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PREPARATION OF FUSED-SILICA POLAR STATIONARY PHASE WALL-COATED OPEN TUBULAR COLUMNS

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SUMMARY

Recent methodology, developed in our laboratory for the preparation of Pyrex (borosilicate) wall-coated open tubular (WCOT) gas chromatography columns with the polar stationary phases Superox™-4 ($4 \cdot 10^6$ molecular weight polyethylene glycol) and SP-1000 (nitroterephthalic acid derivative of Carbowax 20M), was successfully adapted to the preparation of fused-silica WCOT columns with these phases. Pretreatment of the fused-silica tubing with Superox-4 increased the surface energy, improved the wettability of the surface for polar phases and deactivated the fused-silica surface. The polar stationary phases, Superox-4 and SP-1000, were statically coated onto the Superox-4-treated surface. These polar WCOT columns were found to be efficient, thermally stable, and low in surface activity.

INTRODUCTION

The introduction of fused-silica capillary tubing has created a revolution in glass capillary gas chromatography (GC). The high tensile strength and flexibility of fused-silica capillary columns have attracted many chromatographers, who would not have considered venturing into the frail world of Pyrex and soda-lime glass columns. The advantages of sued-silica capillary tubing have been adequately presented in the literature[1–4]. However, the debate over the merits of laboratory preparation of wall-coated open tubular (WCOT) capillary columns as opposed to purchasing columns from commercial suppliers still continues. Some experts insist that the time required for laboratory preparation of columns can only be justified for a limited number of laboratories. Others feel that the advantages of laboratory preparation are sufficient to make column preparation scientifically and economically profitable. About four years ago, our laboratory began its conversion from conventional packed columns to glass capillary columns and most of our WCOT column preparation techniques were developed for Pyrex glass capillary tubing. Experience has indicated that the full power of glass capillary GC can never be realized without preparing columns. The advantages of varying stationary-phase film thicknesses alone dictated its necessity. The number of stationary phases used need only be a few, but the vari-

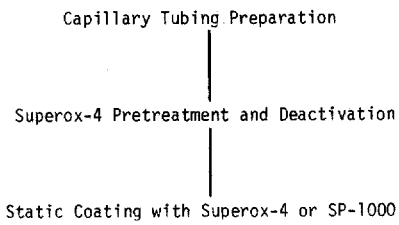

Fig. 1. Procedure for the preparation of fused-silica Superox-4 and SP-1000 WCOT capillary columns by the Superox-4 pretreatment and deactivation procedure.

ation in film thicknesses allows great flexibility in the application of this GC technique. Thus, both volatile and high-molecular-weight components can be separated efficiently and in the shortest possible times on columns of different film thicknesses of the same stationary phase. Each capillary column is thus tailor-made for the specific class of compounds to be separated and efficiency and time of separation are balanced.

The advent of fused-silica capillary tubing has made laboratory preparation of capillary columns even more inviting, since the hazards of column handling during preparation are virtually eliminated. Cost efficiency increases with time, as the polar liquid stationary phases are the best candidates for recoating columns. Polar stationary phase columns, and sometimes even nonpolar phase columns, can be recoated and often are equal to or superior to the original columns[5]. Our recent reports[5,6] have presented details of Pyrex capillary column preparation using Superox™-4, a $4 \cdot 10^6$ molecular weight polyethylene glycol liquid phase. This Superox-4 pretreatment and deactivation procedure is a simple, reproducible technique for the preparation of polar WCOT columns (Fig. 1), as it only involves the static coating of the liquid phase onto a Superox-4 deactivated fused-silica surface. Practical details of preparation will be presented and the surface activities of the columns, determined by analyses of standard activity mixtures, will be described.

Deactivation of glass surfaces with polyethylene glycols, such as Carbowax 20M, has been a common step in many preparation techniques[7]. Due to its gum-like behavior, Superox-4 is also a superior deactivation agent. It adheres to bare glass surfaces and resists droplet formation, even during heating[4,5,8,9]. The Superox-4-treated fused-silica surface has a polar character, in contrast to bare silica, which has a very low surface energy[1–4]. Thus, the low energy of the surface is raised to a level that makes it wetable with polar stationary phases. We consider this to be a pretreatment and refer to this procedure as the "Superox-4 pretreatment and deactivation" technique. The successful adaptation of this methodology to the preparation of fused-silica Superox-4 and SP-1000 WCOT columns will be presented.

EXPERIMENTAL*

Materials

Fused-silica glass capillary tubing was obtained from Hewlett-Packard

* Reference to a company or product name does not imply approval or recommendation by the USDA.

(Avondale, PA, U.S.A.), Superox-4 from Alltech (Deerfield, IL, U.S.A.) and SP-1000 from Supelco (Bellefonte, PA, U.S.A.). All solvents were Burdick and Jackson (Muskegon, MI, U.S.A.) "distilled-in-glass" grade and were redistilled in glass before use.

All connections to vacuum sources, helium sources and coating solution reservoirs were made with heat shrinkable Teflon-TFE-FEP tubing (Alltech, Deerfield, IL, U.S.A.).

Capillary tubing preparation

The fused-silica tubing was rinsed with methylene chloride and dried under nitrogen flow.

Superox-4 pretreatment and deactivation

The fused-silica capillary tubing was rinsed with a 0.2% solution of Superox-4 (2 mg/ml) in methylene chloride by pulling a plug of the solution (to fill about 10% of the capillary tubing length) into the tubing with vacuum and then pushing the plug through the tubing with nitrogen pressure, at a rate of about 1 cm/sec. The tubing was dried under nitrogen flow, placed in an oven, and heated at 330°C for 1 h. During the heating step, air was excluded with a low flow of high-purity nitrogen. Best results were obtained when this process was repeated.

Static coating with Superox-4

The Superox-4-treated fused-silica capillary tubing was filled with a solution of Superox-4 (1–4 mg/ml of methylene chloride) from a reservoir, under nitrogen pressure. After filling, the pressure was released and the tubing was disconnected from the reservoir. Handling the tubing increased its temperature and produced a small flow of solution from the column ends. One end of the column was placed into water-soluble glue, and the other end was connected to vacuum. About 10 cm of the tubing was filled with the water-soluble glue. (It was very important to exclude air bubbles between the glue plug and the coating solution.) The capillary end, connected to the vacuum, was disconnected and elevated so that a small flow from the opposite end induced formation of a tiny droplet of glue. The tubing ends were then leveled and the glue plug was allowed to harden overnight.

Static coating was accomplished by connecting the open end of the column to a vacuum pump and submerging the tubing (all, but the water-soluble glue plug) into a constant temperature water bath, at 40°C[10]. The methylene chloride was evaporated and the liquid phase deposited as a thin film on the tubing surface. After coating, each column was installed in a gas chromatograph, for conditioning and testing, as previously described[6].

Static coating with SP-1000

Static coating of SP-1000 was identical to the procedure for Superox-4. The coating solution contained 4–8 mg of SP-1000 and 1 mg of Superox-4 per ml of methylene chloride.

RESULTS AND DISCUSSION

Superox-4 is an excellent polar liquid phase for a wide variety of components.

Its high thermal stability allows the separation and elution of components, which would not elute from other polyethylene glycol stationary phase columns, even at their maximum operating temperatures[11]. A chromatogram of an activity standard mixture, separated on a fused-silica Superox-4 capillary column showed good peak shapes, indicating low surface activity (Fig. 2). Analysis of a 2,6-dimethylphenol–2,4-dimethylaniline standard mixture, containing equal amounts (g/g) of each compound, indicated low acid–base surface activity. When prepared by our methodology, fused-silica Superox-4 columns are slightly basic in character. However, no adsorption or tailing for the 2,6-dimethylphenol peak was indicated by either standards separation. Superox-4 is also an excellent phase for the separation of basic components. The separation of a standard mixture of major tobacco alkaloids on a fused-

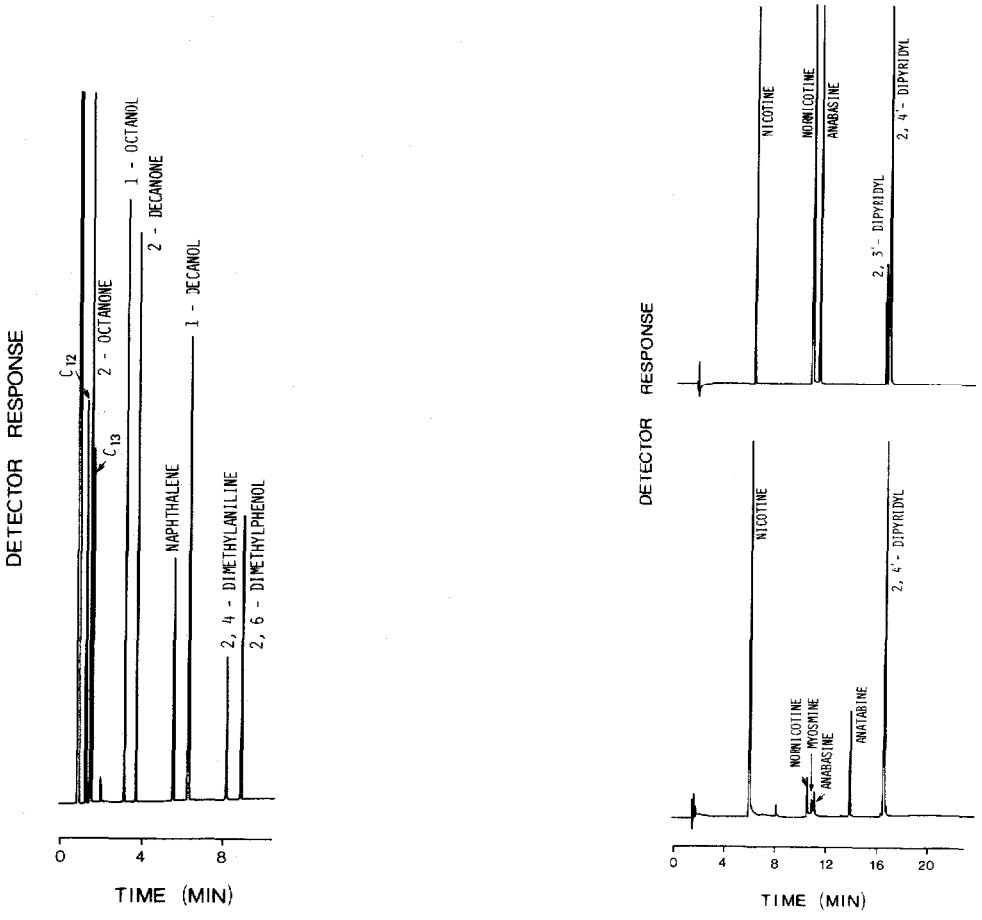

Fig. 2. Chromatogram of an activity standards mixture separated on a fused silica Superox-4 WCOT column. Conditions: 25 m × 0.2 mm I.D.; temperature program, 100–150°C at 4°C/min; H_2 flow, 40 cm/sec; split injection mode; flame-ionization detector.

Fig. 3 GC separation of (A) an alkaloid standards mixture on a fused silica Superox-4 WCOT column and (B) an alkaloids extract from a flue-cured tobacco. Conditions: 25 m × 0.3 mm I.D.; temperature program, 140–220°C at 4°C/min; He flow, 28 cm/sec; split injection mode; NPD.

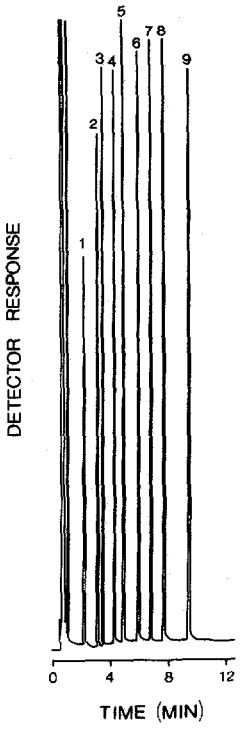

Fig. 4. Chromatogram of a mixture of underivatized, volatile acids on a fused silica SP-1000 WCOT column. Peaks: 1 = acetic acid; 2 = propionic acid; 3 = isobutyric acid; 4 = *n*-butyric acid; 5 = isovaleric and 2-methylbutyric acids; 6 = valeric acid; 7 = β-methylvaleric acid; 8 = caproic acid; 9 = heptanoic acid. Conditions: 10 m × 0.2 mm I.D.; temperature program, 120–220°C at 6°C/min; H_2 flow, 30 cm/sec; split injection mode; flame ionization detector.

silica Superox-4 capillary column with detection by a nitrogen–phosphorus detector (NPD) is shown in Fig. 3A. A similar separation was obtained for alkaloids from cured tobacco leaf (Fig. 3B). We have previously developed methodology for the analysis of tobacco alkaloids on Pyrex glass Superox-4 capillary columns[12]. Since fused-silica Superox-4 columns are lower in surface activity and generally more efficient than the Pyrex columns, this separation of tobacco alkaloids was significantly improved. We have also employed fused-silica Superox-4 WCOT capillary columns to separate other nitrogen-containing compounds, such as nitrosamines, which are known to be highly carcinogenic and have been found in both tobacco leaf and smoke[13]. Carbolines, another class of potentially harmful components of cigarette smoke, have also been successfully separated by capillary GC on fused-silica Superox-4 capillary columns[11].

Fused-silica SP-1000 columns, prepared by our technique, have shown excellent chromatographic characteristics. The separation of our activity standard mixture on a fused-silica SP-1000 WCOT column was almost identical to that on a Superox-4 column (Fig. 2). Stationary phases of the SP-1000 type are slightly acidic in nature and thus produce slightly acidic columns. However, fused-silica SP-1000 columns are not as acidic as Pyrex SP-1000 columns, when prepared with the Superox-4 treatment

procedure. There are two reasons for this difference in acidity. Pyrex glass is acidic in nature and imparts some of this character to Pyrex capillary columns. Secondly, we actually prepare our fused-silica SP-1000 columns with a mixed liquid phase containing 0.1% Superox-4 in the SP-1000 coating solution. This addition of the Superox-4 has increased coating efficiency and reproducibility of the SP-1000 coating procedure.

SP-1000 columns have often been used in analyses of flavor and fragrance compounds. We have also used them for the separation of tobacco-flavor constituents and low-molecular-weight acids. Such a separation of a standard mixture of low-molecular-weight aliphatic acids is shown in Fig. 4. This separation was achieved with the underivatized acids. (Prior to analysis, the sample solution was adjusted to pH 1–2 with concentrated hydrochloric acid.)

We have concluded that our Superox-4 preparation technique is a simple and reproducible procedure that can be recommended to other chromatographers. The flexibility and high tensile strength of the fused-silica tubing greatly facilitate the preparation of GC columns. Good and often excellent columns are produced with this technique. The inherent purity and low surface activity of fused-silica tubing, as compared to Pyrex tubing, results in a marked improvement in column thermal stability and surface activity.

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