobile phase, much shorter retention times and much sharper peaks resulted. The same separation took only about 10 min to finish (Figure 4). In the case of THPA, which contains four saturated C-7 hydrocarbon chains in each molecule, a similar effect was observed but to a lesser degree due to weaker hydrophobic interactions between THPA and these PAH molecules (Figure 5). Similar effects of additives were also observed for alkylbenzenes separations.

Tables II-IV gives the retention times of alkylbenzenes, polycyclic aromatic hydrocarbons and alkylphenols with 50 mM SDS, DOSS, Pluronic L-31, THPA, Brij-30 or CTAB, 28 mM 1,2-decanediol, 40 mM 1-dodecanol or 40 mM Tween-60 as the additive in 60% acetonitrile-water eluent. Retention times of these analytes with 60% acetonitrile alone as the eluent are also shown for comparison. The different retention times shown in Table II-IV when 60% acetonitrile-water alone was the eluent are due to two different analytical columns were used. Table V shows the retention times with and without Brij-30 as the additive to 70% methanol-water mobile phase. Brij-30 caused a much smaller change in retention times in methanol than in acetonitrile. From these tables, we can easily see that the retention of all the analytes was decreased in the presence of the additives but to different degrees. The retention of larger, more hydrophobic molecules was generally reduced more than that of smaller, less hydrophobic ones. Different additives had different modifying powers when the same concentration was used. Their effect on the retention reduction of the analytes is determined by the hydrophobic chain length and the chemical nature of the additive. In the case of alkylphenols, hydrogen bonding formation between the hydroxyl

Table II. Retention times (min.) of various compounds on Supelcosil LC-18 (150 mm x 4.6 mm I.D.) column. Flow rate: 1 ml/min. Detection: UV absorbance @ 254 nm. See Table I for abbreviations.

COMPOUND	60% acetonitrile + No additive	50 mM SDS	50 mM DOSS	50 mM Pluronic L-31	50 mM THPA	40 mM Tween-60
Benzene	4.20	3.09	3.22	2.52	3.28	2.08
Toluene	5.58	4.16	4.23	3.00	4.30	2.36
Ethylbenzene	7.78	5.43	5.57	3.62	5.57	2.64
Propylbenzene	11.78	7.86	8.11	4.80	7.76	3.22
Butylbenzene	18.24	11.65	12.06	6.56	10.98	3.95
Benzene	4.20	3.09	3.22	2.52	3.28	2.08
Naphthalene	7.44	5.46	5.03	3.73	5.04	2.82
Anthracene	16.22	11.96	9.76	7.07	8.81	4.63
Pyrene	22.88	17.98	13.60	9.71	11.34	5.86
Chrysene	33.88	25.27	17.70	13.83	13.80	7.91
Perylene	49.92	39.37	25.66	19.94	17.46	10.06
Phenol	2.56	2.02	1.64	1.85	2.08	1.75
Cresol	2.78	2.24	1.86	1.98	2.34	1.78
Ethylphenol	3.38	2.62	2.08	2.15	2.72	1.92
Propylphenol	4.38	3.34	2.40	2.44	3.40	2.08
Butylphenol	6.02	4.40	3.86	2.94	4.40	2.29
Amylphenol	8.68	6.10	5.24	3.70	5.98	2.63
Heptylphenol	20.84	13.40	11.30	6.96	12.00	3.78

Table III. Retention times (min.) of various compounds on Supelcosil LC-18 (150 mm x 4.6 mm I.D.) column. Flow rate: 1 ml/min. Detection: UV absorbance @ 254 nm. ----: didn't try.

	60% ACN +		
COMPOUND	No additive	50 mM CTAB	50 mM Brij-30
Benzene	3.94	3.00	2.80
Toluene	5.28	4.02	3.46
Ethylbenzene	7.20	5.22	4.34
Propylbenzene	10.80	7.40	6.10
Butylbenzene	16.50	10.76	8.62
Benzene	3.94	3.00	2.80
Naphthalene	6.78	4.86	4.24
Anthracene	14.46	9.26	7.60
Pyrene	20.78	----	10.68
Chrysene	29.38	16.38	12.86
Perylene	44.82	----	18.26
Phenol	2.10	----	1.84
Cresol	2.36	----	2.04
Ethylphenol	2.86	----	2.30
Propylphenol	3.68	----	2.82
Butylphenol	4.96	----	3.54
Amylphenol	7.02	----	4.68
Heptylphenol	15.86	----	9.20

Table IV. Retention times (min.) of various compounds on Alltech Nucleosil C-18 (150 mm x 4.6 mm I.D.) column. Other conditions are the same as in Table II.

COMPOUND	60% ACN +		
	No additive	28 mM 1,2-Decanediol	40 mM 1-Dodecanol
Benzene	4.82	4.62	4.08
Toluene	6.58	6.20	5.58
Ethylbenzene	9.00	8.22	7.46
Propylbenzene	13.30	11.78	10.88
Butylbenzene	20.16	17.20	16.06
Benzene	4.96	4.50	4.12
Naphthalene	8.83	7.62	6.70
Anthracene	19.02	15.42	13.06
Pyrene	27.36	21.78	18.81
Chrysene	39.02	29.78	23.10
Perylene	60.34	46.52	34.26
Phenol	2.58	2.38	2.16
Cresol	2.92	2.72	2.48
Ethylphenol	3.48	3.18	3.00
Propylphenol	4.49	4.04	3.88
Butylphenol	6.02	5.30	5.22
Amylphenol	8.42	7.22	7.28
Heptylphenol	18.61	15.06	15.44

Table V. Retention times (min.) of various compounds on Alltech Nucleosil C-18 (150 mm x 4.6 mm I.D.) column. Other conditions are the same as in Table II.

COMPOUND	70% Methanol +	
	No additive	50 mM Brij-30
Benzene	4.94	5.06
Toluene	7.38	7.38
Ethylbenzene	10.46	10.08
Propylbenzene	16.62	14.88
Butylbenzene	27.43	22.54
Benzene	4.98	4.80
Naphthalene	11.03	10.32
Anthracene	32.88	25.24
Pyrene	52.74	35.64
Chrysene	93.88	50.58
Perylene	162.84	68.64
Phenol	2.42	2.70
Cresol	2.98	3.16
Ethylphenol	3.83	3.94
Propylphenol	5.40	5.28
Butylphenol	8.08	7.46
Amylphenol	12.74	10.78
Heptylphenol	35.18	23.60

groups in the phenols and the head groups in those additives that have hydrogen bond formation capability probably takes place in addition to hydrophobic interactions between the hydrophobic parts of analytes and the additives. Both kinds of interactions help to reduce the retention of phenols and improve their separation.

Retention Mechanism

Micelle Formation:

To investigate whether micelles play an important role in accomplishing the improved separations as they do in micellar HPLC, a literature study was performed. It has been reported that acetonitrile and methanol are among the micelle-inhibiting solvents and that they are able to break all micelles down at concentrations above 15-20% and 10-15%, respectively [8-10]. So, it is probable that no micelles exist in our system where at least 39% acetonitrile or 70% methanol was present. In addition, THPA never forms micelles even in pure water because it has a symmetric tetrahedral geometry [9]. Obviously, micelle formation does not contribute to the improved separations.

Retention Characteristics:

The chromatographic behavior of alkyl homologous series is useful for the investigation of retention mechanisms and for the calibration of retention. According to Guiochon *et al.*[11, 12], a linear relationship is generally observed between log k' and the number of carbons in a homologous series in conventional hydro-organic HPLC. This regular increase of retention due to addition of a methylene group is recognized as a measure of hydrophobic interaction in a given RPLC system. In contrast to this typical relationship,

linearity is found between k', not log k', and the number of carbons of a homogolous series in micellar LC when either a purely aqueous micellar mobile phase or a hybrid mobile phase (micellar mobile phase containing organic solvent) is used [13]. In the present system, a linear relationship was found between log k' of alkylbenzenes and carbon number on their side chains with correlation coefficients between 0.998 and 0.999 (Figure 6). For alkylphenols, correlation coefficients between log k' and carbon number on their side chains were between 0.997 and 1.000 (Figure 7). This was true for all of the additives listed in Table I used at different concentrations. This relationship again suggests that the retention mechanism of our system agrees with conventional hydro-organic HPLC instead of micellar LC.

Interestingly, when log k' of benzene, naphthalene, anthracene and chrysene was plotted against the number of fused benzene rings, a linear relationship was also obtained with correlation coefficients between 0.994 and 0.999 (Figure 8). However, upon adding perylene to the series or replacing chrysene with pyrene, the linearity degraded (Figure 9). This is not surprising, considering the effect of molecular shape on the retention as discussed by Sander and Wise [14]. According to their study, the length-to-breath ratio of a PAH molecule is among the most important parameters affecting its retention. For PAH molecules with the same number of benzene rings, the higher the ratio, the greater its retention will be. Our results seem to agree with them perfectly.

Separation Efficiency:

Compared to conventional hydro-organic HPLC, micellar LC suffers from much lower separation efficiency than conventional hydro-organic HPLC. Generally, broad peaks have

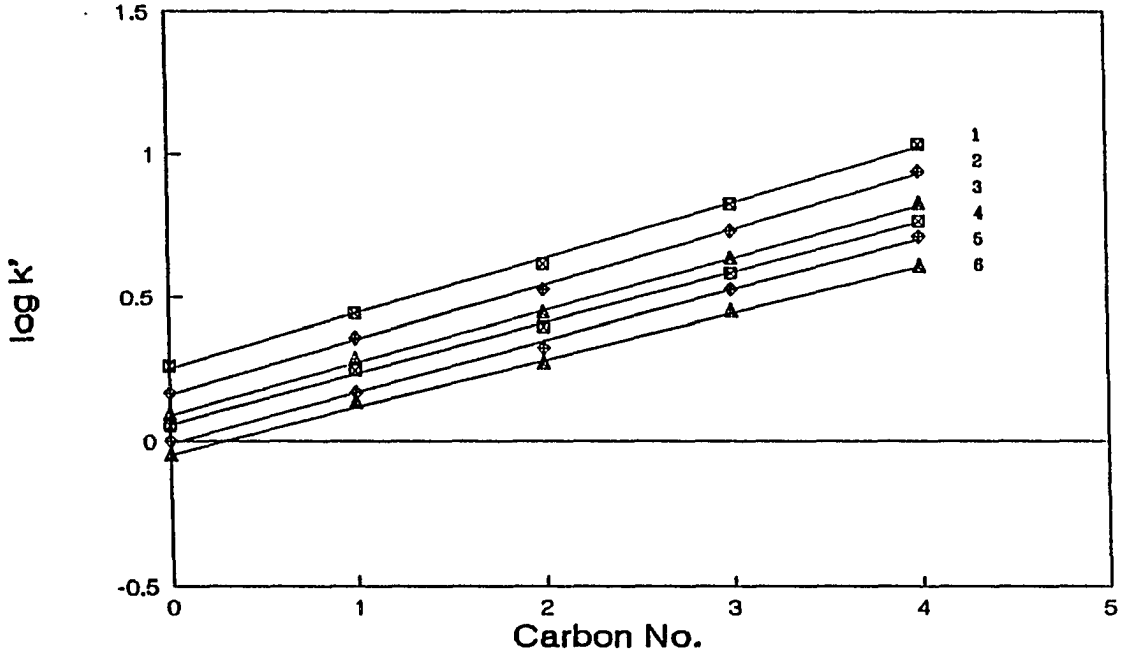


Fig. 6. Linear fits for k' vs. number of carbons on alkylbenzene side chains. symbols, experimental points; lines, linear regression points. (1) 0 mM Brij-30, r^2= 0.999, (2) 5 mM Brij-30, r^2=0.999, (3) 20 mM Brij-30, r^2= 0.999, (4) 35 mM Brij-30, r^2=0.999, (5) 50 mM Brij-30, r^2= 0.998, (6) 100 mM Brij-30, r^2=0.999. Other conditions are the same as in Fig. 1.

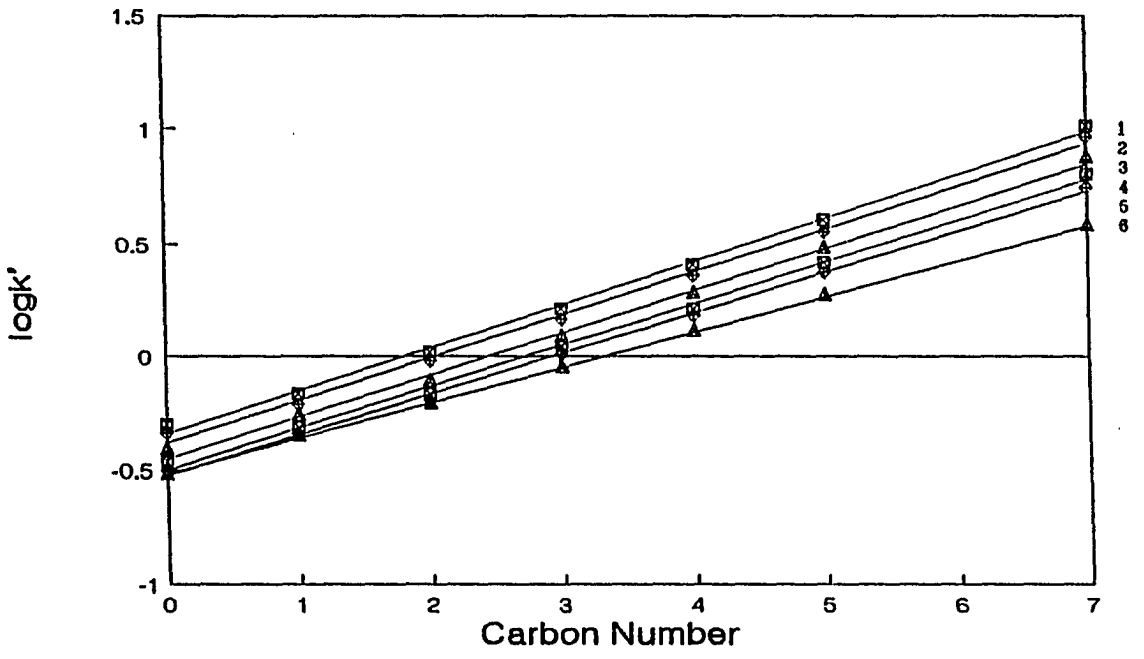


Fig. 7. Linear fits for k' vs. number of carbons on alkylphenol side chains. symbols, experimental points; lines, linear regression points. (1) 0 mM Brij-30, r^2= 0.997, (2) 5 mM Brij-30, r^2=0.997, (3) 20 mM Brij-30, r^2= 0.997, (4) 35 mM Brij-30, r^2=0.997, (5) 50 mM Brij-30, r^2= 0.999, (6) 100 mM Brij-30, r^2=1.000. Other conditions are the same as in Fig. 1.

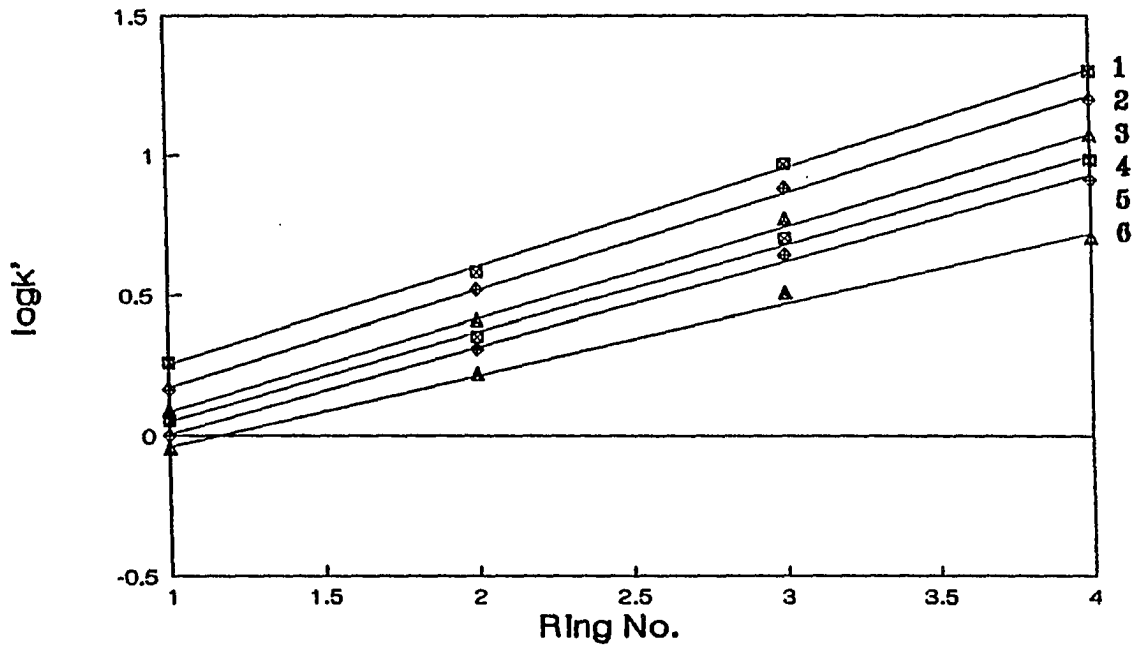


Fig. 8. Linear fits for k' vs. number of fused rings in benzene, naphthalene, anthracene and chrysene. Symbols, experimental points; lines, linear regression points. (1) 0 mM Brij-30, r^2= 0.999, (2) 5 mM Brij-30, r^2=0.999, (3) 20 mM Brij-30, r^2= 0.999, (4) 35 mM Brij-30, r^2=0.998, (5) 50 mM Brij-30, r^2= 0.998, (6) 100 mM Brij-30, r^2=0.994. Other conditions are the same as in Fig. 1.

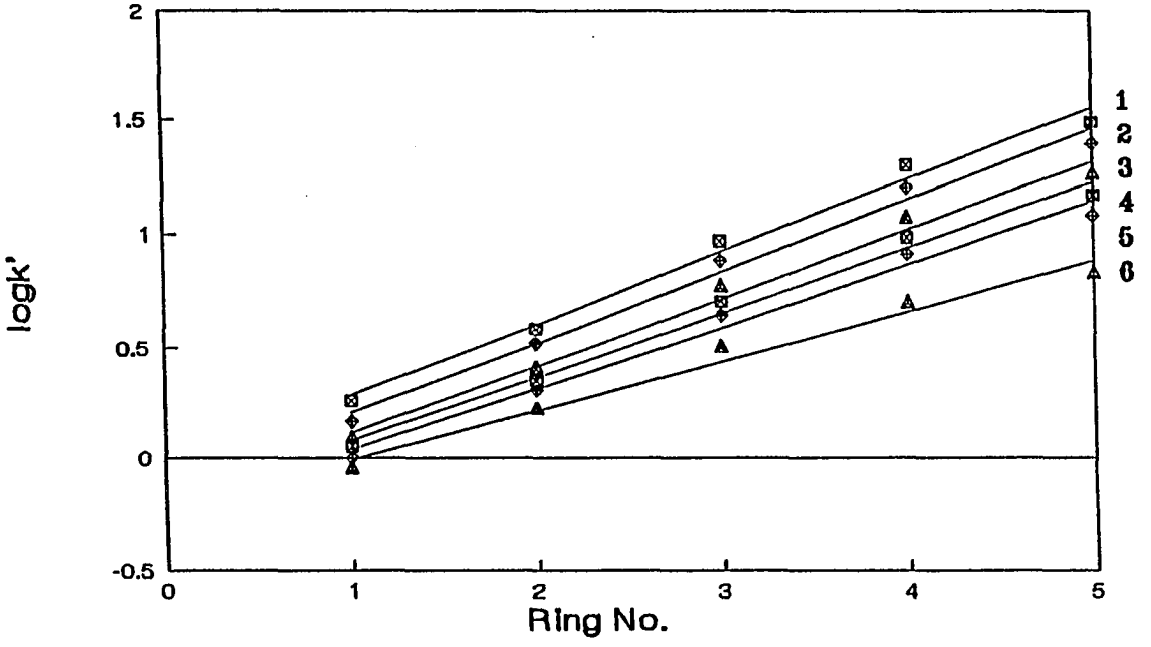


Fig. 9. Linear fits for k' vs. number of fused rings in benzene, naphthalene, anthracene, chrysene and perylene. Symbols, experimental points; lines, linear regression points. (1) 0 mM Brij-30, r^2= 0.989, (2) 5 mM Brij-30, r^2=0.988, (3) 20 mM Brij-30, r^2= 0.989, (4) 35 mM Brij-30, r^2=0.989, (5) 50 mM Brij-30, r^2= 0.986, (6) 100 mM Brij-30, r^2=0.979. Other conditions are the same as in Fig. 1.

resulted with MLC. For example, Hinze *et al.* [7] reported separations of several organic compounds using 50% methanol-water and 0.285 M SDS in water as the eluent. Similar retention times of benzene were obtained in the two cases. But a 75% lower efficiency was obtained in the latter case (N = 1530 compared to N = 6010). This effect was even more dramatic for more hydrophobic solutes such as 2-ethylanthraquinone. This is thought to be the result of slow mass transfer due to (a) adsorption of surfactant molecules in the pores of stationary phase, (b) poor wettability of the stationary phase by the aqueous mobile phase used in MLC, and (c) slow exit rate of analyte molecules from micelles to the bulk aqueous mobile phase.

To study the separation efficiency of our system, 50 mM Brij-30 in 60% acetonitrile was selected as an example with 60% acetonitrile alone as a comparison. With fast chart speed (6 cm/min), each sample peak and dead time marker peak was recorded. Peak width at half maximum, $W_{1/2}$, and half peak width at 10% peak height, A and B, were measured carefully. Peak broadening σ^2 was calculated according to formula $W_{½} = 2.35\sigma$ and the classical plate number, N, was calculated according to: $N = t_o^2(k'+1)^2/\sigma^2$, for each individual compound. By plotting $(1+k')^2$ against σ^2 and taking the slope of it, which is $(k'+1)^2/\sigma^2$, the average classical plate number was calculated from t_o^2/slope. Finally, peak asymmetry B/A of each compound was calculated from measured B and A. The calculated results are shown in Tables VI and VII. Comparison of the results with and without 50 mM Brij-30 indicates that they offer very similar separation efficiencies. Actually, different approaches have been used to improve the separation efficiency MLC. The most common one is using eluent

Table VI. Separation efficiency with 60% ACN/H_2O as eluent on Supelcosil LC-18 (150 mm x 4.6 mm I.D.) column. Average classical plate number N = 6154.

Compound	k'	σ^2 (min^2)	B/A	N
Benzene	1.81	0.00322	1.70	4822
Toluene	2.77	0.00503	1.25	5542
Ethylbenzene	4.14	0.00876	1.40	5915
Propylbenzene	6.71	0.0193	1.30	6036
Butylbenzene	10.78	0.0438	1.20	6220
Naphthalene	3.84	0.00749	1.58	6140
Anthracene	9.33	0.0351	1.27	5964
Chrysene	19.98	0.141	1.16	6132

Table VII. Separation efficiency with 60% ACN/H_2O containing 50 mM Brij-30 as eluent on Supelcosil LC-18 (150 mm x 4.6 mm I.D.) column. Average classical plate number N = 5475.

Compound	k'	σ^2 (min^2)	B/A	N
Benzene	1.09	0.00170	1.06	4633
Toluene	1.58	0.00246	1.26	4857
EthylBenzene	2.24	0.00372	1.26	5063
Propylbenzene	3.55	0.00654	1.11	5692
Butylbenzene	5.43	0.0132	1.15	5629
Naphthalene	2.16	0.00306	1.48	5875
Anthracene	4.67	0.0107	1.23	5387
Chrysene	8.60	0.0304	1.20	5433

containing small percentage of organic solvent such as 3% propanol. However, this method does not improve the separation efficiency when non-ionic surfactant such as Brij-35 is used in the eluent [7]. Besides, the presence of small concentration of organic solvent will break down micelles, which is undesired in MLC. In this aspect, the present system is superior to MLC in that it provides high separation efficiency and no micelle breakdown needs to be worried since the separations are not dependent on the existence of micelles in the mobile phase.

Surface Adsorption:

Since micelle formation is unlikely, the observed additive effect could arise in two ways. First, the additive molecules might be adsorbed on the surface of the stationary phase with the long alkyl or polyoxypropylene chains interacting with the C-18 chains on the stationary phase and the hydrophilic head groups sticking out as described by Morris and Fritz [1], and by Montgomery and Wirth [15]. This would give a more hydrophilic surface and thus reduce the retention times of the analytes. Second, the discrete additive molecules in the mobile phase might interact with analyte molecules by hydrophobic interactions between the analyte molecules and the polyoxypropylene or long alkyl chains of the additives. Hydrogen bonding may play a role when both the analyte and the additive contain potential hydrogen bond formation centers as in alkylphenol separations with Brij-30 or Tween-60 as the additive. To evaluate the first aspect, several experiments were performed.

First, if surface adsorption of the additives exists, the additives should be retained by the stationary phase. However, when a solution of SDS, DOSS, CTAB or THPA was injected

into the system with 60% acetonitrile as the eluent, no apparent retention of any of these compounds was observed. Instead, they came out at almost the same time as the system dead time, which was indicated by the elution time of sodium nitrate. These compounds were detected by a conductivity detector.

Second, a 150-ml solution of 5 mM CTAB in 60% acetonitrile/water was shaken with 2 g of the stationary phase. The mixture was allowed to stand overnight in a tightly closed container, and then was filtered through a Nylon-66 filter of 0.45 μm pore size. The conductivity of both the clear filtrate and the original 5 mM CTAB solution was measured by pumping the solution through a Dionex CDM-3 conductivity detector. No decrease of conductivity was observed. This indicated that no CTAB had been lost from the solution due to the adsorption onto the stationary phase.

As a further check, we observed the retention times of the analyte molecules using 60% acetonitrile/water as mobile phase before and after continuously running mobile phase containing additives. No apparent change in the retention times of the analytes was seen.

When switching the mobile phase to one containing an additive after running without additives, no column re-equilibration time was needed to obtain a stable baseline except a time period of about 0.5 h needed to completely replace the previous eluent left in the system. All of the above suggest that no adsorption of the additive molecules by the surface of the stationary phase occurs.

Thus, the second mechanism is probably correct. In other words, the observed effect of these additives on the retention and separations of the analytes is a result of the interactions

between analyte and discrete additive molecules in the mobile phase. This has also been reported by Jorgenson [3] and Shi and Fritz [16] in CZE. Figure 10 is a cartoon illustration of this mechanism.

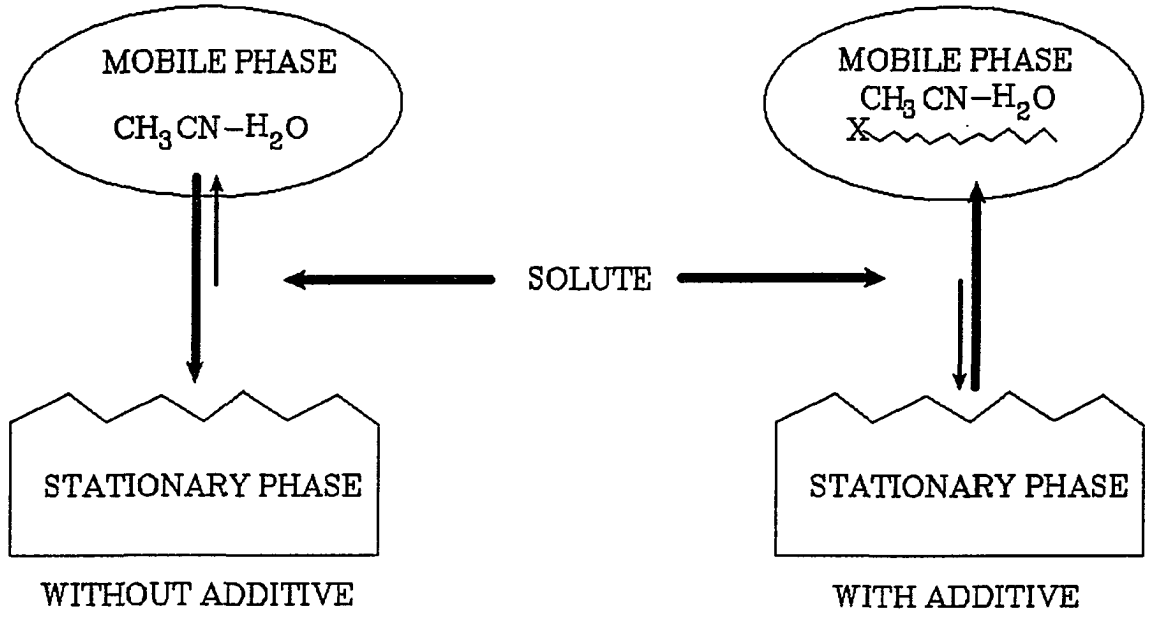
Solvent Strength and Selectivity

Several reports have shown that in micellar LC both solvent strength and selectivity can be controlled by varying the surfactant concentration in mobile phase [6, 7]. This is also true in our system. The capacity factors of various analytes were determined as a function of additive concentration in 60% acetonitrile-water for Brij-30, THPA, DOSS and Tween-60, and in 39% acetonitrile/water for DOSS. Typical plots are shown in Figure 11 for PAH analytes with Brij-30 as the additive. Increasing concentrations of Brij-30 result in progressively lower k' values for all of the analytes, but the magnitude of the decrease in k' varies from one analyte to another. The amount by which k' is decreased for any given analyte depends on the type of additive as well as the concentration of the additive. Numerical values for the difference and ratio of capacity factors with and without additive are shown in Table VIII (D and R). The percent and absolute decrease of k' is generally significantly greater for analytes with larger k' values. Thus, the effect of additives is akin to gradient elution in HPLC, both in percent and in absolute magnitude. Furthermore, gradient elution by varying additive concentration should be possible. Since there is no surface adsorption taking place, gradient elution can be very fast because no re-equilibration of the column would be required.

Selectivity of this type of system on analytes with different functionalities was also

Fig. 10. Pictorial view of the HPLC system using a surfactant as the mobile phase additive.

PICTORIAL VIEW OF THE SYSTEM



* DYNAMIC ASSOCIATION EQUILIBRIUM BETWEEN SOLUTE AND DISCRETE ADDITIVE MOLECULES IN THE MOBILE PHASE

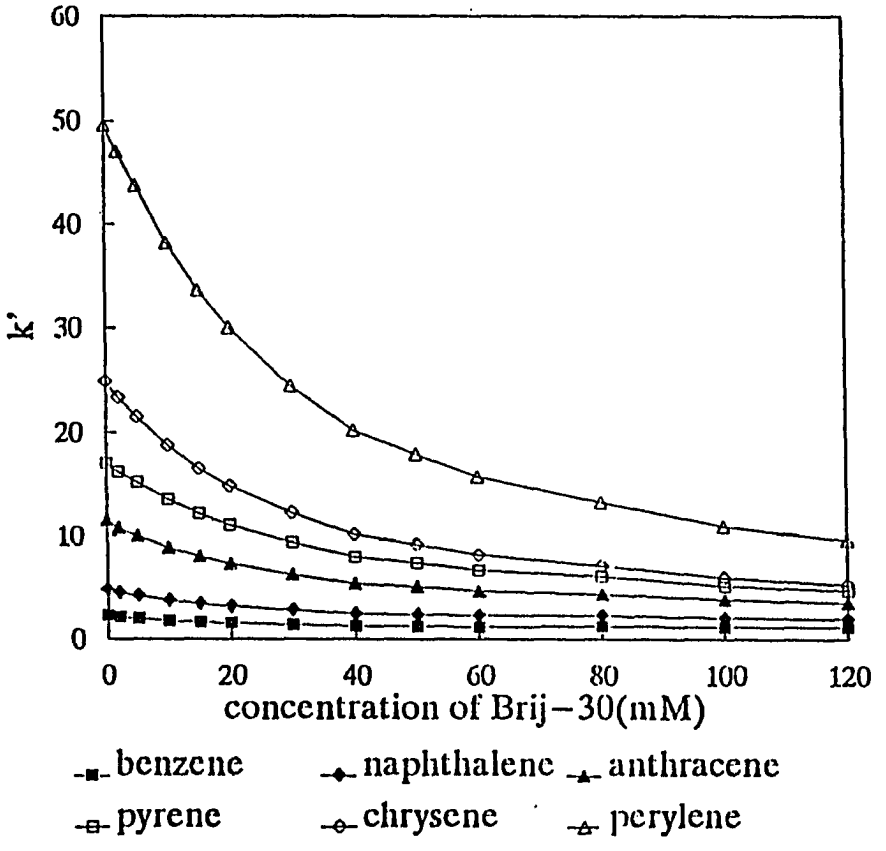


Fig. 11. Effect of Brij-30 on the retention of polycyclic aromatic hydrocarbons. Experimental conditions are the same as in Fig. 1.

Table VIII. Effect of mobile phase additives on the retention of alkylbenzene, polycyclic aromatic hydrocarbon and alkylphenol analytes. Experimental conditions are the same as in Fig. 1, except the eluents are as indicated. D and R: the difference and ratio of capacity factors with and without additives. NA: data not available.

COMPOUND	60% ACN + 40 mM Tween-60		60% ACN + 50 mM Brij-30		60% ACN + 50 mM Pluronic L-31		60% ACN + 50 mM THPA	
	D	R	D	R	D	R	D	R
Benzene	1.75	3.44	0.81	1.81	1.39	2.28	0.76	1.44
Toluene	2.66	3.80	1.30	1.88	2.13	2.43	1.06	1.41
Ethylbenzene	4.25	4.59	2.04	1.97	3.44	2.73	1.83	1.51
Propylbenzene	7.07	5.26	3.36	2.00	5.77	2.94	3.32	1.61
Butylbenzene	11.81	6.22	5.63	2.09	9.65	3.18	6.00	1.74
Benzene	1.75	3.44	0.81	1.81	1.39	2.28	0.76	1.44
Naphthalene	3.82	3.87	1.81	1.89	3.07	2.47	1.98	1.63
Anthracene	9.58	4.39	4.90	2.11	7.56	2.56	6.12	1.98
Pyrene	14.07	4.66	7.21	2.09	10.88	2.55	9.54	2.13
Chrysene	21.05	4.80	11.80	2.44	16.16	2.55	16.18	2.56
Perylene	32.94	5.50	18.97	2.57	24.78	2.60	26.83	3.00
Phenol	0.67	2.50	0.18	1.59	0.59	2.11	0.40	1.55
Cresol	0.85	2.73	0.23	1.50	0.66	2.02	0.36	1.39
Ethylphenol	1.21	3.06	0.40	1.62	1.02	2.31	0.54	1.44
Propylphenol	1.90	3.64	0.61	1.60	1.60	2.58	0.81	1.45
Butylphenol	3.08	4.45	1.04	1.66	2.54	2.78	1.34	1.51
Amylphenol	5.00	5.26	1.67	1.71	4.12	3.00	2.23	1.57
Heptylphenol	14.10	7.62	4.76	1.85	11.47	3.40	7.30	1.82

Table VIII (continued)

COMPOUND	60% ACN + 50 mM SDS		60% ACN + 50 mM DOSS		39% ACN + 40 mM Tween-60		39% ACN + 50 mM DOSS	
	D	R	D	R	D	R	D	R
Benzene	0.92	1.59	0.81	1.49	4.59	3.52	2.31	1.54
Toluene	1.17	1.48	1.12	1.45	9.93	4.89	4.96	1.66
Ethylbenzene	1.94	1.56	1.83	1.51	19.49	6.91	10.28	1.82
Propylbenzene	3.24	1.59	3.03	1.53	41.62	10.63	23.59	2.06
Butylbenzene	5.44	1.63	5.11	1.57	86.51	17.54	52.92	2.36
Benzene	0.92	1.59	0.81	1.49	4.69	3.49	2.31	1.54
Naphthalene	1.64	1.46	1.99	1.63	20.11	6.88	17.51	3.91
Anthracene	3.52	1.40	5.34	1.76	87.67	16.26	81.64	7.94
Pyrene	4.05	1.29	7.67	1.75	NA	NA	NA	NA
Chrysene	6.70	1.34	12.96	1.95	NA	NA	NA	NA
Perylene	8.72	1.28	20.05	1.99	NA	NA	NA	NA
Phenol	0.45	1.67	0.48	1.75	0.82	1.84	0.60	1.50
Cresol	0.43	1.49	0.51	1.65	1.61	2.37	1.14	1.69
Ethylphenol	0.63	1.54	0.78	1.78	3.35	3.22	2.13	1.78
Propylphenol	0.83	1.46	1.16	1.79	7.30	4.74	4.54	1.97
Butylphenol	1.34	1.51	1.78	1.82	15.52	7.41	9.82	2.21
Amylphenol	2.08	1.51	2.84	1.85	32.21	11.92	21.21	2.52
Heptylphenol	5.97	1.58	7.88	1.94	NA	NA	NA	NA

studied. A group of 14 compounds was selected for this purpose. Numerical values for the difference and ratio of capacity factors (D and R) with and without additive are shown in Table IX. The values are generally larger for the compounds with bulkier and more hydrophobic functional groups. However, the values for phenylacetic acid and benzylamine vary greatly from additive to additive probably due to the fact that these two compounds are ionizable while the others are not. When ionic surfactants such as SDS, DOSS and THPA were used as the additives, electrostatic interactions exist between these two analyte compounds and the additives. Secondly, phenylacetic acid and benzylamine contain hydrogen bond formation groups as some of the other compounds. When an additive possessing hydrogen bond formation centers is present, hydrogen bond interaction will exist. Thus, electrostatic interactions, hydrogen bonding as well as hydrophobic interactions must all be taken into consideration in order to explain the"abnormal" behavior of phenylacetic acid and benzylamine. The pH values of all the eluents were measured and listed in Table X for this purpose.

Benzylamine (pKa = 9.33):

When Tween-60, Brij-30 or Pluronic L-31 is used as the mobile phase additive, no electrostatic interaction but only hydrogen bonding between the amine group and the hydroxyl groups in the additives exists since these additives are all non-ionic. Consequently, moderate D and R values result compared to the following cases. When THPA is used as the additive (pH = 3.54), there is electrostatic repulsion between benzylamine and THPA because they both are present in cationic form, and no hydrogen bonding exists. The repulsion will cancel

Table IX. Effect of mobile phase additives on the retention of analytes of different functionalities. Experimental conditions are the same as in Fig. 1, except that the eluents are as indicated. D and R: the difference and ratio of capacity factors with and without additives.

COMPOUND	60% ACN + 40 mM Tween-60		60% ACN + 50 mM Brij-30		60% ACN + 50 mM Pluronic L-31		60% ACN + 50 mM THPA	
	D	R	D	R	D	R	D	R
Phenylacetic acid	0.40	6.33	0.18	1.59	0.07	1.17	-0.66	0.42
Benzyl alcohol	0.45	2.64	0.21	1.79	0.36	1.98	0.20	1.38
Benzyl bromide	0.71	3.61	1.30	1.98	0.60	2.59	0.53	2.16
Benzylamine	1.13	2.38	0.72	2.70	1.43	3.82	-0.64	0.75
Benzyl cyanide	1.31	5.42	0.98	4.45	1.14	3.42	0.79	1.97
Benzyl acetate	1.70	6.28	1.24	3.64	1.49	3.77	0.94	1.87
Benzene	1.75	3.44	0.81	1.81	1.39	2.28	0.76	1.44
Nitrobenzene	2.01	5.50	1.18	3.59	1.77	3.60	1.30	2.12
Benzylacetone	2.01	7.49	0.97	3.27	1.83	4.68	1.21	2.10
Chlorobenzene	2.21	3.08	1.57	1.83	1.60	1.95	0.85	1.35
Phenylacetaldehyde	2.35	9.11	0.40	2.47	2.14	5.27	1.86	3.39
Benzyl chloride	2.91	5.08	1.68	2.40	2.48	3.15	1.82	2.00
Nitrotoluene	3.11	6.78	1.46	2.76	2.77	4.16	2.14	2.42
Bromobenzene	4.24	4.54	1.71	1.77	3.50	2.80	2.69	1.98

Table IX (continued).

COMPOUND	60% ACN + 50 mM SDS		60% ACN + 50 mM DOSS		39% ACN + 40 mM Tween-60		39% ACN + 50 mM DOSS	
	D	R	D	R	D	R	D	R
Phenylacetic acid	0.13	1.39	0.26	2.24	1.09	133.00	0.97	8.31
Benzyl alcohol	0.29	1.67	0.27	1.61	0.21	1.25	0.35	1.49
Benzyl bromide	0.42	1.75	0.51	2.09	0.57	2.01	0.38	1.50
Benzylamine	1.29	2.97	1.23	2.73	1.26	2.16	1.02	1.78
Benzyl cyanide	1.16	3.54	0.98	2.56	1.88	3.10	1.48	2.15
Benzyl acetate	1.37	3.08	1.09	2.17	3.10	4.25	2.23	2.22
Benzene	0.92	1.59	0.81	1.49	4.69	3.52	2.31	1.54
Nitrobenzene	1.51	2.60	1.30	2.14	2.80	3.16	1.94	1.90
Benzylacetone	1.69	3.67	1.40	2.52	3.04	4.42	2.21	2.29
Chlorobenzene	0.83	1.34	0.78	1.31	10.55	4.68	5.42	1.68
Phenylacetaldehyde	2.08	4.76	2.17	5.70	1.47	2.76	1.06	1.85
Benzyl chloride	2.07	2.34	1.96	2.18	8.15	4.62	5.12	1.97
Nitrotoluene	2.50	3.20	2.13	2.41	5.16	4.18	3.40	2.01
Bromobenzene	2.60	1.91	2.56	1.89	13.40	5.20	6.92	1.72

Table X. pH values of the mobile phases used in the chromatographic separations of various organic compounds.

SOLUTION	pH
60% acetonitrile (ACN)	6.76
60% ACN + 40 mM Tween-60	5.87
60% ACN + 50 mM Brij-30	4.34
60% ACN + 50 mM Pluronic L-31	3.41
60% ACN + 50 mM THPA	3.54
60% ACN + 50 mM SDS	6.95
60% ACN + 50 mM DOSS	6.55
39% ACN + 40 mM Tween-60	5.53
39% ACN + 50 mM DOSS	6.83

out the attractive hydrophobic interaction between these two species. Hence, a relatively small additive effect was observed as shown by the small D and R values. On the other hand, in the eluents containing SDS or DOSS (pH < 7), benzylamine is present in cationic form. So, besides the hydrophobic interaction and hydrogen bonding, there also exists electrostatic attraction between these anions and the protonated amine group. So, a relatively larger D and R values result.

Phenylacetic Acid (pKa = 4.28):

Phenylacetic acid exists in mainly neutral form in eluent containing THPA (pH = 3.54), and predominantly anionic form in that containing SDS or DOSS (pH > 6.5). Hydrogen bonding and hydrophobic interactions likely exist between phenylacetic acid and Pluronic L-

31, Brij-30 and Tween-60. Virtually, only hydrophobic interaction exists between it and THPA. Electrostatic repulsion, hydrogen bonding as well as hydrophobic interaction exists between it and SDS or DOSS. The order of the D and R values can probably be explained by taking these interactions into consideration. Of course, these are very rough explanations considering the order of magnitude of each type of interaction should vary from one additive to another.

In short, by choice of additive and/or amount of additive added, the solvent strength and selectivity of our chromatographic system can be varied according to actual needs. Example separations are shown in Figures 12 and 13. In Figure 12, a group of ten compounds with different hydrophobicity and functionality was separated by using 60% acetonitrile alone as the eluent. When 50 mM DOSS was added to the eluent, k' of all the analytes was reduced but to different extents. Most noticeable are peaks 2, 8, 9 and 10. While peaks 2 and 8 move much closer to peaks 1 and 7, and farther away from peaks 3 and 9 respectively, a reversed order of elution resulted for peaks 9 and 10 (Figure 13). This was confirmed by injections of each individual compound.

Determination of Solute-Surfactant Binding Constant

In micellar liquid chromatography, a three-phase equilibrium model relating capacity factor to micellar mobile phase concentration has been proposed and equations have been derived which allow the calculation of binding constants between the solute and the micelle aggregates [6, 17]. A similar approach was found to be valid for the present study. Two equilibria were considered: that of solute in the mobile phase (E_m) combining with stationary

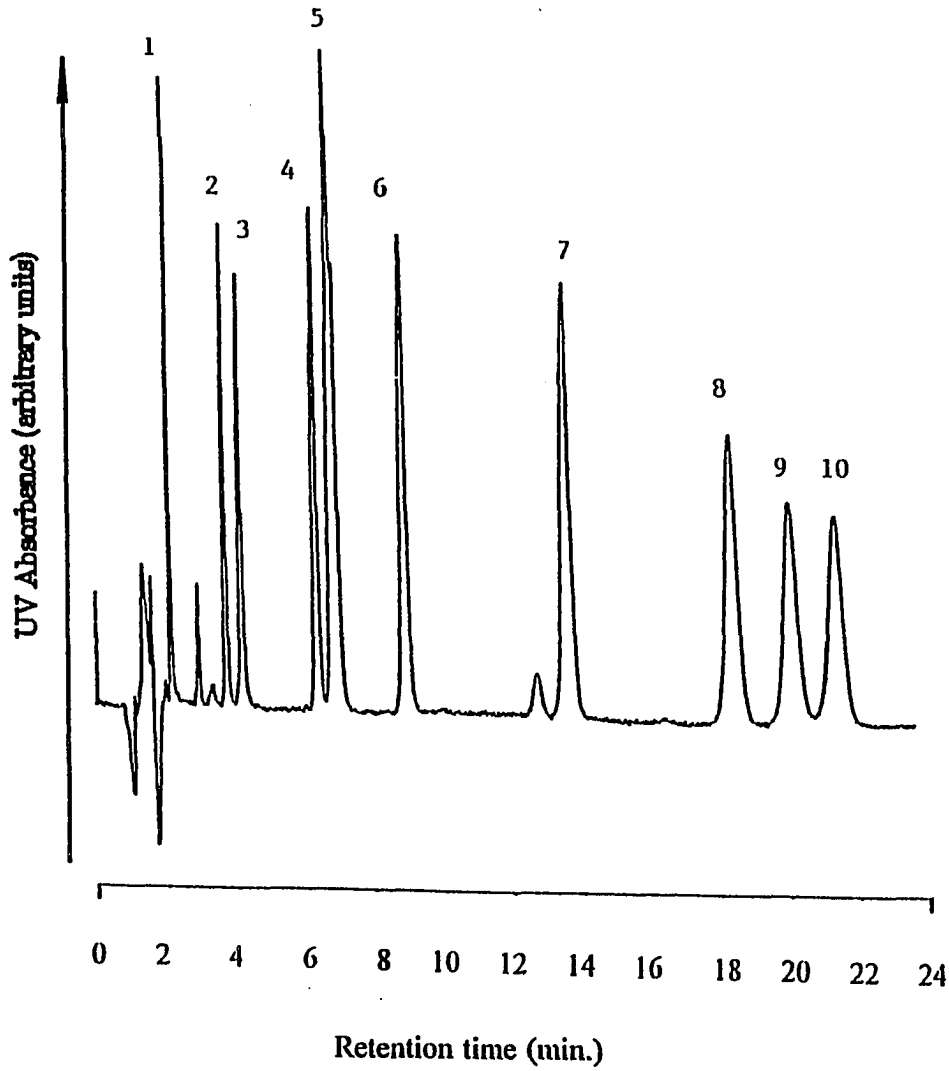


Fig. 12. Chromatographic separation on Supelcosil LC-18 (150 mm x 4.6 mm I.D.) column. Eluent: 60% acetonitrile/water. Flow rate: 1 ml/min. Detection: UV @ 254 nm. Peak identification: 1 = benzyl bromide, 2 = benzyl acetate, 3 =4-n- propylphenol, 4 = toluene, 5 = bromobenzene, 6 = ethylbenzene, 7 = propylbenzene, 8 = anthracene, 9 = butylbenzene, 10 = 4-n-heptylphenol.

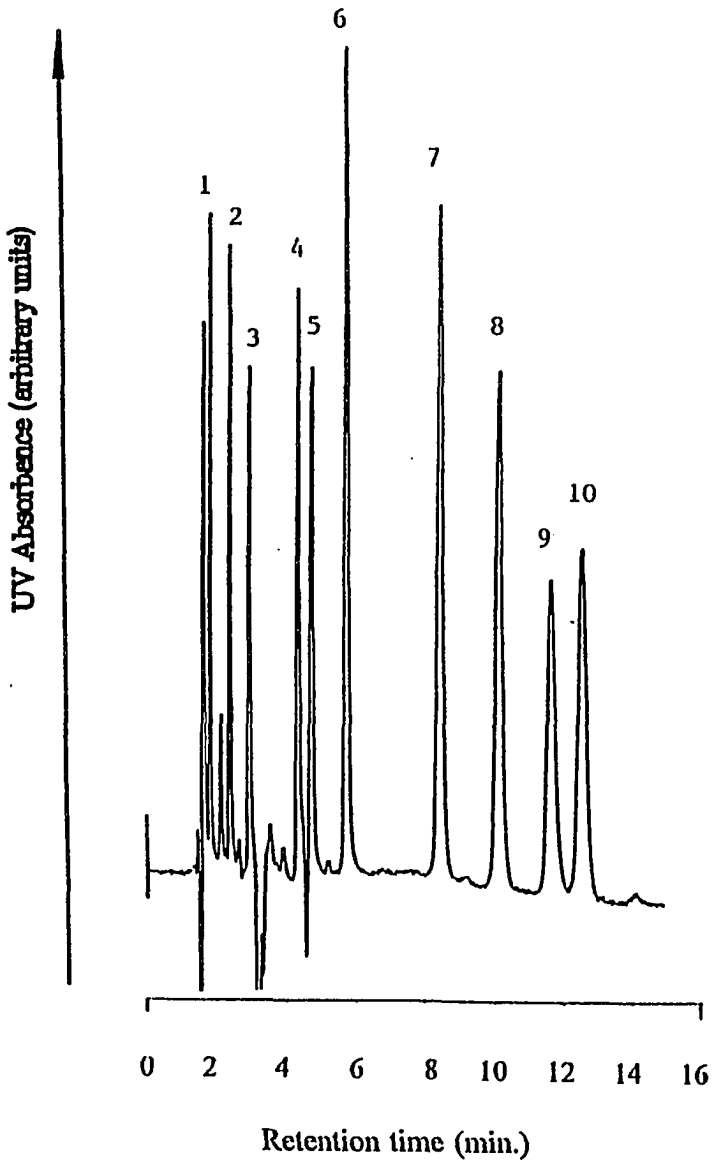


Fig. 13. Chromatographic separation on Supelcosil LC-18 (150 mm x 4.6 mm I.D.) column. Eluent: 60% acetonitrile/water containing 50 mM DOSS. Flow rate: 1 ml/min. Detection: UV @ 254 nm. Peak identification: 1 = benzyl bromide, 2 = benzyl acetate, 3 = 4-n-propylphenol, 4 = toluene, 5 = bromobenzene, 6 = ethylbenzene, 7 = propylbenzene, 8 = anthracene, 9 = 4-n-heptylphenol, 10 = butylbenzene.

phase sites (L_s) and that of solute combining with additive (A_m) to form an association complex.

$$E_m + L_s \overset{K_1}{\rightleftharpoons} EL_s \qquad (1)$$

$$E_m + A_m \overset{K_2}{\rightleftharpoons} EA_m \qquad (2)$$

The equation derived (18) was as follows:

$$\frac{1}{k'} = \frac{[A_m]K_2}{\phi[L_s]K_1} + \frac{1}{\phi[L_s]K_1} \qquad (3)$$

where k' is the capacity factor of the solute and ϕ is the phase ratio. A plot of 1/k' against the concentration of the additive in the mobile phase, $[A_m]$, should be linear. The desired binding constant, K_2, is obtained by dividing the slope by the intercept at $[A_m]$ = zero. The reciprocal of the intercept at $[A_m] = 0$ indicates the affinity of the analyte to the stationary phase when no additive is present in the mobile phase.

It should be pointed out that this treatment assumes 1:1 association between solute and additive. This linear range tends to be limited to the rather low concentrations of additive. This may stem from the fact that any distribution of EA between the stationary and mobile phase is ignored.

Table XI lists the calculated results including the intercepts at $[A_m] = 0$ and binding constants (K) for several additives. The correlation coefficients for linear regression are reasonably good. The calculated binding constants and intercepts follow the expected trend

Table XI. Calculated solute-additive binding constants, K, and the related linear regression results. K is in L/mol. Intercept is at $[A_m] = 0$[1].

COMPOUND	39% ACN + DOSS			60% ACN + Tween-60		
	Intercept	K	Corr. coeff.	Intercept	K	Corr. coeff.
Benzene	0.14	15.44	0.99	0.44	241.28	0.99
Toluene	0.076	16.85	0.99	0.28	311.46	0.9994
Ethylbenzene	0.042	19.55	0.992	0.18	376.84	0.9997
Propylbenzene	0.021	22.49	0.993	0.11	422.42	0.9996
Butylbenzene	0.010	27.05	0.996	0.068	488.00	0.999
Benzene	0.14	15.44	0.99	0.44	241.82	0.99
Naphthalene	0.040	22.52	0.991	0.20	255.50	0.995
Anthracene	0.010	33.13	0.99	0.078	242.59	0.9991
Pyrene	0.0061	36.46	0.98	0.047	291.52	0.965
Chrysene	NA	NA	NA	0.036	168.79	0.995
Perylene	NA	NA	NA	0.022	169.71	0.98
Phenol	0.54	20.45	0.94	0.96	134.86	0.96
Cresol	0.35	20.93	0.96	0.81	162.49	0.98
Ethylphenol	0.20	22.64	0.96	0.59	194.72	0.983
Propylphenol	0.10	24.21	0.96	0.41	227.76	0.98
Butylphenol	0.053	26.58	0.95	0.28	271.27	0.98
Amylphenol	0.027	30.62	0.94	0.18	316.67	0.981
Heptylphenol	0.0066	38.95	0.94	0.067	398.38	0.992

[1] NA: data are not available because the retention times of chrysene and perylene are too long. *: did not detect.

Table XI (continued).

COMPOUND	60% ACN + Brij-30			60% ACN + THPA		
	Intercept	K	Corr. coeff.	Intercept	K	Corr. coeff.
Benzene	0.47	18.64	0.98	0.47	13.22	0.99
Toluene	0.31	19.11	0.985	0.31	12.65	0.99
Ethylbenzene	0.21	21.38	0.99	0.20	13.71	0.97
Propylbenzene	0.13	23.33	0.994	0.13	14.85	0.96
Butylbenzene	0.082	25.85	0.997	0.079	16.98	0.96
Benzene	0.47	18.64	0.98	0.47	13.22	0.99
Naphthalene	0.22	22.89	0.996	0.22	15.24	0.98
Anthracene	0.087	28.67	0.9997	0.09	20.57	0.982
Pyrene	0.058	29.27	0.998	*	*	*
Chrysene	0.039	35.26	0.999	0.041	33.58	0.994
Perylene	0.024	43.64	0.999	*	*	*
				*		
Phenol	1.28	12.54	0.996	*	*	*
Cresol	1.26	13.27	0.992	*	*	*
Ethylphenol	0.70	15.41	0.994	*	*	*
Propylphenol	0.46	17.13	0.999	*	*	*
Butylphenol	0.30	19.68	0.998	*	*	*
Amylphenol	0.19	20.97	0.992	*	*	*
Heptylphenol	0.07	27.29	0.996	*	*	*

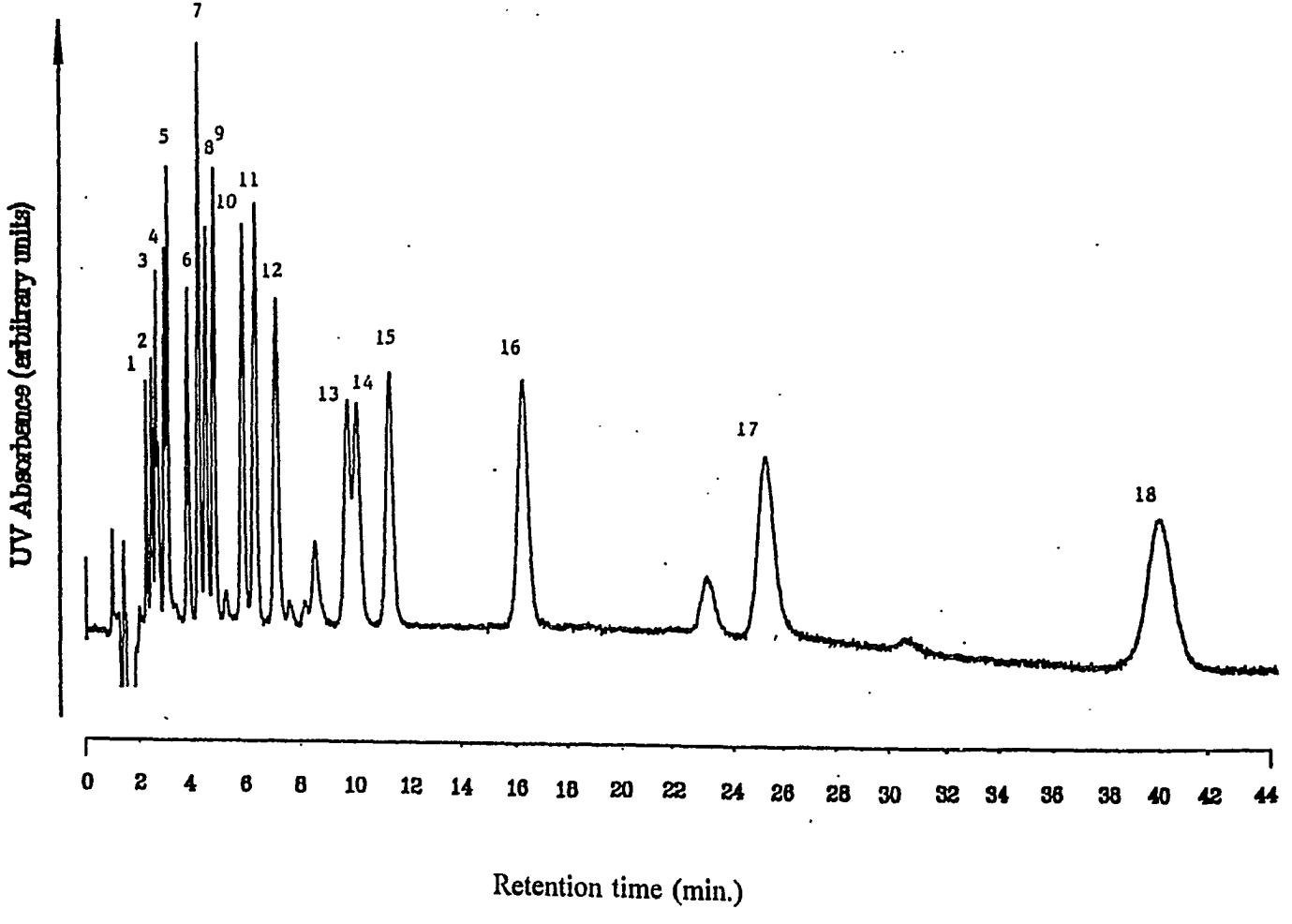
with the bulkier analytes having larger constants and smaller intercepts at $[A_m] = 0$. The intercepts calculated from different additives are the same for each analyte when the concentration of acetonitrile is the same. The values of the binding constants and the intercepts are of course affected by the percentage of acetonitrile in the mobile phase. A decrease in the percentage of acetonitrile should decrease the strength of acetonitrile solvation and lead to higher binding constants for the EA complexes and smaller values of intercepts at $[A_m] = 0$.

Gradient Elution

Gradient elution has been commonly used to separate relatively complicated analyte mixtures. In conventional HPLC, a gradient in the percentage of organic solvent is generally applied since an increase in organic solvent concentration would result in a stronger eluent. The use of mobile phase additives provides similar benefits to conventional solvent gradient elution with a mobile phase of fixed concentration. The additives complex later-eluting analytes more strongly and thereby reduce the retention times by a greater percentage than those of the earlier peaks.

Reduction of analyte retention times was compared using a higher concentration of acetonitrile and by the use of mobile-phase additives. First of all, a separation of eighteen aromatic compounds was obtained using 80% acetonitrile in water as the eluent (Figure 14). The separation took 40 min. While the later peaks are quite far from each other, the early peaks are very crowded, especially, peaks 1, 2 and 3, peaks 4 and 5, and peaks 13 and 14. A decrease of acetonitrile concentration to 70% resulted in a much better separation for the

Fig. 14. Chromatographic separation on Supelcosil LC-18 (150 mm x 4.6 mm I.D.) column. Mobile phase: 80% acetonitrile/water. Flow rate: 1 ml/min. Detection: UV @ 254 nm. Peak identification: 1 = benzophenone, 2 = benzene, 3 = toluene, 4 = naphthalene, 5 = ethylbenzene, 6 = fluorene, 7 = phenanthrene, 8 = anthracene, 9 = butylbenzene, 10 = pyrene, 11 = 2,3-benzofluorene, 12 = chrysene, 13 = benz[e]acephenathrylene, 14 = perylene, 15 = benzo[a]pyrene, 16 = benzo[ghi]perylene, 17 = rubrene, 18 = 3,4,9,10-dibenzopyrene. All the other peaks are impurity peaks.


UV Absorbance (arbitrary units)
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7
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13
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16
17
18
0
2
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6
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12
14
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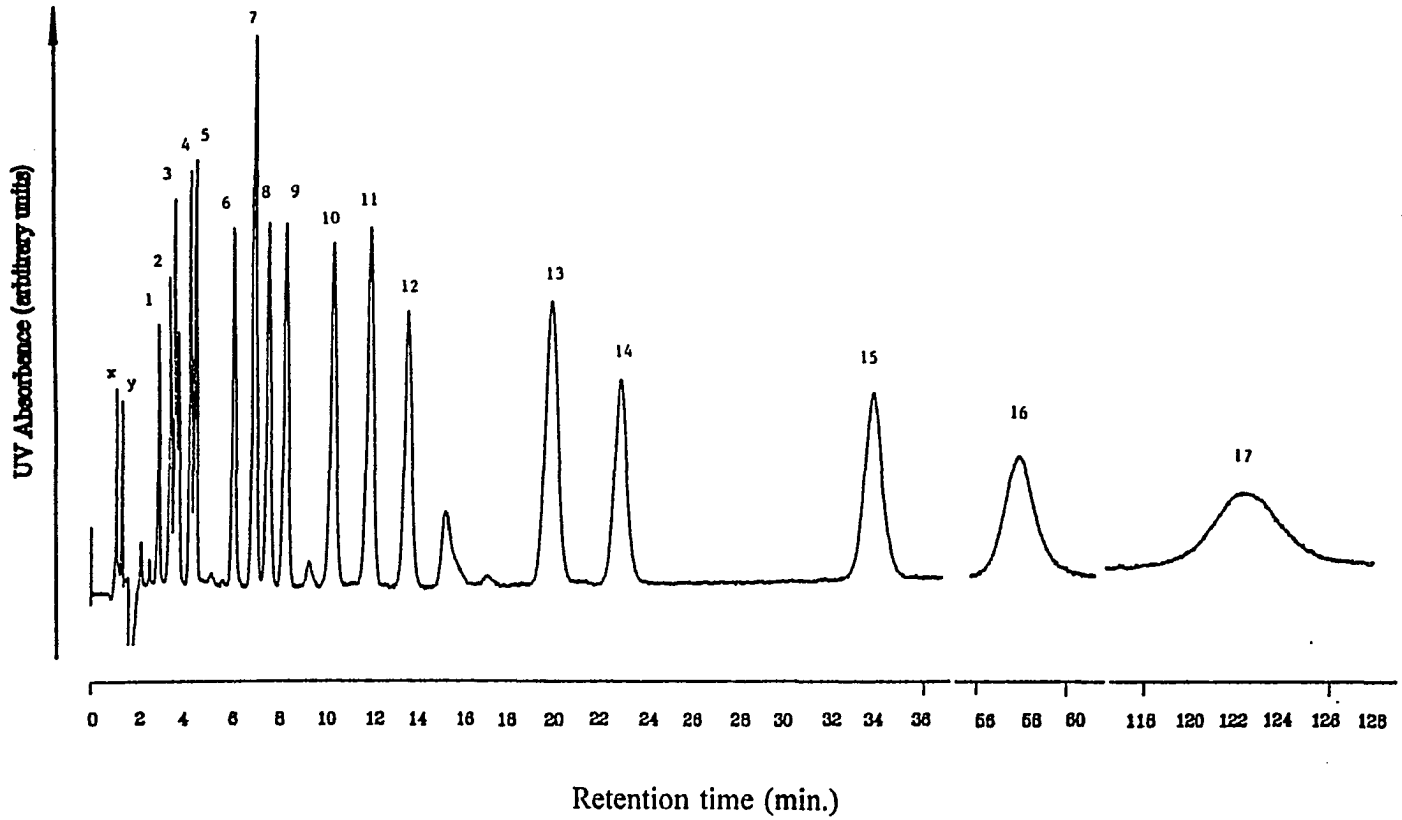
18
20
22
24
26
28
30
32
34
36
38
40
42
44
Retention time (min.)

middle peaks. However, it took more than two hours to elute all eighteen compounds while peaks 2 and 3, 4 and 5 were still not baseline resolved. Besides, benz(e)acephenathrylene and perylene co-elute (Figure 15). With an eluent of 70 mM Brij-30 in 50% acetonitrile, the separation took only around 31 min (Figure 16). Also, the sample peaks were distributed fairly evenly within this time period. This is a noticeable gradient elution feature although no gradient elution was actually used.

CONCLUSIONS

In the present study, a novel type of additive for chromatographic separations of organic compounds is described. This system acts as a bridge between conventional and micellar HPLC in that it uses surfactants as additives as in MLC but does not depend on the presence of micelles to accomplish separations. It offers a better separation window than conventional hydro-organic mobile phases and superior separation efficiency compared to micellar LC. An interaction in solution between the analytes and additives is the basis of the improved separations. Control of solvent strength and selectivity is possible by careful choice of the type of surfactant and concentration. Rapid gradient elution may be possible because no column re-equilibration is required. Binding constants between solute and surfactant molecules in mobile phase can be calculated by relating capacity factor and the surfactant concentration.

Fig. 15. All the conditions are the same as in Fig. 14, except that the eluent is 70% acetonitrile/water. Peaks x and y are the injection peaks and all the other peaks are impurity peaks.


UV Absorbance (arbitrary units)
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y
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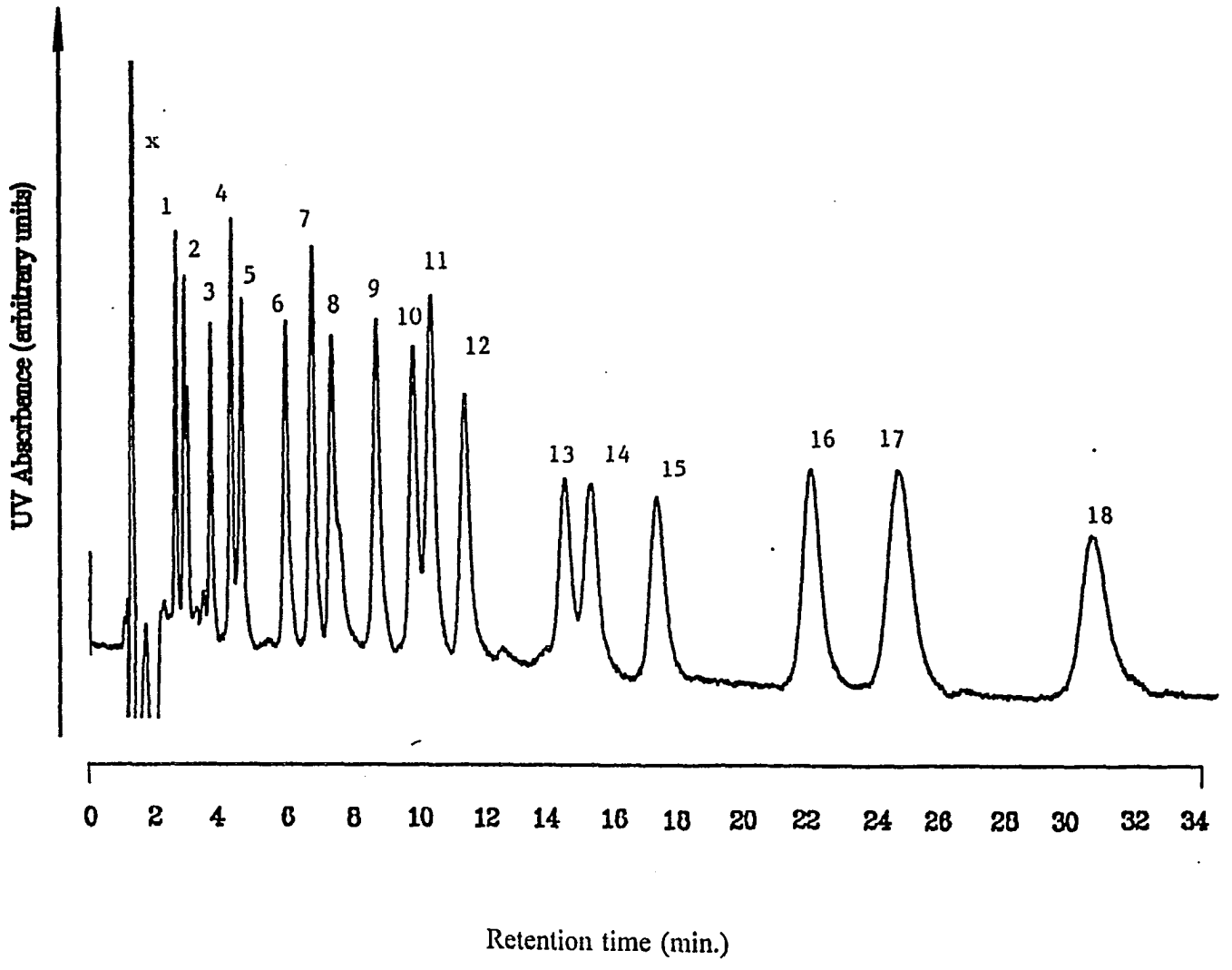
16
17
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 56 58 60 116 120 122 124 126 128
Retention time (min.)

Fig. 16. All the conditions are the same as in Fig. 14, except that the eluent is 50% acetonitrile/water containing 70 mM Brij-30. Peak x is the injection peak and all the other peaks are impurity peaks.


UV Absorbance (arbitrary units)
x
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5
6
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2
4
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8
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18
20
22
24
26
28
30
32
34
Retention time (min.)

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MIXED SURFACTANTS FOR THE SEPARATIONS OF ORGANIC COMPOUNDS BY HPLC

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Xue Li and James S. Fritz
Ames Laboratory, U.S. Department of Energy and
Department of Chemistry, Iowa State University
Ames, Iowa 50011, U.S.A.

ABSTRACT

A low concentration of a surfactant added to a mobile phase containing 60% acetonitrile (+ 40% water) significantly reduces the capacity factors of organic analytes. An even greater reduction in k′ resulted from the use of binary additive systems, particularly those containing a cationic and an anionic surfactant. It is believed that an ion-pair is formed that is more effective in forming solution complexes with organic analytes than either additive alone. Other binary additive systems include mixtures of a neutral and a charged additive or a mixture of two anionic surfactant additives. A ternary mixture of additives is more effective than any of the binary mixtures in modifying the chromatographic behavior of organic solutes

in HPLC.

INTRODUCTION

The idea of using mobile phase additives to improve chromatographic separations has been explored extensively. Addition of a suitable organic modifier results in additional interactions either between analytes and the stationary phase in the case of liquid chromatography, as when a long chain alcohol is used to coat the surface of the stationary phase [1], or between analytes and the mobile phase, as occurs when cyclodextrins are used for chiral separations in both liquid chromatography and capillary electrophoresis [2, 3].

Surfactants have found a wide range of use in analytical techniques [4]. Two important examples are ion pair chromatography (IPC) and micellar liquid chromatography (MLC), in which surfactants are used in the mobile phase of LC systems. In IPC [5, 6], a small concentration of an ion pairing reagent, which has an opposite charge to the ionic solutes, is added to an aqueous mobile phase and its concentration is intentionally kept low in order to avoid formation of micelles. In MLC [7], surfactants are used at micelle-forming concentrations. The popularity of micelle-mediated LC separations has increased since the pioneering work by Armstrong and Henry in 1980 [8]. Despite the advantages of MLC, its column efficiency is inferior to hydro-organic systems, and efficiency deteriorates as the hydrophobicity of solutes increases [7, 9].

Mixed surfactants have been under extensive study by physical chemists for a long time [10-16]. A monograph was devoted entirely to the discussion of mixed surfactant

systems [14]. When an anionic surfactant and a cationic surfactant, such as sodium dodecylsulfate (SDS) and cetyltrimethylammonium bromide (CTAB) are mixed and incorporated into a solution, hydrophobic interactions will exist between their hydrophobic tails and electrostatic interactions between their head groups [10-13]. When an anionic surfactant and a non-ionic surfactant, such as SDS and Brij-30, are mixed and incorporated into a solution, hydrophobic interactions will exist between their hydrophobic tails, ion-dipole interactions between their head groups, and hydrophilic-hydrophilic interactions between the hydrophilic groups which have attached water molecules of hydration [14-16]. These interactions account for the formation of mixed micelles and the deviation from ideality of solution behavior when two surfactants are mixed and incorporated in an aqueous solution. When a solution is made up from three surfactants, cationic, anionic and non-ionic, ternary micelles will be formed as a result of the above interactions.

Mixed surfactant systems have been utilized in liquid chromatography and capillary electrophoresis when the surfactants form as mixed micelles. The formation of mixed micelles was utilized by Sicilia and co-workers for the determination of surfactants [17]. Okada [18] reported the use of mixed micelles formed by ionic and non-ionic surfactants, dodecylsufuric acid and polyoxyethylene (23) dodecyl ether, for the separation of carboxylic acids by ion exclusion chromatography. Co-micellization of sodium dodecylsulfate and chiral surfactants with L-amino acid residues enables chiral recognition of N-acylated amino acid esters by micellar electrokinetic chromatography (MEKC) [19, 20]. Khaledi *et al.* investigated the use of fluorocarbon-hydrocarbon mixed micelles for MEKC [21] separation of small organic

compounds. Mixed fluorocarbon-hydrocarbon anionic surfactants have also been used by Ye, Hadjmohammadi and Khaledi for MEKC separation of small peptides [22]. Mixed SDS and Brij-35 were utilized in MEKC in several studies for the separation of ASTM test mixture [23], for validated pharmaceutical quality control [24], and for the separation of n-alkylphenones and investigation of the possibility of extending the elution window in MEKC [25]. Several other MEKC systems, such as mixed bile salt surfactants and the binary and ternary mixtures of SDS and different bile salts, have also been explored by Khaledi and his colleagues [26]. A higher degree of selectivity control was permitted in these mixed micelle systems because of the presence of additional partition process of analyte compounds.

Surfactants have also been used in primarily aqueous solution for chromatographic separations and also in mobile phases containing high percentage of organic solvent. Recently, Li and Fritz reported a "bridge" system between conventional and micellar liquid chromatographic system [27], in which single surfactants were used as additives without the presence of micelles. It offers a better separation window than conventional hydro-organic mobile phases and superior separation efficiency compared to micellar LC. An interaction in solution between the analytes and additives is believed to be the basis of the improved separations. Solvent strength and selectivity are controlled by careful choice of the type of surfactant and concentration. Rapid gradient elution may be possible because no column re-equilibration is required. Similar dynamic association equilibrium had been proposed by Jorgenson *et al.* between the tetrahexylammonium ion and neutral organic solutes in acetonitrile-water [28], and this association allowed the separation of several neutral organic

compounds by CZE. Shi and Fritz were also able to separate fairly complicated mixtures of neutral aromatic compounds by CZE using buffer solutions containing tetraheptylammonium (THPA) or dioctylsulfosuccinate (DOSS) [29, 30]. Other authors have reported the use of high concentration of organic solvent (e.g., 35-50% acetonitrile) for the separations of steroids [31], n-alkylphenones [32], and Triton X-100 oligomers [33]. However, to the best of our knowledge, mixed surfactants have never been used in separations by reversed phase liquid chromatography.

In the present paper, liquid chromatographic mobile phases containing various binary and ternary mixed surfactants were studied for the first time. Because of the presence of 39-60% acetonitrile/water in the mobile phase, no co-micellization is expected. However, still more powerful mobile phase additives, and hence, stronger eluting solvents have resulted. Unique selectivities compared to single or no surfactant systems were found. Excellent separations of different analyte mixtures containing 18-20 compounds with complex polarities and functionalities were obtained.

EXPERIMENTAL

Chromatographic System

The chromatographic system consists of several components. A Dionex DXP pump (Dionex, Sunnydale, CA, U.S.A.) was used to deliver a flow of 1 ml/min. A 7010 Rheodyne injector (Rheodyne, Berkeley, CA, U.S.A.) delivered 10 μl sample which was detected with a Kratos Spectra flow 783 UV absorbance detector (Kratos Analytical Instrument, Ramsey,

NJ, U.S.A.). Separations were recorded by a Servogor 120 chart recorder (Abb Goerz Instrument, Vienna, Austria). A Supelcosil LC-18 columns (150 mm x 4.6 mm I.D.) was used as the separation column.

Reagents and Chemicals

Acetonitrile was of HPLC grade and used as obtained from Fisher (Fisher Scientific, Pittsburgh, PA, U.S.A.). The mobile phase additives and analyte chemicals were reagent grade and were all used as obtained from Aldrich (Aldrich Chemical Company, Inc., Milwaukee, WI, U.S.A.), J.T.Baker (J.T. Baker Chemical Co., Phillipsburg, NJ, U.S.A.), BASF (BASF Corporation, Parsippany, NJ, U.S.A.) and Sigma (Sigma Chemical Company, St. Louis, MO, U.S.A.). All eluents were prepared daily. Stock solutions were used to prepare all sample solutions by diluting with mobile phase. A Barnstead Nanopure II system (Sybron Barnstead, Boston, MA, U.S.A.) was used to further deionize distilled water for all eluents and sample mixtures.

Chromatographic Procedure

A flow rate of 1 ml/min was selected for all the chromatographic separations. The separation column was equilibrated with mobile phase containing no additives, i.e., 60% or 39% acetonitrile until the baseline was stabilized. Then the desired eluent was used. The baseline was stable after about 0.5 h. Sample injections were made at this point. The eluted species were detected by a UV-vis detector at 254 nm with an output range of 0.010 AUFS.

Capacity factor, k', was calculated according to expression: $k' = (t_r - t_o)/t_o$. The system dead time, t_o, used to calculate capacity factor k', was measured by injecting nitrate solution

into the system. An average of at least three replicates was used to do all the calculations.

RESULTS AND DISCUSSIONS

Single-additive Systems

Capacity factors (k') were measured for alkylbenzenes, PAH compounds and alkylphenols with 60% acetonitrile + 40% water. Capacity factors of these analytes were also measured in 60% acetonitrile containing 50 mM concentrations of a single surfactant as a mobile-phase additive. The results are given in Table I.

Each of the additives reduced the k' values, presumably by forming an association complex with the analytes in the mobile phase. The capacity factors of the larger, more hydrophobic compounds are reduced by a larger percentage than those of the smaller analytes. The effect also varies with the chemical structure of the additive. Brij-30 was generally the most effective of the additives tested. In addition to a long-chain alkyl group ($C_{12}H_{25}$-), Brij-30 has a repeating (n=4) chain of ethoxy groups terminating in a hydroxyl group. This combination of groups may account for the enhanced ability of Brij-30 to form association complexes in solution and thereby lower the capacity factors.

Binary-additive Systems

Cationic-anionic Additive Pairs:

Addition of a lower concentration (25 mM) of each of two additives to the mobile phase was investigated. One of the additives had a positively charged quaternary ammonium group and the other had a negatively charged group (carboxylate or sulfonate). In many cases

Table I. Effect of a single surfactant additive on capacity factors of organic compounds. Separation column is Supelcosil LC-18 (150 mm x 4.6 mm). Eluent is 60% acetonitrile-40% water. Each additive is 50 mM.

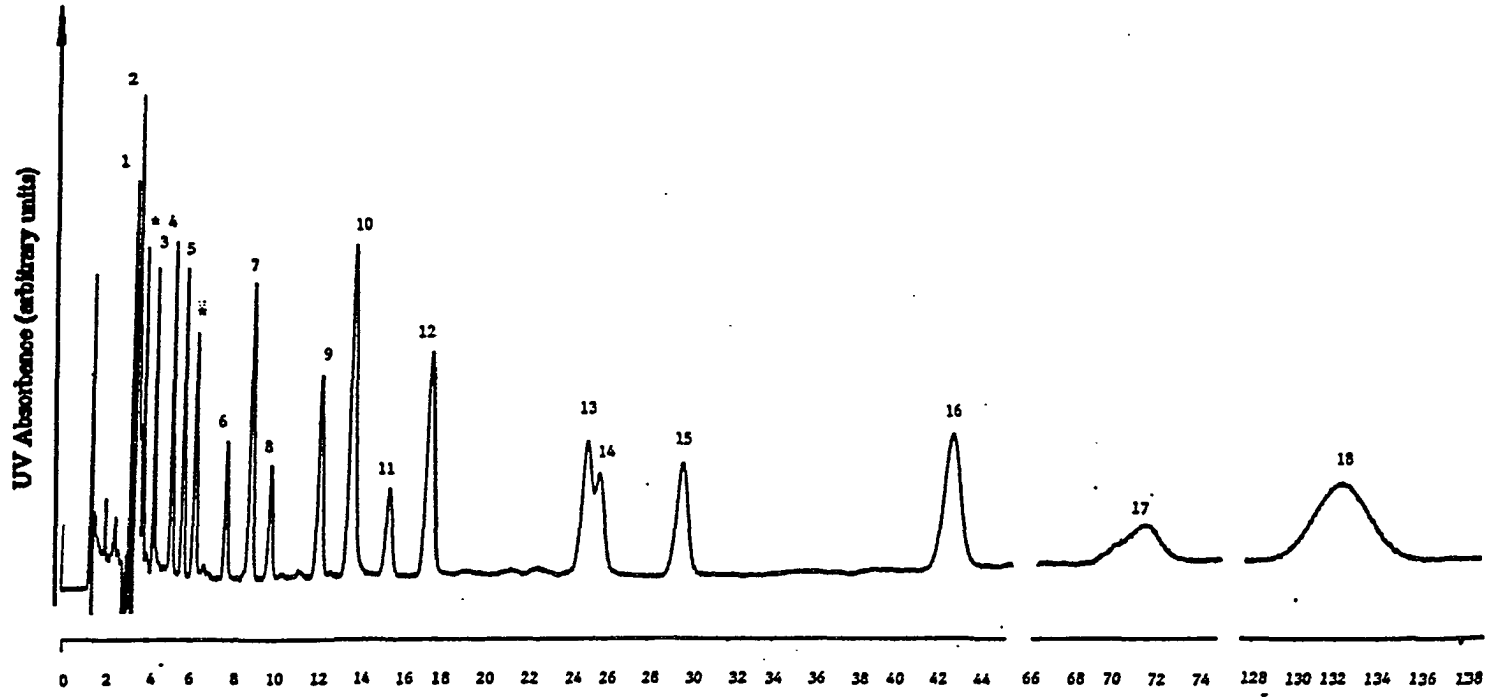
Compounds	Capacity factor, k'						
	No additive	DOSS	SDS	CTAB	THPA	TOMA	Brij-30
Benzene	2.50	1.97	1.97	1.83	2.09	1.73	1.59
Toluene	3.78	3.05	3.03	2.79	3.01	2.77	2.25
Ethylbenzene	5.48	4.39	4.35	4.03	4.25	3.87	3.13
Propylbenzene	8.59	6.93	6.91	6.29	6.59	5.95	4.89
Butylbenzene	13.66	10.95	10.93	9.75	9.83	9.03	7.41
Naphthalene	5.02	3.95	4.53	3.77	4.30	3.13	3.03
Anthracene	11.63	8.63	11.27	8.29	7.73	6.25	6.39
Pyrene	16.78	12.45	17.39	11.95	10.21	8.55	9.47
Chrysene	24.75	16.55	24.73	15.83	12.69	10.41	11.65
Perylene	38.98	24.47	39.11	22.85	16.37	13.97	17.05
Phenol	0.83	0.71	0.83	0.79	0.89	0.63	0.63
Cresol	1.12	0.89	1.07	1.09	1.15	0.83	0.83
Ethylphenol	1.50	1.19	1.47	1.55	1.53	1.21	1.09
Propylphenol	2.16	1.77	2.17	2.33	2.21	1.77	1.61
Butylphenol	3.25	2.71	3.29	3.59	3.19	2.65	2.33
Amylphenol	4.95	4.07	5.11	5.51	4.79	3.95	3.47
Heptylphenol	13.05	10.15	12.79	13.59	10.83	9.19	7.99

the use of two additives resulted in shorter retention times (and lower k′ values) than with a higher concentration of a single additive. Figure 1 shows a chromatographic separation of 18 organic compounds in 60% acetonitrile with 50 mM DOSS as a mobile-phase additive. Peaks 13 and 14 are not resolved and peaks 16, 17 and 18 have very long retention times. Retention times of the various analytes with 50 mM CTAB as the additive are similar to those obtained with DOSS.

In Figure 2 the same separation is shown in 60% ACN but with 25 mM CTAB + 25 mM DOSS as the additives. Now peaks 13 and 14 are well resolved and the retention times of peaks 16-18 are much shorter. The use of two additives has resulted in a very favorable synergistic effect.

The effect of mixed additives on the HPLC behavior of several earlier-eluting analytes is shown in Figure 3 and 4. With 60% ACN containing 25 mM CTAB and 25 mM DOSS as the mobile phase, complete resolution of five analytes was obtained in only 8 min (Fig. 3). With 60% ACN alone, excellent resolution of the sample compounds was obtained but the separation required 18 min (Fig. 4). In addition to the synergistic effect related to this system, different selectivity was observed compared with single or no surfactant systems. In Figure 5A, a group of ten compounds with different hydrophobicity and functionalities were separated by using 60% acetonitrile alone as the eluent. When 50 mM DOSS was added to the eluent, the retention of all the analytes were reduced but to different extents. Most noticeable are peaks 2, 8, 9 and 10. While peaks 2 and 8 move much closer to peaks 1 and 7, and farther away from peaks 3 and 9 respectively, a reversed order of elution resulted for

Fig. 1. Chromatographic separation on Supelcosil LC-18 (150 mm x 4.6 mm I.D.) column. Mobile phase: 60% acetonitrile/water containing 50 mM DOSS. Flow rate: 1 ml/min. Detector: UV @ 254 nm. Peak identification: 1 = benzophenone, 2 = benzene, 3 = toluene, 4 = naphthalene, 5 = ethylbenzene, 6 = fluorene, 7 = phenanthrene, 8 = anthracene, 9 = butylbenzene, 10 = pyrene, 11 = 2,3-benzofluorene, 12 = chrysene, 13 = benz[e]acephenathrylene 14 = perylene, 15 = benzo[a]pyrene, 16 = benzo[ghi]perylene, 17 = rubrene, 18 = 3,4,9,10-dibenzopyrene. Peak x is the injection peak and all the other peaks are impurity peaks.


UV Absorbance (arbitrary units)
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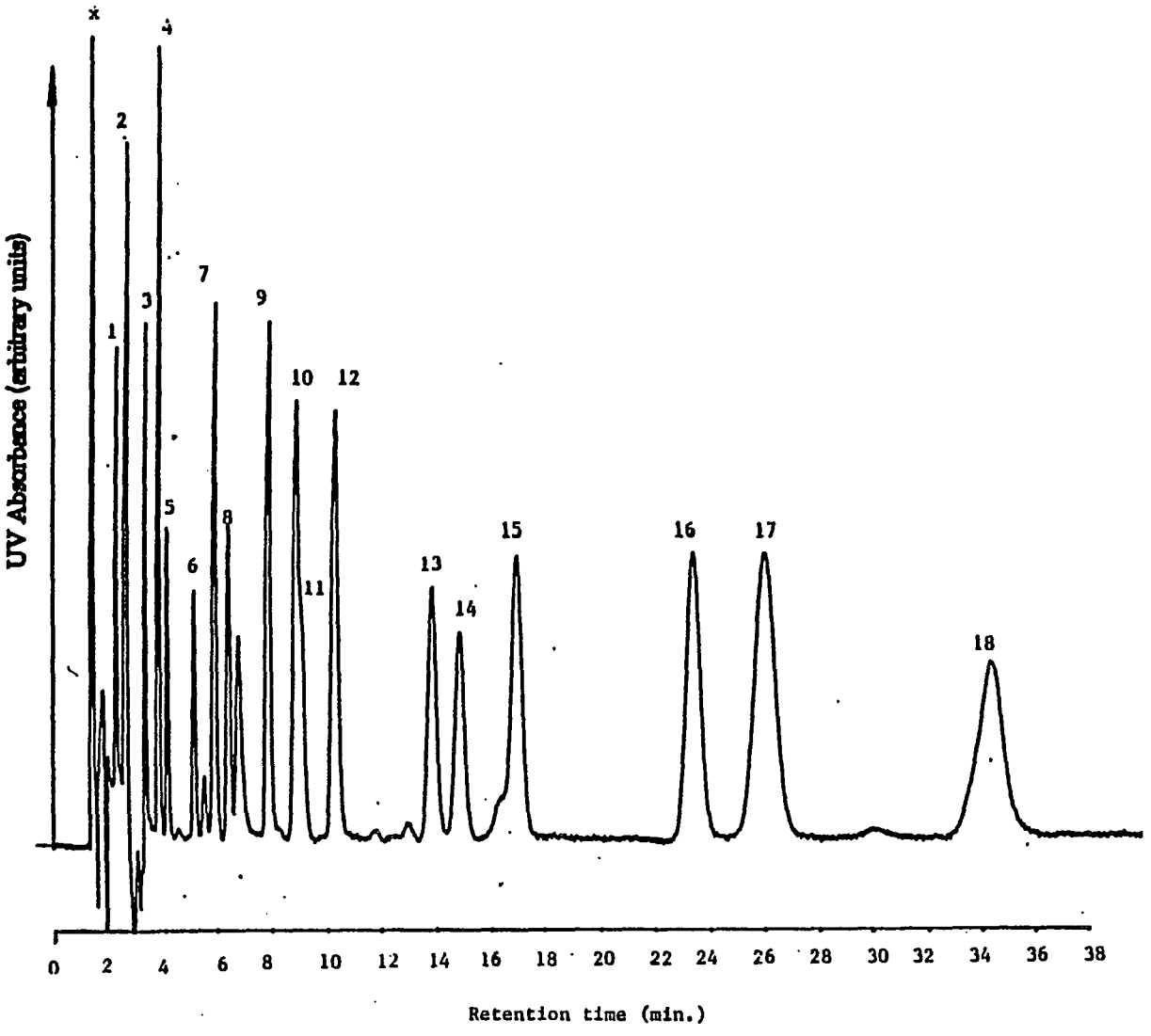
Retention time (min.)

Fig. 2. All conditions are the same as Fig. 1., except that the eluent is 60% acetonitrile/water containing 25 mM DOSS and 25 mM CTAB.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0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
Retention time (min.)

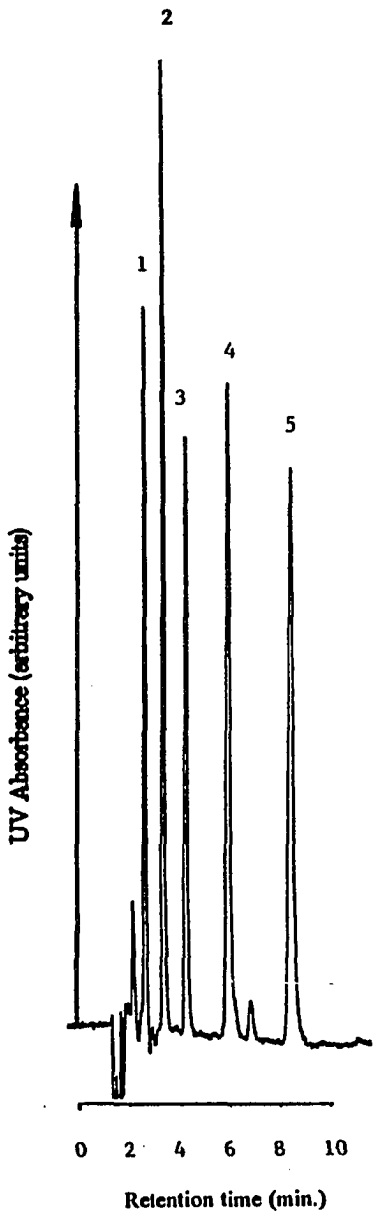


Fig. 3. Chromatographic separation on Supelcosil LC-18 (150 mm x 4.6 mm I.D.) column. Eluent: 60% acetonitrile/water containing 25 mM DOSS and 25 mM CTAB. Flow rate: 1 ml/min. Detector: UV @ 254 nm. Peak identification: 1 = benzene, 2 = toluene, 3 = ethylbenzene, 4 = propylbenzene, 5 = butylbenzene.

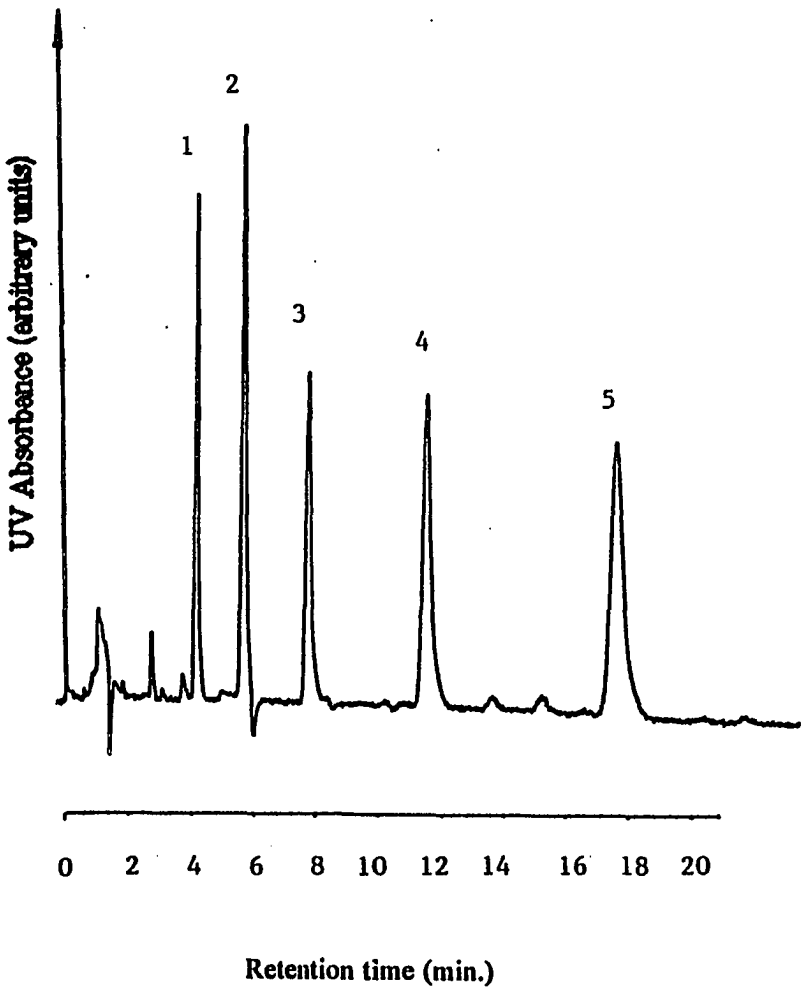
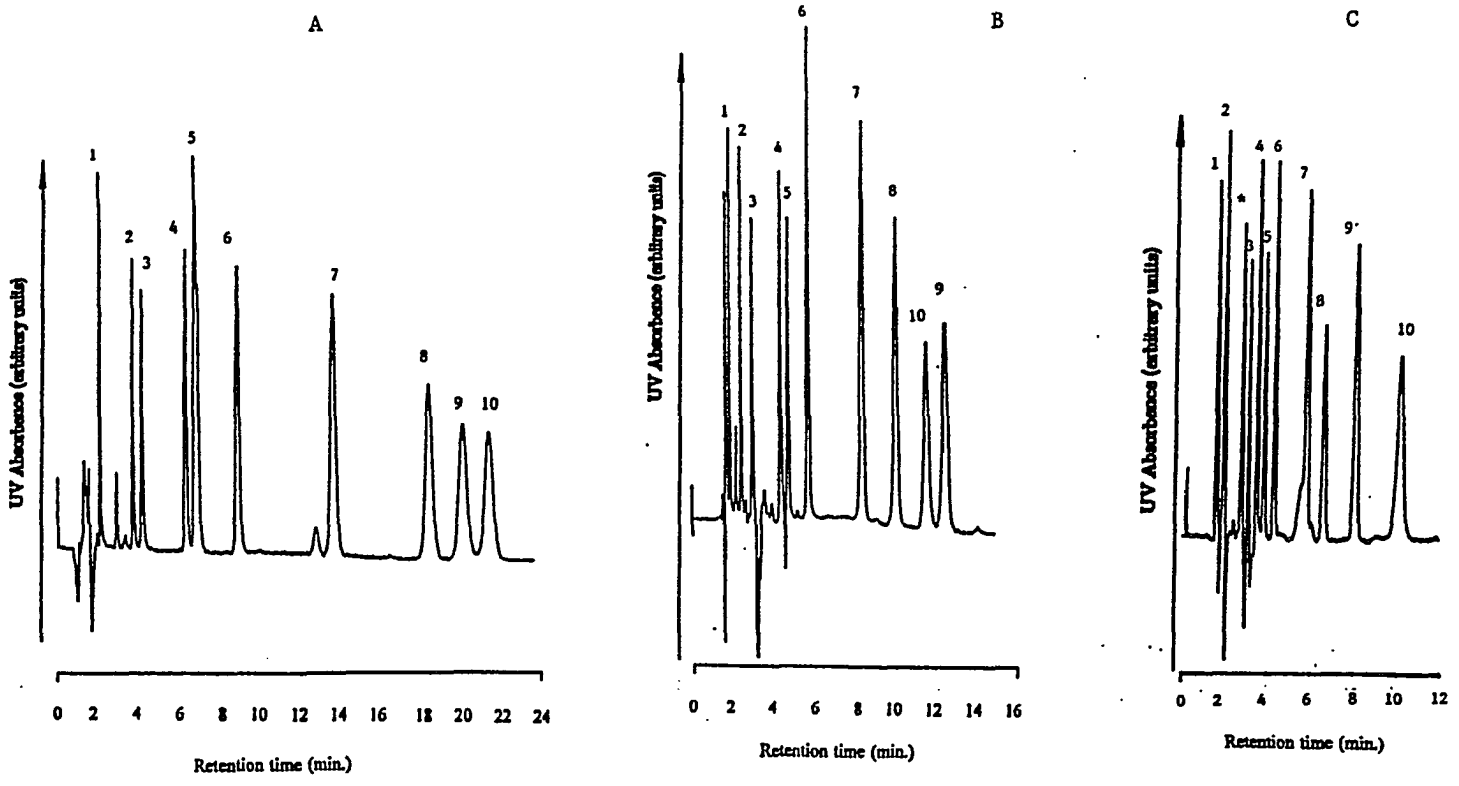


Fig. 4. All conditions are the same as Fig. 3., except that the eluent is 60% acetonitrile/water.

Fig. 5. Chromatographic separation on Supelcosil LC-18 (150 mm x 4.6 mm I.D.) column. Eluent: A: 60% acetonitrile/water, B: 60% acetonitrile/water containing 50 mM DOSS, C: 60% acetonitrile/water containing 25 mM DOSS and 25 mM CTAB. Flow rate: 1 ml/min. Detection: UV @ 254 nm. Peak identification: 1 = benzyl bromide, 2 = benzyl acetate, 3 = 4-n-propylphenol, 4 = toluene, 5 = bromobenzene, 6 = ethylbenzene, 7 = propylbenzene, 8 = anthracene, 9 = butylbenzene, 10 = 4-n-heptylphenol.


A
B
C
UV Absorbance (arbitrary units)
Retention time (min.)
0 2 4 6 8 10 12 14 16 18 20 22 24
0 2 4 6 8 10 12 14 16
0 2 4 6 8 10 12

peaks 9 and 10 (Fig. 5B). When 25 mM CTAB was added to replace 25 mM of the DOSS present in the eluent, retention of all the analytes were reduced further but again to different degrees. Most noticeable is another reversal of the elution order of butylbenzene and heptylphenol (peaks 9 and 10) (Fig. 5C).

Capacity factors were measured for organic analytes in several cationic-anionic binary additive systems. The results are summarized in Tables II-V.

A mixture of CTAB and DOSS was the best two-additive system by far. Capacity factors of most alkylbenzenes and PAH compounds were reduced by >40% compared to either CTAB or DOSS alone. Substantial reductions in k′ of alkyl phenols were also noted.

The additive pairs THPA-DOSS, TOMA-DOSS and THPA-DOSS behaved very similarly, and they produced little, if any, synergistic effects for the majority of the analytes studied. For alkylbenzenes and PAHs the k′ values were similar to or intermediate between the corresponding single-additive system. For alkylphenols these three additive pairs resulted in a very substantial increase in k' compared to the single additive systems (Tables III-V). For example, heptylphenol was eluted later than both pyrene and chrysene in these three binary systems while it has similar retention to butylbenzene in all the other systems attempted. This unique selectivity feature permits a separation of 14 compounds using any of the three eluents (Fig. 6-8).

Figure 6 shows the HPLC separation of several organic compounds using 60% acetonitrile containing 25 mM each of TOMA and DOSS. Figure 7 shows the same separation with 25 mM each of THPA and SDS as additives. The latter separation is a little faster and

Table II. Effect of binary additives (25 mM each) on capacity factors (k') of organic compounds. Experimental conditions are the same as in Table I. Δk' (%) is the percentage change of the capacity factor with two additives (k'_{mix}) from the k' with each additive alone (k'_1 and k'_2). See Table I for k' values with a single additive.

Compounds	k'_{mix} 1 = CTAB 2 = DOSS	Δk' (%) from 1 alone	Δk' (%) from 2 alone
Benzene	1.09	-40	-45
Toluene	1.79	-36	-41
Ethylbenzene	2.51	-38	-43
Propylbenzene	3.93	-38	-43
Butylbenzene	5.91	-39	-46
Naphthalene	2.21	-41	-44
Anthracene	4.79	-42	-45
Pyrene	7.17	-40	-42
Chrysene	8.53	-46	-48
Perylene	12.91	-44	-47
Phenol	0.41	-48	-42
Cresol	0.59	-46	-34
Ethylphenol	0.83	-46	-30
Propylphenol	1.37	-41	-23
Butylphenol	2.13	-41	-21
Amylphenol	3.29	-40	-19
Heptylphenol	8.19	-40	-19

Table III. Effect of binary additives (25 mM each) on capacity factors (k') of organic compounds. Experimental conditions are the same as in Table I. $\Delta k'$ (%) is the percentage change of the capacity factor with two additives (k'_{mix}) from the k' with each additive alone (k'_1 and k'_2). See Table I for k' values with a single additive.

Compounds	k'_{mix} 1 = THPA 2 = DOSS	$\Delta k'$ (%) from 1 alone	$\Delta k'$ (%) from 2 alone
Benzene	2.35	12	19
Toluene	3.29	9	8
Ethylbenzene	4.47	5	2
Propylbenzene	6.41	-3	-8
Butylbenzene	9.17	-7	-16
Naphthalene	4.31	0.2	9
Anthracene	8.43	9	-2
Pyrene	11.61	14	-7
Chrysene	14.33	13	-13
Perylene	19.75	21	-19
Phenol	1.63	83	130
Cresol	2.11	83	137
Ethylphenol	2.79	82	134
Propylphenol	3.97	80	124
Butylphenol	5.75	80	112
Amylphenol	8.37	75	106
Heptylphenol	17.83	65	76

Table IV. Effect of binary additives (25 mM each) on capacity factors (k') of organic compounds. Experimental conditions are the same as in Table I. $\Delta k'$ (%) is the percentage change of the capacity factor with two additives (k'_{mix}) from the k' with each additive alone (k'_1 and k'_2). See Table I for k' values with a single additive.

Compounds	k'_{mix} 1 = TOMA 2 = DOSS	$\Delta k'$ (%) from 1 alone	$\Delta k'$ (%) from 2 alone
Benzene	2.27	31	15
Toluene	3.33	20	9
Ethylbenzene	4.35	12	-1
Propylbenzene	6.23	5	-10
Butylbenzene	9.07	0.4	-17
Naphthalene	4.11	31	4
Anthracene	8.19	31	-5
Pyrene	11.59	36	-7
Chrysene	14.17	36	-14
Perylene	20.17	44	-18
Phenol	1.49	136	110
Cresol	1.95	135	119
Ethylphenol	2.59	114	118
Propylphenol	3.79	114	114
Butylphenol	5.75	117	112
Amylphenol	7.99	102	96
Heptylphenol	17.15	87	69

Table V. Effect of binary additives (25 mM each) on capacity factors (k') of organic compounds. Experimental conditions are the same as in Table I. $\Delta k'$ (%) is the percentage change of the capacity factor with two additives (k'_{mix}) from the k' with each additive alone (k'_1 and k'_2). See Table I for k' values with a single additive.

Compounds	k'_{mix} 1 = THPA 2 = SDS	$\Delta k'$ (%) from 1 alone	$\Delta k'$ (%) from 2 alone
Benzene	2.01	-4	2
Toluene	2.95	-2	-3
Ethylbenzene	3.93	-8	-10
Propylbenzene	5.79	-12	-16
Butylbenzene	8.55	-13	-22
Naphthalene	3.77	-12	-17
Anthracene	8.11	5	-28
Pyrene	11.49	12	-34
Chrysene	14.45	14	-42
Perylene	20.43	25	-48
Phenol	1.57	76	89
Cresol	1.97	71	84
Ethylphenol	2.71	77	84
Propylphenol	3.85	74	77
Butylphenol	5.61	76	70
Amylphenol	8.21	71	61
Heptylphenol	18.07	67	41

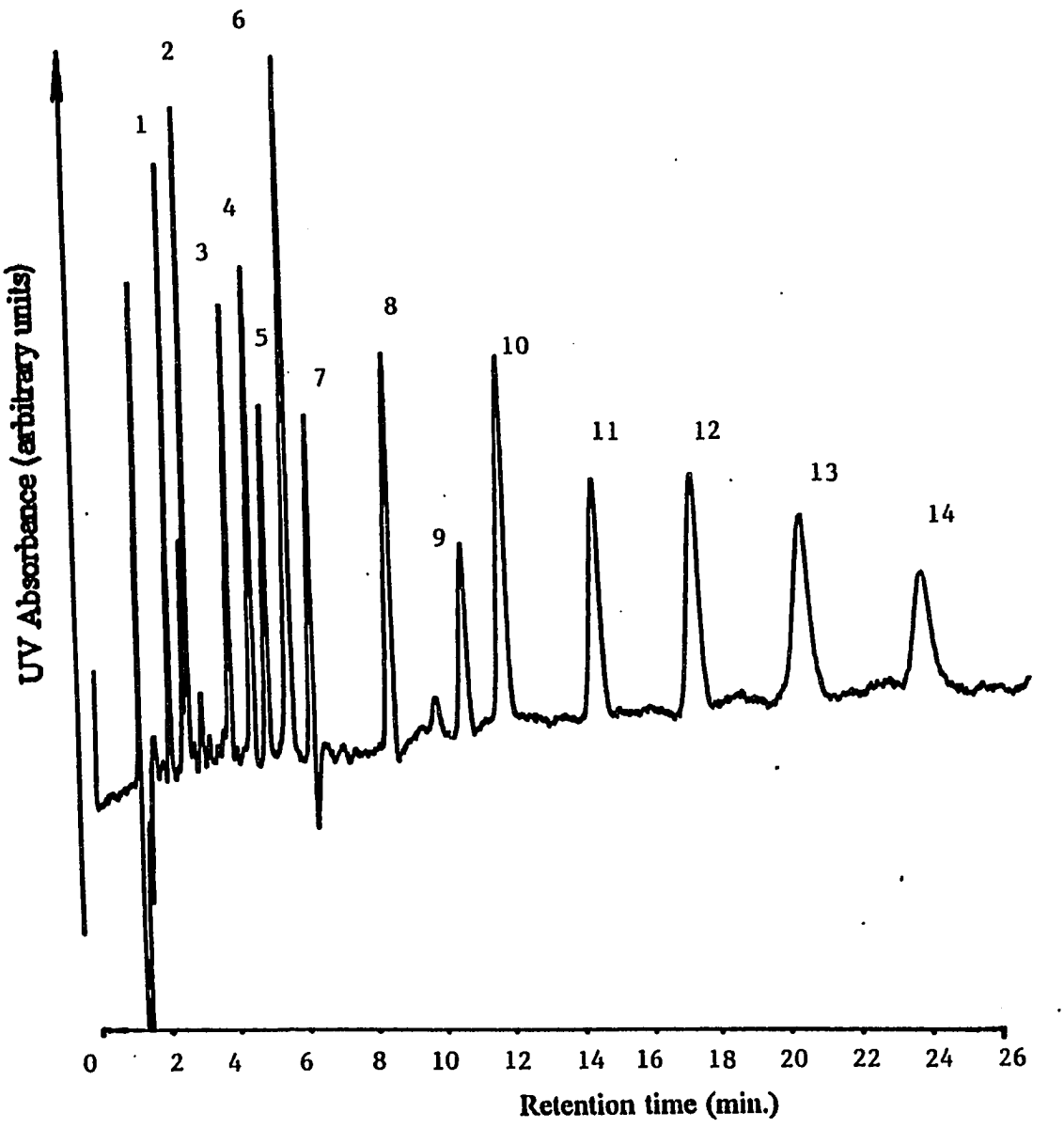


Fig. 6. Chromatographic separation on Supelcosil LC-18 (150 mm x 4.6 mm I.D.) column. Eluent: 60% acetonitrile/water containing 25 mM TOMA and 25 mM DOSS. Flow rate: 1 ml/min. Detection: UV @ 254 nm. Peak identification: 1 = benzylbromide, 2 = benzylacetate, 3 = benzene, 4 = toluene, 5 = bromobenzene, 6 = propylphenol, 7 = ethylbenzene, 8 = propylbenzene, 9 = anthracene, 10 = butylbenzene, 11 pyrene, 12 = chrysene, 13 = heptylphenol, 14 = perylene.

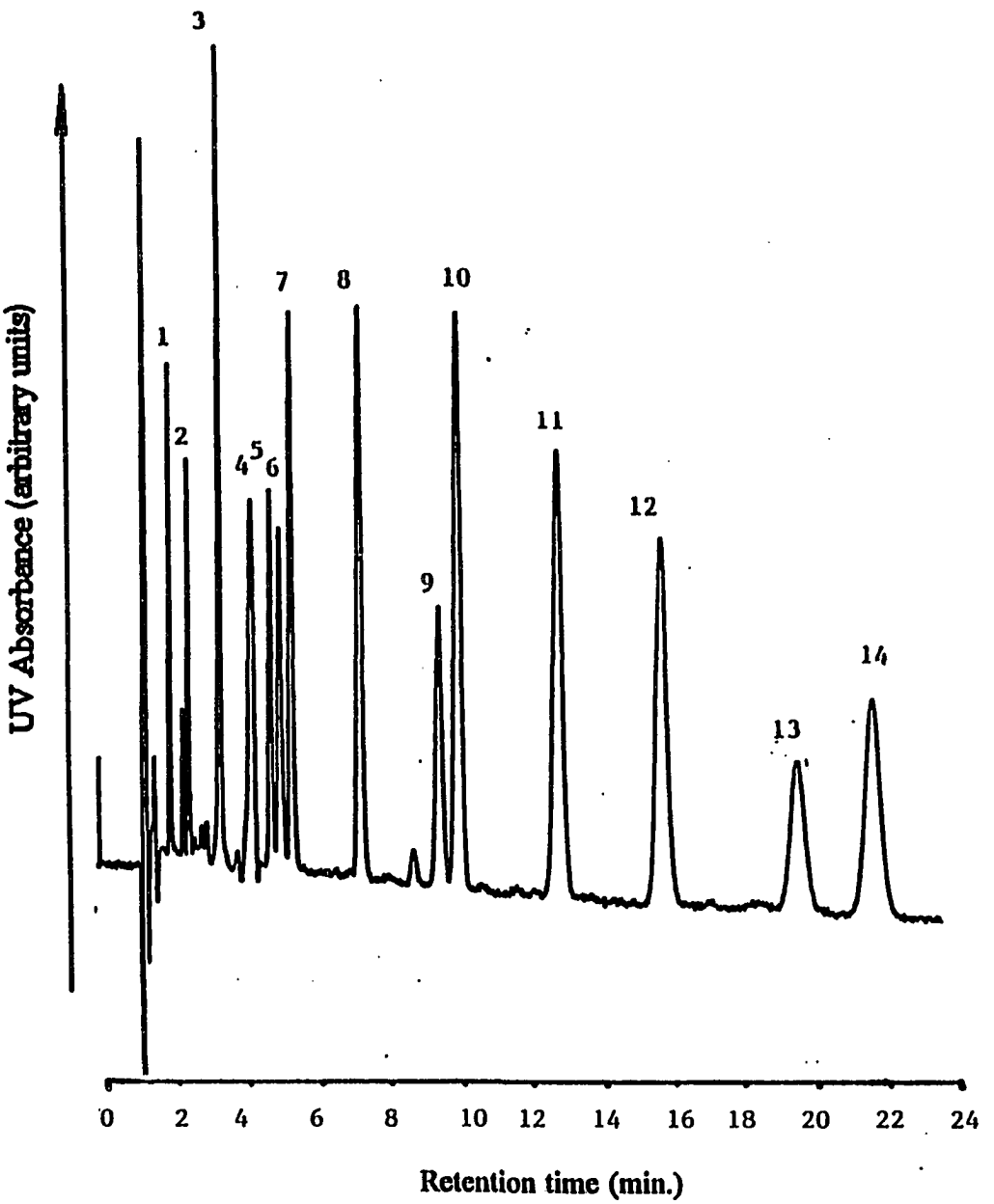


Fig. 7. All the conditions are the same as Fig. 6, except that the eluent is 60% acetonitrile/water containing 25 mM THPA and 25 mM SDS.

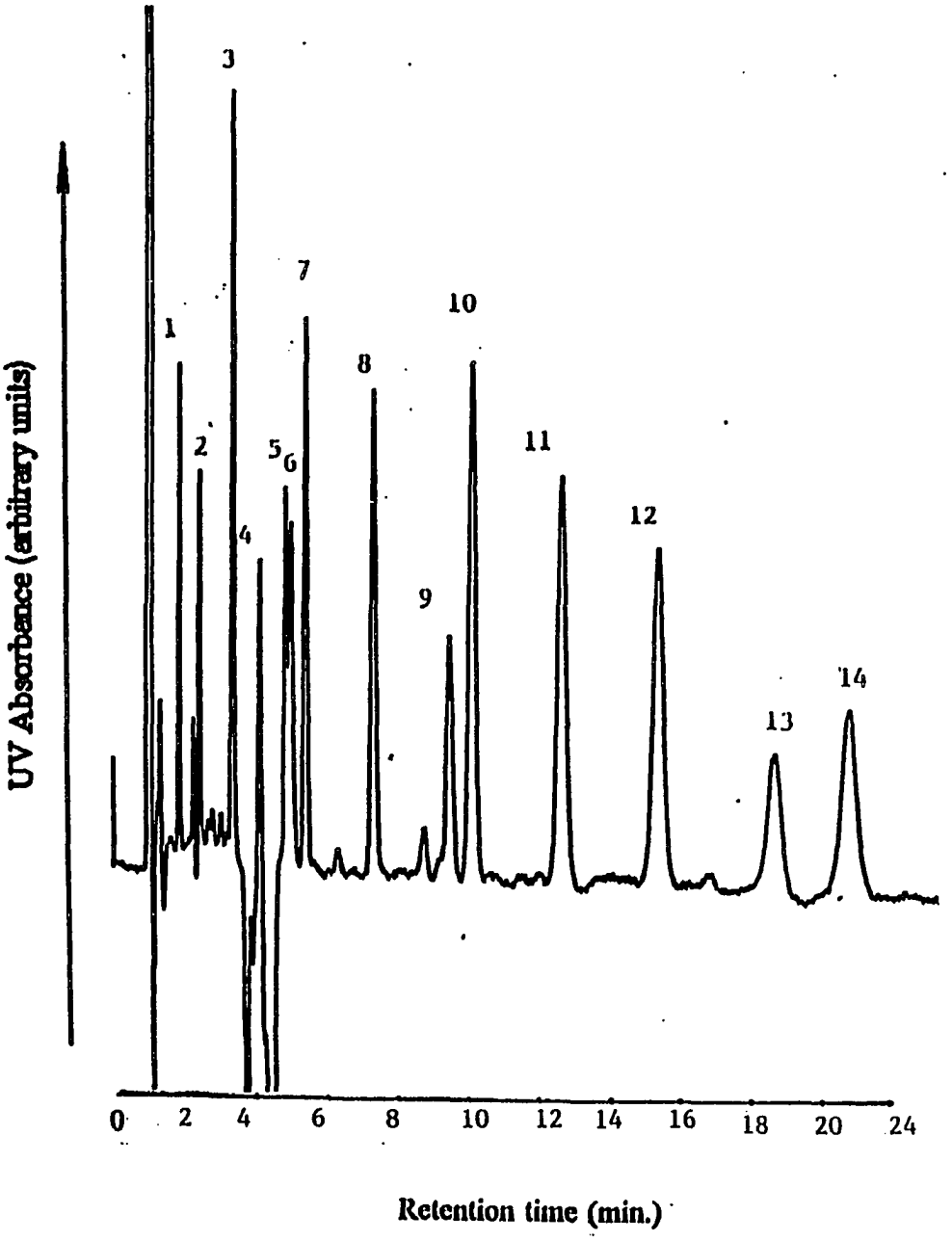


Fig. 8. All the conditions are the same as Fig. 6, except that the eluent is 60% acetonitrile/water containing 25 mM THPA and 25 mM DOSS.

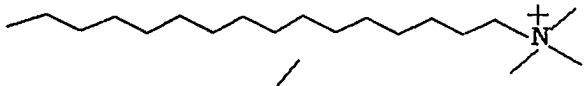
the baseline is flatter. A similar separation was obtained with THPA and DOSS as additives but peaks 5 and 6 were only partially resolved (Fig. 8). It is worth pointing out that when single or no surfactant was added to the eluent, propylphenol (peak 6) always comes out before toluene and bromobenzene (peaks 4 and 5), and heptylphenol (peak 13) always comes out before pyrene and chrysene (peaks 11 and 12).

All of these additive pairs consist of a cationic surfactant and an anionic surfactant. The reason for the synergistic effects observed is believed to be that when an anionic surfactant and a cationic surfactant are mixed, their head groups will be attracted to each other by electrostatic attraction. There will also be hydrophobic attractions between the hydrocarbon chains [9-12]. As a result of these interactions ion pairs will be formed. Each ion pair subsequently interacts with the analyte molecules as if it were a larger and more powerful additive with regard to reducing the retention times of the analytes.

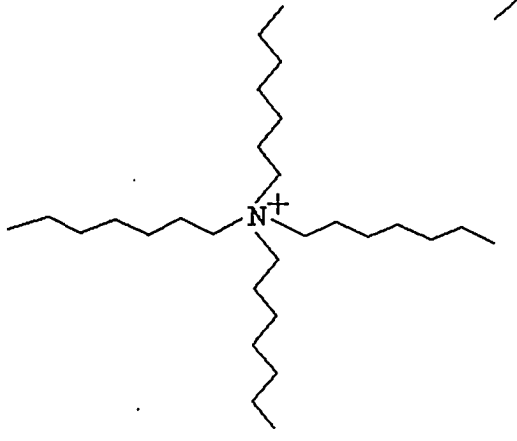
To explain the difference in the behavior of these three systems and CTAB and DOSS, the geometrical structures of the additives used in the mixed surfactant systems were examined (Fig. 9). The structural difference between CTAB and THPA, or CTAB and TOMA is that CTAB has only one long hydrocarbon chain while THPA and TOMA have four or three long hydrocarbon chains. The molecules containing three or four long chains are much bulkier and can sterically hinder the approaching of other molecules. Because of this steric hinderance, SDS or DOSS can not approach THPA or TOMA effectively so that the electrostatic and hydrophobic interactions between them will be far less effective than those between DOSS and CTAB, or SDS and CTAB. In fact, when SDS and CTAB was mixed in

GEOMETRIC STRUCTURE OF MOBILE PHASE ADDITIVES

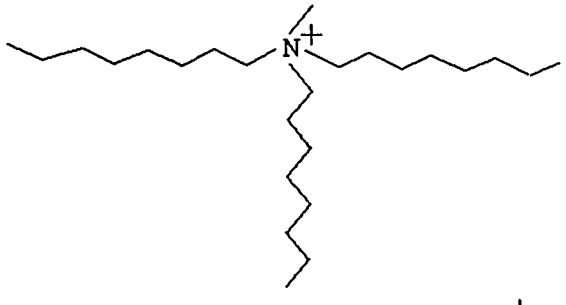
1. Cetyltrimethylammonium



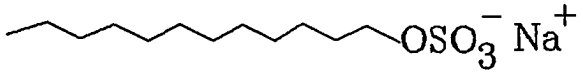
2. Tetraheptylammonium:



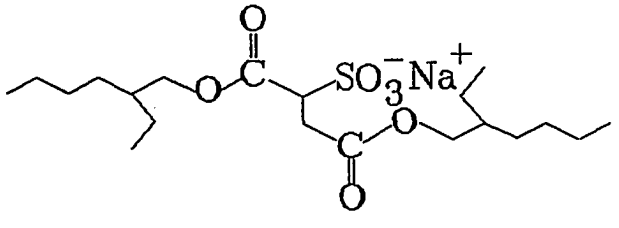
3. Trioctylmethylammonium:



4. Dodecylsulfate



5. Dioctylsulfosuccinate



6. Brij-30

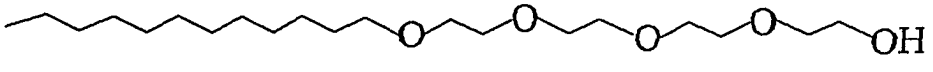


Fig. 9. Geometric structure of mobile phase additives used in the binary and ternary surfactant systems.

60% acetonitrile/water at 25 mM each, a precipitate was formed right away. Considering SDS has only one long chain around the anionic head group and DOSS has two, the steric hinderance of SDS to CTAB will be less than DOSS to CTAB. The approaching of CTAB to SDS was so effective that the interactions between them are very strong resulting in virtually the formation of a larger molecule with neutral charge on it.

DOSS and TOMA alone each decreases the chromatographic retention of phenols compared to retention times in 60% acetonitrile containing no surfactant. But a mixture of DOSS and TOMA increases the k of alkylphenols by an average of >100%. DOSS and THPA behave in a similar manner. Why is the effect of these additive pairs on phenols so much different from DOSS + CTAB which causes a substantial decrease in the retention of phenols? The only obvious difference is that CTAB has a single, long hydrocarbon chain whereas both THPA and TOMA have multiple C_7 or C_8 hydrocarbon chains within their molecules. The more bulky ion pairs that THPA and TOMA form with DOSS may reduce the ability of acetonitrile to solvate the phenolic analytes.

Other Binary Additive Systems:

In addition to the systems containing anionic and cationic surfactants, those containing anionic and non-ionic ones were also studied. No significant synergistic effect was generally observed; instead, the resulting eluent behaves close to that containing only the stronger one of the two additives (Tables VI-VII). This is not surprising considering the ion-dipole interactions between an anionic and non-ionic surfactant are much weaker than the electrostatic interactions between an anionic and cationic surfactant. Nevertheless, Brij-30 and

Table VI. Effect of binary additives (25 mM each) on capacity factors (k') of organic compounds. Experimental conditions are the same as in Table I. Δk' (%) is the percentage change of the capacity factor with two additives (k'_{mix}) from the k' with each additive alone (k'_1 and k'_2). See Table I for k' values with a single additive.

Compounds	k'_{mix} 1 = Brij-30 2 = DOSS	Δk' (%) from 1 alone	k' (%) from 2 alone
Benzene	1.77	11	-10
Toluene	2.68	19	-12
Ethylbenzene	3.83	22	-13
Propylbenzene	5.93	21	-14
Butylbenzene	9.14	23	-16
Naphthalene	3.47	14	-12
Anthracene	7.68	20	-11
Pyrene	11.45	21	-8
Chrysene	14.55	25	-12
Perylene	21.99	29	-10
Phenol	0.73	16	3
Cresol	0.92	11	3
Ethylphenol	1.20	10	1
Propylphenol	1.89	17	7
Butylphenol	2.82	21	4
Amylphenol	4.32	24	6
Heptylphenol	10.34	29	2

Table VII. Effect of binary additives (25 mM each) on capacity factors (k') of organic compounds. Experimental conditions are the same as in Table I. Δk' (%) is the percentage change of the capacity factor with two additives (k'_{mix}) from the k' with each additive alone (k'_1 and k'_2). See Table I for k' values with a single additive.

Compounds	k'_{mix} 1 = Brij-30 2 = SDS	Δk' (%) from 1 alone	Δk' (%) from 2 alone
Benzene	1.53	-4	-22
Toluene	2.36	5	-22
Ethylbenzene	3.38	8	-22
Propylbenzene	5.28	8	-24
Butylbenzene	8.14	10	-26
Naphthalene	3.18	5	-30
Anthracene	7.28	14	-35
Pyrene	10.93	15	-37
Chrysene	14.29	23	-42
Perylene	21.54	26	-45
Phenol	0.63	0	-24
Cresol	0.81	-2	-24
Ethylphenol	1.08	-1	-26
Propylphenol	1.67	4	-23
Butylphenol	2.53	9	-23
Amylphenol	3.88	12	-24
Heptylphenol	9.48	19	-26

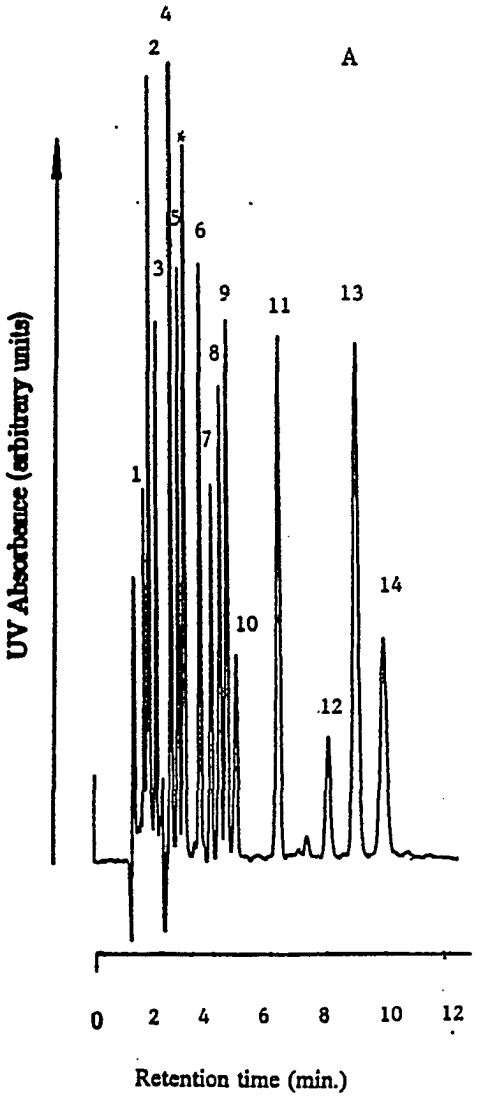
SDS system shows very different selectivity from any other system studied. As a result, a separation of 14 compounds was obtained in 10 min. (Fig. 10A). Separation of this mixture in 60% acetonitrile alone is shown in Figure 10B. It took longer to elute all these 14 compounds from the column with peaks 8 and 9 not completely resolved, and peaks 9 and 10, 13 and 14 co-eluted.

To investigate the behavior of a mixed anionic-anionic surfactant system, a mixture of 25 mM SDS and DOSS was incorporated in 60% acetonitrile. It was found that the resulted eluent behaves very similarly to DOSS, which is a stronger additive than SDS, in terms of the selectivity and the reduction in retention of the analytes (Table VIII). This can be explained by taking the potential interactions between DOSS and SDS into account. There can be three types of interactions: electrostatic repulsion, hydrophobic interactions and hydrophilic-hydrophilic interactions. The net interaction is probably repulsive or zero because of the order of magnitude of these three interactions.

Ternary Additive Systems

Ternary surfactant systems containing DOSS, CTAB and Brij-30 were also investigated. Table IX shows the capacity factors of alkylbenzenes, PAHs and alkylphenols in systems containing 16.7 mM each of the three surfactants, and the change of the capacity factors from the systems containing any of the three surfactants at 50 mM or containing CTAB + DOSS at 25 mM each. Synergistic effect was observed again in the ternary system compared to the single surfactant systems. A stronger solvent resulted by replacing a third of one additive by a second additive, and another third by the third one. As a result, the

Fig. 10. Chromatographic separation on Supelcosil LC-18 (150 mm x 4.6 mm I.D.) column. Mobile phase: A: 60% acetonitrile/water containing 25 mM Brij-30 and 25 mM SDS, B: 60% acetonitrile/water. Flow rate: 1 ml/min. Detection: UV @ 254 nm. Peak identification: 1 = benzyl cyanide, 2 = benzyl acetate, 3 = methyl benzoate, 4 = 4-bromo-1-nitrobenzene, 5 = phenetole, 6 = chlorobenzene, 7 = bromobenzene, 8 = naphthalene, 9 = ethyl benzene, 10 = amylphenol, 11 = propyl benzene, 12 = anthracene, 13 = butyl benzene, 14 = heptylphenol, * = impurity from anthracene.


A
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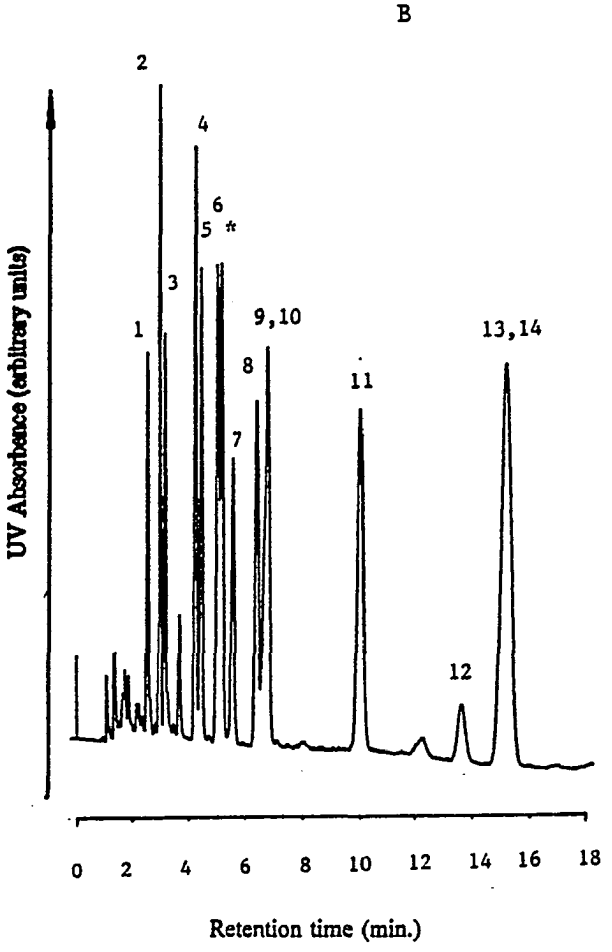
Retention time (min.)


B
UV Absorbance (arbitrary units)
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0 2 4 6 8 10 12 14 16 18
Retention time (min.)

Table VIII. Effect of binary additives (25 mM each) on capacity factors (k') of organic compounds. Experimental conditions are the same as in Table I. $\Delta k'$ (%) is the percentage change of the capacity factor with two additives (k'_{mix}) from the k' with each additive alone (k'_1 and k'_2). See Table I for k' values with a single additive.

Compounds	k'_{mix} 1 = DOSS 2 = SDS	$\Delta k'$(%) from 1 alone	$\Delta k'$ (%) from 2 alone
Benzene	2.09	6	6
Toluene	3.21	5	6
Ethylbenzene	4.54	3	4
Propylbenzene	7.11	3	3
Butylbenzene	11.05	1	1
Naphthalene	3.92	-1	-13
Anthracene	8.74	1	-22
Pyrene	12.48	0.2	-28
Chrysene	16.91	2	-32
Perylene	24.83	1	-36
Phenol	0.72	1	-13
Cresol	0.95	7	-11
Ethylphenol	1.22	2	-17
Propylphenol	1.81	2	-17
Butylphenol	2.80	3	-15
Amylphenol	4.19	3	-18
Heptylphenol	10.52	4	-18

Table IX. Effect of ternary additives (16.7 mM each) on capacity factors (k') of organic compounds. Experimental conditions are the same as in Table I. $\Delta k'$ (%) is the percentage change of the capacity factor with three additives (k'_{mix}) from the k' with each additive alone (k'_1, k'_2 and k'_3) or from k' with CTAB + DOSS at 25 mM each . See Tables I and II for k' values with single and binary additives.

Compounds	k'_{mix} 1 = CTAB 2 = DOSS 3 = Brij-30	$\Delta k'$ (%) from 1 alone	$\Delta k'$ (%) from 2 alone	$\Delta k'$ (%) from 3 alone	$\Delta k'$ (%) from 2 + 3
Benzene	0.98	-46	-50	-38	-10
Toluene	1.45	-48	-52	-36	-19
Ethylbenzene	1.95	-52	-56	-38	-22
Propylbenzene	2.91	-54	-58	-40	-26
Butylbenzene	3.94	-60	-64	-47	-33
Naphthalene	1.91	-49	-52	-37	-14
Anthracene	4.36	-47	-49	-32	-9
Pyrene	6.59	-45	-47	-30	-8
Chrysene	9.42	-40	-43	-19	10
Perylene	14.45	-37	-41	-15	12
Phenol	0.61	-23	-14	-3	49
Cresol	0.64	-41	-28	-23	8
Ethylphenol	0.95	-39	-20	-13	14
Propylphenol	1.25	-46	-29	-22	-9
Butylphenol	1.79	-50	-34	-23	-16
Amylphenol	2.52	-54	-38	-27	-23
Heptylphenol	5.73	-58	-44	-28	-30

separation of alkylbenzenes in Figures 3 and 4 only takes about 5.3 min, with all of them still completely resolved (Fig. 11).

However, the eluent containing three surfactants is not necessarily stronger than the binary mixture. Table IX indicates that the solvent containing CTAB, DOSS and Brij-30 has less eluting power for chrysene, perylene, phenol, cresol, ethylphenol and propylphenol than that containing CTAB and DOSS. In a way, Brij-30 acts as a diluent to the ion pair formed between CTAB and DOSS [15]. This is verified by the following observation. When 10 mM CTAB and DOSS was dissolved in 39% acetonitrile, the resulted solution remained cloudy despite the continuous stirring for more than 24 hours. However, the solution completely cleared up as soon as 10 mM Brij-30 was added. When this solution was used as eluent, it took about a hour to finish the separation in Figure 2. While the latter allows a complete separation of this mixture except peaks 10 and 11, the former cannot separate 4 and 5, 8 and 9, 16 and 17. As a result, separation of only 15 of the 18 compounds was possible (Fig. 12). However, another 18 compounds, many of which are more polar, were nicely separated using this ternary-additive system (Fig. 13).

It is believed that the additive-analyte interactions and acetonitrile-analyte interactions compete with each other in the solution phase. If so, a decrease in the percentage of acetonitrile present in the eluent would result in a stronger interaction between the additive and analyte resulting in a more prominent change in selectivity. This was confirmed by the separation of 20 compounds using 39% acetonitrile containing 10 mM each of Brij-30, CTAB and DOSS (Fig. 14A). This separation took 26 min. without significant peak overlapping

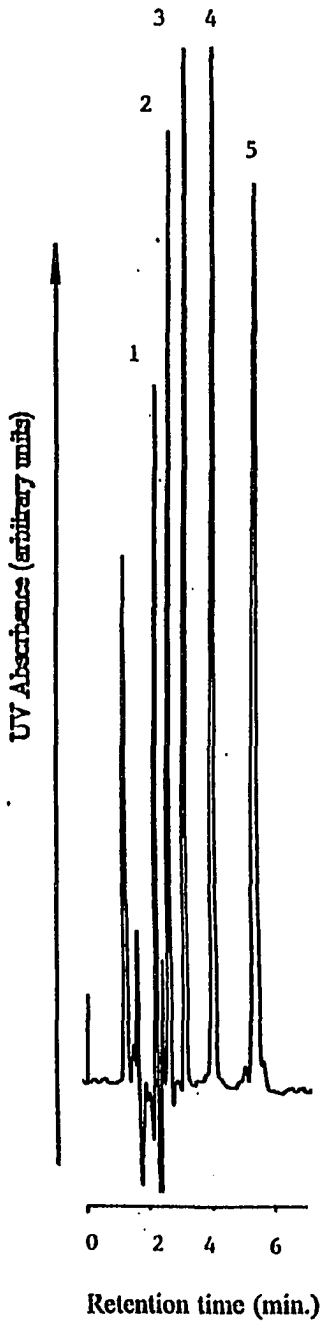
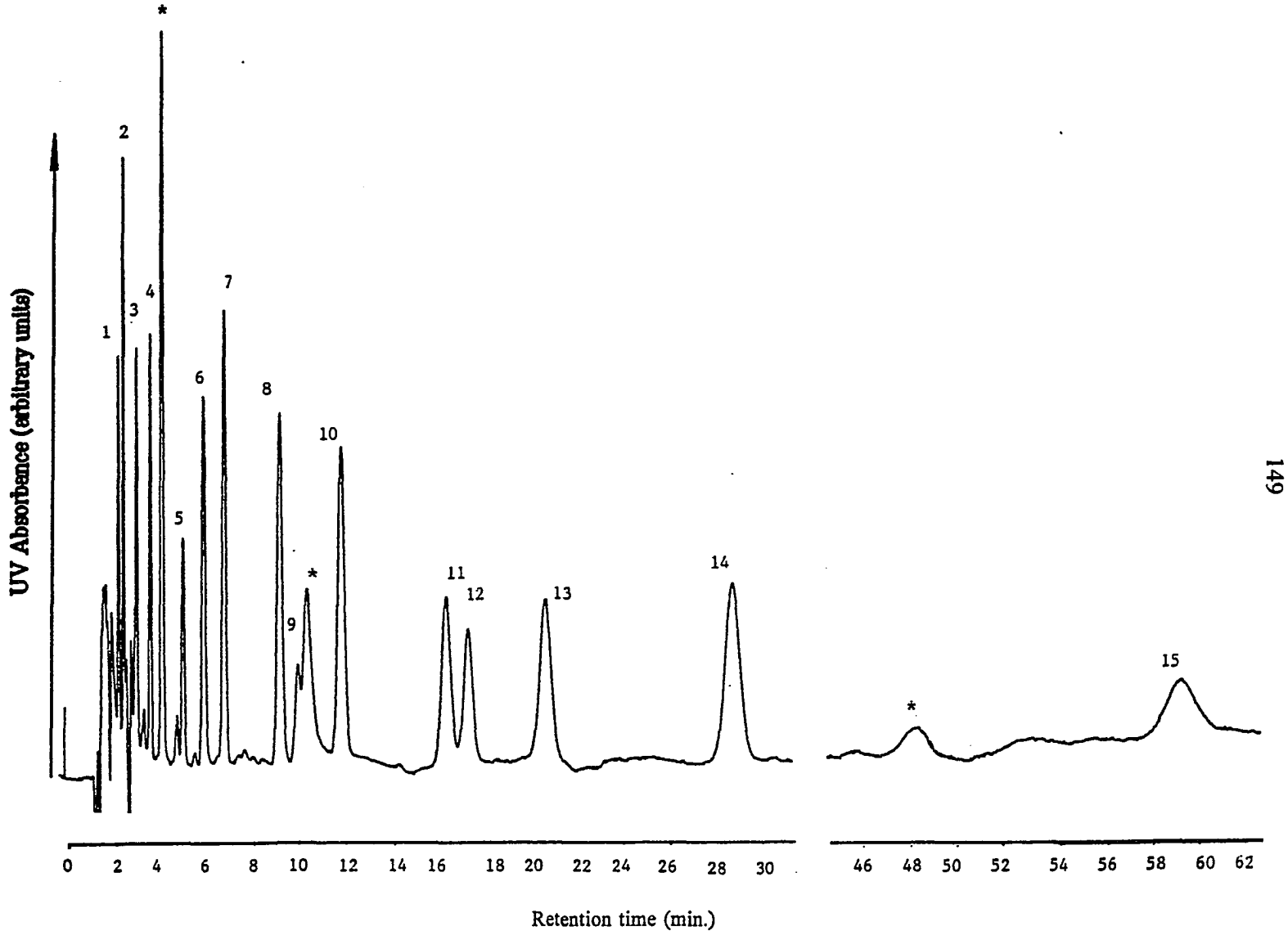


Fig. 11. All conditions are the same as in Fig. 3, except that the eluent is 60% acetonitrile/water containing 16.7 mM Brij-30, DOSS and CTAB ea.

Fig. 12. Chromatographic separation on Supelcosil LC-18 (150 mm x 4.6 mm I.D.) column. Eluent: 60% acetonitrile/water containing 16.7 mM Brij-30, DOSS and CTAB ea. Flow rate: 1 ml/min. Detection: UV @ 254 nm. Peak identification: 1 = benzophenone, 2 = benzene, 3 = toluene, 4 = naphthalene, 5 = fluorene, 6 = phenanthrene, 7 = butyl benzene, 8 = pyrene, 9 = 2,3-benzofluorene, 10 = chrysene, 11 = benz[e]acephenanthrylene, 12 = perylene, 13 = benzo[a]pyrene, 14 = benzo[ghi]perylene, 15 = 3,4,9,10-dibenzopyrene. Other peaks are injection and impurity peaks.


UV Absorbance (arbitrary units)
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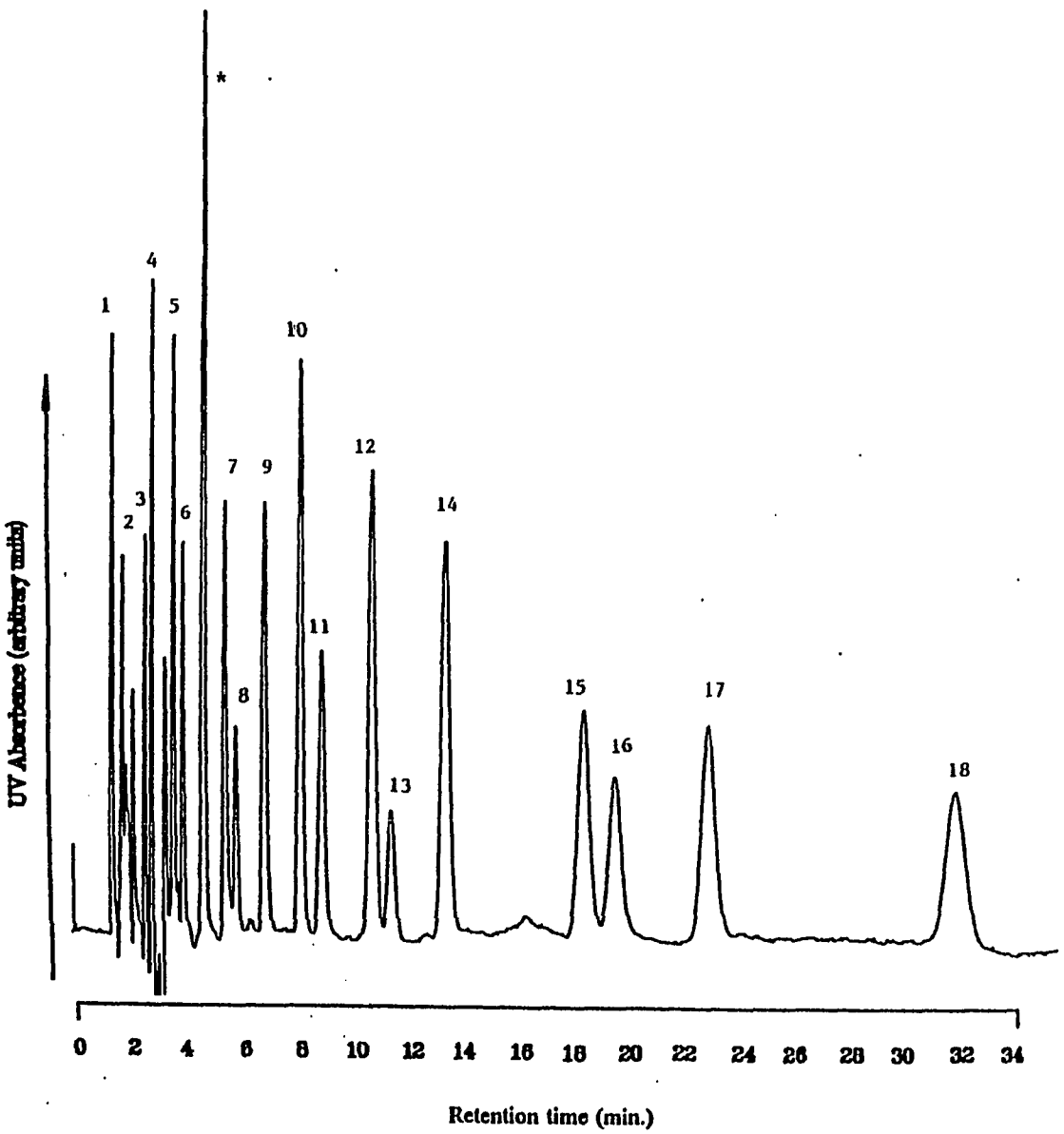
46 48 50 52 54 56 58 60 62
Retention time (min.)

Fig. 13. Chromatographic separation on Supelcosil LC-18 Column (15 cm x 4.6 mm i.d.). Eluent: 60% acetonitrile/water containing Brij-30, DOSS and CTAB at 16.7 mM each. Flow rate: 1 ml/min. Detection: UV absorbance @ 254 nm. Peak identification: 1 = benzyl bromide, 2 = methyl benzoate, 3 = benzophenone, 4 = benzene, 5 = chlorobenzene, 6= bromobenzene, 7 = 1-methylnaphthalene, 8 = fluorene, 9 = phenanthrene, 10 = butyl benzene, 11 = fluoranthene, 12 = p-terphenyl, 13 = 2,3-benzofluorene, 14 = chrysene, 15 = benz[e]acephenanthrylene, 16 = perylene, 17 = benzo[a]pyrene, 18 = benzo[ghi]perylene. Others are impurity or injection peaks.


UV Absorbance (arbitrary units)
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Retention time (min.)

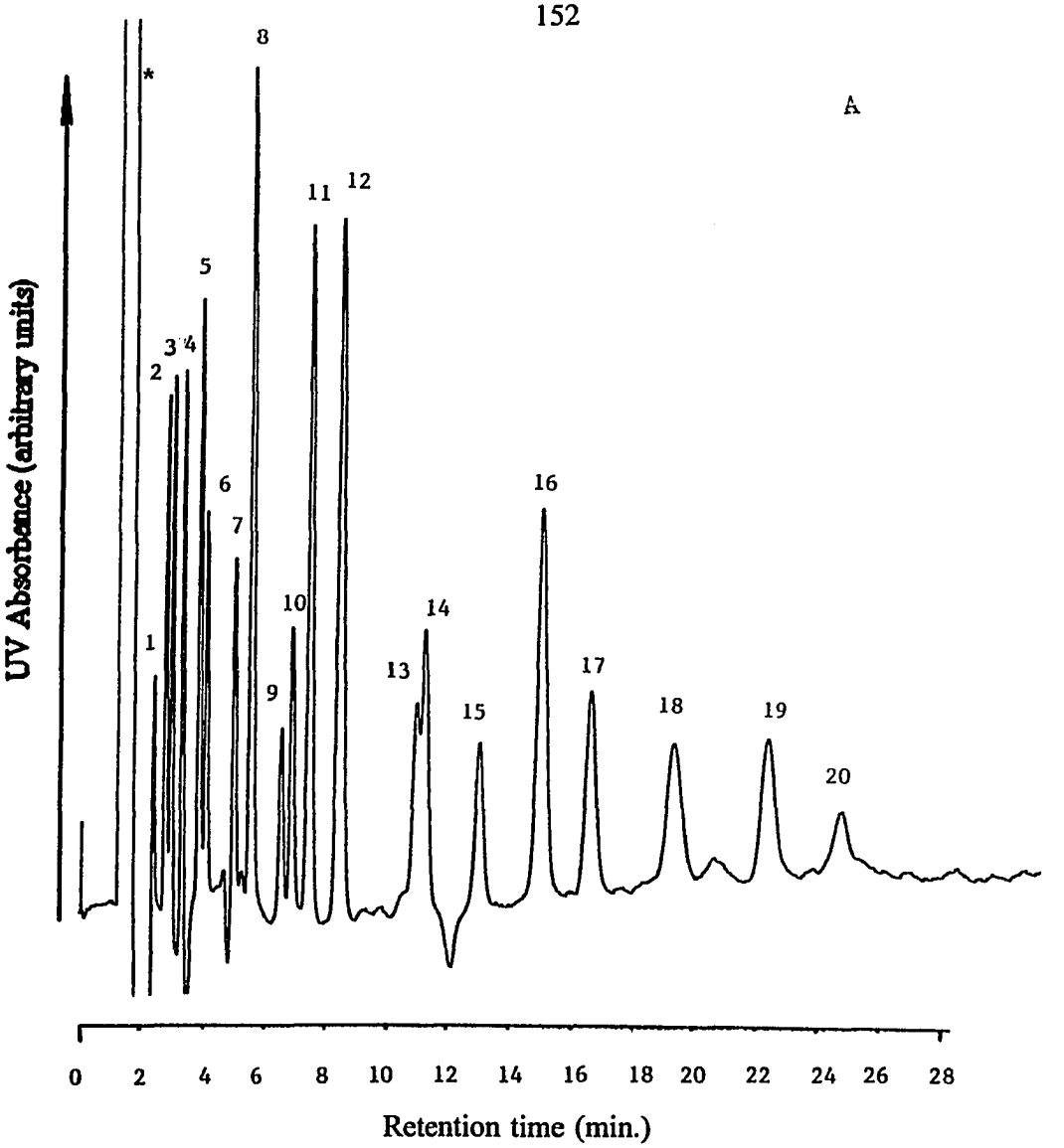

Fig. 14. Chromatographic separation on Supelcosil LC-18 (150 mm x 4.6 mm I.D.) column. Eluent: A: 39% acetonitrile/water containing 10 mM Brij-30, DOSS and CTAB ea., B: 39% acetonitrile/water. Flow rate: 1 ml/min. Detection: UV @ 254 nm. Peak identification: 1 = benzyl bromide, 2 = benzyl cyanide, 3 = benzyl nitrile, 4 = benzyl acetate, 5 = nitrobenzene, 6 = methyl benzoate, 7 = nitrotoluene, 8 = ethylphenol, 9 = 4-bromo-nitrobenzene, 10 = benzene, 11 = phenetole, 12 = propylphenol, 13 = toluene, 14 = chlorobenzene, 15 = bromobenzene, 16 = naphthalene, 17 = ethyl benzene, 18 = amylphenol, 19 = 1-methylnaphthalene, 20 = fluorene. * = injection peak.

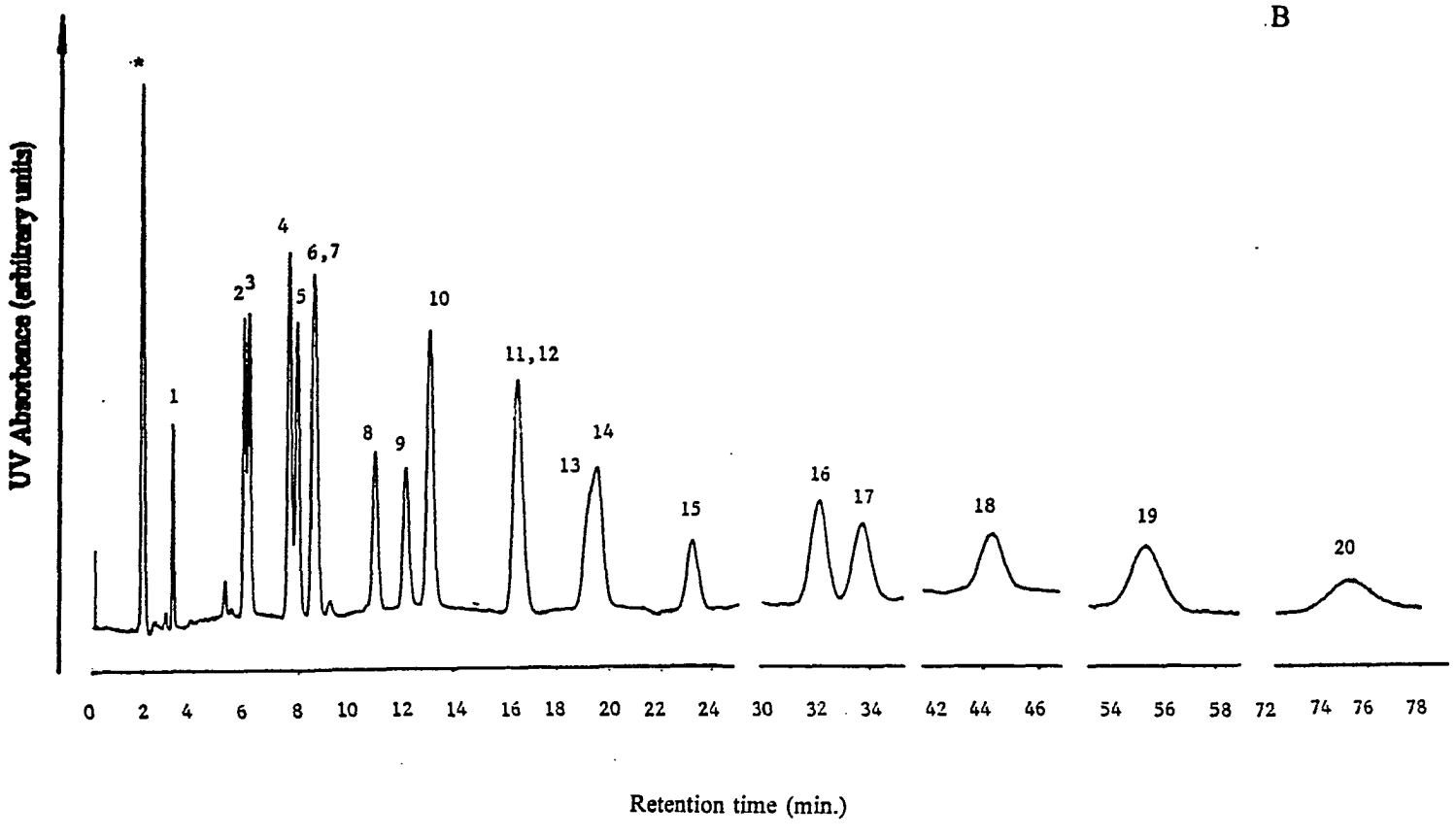

Fig. 14 (continued).

except that between peaks 13 and 14. When 39% acetonitrile alone was used as the eluent (Fig. 14B), the separation took 76 min with peak overlapping or co-elution between peaks 2 and 3, 5 and 8, 4 and 6, 13 and 14 and the elution order was significantly different.

CONCLUSIONS

For the first time, mixed surfactants including binary anionic and cationic, anionic and non-ionic, and ternary mixtures were successfully applied for reversed phase liquid chromatographic separations. A synergistic effect was observed. Unique selectivity control was possible by adjusting the choice and concentration of the additives used. Excellent separations were obtained of mixtures containing compounds with various polarities and functionalities.

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EFFECT OF PACKING CONDITIONS ON THE PERFORMANCE OF HPLC COLUMNS

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

Xue Li and James S. Fritz
Ames Laboratory, U.S. Department of Energy and
Department of Chemistry, Iowa State University
Ames, Iowa 50011, U.S.A.

ABSTRACT

This paper proposes an HPLC column-packing method, which is to pack polymeric- or silica-based columns under low pressure using 2-ethoxyethanol as the slurry and packing solvent. Our method development study reveals that it is necessary for the slurry and packing solvents to wet the stationary phase well in order to obtain columns with good chromatographic performance. Low packing pressure (500 psi) yields columns with better performance than high pressure (3000 psi) when such a solvent is employed. 2-ethoxyethanol was found to be the best choice among methanol/water mixture, methanol, ethanol, acetonitrile, acetone, 2-propanol, toluene, tetrahydrofuran, 2-methoxyethanol and 2-

ethoxyethanol. 2-ethoxyethanol was also found to be a good choice to reduce the size distribution of 5 μm polystyrene/divinylbenzene resin. This was accomplished by simply settling the resin in 2-ethoxyethanol four consecutive times. Columns packed with the resulting resin gave better performance than those with the resin without in-house sizing. Silica C-18 packings seem to have less stringent requirement in the solvent choice, however the best solvent found for polymeric resin also applies.

INTRODUCTION

Column packing and evaluation continue to be the weak link in high performance liquid chromatography, although it has been three decades since the inception of high performance liquid chromatography. There are generally three types of column packing methods: dry packing [1-3], high pressure slurry packing [1, 2, 4] and compression slurry packing [5-7]. Because the attraction between the particles is drastically reduced as a result of the dispersion of the stationary phase particles in a solvent, slurry packing results in more favorable packing conditions compared to dry packing method [8, 9].

Among the column packing techniques, high pressure slurry packing has been the most successful and the most widely used. In this approach, the suspension of packing materials is stored in a reservoir which is connected to the column. A packing solvent is then introduced under high pressure and at high flow rate into the reservoir to push the slurry into the column and form the resin bed by filtration.

The selection of solvents used in slurry packing is very important. The small particles

of the packing materials tend to form small aggregates. The co-existence of aggregates and discrete particles is analogous to using a packing with a wide particle size range. Packing agglomeration causes nonuniform compaction during the packing process resulting in a wide variation of flow velocity through the column and thus very poor column efficiency [2, 10, 11]. So, it is important to have a solvent which can disperse the particles well and prevent the formation of aggregates. For this purpose, dilute ammonium hydroxide solution was used to slurry silica microspheres by Kirkland [12]. Organic acids such as n-heptanoic and dichloroacetic acid have also been successfully used to slurry HPLC packing materials [13]. Sedimentation packing technique was recently introduced by Wang, Hartwick, Miller and Shelly [14]. With this approach, a slurry consisting of the packing and a deflocculating solvent, acetone, was poured into the column and the bed was formed by sedimentation. Then, the bed was solidified by a flocculating solvent, methanol/water (1:1). The function of the deflocculating solvent is to disperse the particles and prevent the agglomeration of them, and the function of the flocculating solvent is to solidify the packing bed. Vissers, *et al.* [15] reported that the performance of slurry-packed microcolumns is predominantly determined by the selection of the packing solvent, which preferably has to be coagulating solvent, and the selection of the slurry solvent is of minor importance.

The column packing protocol for the Shandon column packing pump used in our laboratory is that: 2-propanol or methanol is used as the slurry solvent and packing solvent for silica-based reverse-phase stationary phase [16]. Methanol or methanol/water (1:1) has long been used as the slurry and packing solvent, and 3000 psi as the pressure to perform the

column packing in our laboratory.

The questions that were asked at the start of this study were:

(1) Can the methanol/water (1:1) or methanol wet the PS/DVB stationary phase and disperse the formation of resin agglomeration?

(2) Why is it necessary to use high packing pressure like 3000 psi to pack a HPLC column? It was felt that if the particles can be dispersed well by the solvent, they should be able to form a resin bed allowing high efficiency separations without applying high packing pressure.

The present study shows that neither methanol/water nor methanol can wet the particles well and prevent the agglomeration of the resin particles. As a result, broad, asymmetrical chromatographic peaks are obtained. High packing pressure is not needed to produce well packed columns if a good solvent is chosen. The function of the high pressure might be to force the particles to hit the packed resin bed at high speed and break up the agglomerates formed in the slurry.

EXPERIMENTAL

Chromatographic System

The chromatographic system consists of several components. A Dionex DXP pump (Dionex, Sunnydale, CA, U.S.A.) was used to deliver a flow of 1 ml/min. A 7010 Rheodyne injector (Ryeodyne, Berkeley, CA, U.S.A.) delivered 10 µl sample which was detected with a Kratos Spectra flow 783 UV absorbance detector (Kratos Analytical Instrument, Ramsey,

NJ, U.S.A.). Separations were recorded by a Servogor 120 chart recorder (Abb Goerz Instrument, Vienna, Austria) and retention times were recorded by a Shimadzu Chromatopac C-R3A integrator (Shimadzu Corporation, Kyoto, Japan). Analytical columns (50 mm x 4.6 mm I.D.) were packed with a Shandon HPLC packing pump (Shandon Southern, Sewickley, PA, U.S.A.). A Galen III microscope equipped with a MF-10 camera (Cambridge Instruments, Buffalo, NY, U.S.A.) was employed to observe the aggregation behavior of the particles in slurry/packing solvents.

Materials

The solvents used in this work: acetonitrile, methanol, ethanol, 2-propanol, acetone, toluene, tetrahydrofuran and 2-methoxyethanol were of HPLC grade and 2-ethoxyethanol was of spectrophotometric grade. They were all used as obtained from Fisher (Fisher Scientific, Pittsburgh, PA, U.S.A.) or Aldrich (Aldrich Chemical Company, Inc., Milwaukee, WI, U.S.A.). The eluent, 60% acetonitrile/water, was prepared daily. The test compounds, benzene, toluene, ethylbenzene, propylbenzene and butylbenzene were all of reagent grade and used as obtained from Aldrich (Aldrich Chemical Company, Inc., Milwaukee, WI, U.S.A.). Stock solutions were used to prepare all sample solutions by diluting with mobile phase. A Barnstead Nanopure II system (Sybron Barnstead, Boston, MA, U.S.A.) was used to further deionize distilled water for all eluents and sample mixtures. 5 μm macroporous polystyrene/divinylbenzene (PS/DVB) resin from Sarasep (Sarasep Inc., Santa Clara, CA, U.S.A.) or 5 μm Alltech Nucleosil C18 5U (Alltech Associates, Inc., Deerfield, Illinois, U.S.A.) was used as the column packing materials.

Column Packing Procedure

A slurry made from 1.2 grams of the packing materials in 40 ml of the packing solvent was stirred for 10 min on a hot plate. Then the slurry was poured into a stainless steel reservoir. Downward mode packing was used for half an hour at desired packing pressure (500 psi or 3000 psi) using the desired packing solvent as the carrier solvent. Then acetonitrile was used to condition the column for 0.5 hour since 60% acetonitrile/water was the eluent. After the pressure has dissipated, the column was carefully taken off the packing device to avoid disturbing the packing.

Column Evaluation

The separation column was equilibrated with 60% acetonitrile until the baseline was stabilized. Sample injections were made at this point. The eluted species were detected by a UV-vis detector at 254 nm with an output range of 0.010 AUFS.

Capacity factor, k', was calculated according to expression: $k' = (t_r - t_o)/t_o$. The system dead time, t_o, used to calculate capacity factor k', was measured by injecting nitrate solution into the system. The column was evaluated in terms of the asymmetry factor, B/A, and theoretical plate number, N. B/A is the ratio of the half-peak width at 10% peak height. N was calculated from the equation: $N = 5.54(t_r/w_{1/2})^2$. A relatively fast chart speed (6 cm/min) was used to ensure accurate measurements of the peak width at half peak height and the half peak width at 10% of the peak height. An average of at least three replicates was used to do all the calculations.

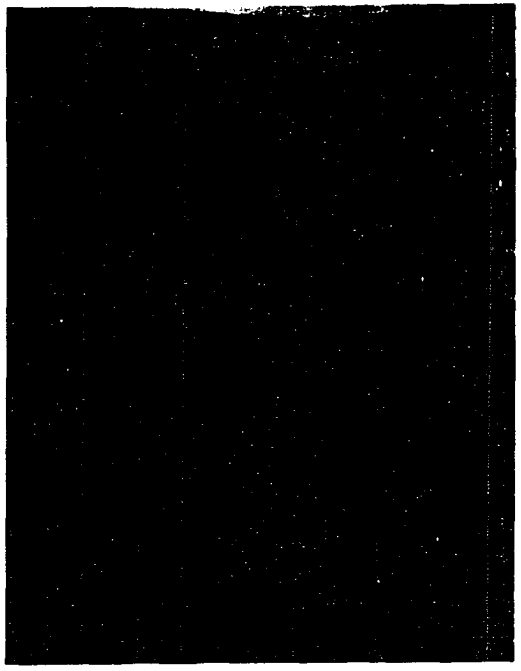
RESULTS AND DISCUSSIONS

Slurry/packing Solvent and Wetting of PS/DVB

Because of the convenience of obtaining the PS/DVB resin, the macroporous PS/DVB resin (5 μm) was used for the majority of this study although the proposed column packing method was also validated for silica-based packing materials.

One of the commonly used packing solvents is methanol/water (1:1). So, this was the first solvent studied. Nine other common organic solvents were also studied as the slurry and packing solvent. To observe the aggregation behavior of packing materials (PS/DVB resin or silica C18) in a solvent, the packings were put into some solvent and a slurry was formed. Visual observation was made to see if the resin would be floating on top of the solution or dispersed in the solvent. Also, a small amount of slurry was put on a glass slide and observed under an optical microscope, and the photomicrographs were taken. Well-dispersed particles would exist as individual particles in dilute slurry and form a nicely distributed layer of particles on the slide as the slurry becomes more concentrated. On the other hand, incompletely wetted particles would form three dimensional aggregates in the slurry and in the extreme cases, some of the particles would stay dry and float on top of the solution. In methanol/water (1:1), the PS/DVB was almost completely unwetted. As a result, the particles were not dispersed; instead, they stayed as big aggregates (Fig. 1A). Pure organic solvents, however, wet the resin better than methanol/water mixture. Methanol, acetonitrile and ethanol were found to be better wetting solvents. Fewer and smaller resin aggregates were formed in these solvents compared with in methanol/water. A photo is shown in Fig. 1B. Acetone,

A.



B.

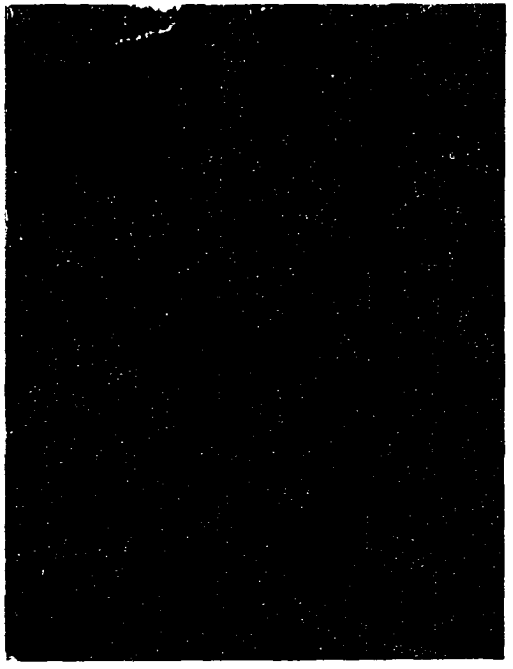
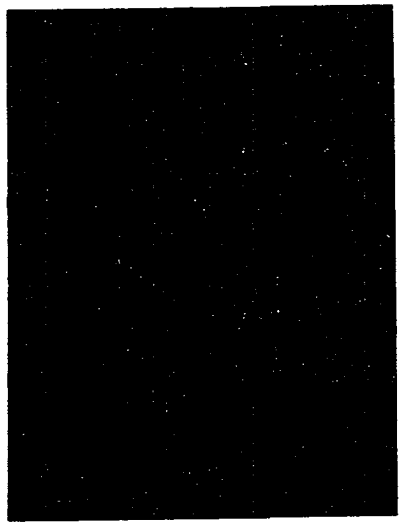


Fig. 1. Photomicrographs of 5 μm Sarasep PS/DVB resin in various slurry/packing solvents. Magnification: 400 x. Slurry solvent: A. methanol/water (1:1), B. acetonitrile, C. toluene and D. 2-ethoxyethanol.

C.



D.

Fig. 1 (continued).

2-propanol, tetrahydrofuran, toluene, 2-methoxyethanol and 2-ethoxyethanol turned out to be better wetting solvent for PS/DVB resin with only very few or no aggregates existing in the slurry at all. The photomicrographs of resin-toluene and resin-2-ethoxyethanol mixtures are as shown in Figs. 1C and 1D.

Packing Pressure and Column Performance

As mentioned in the experimental section, two packing pressures: 3000 psi and 500 psi were chosen as the examples of high and low packing pressures to study the effect of pressure on column performance. 3000 psi has been the typical packing pressure utilized in this laboratory. Each column has the dimensions of 5 cm in length and 4.6 mm in internal diameter. For each of the seven solvents studied (Table I), one column was packed at 3000 psi packing pressure and another at 500 psi. Separations were obtained with alkylbenzenes as the test compounds, and theoretical plate numbers and peak asymmetry were calculated. The results are as shown in Figs. 2 and 3, and Table I.

It is evident that 3000 psi gave a better column than 500 psi when methanol/water, methanol or acetonitrile was the solvent, and that these three are not good wetting solvents. It appears that the high pressure generally used in HPLC column packing might be breaking down the aggregates formed by the particles when they cannot be wetted and dispersed enough to exist as discrete particles. If this is true, it would not be necessary to apply high pressure to obtain high efficient columns provided that an appropriate solvent is used.

On the other hand, when the solvent can wet the particles well, better columns resulted from lower packing pressure. According to Freitag *et al.*, high packing pressure would deform

Fig. 2. Chromatographic separation on a 5 cm x 4.6 mm i.d. column packed with 5 μm PS/DVB particles. Packing pressure: 3000 psi. Packing solvents are as indicated at the bottom of each chromatogram. Eluent: 60% acetonitrile/water, Peak identification: 1 = benzene, 2 = toluene, 3 = ethylbenzene, 4 = propylbenzene, 5 = butylbenzene. Others are impurity or injection peaks.

Fig. 3. All the conditions are the same as Fig. 2 except that packing pressure is 500 psi.

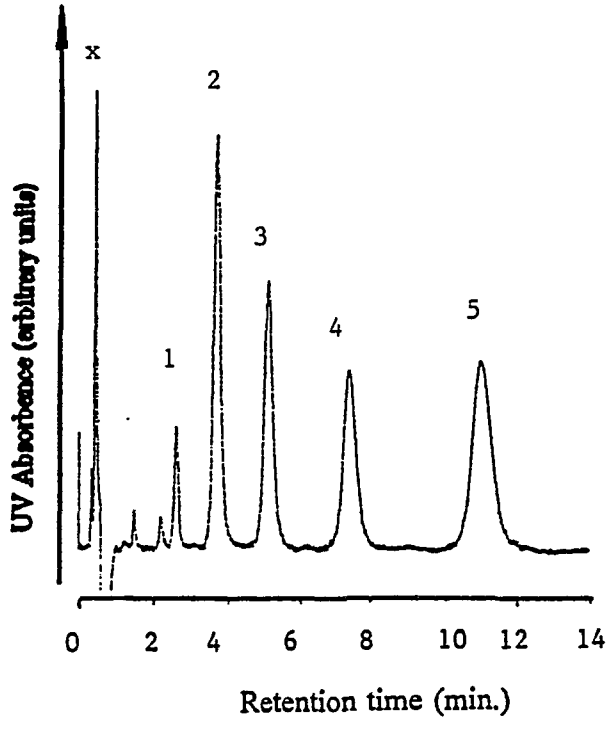


Fig. 2A. methanol/water (1:1), 3000 psi

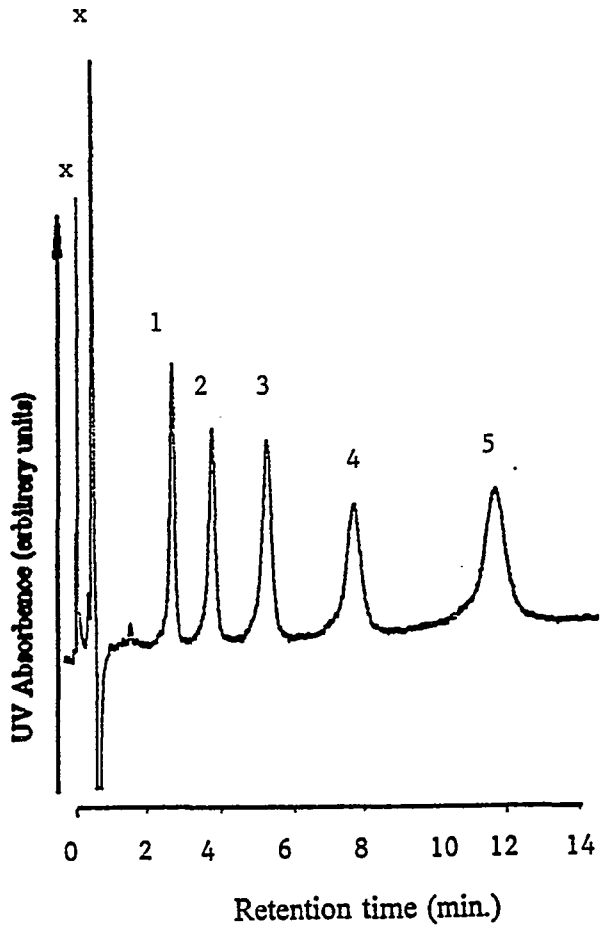


Fig. 3A. methanol/water (1:1), 500 psi

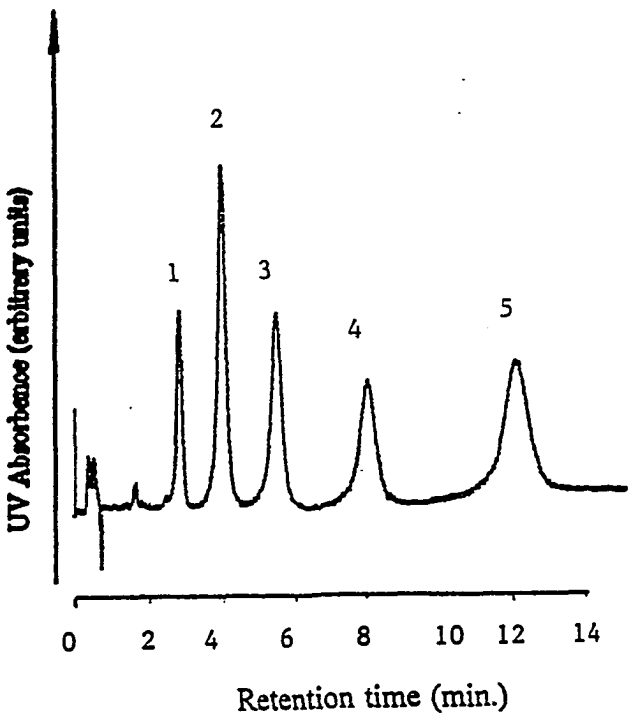


Fig. 2B. methanol, 3000 psi

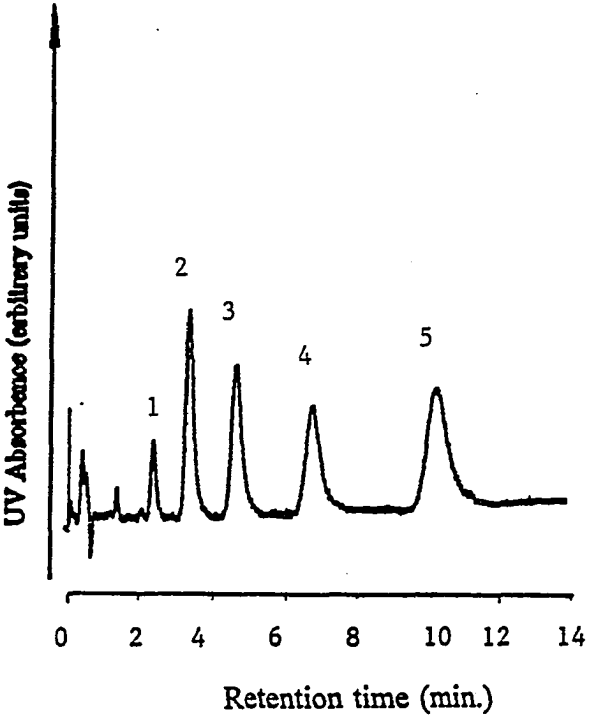


Fig. 3B. methanol, 500 psi

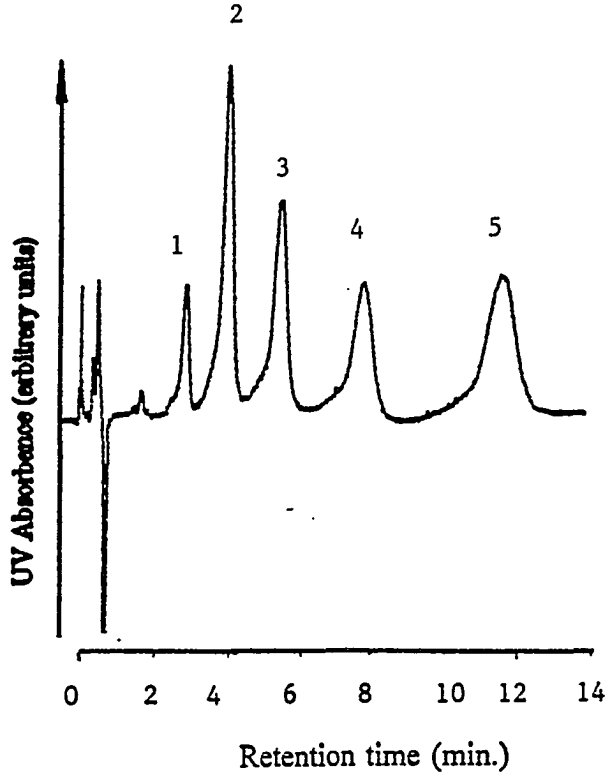


Fig. 2C. acetonitrile, 3000 psi

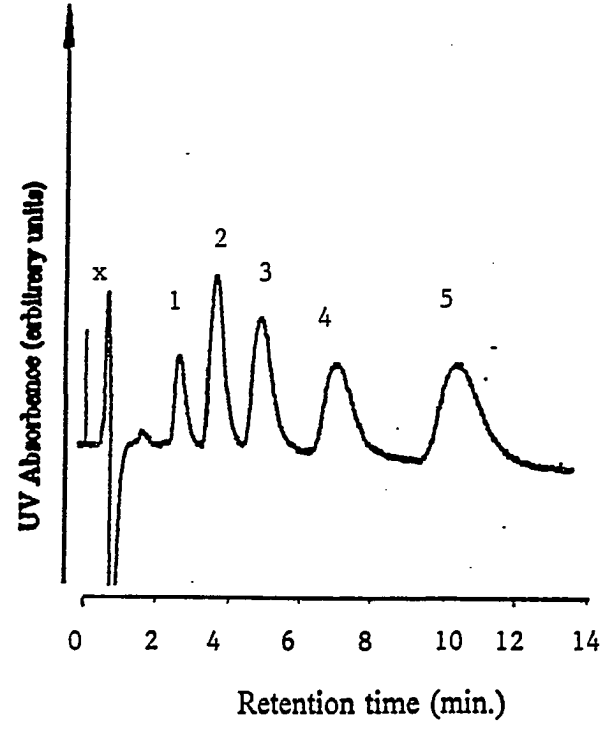


Fig. 3C. acetonitrile, 500 psi

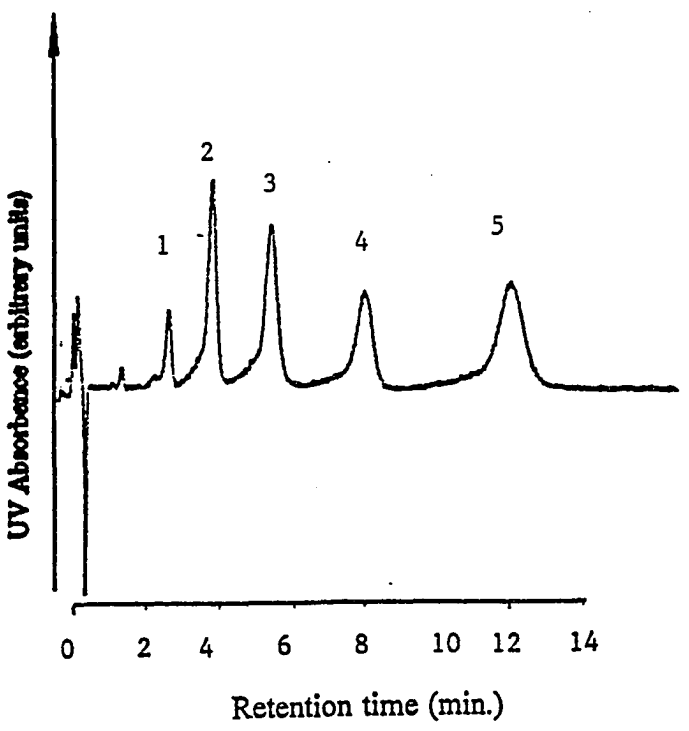


Fig. 2D. acetone, 3000 psi

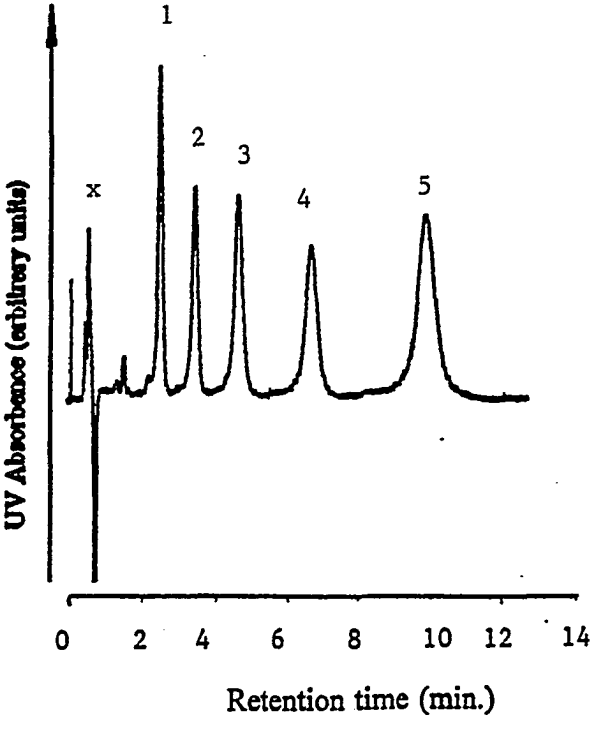


Fig. 3D. acetone, 500 psi

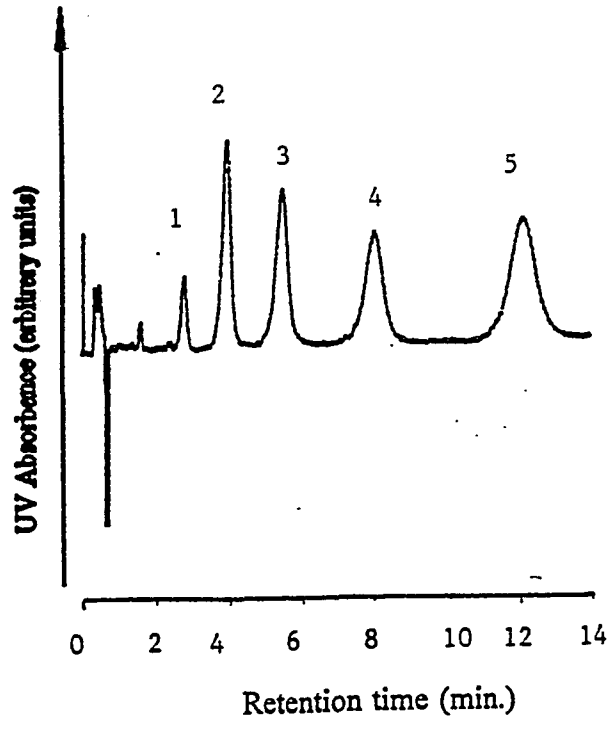


Fig. 2E. ethanol, 3000 psi

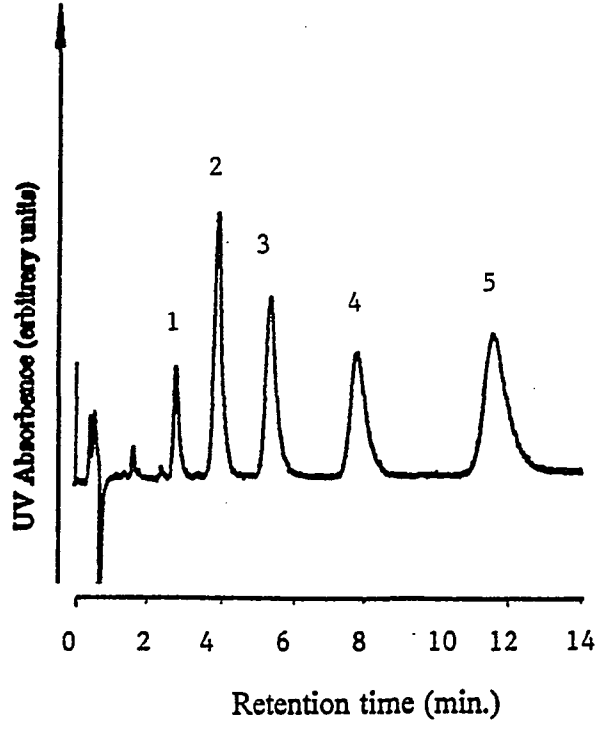


Fig. 3E. ethanol, 500 psi

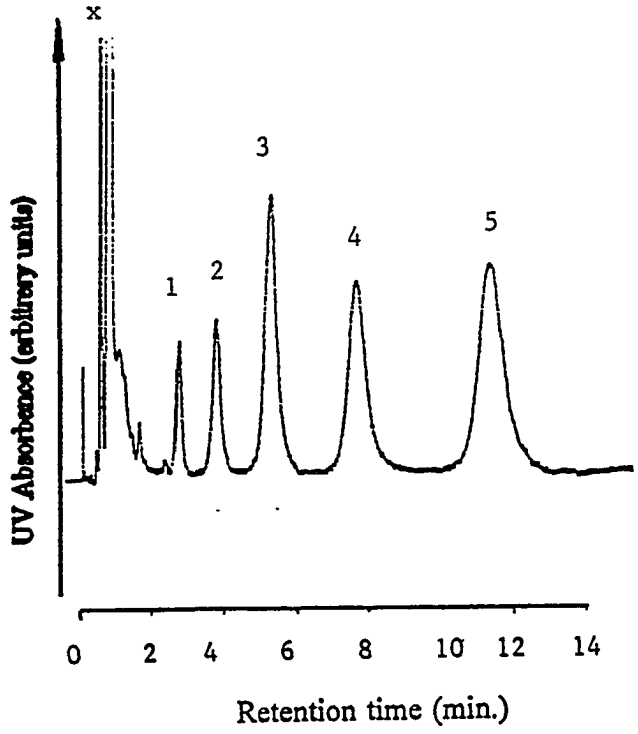


Fig. 2F. toluene, 3000 psi

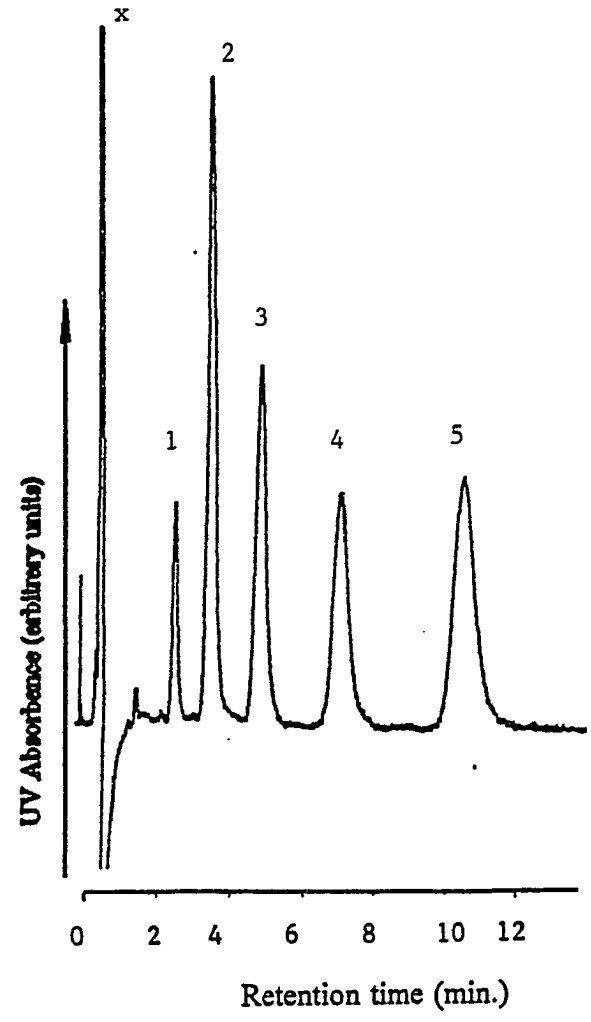


Fig. 3F. toluene, 500 psi

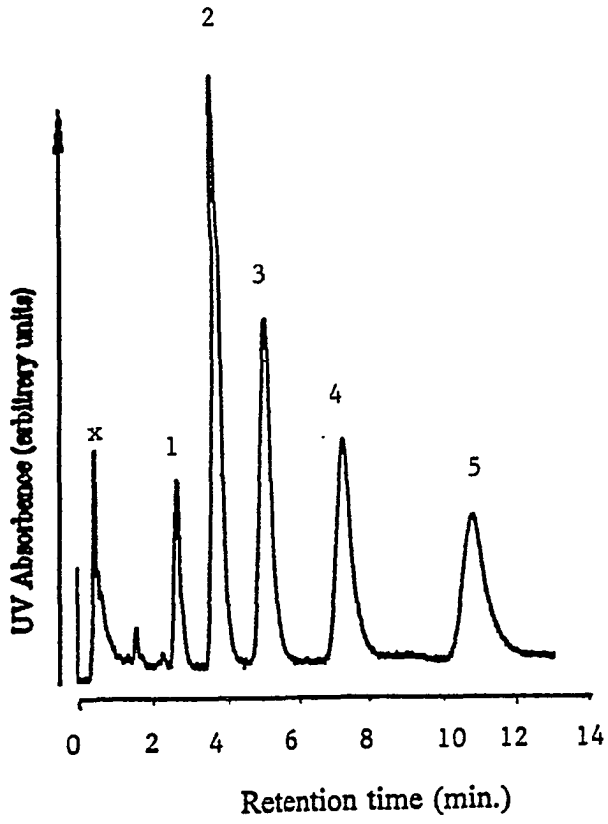


Fig. 2G. 2-ethoxyethanol, 3000 psi

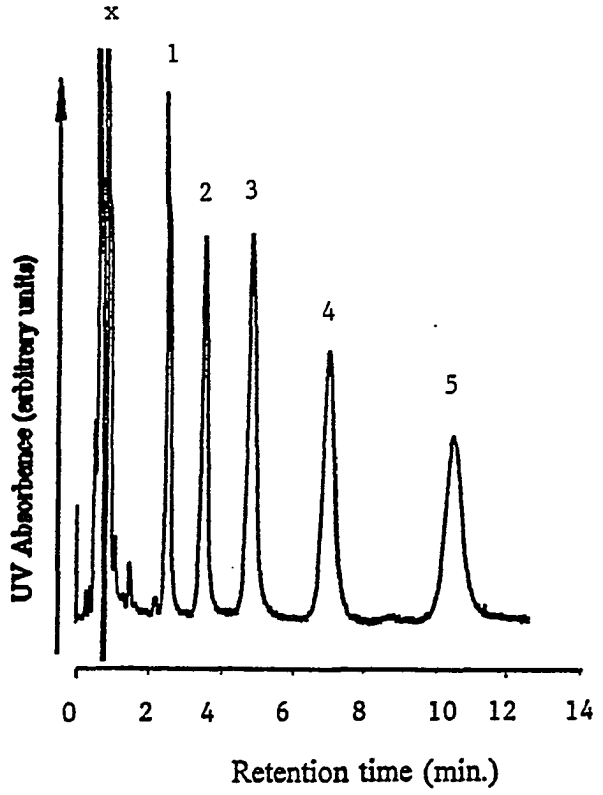


Fig. 3G. 2-ethoxyethanol, 500 psi

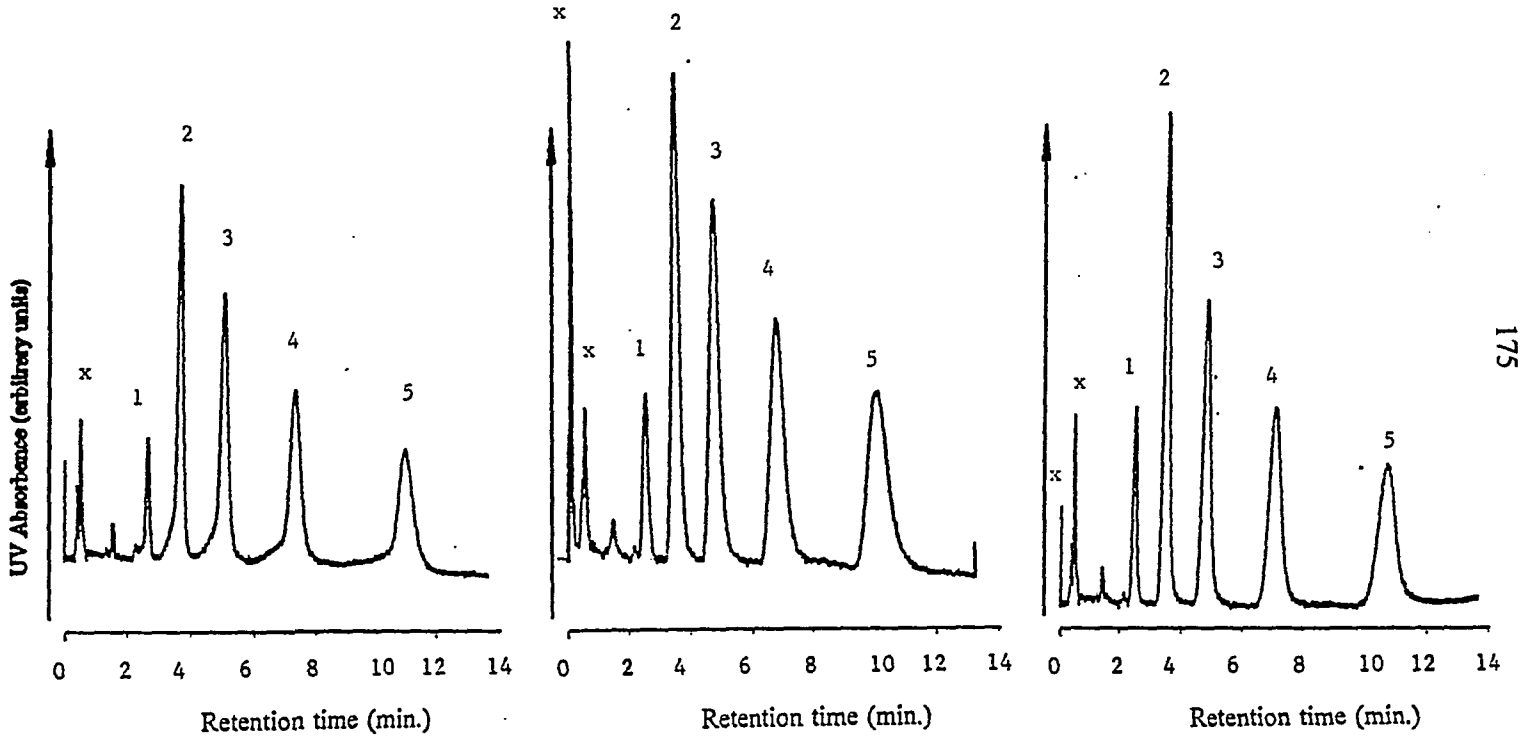


Fig. 3H. 2-propanol, 500 psi

Fig. 3I. tetrahydrofuran, 500 psi

Fig. 3J. 2-methoxyethanol, 500

Table I. Effect of column packing pressure on the performance of HPLC columns. Column dimensions: 5 cm x 4.6 mm, Packing materials: 5 μm PS/DVB. k': capacity factors, N: theoretical plate number per meter, B/A: peak asymmetry factor.

Packing solvent	3000 psi			500 psi		
	k'	N	B/A	k'	N	B/A
	Benzene					
Methanol/water (1:1)	5.93	44000	1.22	5.99	45000	0.74
Methanol	6.14	36000	0.78	5.18	29000	1.68
Acetonitrile	6.07	40000	0.63	5.56	14000	1.79
Acetone	6.70	35000	0.60	5.27	46000	0.85
Ethanol	6.22	30000	1.03	6.07	29000	2.71
Toluene	5.75	32000	1.41	4.43	32000	1.33
2-ethoxyethanol	5.79	27000	1.85	5.54	55000	1.28
	Toluene					
Methanol/water (1:1)	8.73	47000	1.29	8.80	43000	0.64
Methanol	9.14	33000	0.67	7.66	26000	1.89
Acetonitrile	8.93	38000	0.62	8.00	16000	2.02
Acetone	9.82	33000	0.66	7.70	47000	0.80
Ethanol	9.21	32000	0.99	8.87	36000	1.85
Toluene	8.39	32000	1.27	6.42	34000	1.62
2-ethoxyethanol	8.47	29000	1.84	8.11	48000	1.18

Table I (continued).

Packing solvent	3000 psi			500 psi		
	k'	N	B/A	k'	N	B/A
	Ethylbenzene					
Methanol/water (1:1)	12.37	43000	1.20	12.71	36000	0.88
Methanol	13.05	30000	0.82	10.90	27000	1.75
Acetonitrile	12.70	34000	0.61	11.22	16000	1.84
Acetone	13.88	31000	0.64	10.85	45000	0.96
Ethanol	13.13	25000	0.99	12.62	36000	2.13
Toluene	12.12	25000	1.29	9.25	29000	1.36
2-ethoxyethanol	12.00	27000	1.80	11.54	44000	1.44
	Propylbenzene					
Methanol/water (1:1)	18.27	46000	1.27	19.02	33000	0.74
Methanol	19.49	29000	0.76	16.29	27000	1.57
Acetonitrile	18.90	34000	0.66	16.43	16000	1.64
Acetone	20.54	33000	0.72	16.06	41000	0.90
Ethanol	19.61	25000	1.06	18.80	30000	1.96
Toluene	18.13	23000	1.04	13.78	27000	1.52
2-ethoxyethanol	17.77	27000	2.09	17.20	47000	1.40
	Butylbenzene					
Methanol/water (1:1)	27.62	42000	1.26	29.20	38000	0.78
Methanol	29.79	30000	0.71	24.85	25000	1.74
Acetonitrile	28.77	32000	0.80	24.77	18000	1.90
Acetone	31.14	28000	0.56	24.27	38000	0.92
Ethanol	29.94	24000	1.11	28.58	32000	1.64
Toluene	27.66	24000	1.35	20.94	32000	1.43
2-ethoxyethanol	26.99	29000	1.92	26.06	45000	1.50

the packing materials to make them less than perfect spherical [17]. This may be the reason why columns packed under high pressure has lower efficiency.

Slurry/packing Solvent and Column Performance

To evaluate the effect of slurry/packing solvent on the performance of the resulting chromatographic columns, ten columns (5 cm x 4.6 mm) were packed with each of ten chosen solvents under 500 psi packing pressure. The results are as shown in Fig. 3 and Table II. These results indicated that when organic solvents which can wet the polymer particles completely were used as the slurry and packing solvents, HPLC columns with higher theoretical plate numbers, lower peak asymmetry values and hence better efficiency were generally obtained. The column packed with 2-ethoxyethanol, the best wetting solvent, is proved to be the best column. However, the calculated results from THF, ethanol and toluene are not satisfying for all the test compounds although their peak shapes look much better than when using methanol/water, methanol or acetonitrile (Fig. 3). Toluene is not a good choice of packing solvent since it took a long time for the baseline to stabilize This is due to the fact that toluene is not UV transparent and a UV visible absorbance detector was used to detect the analytes.

Resin Sizing

Although the PS/DVB resin from Sarasep was labeled to be good in particle size distribution, an examination of the photomicrographs revealed that the size distribution of the resin is actually quite broad, with some large particles and a lot of fines (Fig. 1). As is well accepted, in order for a HPLC column to yield high efficiency, the stationary phase should

Table II. Effect of slurry/packing solvent on the performance of HPLC columns. Column packing pressure is 500 psi. Other conditions are the same as in Table I.

Solvent	Benzene			Toluene			Ethylbenzene		
	k'	N	B/A	k'	N	B/A	k'	N	B/A
Methanol/water (1:1)	5.99	45000	0.74	8.80	43000	0.64	12.7	36000	0.88
Methanol	5.18	29000	1.68	7.66	26000	1.89	10.90	27000	1.75
Acetonitrile	5.56	14000	1.79	8.00	16000	2.02	11.22	16000	1.84
Acetone	5.27	46000	0.85	7.70	47000	0.80	10.85	45000	0.96
Ethanol	6.07	29000	2.71	8.87	36000	1.85	12.62	36000	2.13
Toluene	4.43	32000	1.33	6.42	34000	1.62	9.25	29000	1.36
2-ethoxyethanol	5.54	55000	1.28	8.11	48000	1.18	11.54	44000	1.44
2-propanol	5.80	54000	0.90	8.46	52000	0.81	12.02	45000	0.76
2-methoxyethanol	5.48	39000	1.25	8.11	33000	1.41	11.65	34000	1.26
Tetrahydrofuran	4.33	20000	1.80	6.29	22000	1.70	8.96	21000	1.78
	Propylbenzene			Butylbenzene					
Methanol/water (1:1)	19.02	33000	0.74	29.20	38000	0.78			
Methanol	16.29	27000	1.57	24.85	25000	1.74			
Acetonitrile	16.43	16000	1.64	24.77	18000	1.90			
Acetone	16.06	41000	0.90	24.27	38000	0.92			
Ethanol	18.80	30000	1.96	28.58	31000	1.64			
Toluene	13.78	27000	1.52	20.94	32000	1.43			
2-ethoxyethanol	17.20	47000	1.40	26.06	45000	1.50			
2-propanol	17.87	43000	0.70	27.19	41000	0.78			
2-methoxyethanol	17.49	33000	1.28	26.87	34000	1.32			
Tetrahydrofuran	13.24	20000	1.93	20.08	19000	1.57			

have as narrow a size distribution as possible, ideally a uniform size. Thus, attempts were made to separate the particles by sizes. It is well known that when a solvent with low density and viscosity was used to slurry packing materials, both the large and small resin particles would settle down to the bottom of the container at fast speed so that no separation of large and small particles could be achieved. This is what happened when acetone, acetonitrile or methanol was used as the slurry solvent. However, as 2-ethoxyethanol was used as the slurry solvent, it takes much longer time for the resin particles to settle down due to its high density and viscosity. So, 2-ethoxyethanol was selected as the slurry solvent to further size the PS/DVB resin particles.

The process of resin sizing is as shown in Fig. 4. First, 2-3 grams of resin was put into 60 ml 2-ethoxyethanol. After stirring for several minutes, the slurry was tested under microscope to make sure the particles were well dispersed (Fig. 1D). Then, the slurry was allowed to settle by gravity for 2-3 hours and the suspension separated into two layers. Observed under the microscope after being carefully taken out with a disposable pipet, the top layer was found (2T) to contain mainly the fine particles. So, it was discarded. Because the bottom part (2B) was mainly consolidated resin, 40 ml 2-ethoxyethanol was added to make a second slurry by stirring. Observations under microscope showed that this part contains mainly big and regular sized particles with some fine ones left. So, a second settling was made by allowing this slurry to settle for another 2-3 hours and it separated into two layers again (3T and 3B). Similar observations to 2T and 2B were found for these two layers. Again the top part (3T) was discarded and another slurry was made of the bottom part (3B).

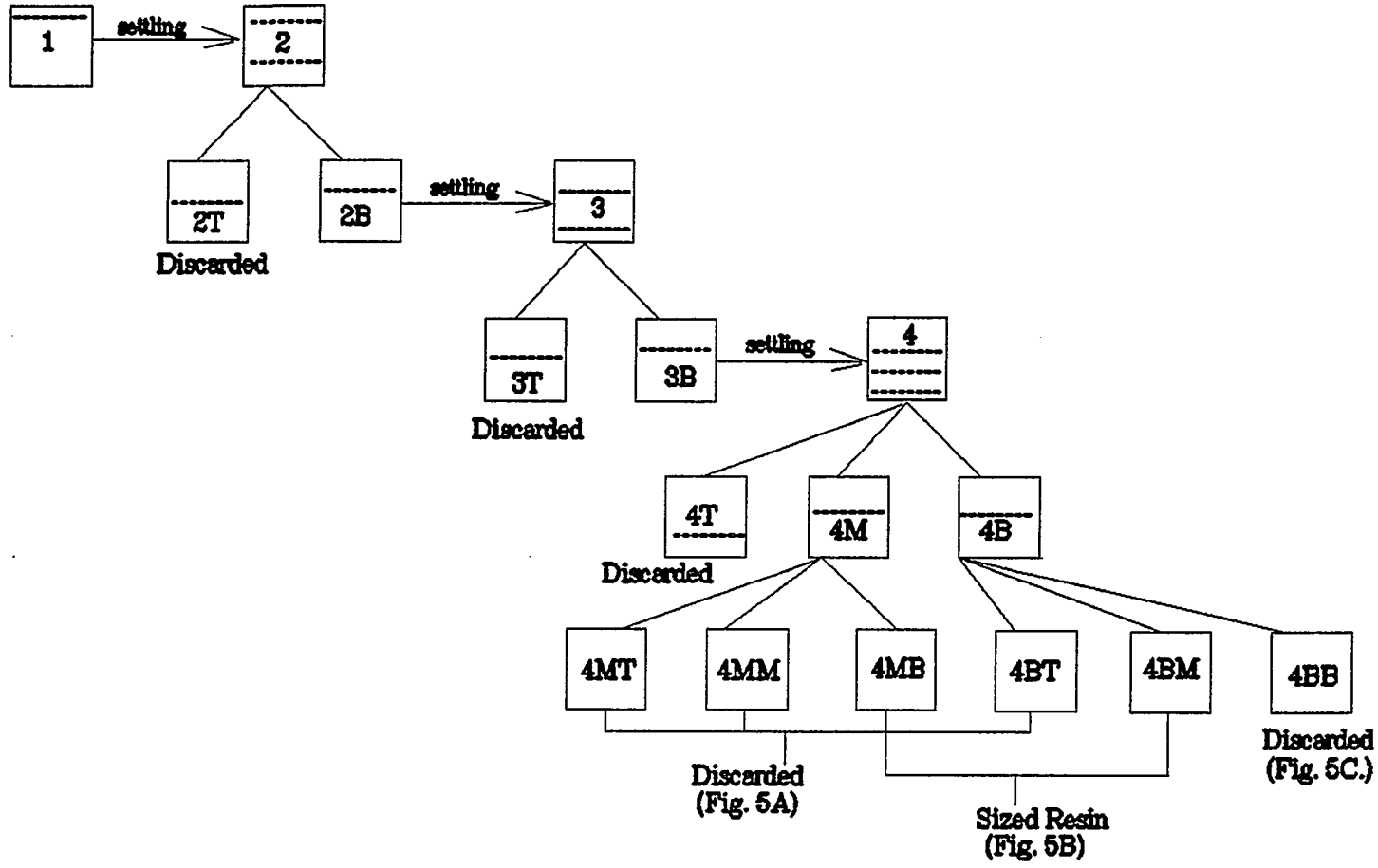


Fig. 4. Flow chart of PS/DVB resin sizing.

A third settling following the same procedure resulted in three layers (4T, 4M and 4B). 4B and 4M were retained and further sized since 4T mainly contains the fines. As 4M and 4B were allowed for further settling by the same procedure, each of the two suspensions again separated into three layers. After examining under microscope, the top layer of both and the middle layer of 4M (4BT, 4MT and 4MM) were discarded since they contained mainly fines (Fig. 5 A), the bottom layer of 4M and middle layer of 4B (4MB and 4BM) were combined and the picture is shown in Fig. 5B and the picture of the bottom layer of 4B (4BB) is shown in Fig. 5C. It is obvious that after the above simple settling, the fine and large particles were both separated from the particles with regular size so that the size distribution of the polymer resin was narrowed. A column then was packed using the sized resin (4MB and 4BM) with 2-ethoxyethanol as the slurry and packing solvent under 500 psi packing pressure. The chromatogram from this column is shown in Fig. 6. The theoretical plate number and peak asymmetry of this column and the column packed under the same conditions using unsized PS/DVB as the stationary phase are as shown in Table III. Comparison of the performance of these two columns shows the improvement in plate number as well as peak asymmetry for the column packed with sized *versus* unsized resin.

Column-packing Conditions for Silica-based Packings

Work was also done for silica-based packings. Alltech Nucleosil 5U was selected for this part of the study. Examination of the wetting of this packing material under microscope shows that methanol/water (1:1) does not wet the particles well although all of the previously mentioned pure solvents seemed to wet them almost completely. And, wetting of this silica

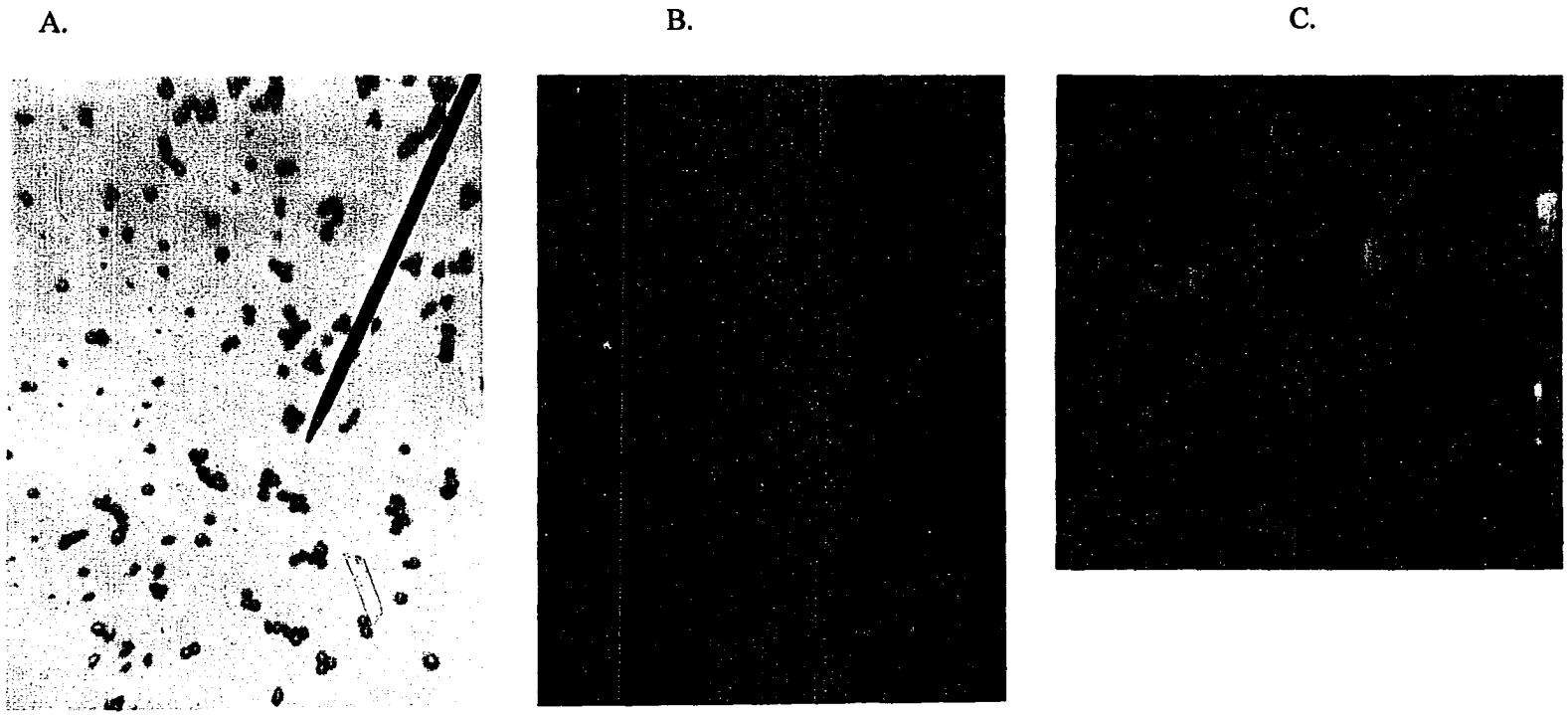


Fig. 5. Photomicrographs of 5 μm Sarasep PS/DVB resin in 2-ethoxyethanol. Magnification: 400 x. A. 4BT, 4MT and 4MM; B. 4MB and 4BM and C. 4BB.

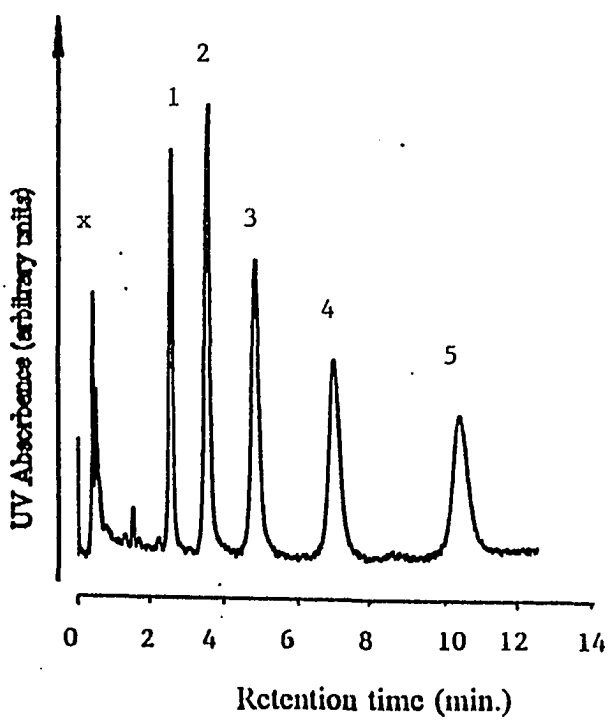


Fig. 6. Chromatographic separation on a 5 cm x 4.6 mm i.d. column packed with 5 µm PS/DVB particles with reduced size distribution. Other conditions are the same as Fig. 3G.

Table III. Effect of resin sizing on the performance of HPLC columns. Slurry/packing solvent: 2-ethoxytheanol Packing pressure: 500 psi. Other conditions are the same as in Table I.

	Unsized PS/DVB			Sized PS/DVB (4MB+4BM)		
Compound	k'	N	B/A	k'	N	B/A
Benzene	5.54	55000	1.28	5.52	65000	1.14
Toluene	8.11	48000	1.18	8.08	62000	1.24
Ethylbenzene	11.54	44000	1.44	11.43	62000	1.22
Propylbenzene	17.20	47000	1.40	16.95	53000	1.28
Butylbenzene	26.06	45000	1.50	25.72	52000	1.31

C-18 phase seems to be better than the polymeric resin when using the same slurry solvent. Example photomicrographs are shown in Fig. 7. This observation agrees with the fact that the silica C-18 surface is less hydrophobic than the polystyrene/divinylbenzene surface so that it has less stringent requirement for the slurry/packing solvent than the PS/DVB packings.

Also did was packing columns using this silica C-18 packing with methanol/water (1:1) and 2-ethoxyethanol as the slurry/packing solvents. Packing pressure was 500 psi in both cases (Fig. 8). Calculated theoretical plate number and peak asymmetry are shown in Table IV. It is evident that 2-ethoxyethanol, a better wetting solvent gives a column with better performance.

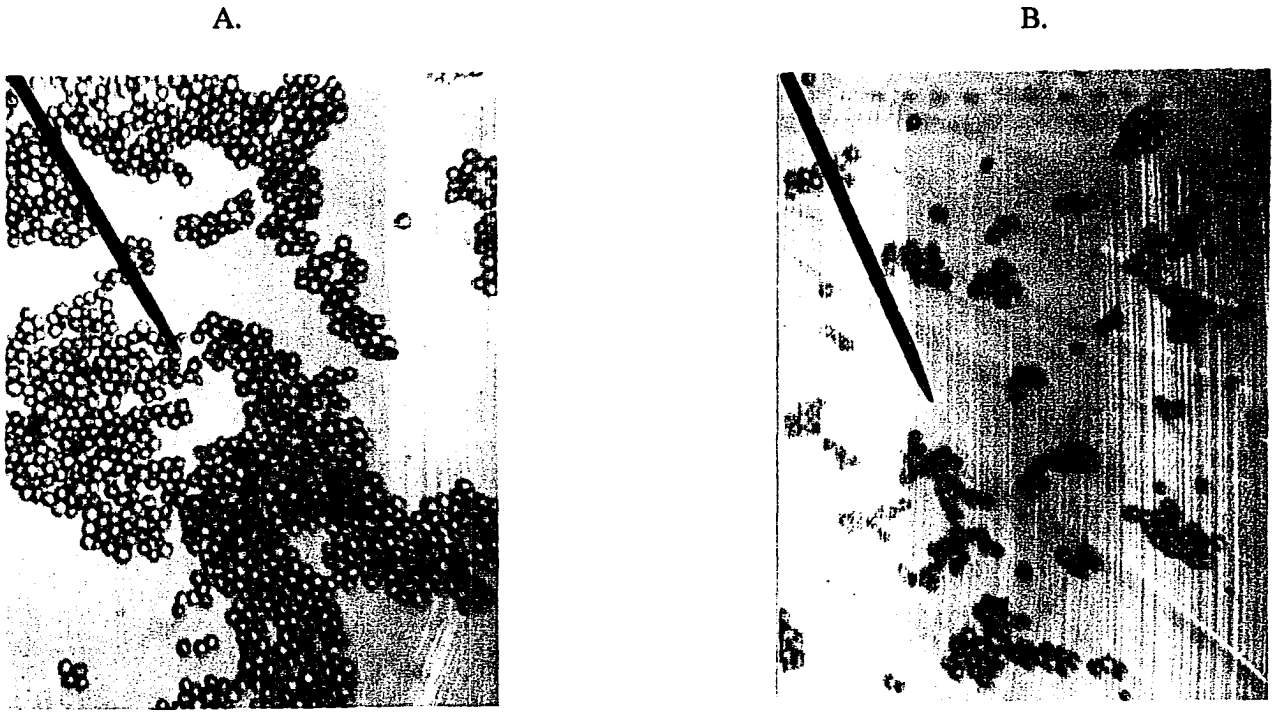


Fig. 7. Photomicrographs of Alltech Nucleosil 5U in various slurry/packing solvents. Magnification: 400 x. Slurry solvent: A. methanol/water (1:1) and B. 2-ethoxyethanol.

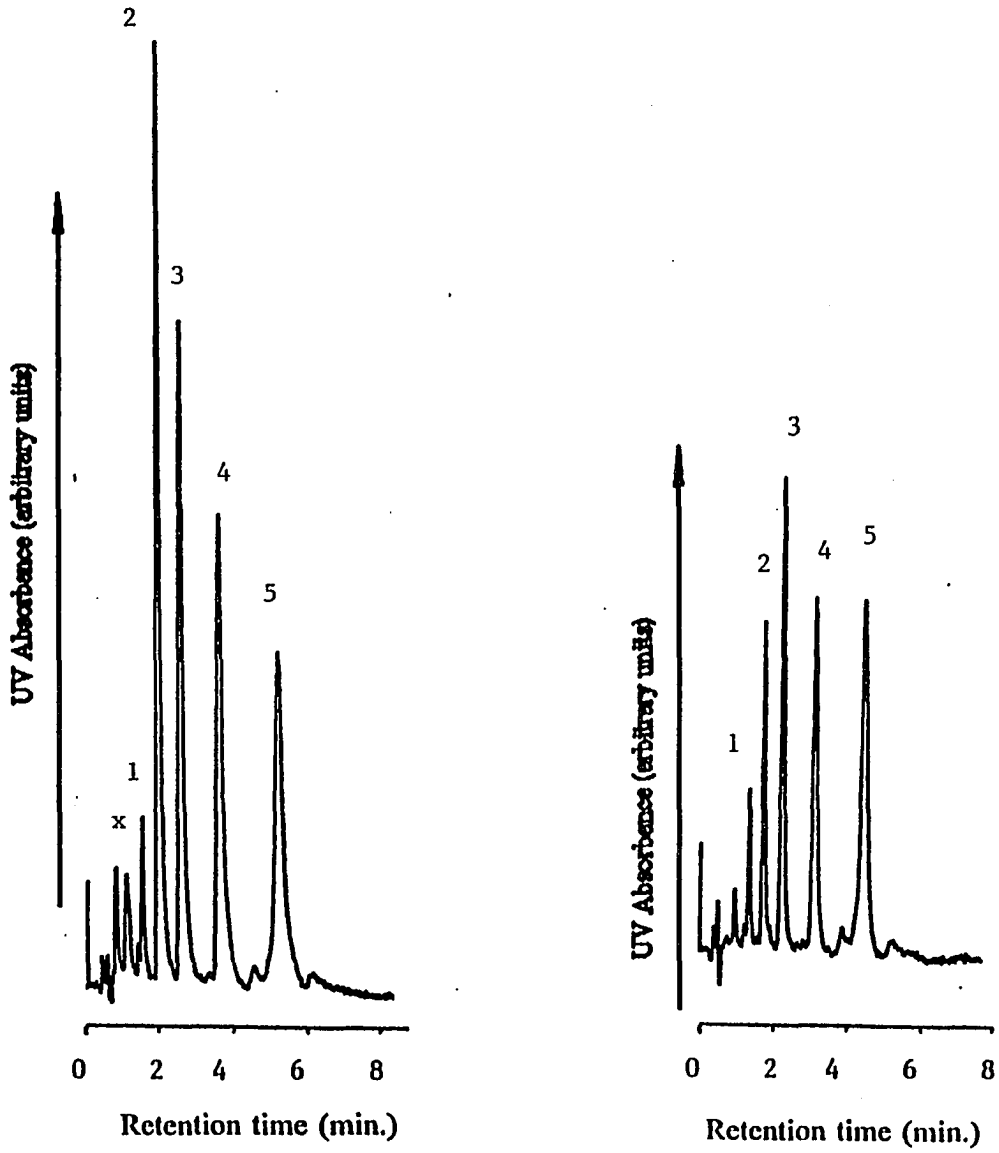


Fig. 8. Chromatographic separation on a 5 cm x 4.6 mm i.d. column packed with 5 μm Alltech Nucleosil 5U particles. Packing pressure: 500 psi. Packing solvent: A. methanol/water (1:1), B. 2-ethoxyethanol. Eluent: 60% acetonitrile/water, Peak identification: 1 = benzene, 2 = toluene, 3 = ethylbenzene, 4 = propylbenzene, 5 = butylbenzene.

Table. IV. Effect of column packing conditions on the performance of HPLC columns. Column dimensions: 5 cm x 4.6 mm, Packing materials: Alltechsil 5U. k': capacity factors, N: theoretical plate number per meter, B/A: peak asymmetry factor. See text for other conditions.

	Methanol/water (1:1)			2-ethoxyethanol		
Test Compounds	k'	N	B/A	k'	N	B/A
Benzene	2.80	48000	1.80	2.46	59000	2.00
Toluene	4.05	48000	1.90	3.45	68000	1.61
Ethylbenzene	5.60	54000	2.42	4.76	69000	1.72
Propylbenzene	8.33	56000	2.28	7.04	68000	1.61
Butylbenzene	12.51	53000	1.26	10.52	70000	1.15

CONCLUSIONS

This column-packing condition study demonstrated that the choice of slurry and packing solvent is critical to obtain a column with high efficiency. The ability of the solvent to wet the surface of column packing materials appears to be the key parameter. 2-ethoxyethanol was found to be the best choice among ten commonly used solvents. Low packing pressure (500 psi) yields columns with better performance than high pressure (3000 psi) when an appropriate solvent is chosen. Settling by gravity using 2-ethoxyethanol reduces the size distribution of polymeric resin resulting in HPLC columns with higher efficiency. Silica C-18 seems to have less stringent requirement in the choice of solvent. The best solvent for polymer resin is also a good choice for silica C-18 stationary phase.

The proposed column packing method has been applied in this laboratory for several

months and was proved to be practically applicable for polymeric-based as well as silica-based stationary phases.

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GENERAL CONCLUSION

Straight-chain alcohols, diols and animo alcohols were successfully used as organic modifiers for the separations of carboxylic acids and organic bases. They were either incorporated at a low percentage into the eluent or used in a small amount to pretreat the separation column.

The function of these modifiers is to coat the surface of the stationary phase by hydrophobic or electrostatic interactions. The hydroxyl groups stick out from the stationary phase to make it less hydrophobic. The hydrophobic interactions between analytes and the stationary phase were reduced and so were the retention times of the analytes. Those modifiers with longer hydrocarbon chains are more effective than those with shorter chains. The former have a greater tendency to be adsorbed onto the surface of the stationary phase, and lower concentrations in the eluent are therefore needed to produce an effective coating. The retention times of the analytes on the columns treated with amino alcohols were affected both by the sulfonation capacity of the stationary phase and the hydrocarbon-chain length of the amino alcohols used to treat the column.

This concept (dynamic coating) should have far reaching possibilities for HPLC. Use of surface modifiers has clear advantages over chemically derivatized stationary phases in that the properties, and thus selectivity, of the stationary phase can be altered and fine tuned by carefully choosing the chemical nature of the modifier.

A novel type of additive, surfactant, for chromatographic separations of organic compounds is also described in this work. Systems containing the additives act as a bridge

between conventional and micellar HPLC in that they use surfactants as additives as in MLC but do not depend on the presence of micelles to accomplish separations. Surfactants offer a better separation window than conventional hydro-organic mobile phases and superior separation efficiency compared with micellar LC. Dynamic association in solution between the analyte and discrete additive molecules is believed to be the basis of the improved separations. Control of solvent strength and selectivity are possible by careful choice of the type and concentration of the surfactant. Rapid gradient elution may be possible because no column re-equilibration is required. Binding constants between solute and surfactant molecules in mobile phases were calculated from capacity factor and the surfactant concentration.

Mixed surfactants including binary anionic and cationic, anionic and non-ionic, and ternary mixtures were also successfully applied for reversed phase liquid chromatographic separations. Synergistic effect and greater reduction in retention of the analytes was observed compared to the systems using single surfactant as mobile phase additives. Unique selectivity control was possible by adjusting the choice and concentration of the additives used. Excellent separations were obtained of mixtures containing 18-20 compounds with various polarities and functionalities.

A study of column packing conditions shows that it is crucial for the stationary phase to be completely wettable by the packing solvent. A commonly used organic-water mixture (e.g. 50% methanol-water) cannot wet underivatized polystyrene/divinylbenzene resin or silica C-18 particles completely. Instead, a pure organic solvent should be used as the packing solvent. Low packing pressure yields better-performing columns than high pressure, provided

that a good packing solvent was chosen. Optimal packing conditions were found to be 500 psi as the packing pressure and 2-ethoxyethanol as the packing solvent. Polymeric resins often have a broader size distribution, thus giving broader peaks and lower efficiency than silica-based columns. This is the case for the PS/DVB resin used in our laboratory. It was found that the resin size distribution can be improved by sedimentation in a solvent with appropriate density and viscosity, and 2-ethoxyethanol is a good choice for sizing the 5 μm PS/DVB resin used in this study.

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