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Open Tubular HPLC Columns Using Chemically Etched Poly(Tetrafluoroethylene) Serpentine Tubing

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Open Tubular HPLC Columns Using Chemically Etched Poly(Tetrafluoroethylene) Serpentine Tubing

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Abstract

A novel open tubular liquid chromatographic column has been developed and demonstrated. Narrow bore poly(tetrafluoroethylene) tubing was knitted into a serpentine configuration using an electronic breadboard as the supporting framework. Studies with unretained solutes showed that these tubes give much less band broadening per unit length than conventional capillary tubes of the same i.d. due to enhanced radial mixing. When such tubes are treated with a sodium/naphthalene fluorocarbon etching reagent, a carbonaceous surface is formed on the inner wall which provides a stable chromatographic stationary phase. The resulting columns can exhibit either normal or reversed phase behavior, depending on the mobile phase used, and are amenable to both isocratic and gradient elution chromatography.

INTRODUCTION

The ideal chromatographic column should be capable of yielding high efficiency, i.e., a large number of theoretical plates, in the shortest possible time. While the use of microparticulate packings has allowed large increases in the efficiency of liquid chromatographic columns, there are limits to the use of smaller and smaller particles. Since the backpressure at a given linear velocity is proportional to the inverse square of the particle size, the use of submicron particles at reasonable linear velocities becomes economically unfeasible. Also, the tendency of particles to aggregate increases greatly as the average particle size decreases. This makes efficient slurry packing of very small particles

difficult. For these reasons, open tubular columns, which have been used extensively in gas chromatography, should be the preferred means of achieving very high efficiencies in HPLC. However, conventional capillaries are impractical for constructing HPLC columns due to the slow diffusion of solutes in liquids compared to gasses. The resulting slow radial mass transport within the capillary gives unacceptably low column efficiencies. Jorgensen and coworkers (*1–3*) overcame this problem by using extremely narrow bone capillaries (10–40 μm i.d.) where mass transfer across the small radius is sufficiently fast to give reasonable column efficiency. The extremely severe dead volume constraints that these microcapillary columns place on the rest of the chromatographic system constitute a major drawback. The radial mass transfer in capillaries can be greatly enhanced by various geometric deformations. Hofmann and Halasz (*4*) investigated band broadening of unretained solutes in squeezed, twisted, and coiled tubes made from various materials using liquid mobile phases. They observed large reductions in plate height relative to straight capillaries, especially at higher flow rates. Katz and Scott (*5*) fabricated serpentine tubing by passing a metal capillary between a pair of toothed gear wheels. These tubes gave lower band dispersion per unit length than either straight or helically coiled tubes of identical bore and were subsequently shown to be useful as low dispersion connectors for HPLC (*6*). Capillaries deformed by both coiling and knitting, the latter being made from plastic tubing, have been used as reactors in postcolumn derivitization detectors due to their favorable band dispersion characteristics (*7, 8*). Englehardt and Lillig (*9*) used stainless steel capillaries stitched through a steel mesh framework for postcolumn reactors. Tijssen (*10*) attempted to use helically coiled metal capillaries as open tubular HPLC columns. Although minimal band dispersion was observed for unretained components, liquid chromatographic separations were not attained since a stable coating of stationary phase onto the column wall could not be prepared.

In this work, poly(tetrafluoroethylene) (PTFE) tubing was knitted into a serpentine configuration using a commercially available electronic breadboard as the supporting framework. Studies with unretained solutes demonstrated the greatly reduced band dispersion of the serpentine tubes relative to undeformed tubes. When the serpentine PTFE tubing was etched using a sodium/naphthalene solution, a carbonaceous surface was formed on the inner wall which exhibited useful chromatographic retention for some compounds. The resulting columns could be used for either reversed phase or normal phase separations, depending on the mobile phase used; and gradient elution could also be performed.

EXPERIMENTAL

All solutes used to prepare samples were of reagent grade. HPLC grade *n*-hexane, cyclohexane, acetonitrile, tetrahydrofuran (THF), methylene chloride, diethyl ether (all from Burdick and Jackson, Muskegon, Michigan), and in-house deionized water prepared using a Milli-Q system (Millipore Corp., Bedford, Massachusetts) were used to prepare samples and mobile phases. A Gilson Model 303 pump with a Model 802 manometric module (Gilson Medical Electronics, Middleton, Wisconsin) or a SP8700XR pump (Spectra Physics, San Jose, California) were used to deliver mobile phases. A Rheodyne Model 7125 manual loop injector (Rheodyne, Cotati, California) with a 5-μL loop was used for sample introduction. For plate height measurements, a Rheodyne Model 7520 0.5-μL injector was used. The detector was a Kratos Model 783 variable wavelength detector operated at a 20-ms response time and fitted with a 12-μL flow cell or, for plate height measurements, a 2.4-μL flow cell. The internal plumbing connections of the detector were modified so as to minimize the dead volume between the column outlet and the flow cell. The detector signal was digitized using a Hewlett-Packard Model 18652A analog-to-digital converter, operated at a sampling rate of 16 Hz, and integrated using a Hewlett-Packard Model 3357 Laboratory Automation System.

Column Fabrication

The serpentine columns were constructed from 1/16″ o.d. × 0.01″ (0.025 cm) i.d. PTFE tubing (Upchurch Scientific, Oak Harbor, Washington). An epoxy glass electronic breadboard (Vectorboard brand, type 170H85WE, Newark Electronics, King of Prussia, Pennsylvania) was used as the framework to hold the PTFE tubing in the serpentine configuration. This breadboard had 1/16″ diameter holes with a spacing of 10 holes per inch. To facilitate knitting of the tubing on these boards, the holes were reamed using a drill press with a 1/16″ bit prior to knitting the column. The columns were knitted by pushing a small portion of one end of the tubing (approximately 1 to 1.5 in.) through a hole near the corner of a 6-in.^{2} piece of breadboard and drawing the remainder of the tubing through an adjacent hole. As the tubing was drawn tight, a semicircular loop was formed. The remainder of the tubing was then drawn through the next hole in the row to form a second semicircular loop on the opposite face of the board from the first loop. This knitting

process was continued with the direction through which the tube enters the hole alternating between adjacent holes. In this manner a row of knitted tubing was formed which had a wavelike appearance when the breadboard was viewed edgewise (see Fig. 1a). Care was taken to draw each loop tightly before starting the next loop. This made the radius of the loops as small as possible which maximizes the secondary flow for optimum performance of the tube (*6*). After the first row of 50 holes was formed, a new row was started using the holes next to the row just finished. This procedure was continued until only a short piece of 1 to 1.5 in. remains. Stainless steel fittings were then installed on the two free ends for connection to the injector and detector. Figure 1(b) shows a completed serpentine column.

Column Etching and Conditioning

Initially, attempts were made to use the sodium/naphthalene reagent directly by either drawing the reagent through the serpentine column with a vacuum pump or forcing the solution through manually using a syringe. These attempts were unsuccessful due to plugging of the tubes by particulate matter (presumably naphthalene crystals) in the reagent. Discussions with the manufacturer indicated that using slightly elevated temperatures and diluting the reagent in an ether might eliminate the plugging.

The following procedure was developed by trial and error and consistently gave a good etch without plugging the serpentine column. The closed container of etching reagent was warmed to about 40°C using a water bath. (CAUTION: Do not heat the reagent above 50°C!) Shake the bottle thoroughly and wipe off excess moisture from the outside. Pipet 15 mL THF into a 25-mL volumetric flask; then pipet 5 mL heated etching reagent into the same flask. Stopper the flask quickly and shake the mixture thoroughly. It should be noted that the etching reagent, both in neat and diluted form, is susceptible to degradation by atmospheric light and moisture. Therefore, the stock reagent bottle should be closed tightly when not in use and the diluted reagent should be prepared fresh daily. Active reagent, both the stock solution and that diluted in THF, has a blue-black appearance. The reagent becomes light brown or clear as the sodium metal is depleted and should be discarded at this point. All glassware and other materials with which the reagent comes in contact should be dried thoroughly before use. The volumetric flask allows a solvent blanket to form in the neck which protects the reagent from atmospheric degradation during short-term handling. A portion of the

diluted reagent was withdrawn into a glass syringe using a piece of plastic tubing and a Luer-Lok adapter. Excess air was expelled from the syringe which was then connected to the serpentine column using a suitable adapter. Manual pressure on the plunger forced the etching reagent through the column. The leading edge of the reagent zone changed from blue-black to light brown as the sodium was depleted. The plunger was depressed until black (unreacted) reagent emerged from the column outlet. The reagent-filled column was allowed to stand for 5 min, then flushed using 3 mL fresh THF. The inner wall of the etched tube had a dark grey appearance. The column was then flushed with air, followed by a second 3 mL portion of THF. After the second THF rinse was removed with air from the syringe, the column was conditioned by forcing 3 mL water through it. The leading edge of the water zone developed a brown discoloration from substances leached from the etched surface, and the etched inner wall became slightly less dark upon this initial contact with water. The water behind this discolored zone remained clear. If this water conditioning step was omitted, the column exhibited irreversible adsorption for many solutes. The remaining water was flushed out with air, and the column was then ready for equilibration with the mobile phase of choice.

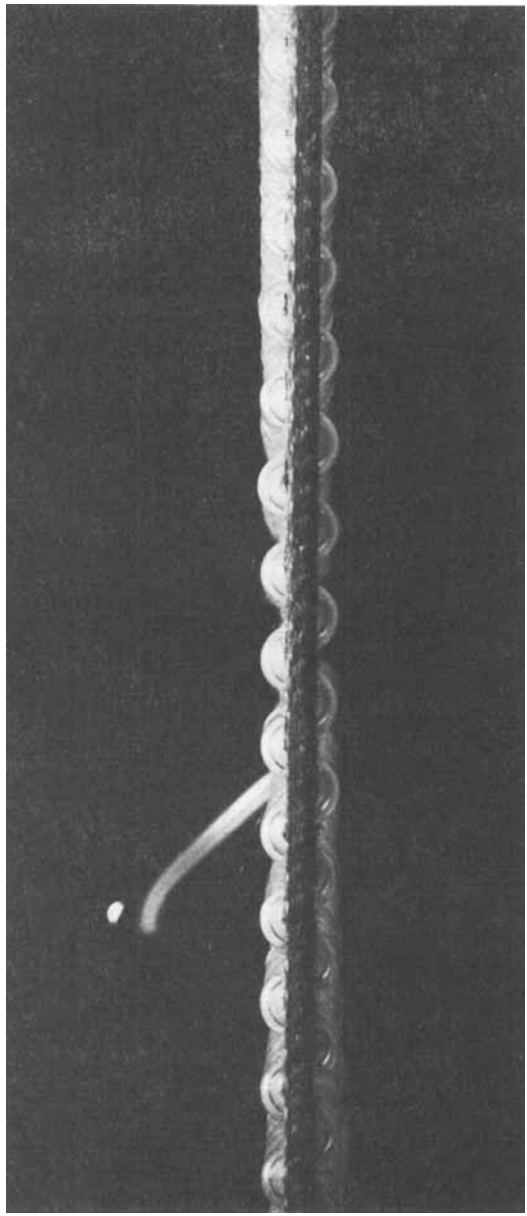
Serpentine Plate Height Measurements

Two PTFE open tubular columns of identical dimensions (0.025 cm i.d. × 511 cm in length) were used. One was woven into a serpentine column having a total of 1161 semicircular turns. The other tube, hereafter referred to as the nonserpentine column, was placed around the laboratory bench to form a single large loop without kinks or sharp turns and the ends connected to the injector and detector. A 0.5-μL injector and a 2.4-μL flowcell were used for these experiments. Both *n*-hexane and cyclohexane were used as mobile phases, and in each case the sample was a 1% v/v solution of toluene ($k' = 0$) in the mobile phase. Theoretical plate numbers were calculated by computer from the digitized chromatograms using the peak width at half-height method. All measurements were taken as the average of three separate injections, and the experiment was conducted at an ambient temperature of 24°C.

RESULTS AND DISCUSSION

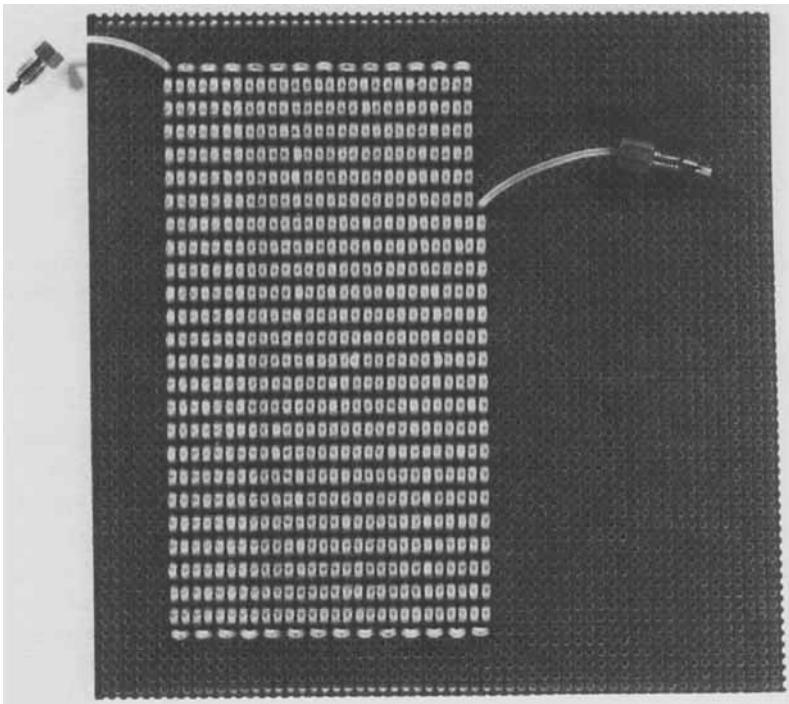
Plate Height Measurements

Figure 2 shows van Deemter plots (HETP vs linear velocity) for toluene on the serpentine and control columns using *n*-hexane and cyclohexane



(a)

FIG. 1. (a) Edgewise view of PTFE serpentine column showing detail of one row. (b) Completed serpentine column with fittings installed.



(b)

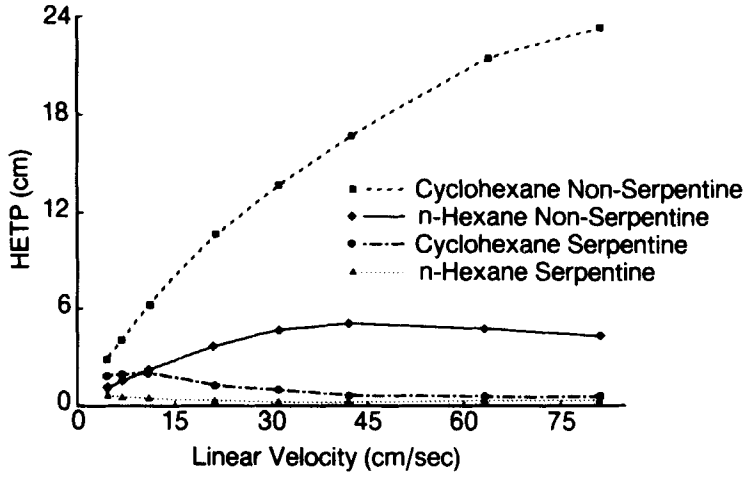


FIG. 2. Van Deemter plot of toluene ($k' = 0$) on serpentine and control columns in both using either *n*-hexane or cyclohexane as mobile phase. Sample 1% v/v in mobile phase, 0.5 μL injection.

as mobile phases. Several points should be noted. The serpentine configuration gave a dramatic decrease in zone dispersion relative to the nonserpentine column, especially at linear velocities above 10 cm/s. This trend of decreased dispersion with increasing velocity was also observed in serpentine and coiled tubes by Katz and Scott (*5*) and was attributed to increased disruption of the parabolic flow profile by increased radial mixing. Greater efficiency (lower HETP) was obtained in *n*-hexane than in cyclohexane as expected from their relative viscosities, viz., 0.31 cP for *n*-hexane vs 1.0 cP for cyclohexane at 20°C. However, efficiency was much less dependent on solvent viscosity and more uniform across the range of flow rates studied for the serpentine column than for the nonserpentine. The reduction in band dispersion obtainable with the serpentine configuration is shown dramatically in Fig. 3, where an injection of toluene in *n*-hexane gives a much narrower and sharper peak on the serpentine than on the nonserpentine column of identical length.

One drawback of the serpentine tubing is its increased backpressure relative to straight tubes. The pressure drop across the 511 cm serpentine tube (184 psi) using water at 2.0 mL/min was 1.9 times greater than that of the control tube (96 psi) of identical length under the same conditions. This agrees with the work of Englehardt and Lillig (*9*) who reported a

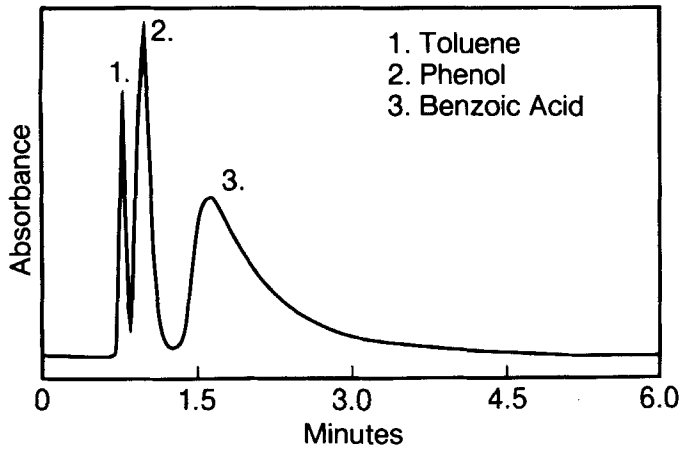


FIG. 3. Peaks of 1% v/v toluene in *n*-hexane (0.5 μL) after passing through 511 cm × 0.25 mm i.d. PTFE serpentine and control columns at 1.5 mL/min (31 cm/s).

factor of 2 increase in backpressure for knitted capillaries relative to straight tubes of the same dimensions.

CHROMATOGRAPHIC SEPARATIONS ON ETCHED COLUMNS

Solutions of sodium metal in liquid ammonia or sodium metal in mixtures of naphthalene and various ethers have been used extensively for etching fluorocarbon surfaces, usually to make them bondable to other substances (*11*). These solutions are believed to contain unpaired electrons which extract fluorine atoms from the polymer leaving a carbonaceous surface (*11*, *12*). Dwight and Riggs (*13*) used ESCA and other techniques to characterize sodium-etched PTFE surfaces. They found evidence for the presence of carbonyl and carboxylic as well as olefinic functional groups within the carbonaceous surface. Examination of the polymer using electron microscopy before and after etching showed a large increase in surface roughness upon etching. The hydrophilic nature of the etched surface, as evidenced by contact angle measurements with water, supports the theory that polar functional groups are present (*14*). It was therefore thought that sodium/naphthalene etching of the inner wall of a PTFE serpentine would produce a workable normal phase column. A separation of toluene, phenol, and benzoic acid on an etched serpentine column using a hexane mobile phase is shown in Fig. 4. The compounds elute in order of

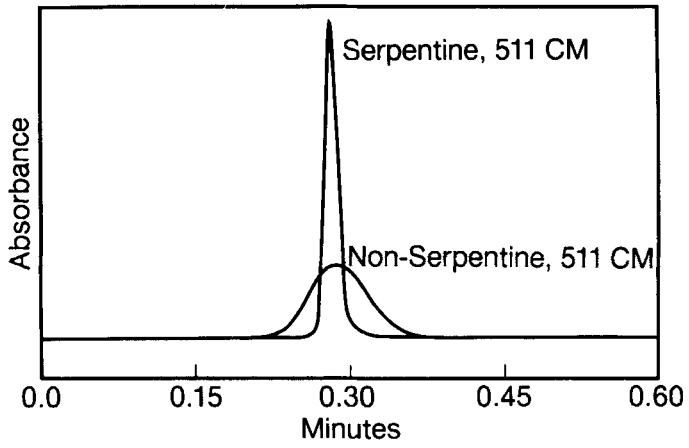


FIG. 4. Separation of toluene (100 ng), phenol (100 ng), and benzoic acid (400 ng) on sodium/naphthalene-etched PTFE serpentine column (5.1 m × 0.25 mm i.d.) using *n*-hexane mobile phase at 0.75 mL/min.

increasing polarity, as expected in a normal phase system. Severe tailing was exhibited by benzoic acid which may be due to interaction of the carboxylic moiety of this species with the strongly polar sites on the etched surface. The retention of this column is admittedly low since even with a very weak mobile phase (*n*-hexane) only slight retention was obtained with two very polar solutes (phenol and benzoic acid). This should be expected since an open tubular column of these dimensions would have an unfavorable phase ratio. If increased roughening could be obtained by more vigorous etching procedures, this drawback might be offset. As mentioned previously, water was used to condition the column after etching. When this step was deleted, phenol and benzoic acid were irreversibly adsorbed using hexane as the mobile phase. Hexane/diethyl ether mobile phases could elute these species, but only as extremely broad (several minutes wide) peaks. The water conditioned columns, although much less retentive, gave greater efficiency. It was also found that the retention of phenol and benzoic acid in the water conditioned columns gradually decreased over a 2-day period. However, rinsing the column with dry THF restored the original retention, which suggests that polar contaminants had accumulated during use.

The fact that the etched surface has a hydrocarbon structure in addition to polar functional groups suggested that it might exhibit reversed phase properties with appropriate aqueous mobile phases. This

was found to be the case. Figure 5 shows a separation of acetone and naphthalene (the former being nonretained) in a methanol/water mobile phase. The retention of naphthalene on the column decreased fairly rapidly with mobile phases containing significant levels of water. The separation shown in Fig. 5 could not be reproduced 2 days later even when the concentration of organic modifier was decreased by 50%. This decrease was accompanied by a fading of the gray color of the etched surface. Unlike the retention decrease with the hexane mobile phase, the water-induced change could only be reversed by re-etching the column. This restored column retention to approximately its original value. Dwight and Riggs noted changes in sodium-etched PTFE after prolonged exposure to boiling water (*13*). However, the effects of water at room temperature were not reported.

Etched PTFE serpentine columns are also amenable to gradient elution in both normal and reversed phase modes. Figure 6 shows a separation of toluene, phenol, benzoic acid, and *p*-hydroxybenzoic acid using a hexane/methylene chloride/THF gradient. All four components are well resolved from one another. Figure 7 shows a separation of acetone, toluene, and naphthalene using a water/acetonitrile gradient. Due to the instability of the etched surface in water, partial resolution of acetone and toluene could only be obtained on a freshly etched column. When this mixture was chromatographed isocratically using water as the mobile phase, the naphthalene did not elute.

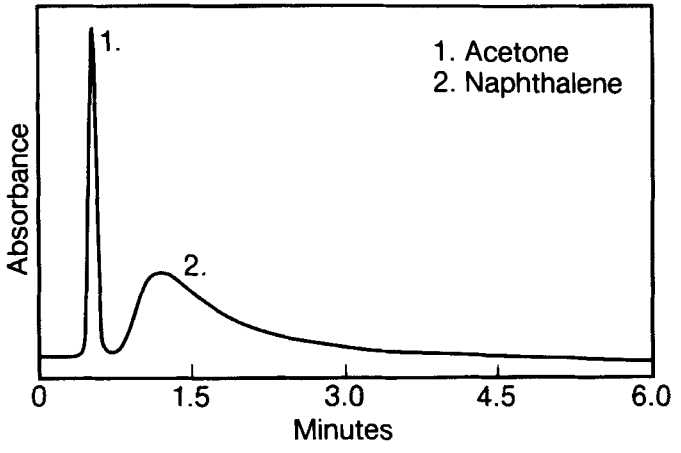


FIG. 5. Separation of acetone (4 μg) and naphthalene (250 ng) on sodium/naphthalene-etched PTFE serpentine (5.1 m × 0.25 mm i.d.) using 20% v/v methanol in water as the mobile phase at 1.0 mL/min. Sample in 10% v/v methanol in water.

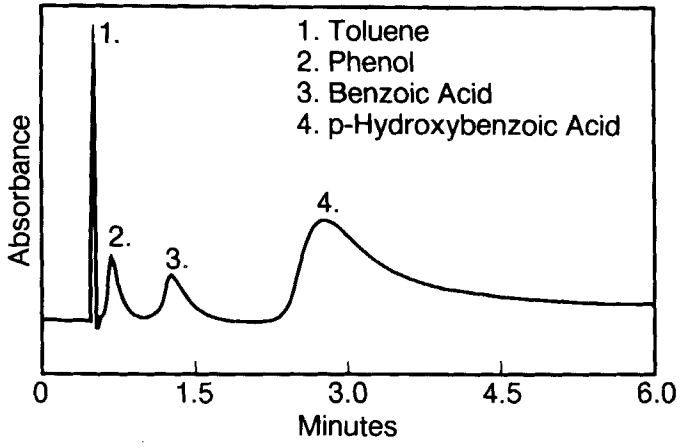


FIG. 6. Separation of toluene (200 ng), phenol (50 ng), benzoic acid (50 ng), and *p*-hydroxybenzoic acid (200 ng) on sodium/naphthalene-etched PTFE serpentine column (5.1 m × 0.25 mm i.d.) by gradient elution. Solvent gradient: 5% v/v CH_2Cl_2 in *n*-hexane to 20% v/v CH_2Cl_2/10% v/v THF in *n*-hexane. Flow rate, 1.0 mL/min. Sample solvent: 5% v/v CH_2Cl_2 in *n*-hexane.

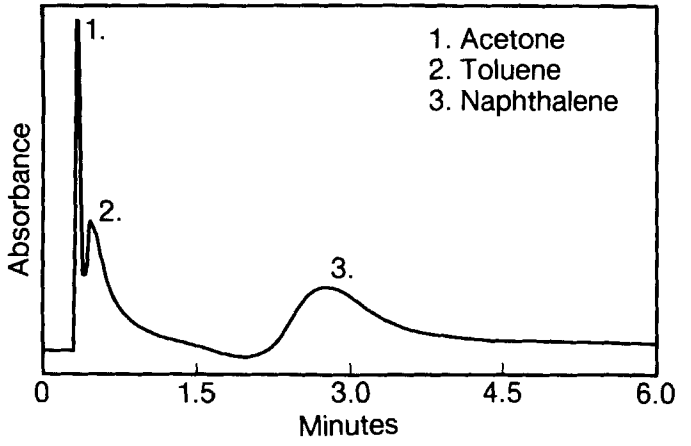


FIG. 7. Separation of acetone (4 μg), toluene (2 mcg), and naphthalene (250 ng) on sodium/naphthalene-etched PTFE serpentine column (5.1 m × 0.25 mm i.d.) by gradient elution. Solvent gradient: 0–20% v/v acetonitrile in water in 1 min at 1.5 mL/min. Sample solvent: 10% v/v acetonitrile in water.

SUMMARY AND CONCLUSIONS

The feasibility of liquid chromatographic separations on an open tubular serpentine column has been demonstrated. The serpentine geometry overcomes the high dispersion characteristic of open tubes, and the sodium/naphthalene etching produces a stationary phase resistant to the shear forces encountered with liquid mobile phases. While this work provides a useful starting point for further investigations into serpentine open tubular liquid chromatography, several limitations to the current apparatus exist. First, the current design is mechanically rigid and can maintain the relatively stiff, thick-walled tubing in a serpentine configuration having minimal loop radius but is time consuming to construct (each row requires approximately 15 min of manual labor). A different framework design may make automated column construction feasible. Also, retention of solutes on these columns tends to be slight, which severely limits the applicability of the system. This may be due to the unfavorable phase ratio and/or the nature of the etched surface. Different etching procedures may overcome this problem. Also, the use of somewhat smaller i.d. tubing would improve the phase ratio while still avoiding the problems inherent in extremely small capillaries. Finally, the etched surface is labile with respect to water, which is a serious limitation. However, the introduction of various functional groups upon etching should make the fluorocarbon surface amenable to derivitization for the preparation of chemically bonded stationary phases. These should be more stable than the columns used in this work and may also exhibit better retention characteristics. The characterization of serpentine columns produced by the use of different etching procedures in conjunction with chemical derivitization of the functional groups will be the primary focus of future work.

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