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SOME PRACTICAL ASPECTS OF COLUMN DESIGN FOR PACKED-COLUMN SUPERCRITICAL-FLUID CHROMATOGRAPHY

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SUMMARY

Different column configurations and column packings are evaluated for packed-column supercritical-fluid chromatography under pressure-programmed conditions. The best chromatographic performance was observed for columns of about 15–25 cm in length, packed to a moderate density with small-diameter particle packings of low surface area. Chemical and physical interactions of polar analytes with silanol groups were identified as a significant problem with available column packings that calls for the use of low-surface-area chemically bonded phases. Further deactivation by coating with a non-extractable, liquid organic salt is shown to modify the selectivity and activity of silica and chemically bonded silica phases. Recommendations are made for the selection of different colunm configurations for analytes of different kinds.

INTRODUCTION

The current revived interest in supercritical-fluid chromatography (SFC) is related to its ability to fill the gap between the capabilities of gas chromatography (GC) and liquid chromatography (LC) for the separation of middle-molecular-weight analytes, especially those of low thermal stability[1,2]. Supercritical fluids have viscosities somewhat similar to those of gases and diffusion coefficients approaching those of liquids. However, their most important characteristic is that their density is easily changed by changing pressure or temperature and in so doing the solubility characteristics of the analytes are dramatically changed. At temperatures above the critical temperature pressure or density programing permits a controlled change in chromatographic characteristics from GC-like at low pressure to LC-like at high pressures. For carbon dioxide, the most popular mobile phase in contemporary practice, the critical temperature is 31.05°C, thus enabling low temperatures to be used for the separation of thermally labile analytes. In terms of efficiency and speed of analysis, GC will always outperform SFC but for those samples of limited volatility and/or thermal stability SFC has the potential for faster analyses than LC.

Unlike the situation in GC and LC there is no kinetic model available to optimize packed-column SFC[2,3]. Also, in contemporary practice, most separations are performed with pressure or density programming, and it is conceivable that a dis-

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tinctly different threshold density exists for individual analytes before a retentive distribution mechanism begins to operate[4,5]. Since supercritical fluids are compressible, appreciable density drops may exist along the column, dependent on the column permeability and length. The column pressure drop (density gradient) can cause peak broadening due to solubility differences along the column[6–14]. Pressure programing produces significant velocity and viscosity gradients along the column[15–17]. This situation is further complicated in practice when pressure regulation is obtained by using a flow restrictor that results in a complex variation in linear velocity with pressure[18]. Pressure programing can also cause significant peak compression, leading to an increase in the quality of the separation. This can result from different mechanisms, for example, from the imbalance of mobile phase mass flow-rate in and out of the column under programed conditions or from the continuous precipitation and dissolution of the analyte along the column due to the existence of a column pressure drop, and the dependence of analyte solubility on density (pressure) and, therefore, migration rate. In both cases, the net result is that the tailing edge of the analyte peak moves faster than the leading edge thus reducing the natural zone broadening.

Several complex factors influence zone broadening in packed-column SFC, and the situation is much more difficult to model than GC or LC. Simply borrowing established models from GC or LC can be expected to represent only crude approximations. This led us to question the reasoning behind using columns optimized for LC in SFC. In the absence of a theoretical model we will take a phenomenological approach to column design under pressure-programed conditions and use the results from carefully considered experiments to establish reasonable practical operating conditions for SFC. When this is done a reasonable picture emerges as to the usefulness of the most common LC columns and column packing types for packed-column SFC. The question is a significant one, as commercially available LC columns are used almost universally in the contemporary practice of SFC and the results obtained would be prejudicial to the growth of SFC if this choice proves inappropriate.

EXPERIMENTAL

All chemicals and solvents were of the highest purity available. Polyethylene glycol and polypropylene glycol standards were obtained from BASF (Wyandotte, MI, U.S.A.). Spherisorb alumina (20 μm and 100 μm) and Spherisorb ODS1 (5, 10, and 100 μm) octadecylsilanized silica were obtained from Phase Separations, (Queensferry, U.K.). Octadecylsilanized silica (40 μm) was obtained from J. T. Baker (Phillipsburg, NJ, U.S.A.). Corasil and PC18 pellicular silica and octadecylsilanized silica (all 37–55 μm) were obtained from Waters Assoc. (Framingham, MA, U.S.A.) and Alltech (Deerfield, IL, U.S.A), respectively. Carbopack B packings of various mesh sizes were obtained from Supelco (Bellefonte, PA, U.S.A.). Porapak Q polymeric beads (100–120 mesh), Seragen latex particles (6.4 ± 1.9 μm), Vydac wide-pore octadecylsilica (330 Å, 10 μm), Nucleosil silica (500 Å, 10 μm) and parafilm tape, were obtained from Anspec (Ann Arbor, MI, U.S.A.). Delta bond cyanopropyl- and octadecylsilica prepacked columns of 10 cm x 1 mm I.D. and 5 and 10 μm particle diameters were obtained from Keystone Scientific (State College, PA, U.S.A.). Ethoquad 18/25 was obtained from Armak Industrial Chemicals (Chicago, IL, U.S.A.).

All chromatographic experiments were performed using a Suprex 200A (Pitts-

burgh, PA, U.S.A.) supercritical-fluid chromatograph with either a tapered, fused-silica restrictor or a stainless-steel restrictor, prepared as described below. Chromatograms were recorded with either a Hewlett-Packard 3396A computing integrator (Avondale, PA, U.S.A.) or a Shimadzu R-111 chart recorder (Columbia, MD, U.S.A.). The mobile phase was supercritical-fluid-grade carbon dioxide from Scott Speciality Gases (Plumsteadville, PA, U.S.A.).

Since this project required installing and removing a large number of columns a rugged restrictor was required that incorporated a standard column end fitting. All results reported in this paper were obtained with the same restrictor, prepared from a 30 cm × 0.01 in. I.D. 1/16 in. O.D. stainless-steel capillary tubing. One end of the tube was symmetrically crimped with a pair of compound pliers. The flow-rate was adjusted by placing the tip of the restrictor in a small vial, filled with water, and adjusting the degree of crimping until the desired bubble rate was obtained. This corresponded to an atmospheric flow-rate of 8 ml/min of carbon dioxide at a pressure of 80 atm, set at the pump. A standard 1/16-in. fitting was then attached to the restrictor, which was positioned so that its tip was about 1 in. below the flame tip and the flame was not extinguished when the pump was operating at its maximum pressure. Excess capillary tubing was cut off. The detector base heater, which also heats the restrictor, was set to 300°C and remained at that temperature for all experiments.

Columns for SFC were prepared by slurry packing under high pressure, as used for LC and described in detail elsewhere[19], by the tap-and-fill method, similar to columns prepared for GC[20], and by displacement with supercritical carbon dioxide as described below. Standard Valco fittings, 2-μm screens, and precut, smoot-bore, stainless-steel tubing were used for all columns (Valco Instruments, Houston, TX, U.S.A.).

For the displacement packing method, the analytical column was connected directly to an appropriately sized reservoir, using a Valco union of larger bore than the internal diameter of the analytical column. Initially the reservoir was filled with excess bulk packing material by tap-and-fill using an electronic engraving tool for vibration; a water aspirator vacuum was applied to the base of the reservoir if necessary. The reservoir and the column were then connected and this assembly was connected to the supercritical-fluid chromatograph at its inlet as if it were a column (Fig. 1). At this point the packing assembly was still isolated from the supercritical fluid. The syringe pump was charged to 480 atm, and the pump isolation valve was opened, thereby flooding the packing assembly with high-velocity carbon dioxide. For a time of several minutes after the isolation valve was opened, the packing assembly was vibrated with the engraving tool. The initial flow-rate of carbon dioxide is relatively slow (the pump can almost recover the preset pressure setting), but as the bed begins to consolidate (usually < 1 min) the flow-rate of carbon dioxide will begin to increase and finally reach a constant value. After several milliliters of carbon dioxide (as read at the pump, high pressure) had passed through the column, a plug was placed in the outlet of the column so that the flow was restricted. The flow was maintained slow enough so that the pump could recover to the set point, 480 atm. This condition was maintained for a short period, and then the plug was removed and the syringe pump isolation valve was closed. After the packing assembly had depressurized through the column exit, the packing assembly was removed from the chromatograph and disassembled. Surplus packing at the top of the column was removed with a razor blade,

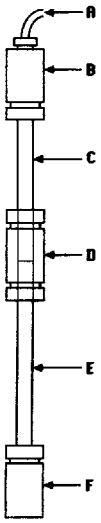


Fig. 1. Assembly for packing columns by the displacement method. A = high pressure carbon dioxide from SFC pump; B = Valco 1/8 in. to 1/16 in. reducing union; C = packing reservoir, 2.1 mm I.D., 1/8 in. O.D. stainless-steel tube; D = Valco bored through 1.8 in. union; E = column; and F = Valco column end fitting 1/8 in. to 1/16 in. fitted with a 2-μm screen.

and a screen and an end fitting were attached to the column. We have used the above method to pack columns with spherical particles from 5 to 40 μm in diameter, lengths from 3 to 30 cm, and column internal diameters from 0.5 to 5.0 mm. We have had columns in continuous service for several months without observable loss in efficiency.

Packings deactivated with Ethoquad 18/25 were prepared with the aid of a rotary evaporator. A dichloromethane solution of Ethoquad 18/25 was added to the required weight of packing material, and the solvent was very carefully evaporated. For particles less than 10 μm in diameter, some loss of material in the solvent vapors is unavoidable, and a different coating procedure may be more appropriate.

RESULTS AND DISCUSSION

In studies of column design for GC or LC, it is conventional practice to construct Van Deemter curves, curves employing reduced parameters according to Knox, or to make measurements of plate heights etc., and to relate the magnitude of these parameters to column properties of realistic column models. This approach is not successful with SFC as the relationship between mobile-phase density, viscosity, velocity, etc. are very complex for packed columns and are still poorly understood, even for the simplest case of operation at constant inlet density[9–14]. Since operation at constant density is not very rewarding in terms of the quality of separations obtained, pressure or density programming is virtually always used in practice, even for relatively simple mixtures. However, under these conditions, we know even less concerning the relationships between mobile phase variables and their changes with position along the column. Solution precipitation/dissolution and peak-compression phenom-

ena also have to be accounted for in a way that is unique. Thus, in this paper we will adopt an empirical approach to establish the influence of column length, column internal diameter, particle size, pore size, packing density, phase ratio, and surface activity on the properties of packed columns under pressure programed conditions.

Fig. 2 shows three separations of the dichloromethane-extractable portion of parafilm (a mixture of hydrocarbons from *ca.* C_{19} to C_{36}) on three columns, packed with pellicular, octadecylsilanized packing of different lengths. Increasing the column length from 4 to 15 cm produces a dramatic increase in resolution but increasing the column length from 15 to 30 cm results in only a small further increase in resolution, gained at the expense of increased analysis time. With decreasing particle size a similar effect is observed, and very long columns seem to offer few advantages in SFC. Columns in the range 10–25 cm are a reasonable compromise. Resolution for a fixed particle size is independent of the column diameter, at least in the range investigated, 0.5–5.0 mm. At larger internal diameters, the high linear velocity and mass flow-rate of the decompressed carbon dioxide at the detector causes problems in maintaining a steady flame. Columns of 1.0–2.0 mm I.D. are convenient to work with and will accept sample sizes in the microliter range without loss of column performance.

Fig. 3 shows the influence of particle size at a fixed column length on the chromatograms of the parafilm sample. The coarse 100-μm diameter particles produce very poor separations, while the difference between the slurry-packed 5-μm particle column and the tap-and-fill pellicular column, containing 37–55 μm particles

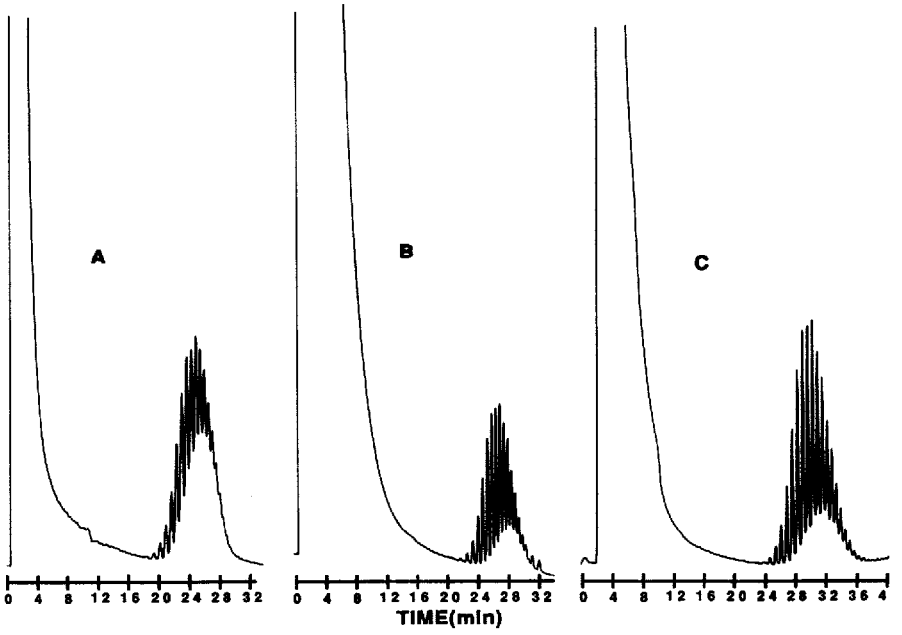


Fig. 2. The influence of column length on the resolution of a mixture of hydrocarbons extracted from parafilm with dichloromethane. The column lengths were A = 4 cm, B = 15 cm, and C = 30 cm, all of 1 mm I.D. Each column was packed with Alltech PC18 (37–55 μm) by the tap-and-fill method. The samples were separated at 80°C, using carbon dioxide as the mobile phase and the following pressure program: 80 atm for 10 min and then linearly increased to 400 atm over 30 min.

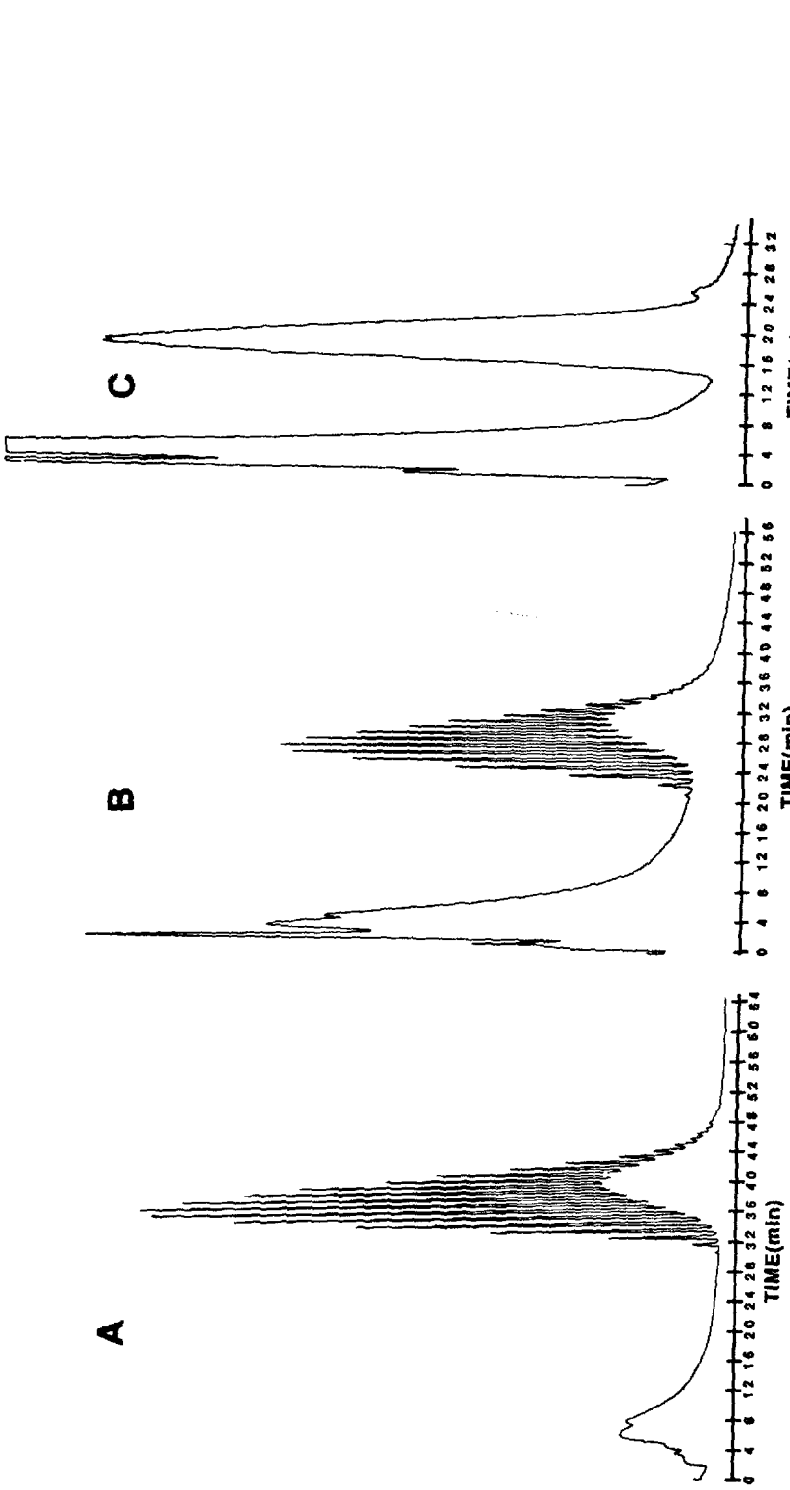


Fig. 3. The influence of particle size on the resolution of a mixture of hydrocarbons, extracted with *dichloromethane* from parafilm. Each column was 10 cm × 1.0 mm I.D. and carbon dioxide was used as the mobile phase with the following pressure program: 80 *atm for* 10 min and then increased linearly to 400 atm over 30 min at 80°C. Column A was obtained from Keystone and was packed with 5 *μm* particles using a *liquid slurry technique*; column B contained pellicular packing (37–50 *μm*) and column C 100 *μm* particles. Columns B and C were packed by the tap-and-fill method.

is not so great. Very large particle diameters are not very useful, even though they provide the lowest column pressure drop, while the smallest particles are not necessarily a great improvement over particles of intermediate size, because they increase the column pressure drop. Particle diameters in the range $5 < d_p < 10$ offer the highest separation capability, but with particle diameters up to 50 μm the differences are not as large as those commonly observed in LC.

To test the possibility that better use of the column pressure drop might be oberved by using a short, narrow, small-particle-diameter column connected to a coarser-particle-diameter column to provide a longer coupled column, two columns containing 10- and 40-μm diameter packings were connected in series in both combinations, Fig. 4. As can be seen the chromatograms are virtually identical. The separation is poor because the particle-size distribution of the 40-μm material is larger than is desirable. The entire column is clearly involved in the separation process, and segregated columns with a short, high-efficiency section, backed by a section of low

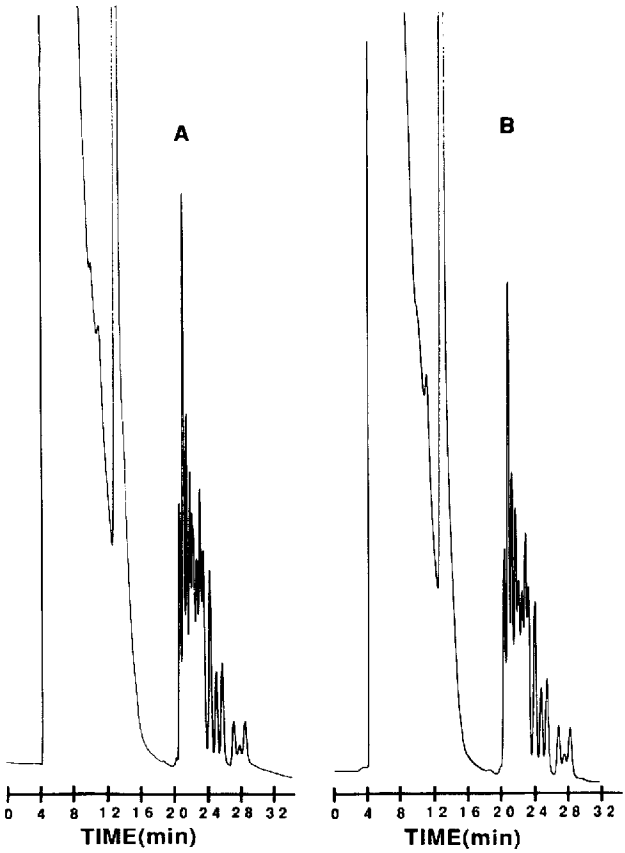


Fig. 4. Coupled columns: A = 10 cm (10 μm) + 10 cm (40 μm) and B = 10 cm (40 μm) + 10 cm (10 μm). Both columns (1.0 mm I.D.) were packed by tap-and-fill with Spherisorb ODS1 (10 μm) and Baker ODS silica (40 μm). The sample was a synthetic mixture of C_{19} to C_{40} *n*-alkanes. The temperature was 100°C. The mobile phase was carbon dioxide with the following pressure program: 80 atm for 10 min then increased linearly to 400 atm over 30 min.

pressure drop, in which the resolution of the sample is enhanced by its migration through a zone of low or no interaction with the stationary phase are not a tenable or useful model. Columns should be homogeneously packed with a single particle size for optimal results, and, as in GC, and LC, the performance of series coupled column most closely resembles that of the lowest performance obtained for the individual columns.

Many separations in SFC have been performed to separate oligomeric mixtures. Fig. 5 shows the separation of a series of polyethylene glycols with an average molecular weight of 400, 600, and 1000 on a pellicular octadecylsilanized packing, deactivated with Ethoquad 18/25. The low-molecular-weight standard is resolved normally, of the next higher-molecular-weight standard only the low-molecular-weight fraction is resolved, and the high-molecular-weight standard is unresolved. The hydrodynamic radius of the oligomers is much smaller than the pore size of the packing (assuming that the same meander spiral model for the oligomers in solution is applicable to their structure in a supercritical fluid) and it can reasonably be assumed that the oligomers of higher-molecular-weight are not excluded by size[21,22]. Fig. 6 shows the separation of polyethylene glycols of average molecular weight 600 and 1000 on the wide-pore (330 Å) octadecylsilanized silica packing deactivated with Ethoquad 18/25. The 600-molecular-weight standard is now normally resolved, while the 1000-molecular-weight standard shows normal resolution of the early fraction

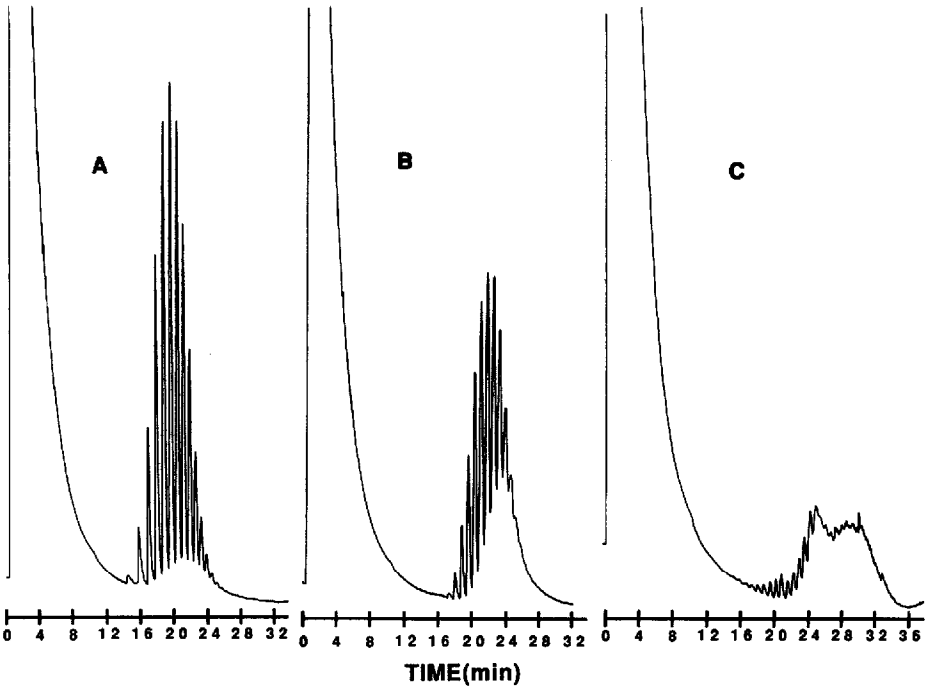


Fig. 5. The separation of polyethylene glycols of average molecular weight, A = 400, B = 600, and C = 1000. The column was 15 cm × 1 mm I.D. packed by tap-and-fill with Alltech PC18 + 0.4% (w/w) Ethoquad 18/25. The mobile phase was carbon dioxide; temperature 80°C, and the pressure program, 10 min at 100 atm, increased linearly to 400 atm over 30 min.

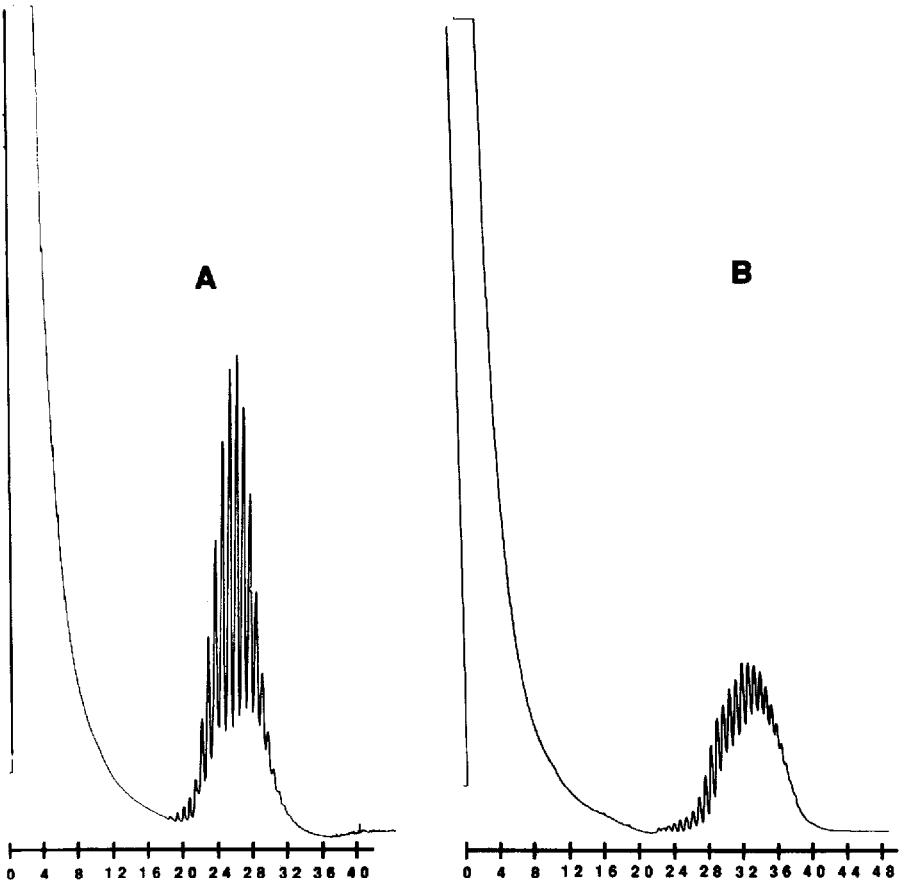


Fig. 6. Separation of standards of polyethylene glycol with an average molecular weight A = 600 and B = 1000 on a wide-pore octadecylsilanized packing (Vydac, 330 Å) coated with 3.5% (w/w) Ethoquad 18/25 and particle size 10 μm. The column, 10 cm × 1 mm I.D. was packed by the displacement method. The mobile phase was carbon dioxide, temperature 80°C, and the pressure program 10 min at 100 atm increased linearly to 400 atm over 30 min.

and poor resolution of the high-molecular-weight fraction. Since the elution density range and temperature are the same the improved separation capacity must result from an increase in efficiency, but more importantly, an increase in the selectivity of the chromatographic system between the two types of column packings. Fig. 7 shows the separation of a polypropylene glycol standard with an average molecular weight of 3010, contaminated with a small amount of polypropylene glycol with an average molecular weight of 1000. In this case, the low-molecular-weight contaminated is well resolved, but the high-molecular-weight major component is unresolved. More selective chromatographic conditions are needed to distinguish between oligomers in the high-molecular-weight range, although the polymers themselves are easily eluted (unseparated) to well over 4000 in molecular weight. For perspective, polypropylene glycol of molecular weight 3010, has an oligomer number of 51, and thus, the oligomer range with an average number of 51 repeat units represents very small changes in

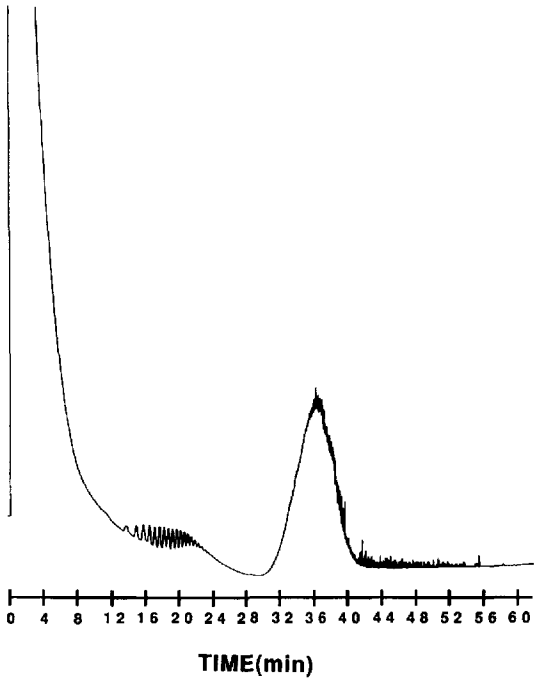


Fig. 7. Separation of a polypropylene glycol standard of average molecular weight 3010, contaminated with some oligomers of lower-molecular-weight material. The column was 7 cm × 1 mm I.D. The other conditions are identical with those for Fig. 6.

molecular characteristics. It will be very difficult for oligomer separations to match the elution capability and separation capacity of the column for high-molecular-weight polymers with large oligomer numbers.

To make the most practical use of the available elution density the density drop along the column must be minimized. To maintain reasonable efficiency small-particle packings are required and this leaves the packing density as the only variable that can be changed. The tap-and-fill method provides good columns of high permeability for particles down to about 20 μm and, with increasing experimental difficulty in packing, down to about 10 μm. The displacement method with the use of supercritical-fluid carbon dioxide is convenient for preparing columns of moderate packing density in the 5–20 μm particle-size range that are difficult to pack consistently by the tap-and-fill method. Fig. 8 shows a separation of hydrocarbon standards on a liquid-slurry-packed and a supercritical-fluid-packed column of 5 μm particles. The small differences in resolution between the two columns are due to the use of different packing materials. The supercritical-fluid-packed columns are more permeable and as efficient as the slurry-packed columns. The former column type is stable to supercritical-fluid conditions, indicating that columns need only be packed as densely as is dictated by the stress they will experience in operation. Supercritical-fluid-packed columns sediment too rapidly to be of use in LC or to permit any comparison of reduced-plate height- and flow-resistance measurements to be made. For SFC applications they provide more economic use of the available column inlet pressure, pro-

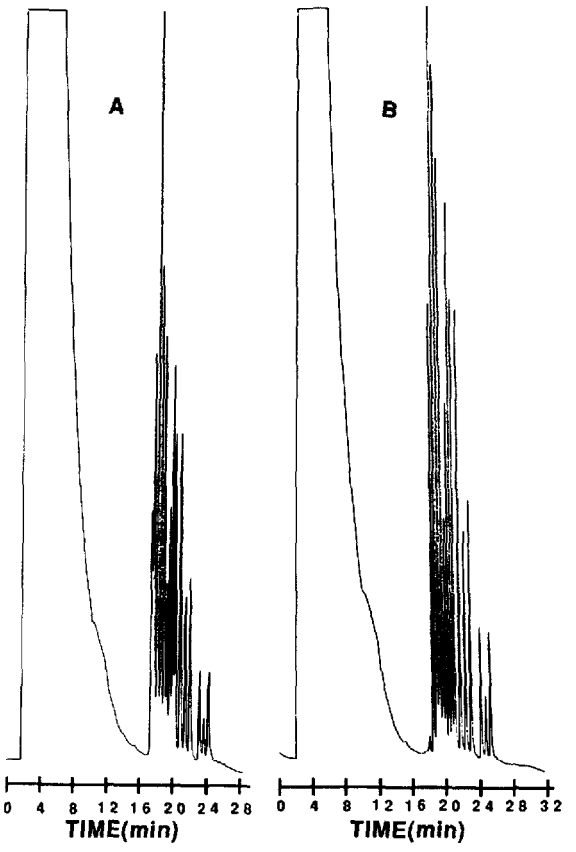


Fig. 8. Comparison of column types, packed by the displacement method (A) with supercritical carbon dioxide, and (B) by the liquid slurry technique. Column A was 10 cm × 1 mm I.D. packed with 5 μm Spherisorb ODS1, Column B was 10 cm × 1 mm I.D. packed with 5 μm Nucleosil ODS. The sample was a synthetic mixture of *n*-alkanes, C_{19}–C_{40}, mobile phase, carbon dioxide, temperature, 100°C, and pressure program, 10 min at 100 atm increased linearly to 400 atm over 40 min.

vide a lower pressure (density) drop per unit column length without loss of column performance, and should, at least in theory, permit the elution of higher-molecular-weight analytes than densely packed columns containing particles of the same size.

Column packings commonly used in LC have large surface areas which may not be required for separations in SFC. Large surface areas generate low phase ratios, and this leads to high retention. This may not be ideal for eluting middle-molecular-weight analytes. Secondly, packings with a large surface area contain a greater number of active sites per unit weight and will be more difficult to deactivate. SFC is being championed for the separation of polar and labile molecules that are likely to interact unfavorably with active column packings, resulting in peak asymmetry and possibly adsorption or catalytic transformation. Hirata[23,24] has reported that silica becomes irreversibly modified when ethanol-containing mobile phases are used in SFC and Schmitz *et al.*[25] have shown that silica reacts chemically with 1,4-dioxane under supercritical-fluid conditions. Doehl *et al.*[26] found that carboxylic acids, amines, and

amides are strongly adsorbed on commercially available column packings in SFC. Most of these reactions are associated with the silanol groups which are known to react chemically with alcohols, amines, and isocyanates at moderate temperatures to form bonded ligands[27]. An interesting example of chemical reactions occurring on silica is shown in Fig. 9 for the separation of cholestane, 5-cholestene, 3,5-cholestadiene and cholesterol at two different temperatures on a low-surface-area pellicular silica packing. Adsorption interactions are useful for the separation of the three cholestane hydrocarbons which are difficult to resolve on bonded-phase packings. On the other hand, the peak width for cholesterol is substantially broadened and shows tailing on its leading edge. Increasing the column temperature causes an increase in tailing and a loss of injected mass. Increasing the column temperature further can result in the complete abstraction of cholesterol from the chromatogram. The interaction of proton-donor solutes with residual silanol groups occurs in our experience on the most chemically deactivated bonded-phase supports commercially available, and is due to chemical interaction rather than adsorption, since the effect is more pronounced at elevated temperatures. More inert packing materials than are generally available at present will be needed for the separation of polar solutes in packed-column SFC. Packings of low surface area should be used, as they provide a lower column activity after deactivation.

Liquid organic salts have been used in GC for the analysis of polar com-

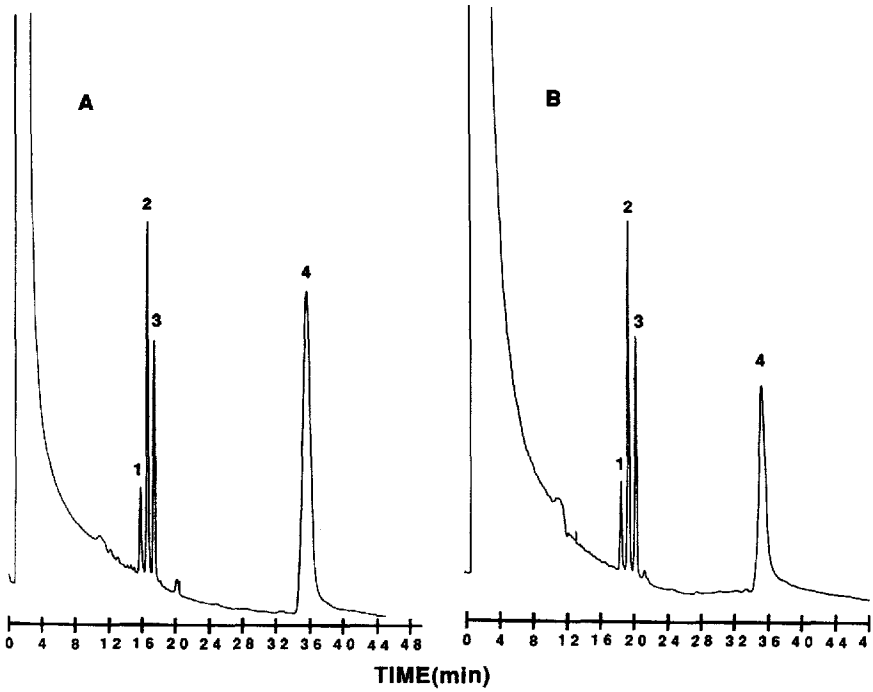
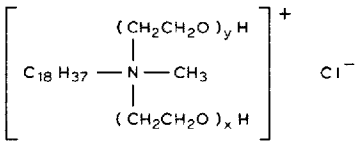


Fig. 9. Influence of silanol groups on the separation and recovery of 1 = cholestane; 2 = 5-cholestene; 3 = 3,5-cholestadiene, and 4 = cholesterol at A = 80°C and B = 100°C. The column was 10 cm × 1 mm I.D. packed with Corasil (II) by the tap-and-fill method. The mobile phase was carbon dioxide and the pressure program 10 min at 80 atm increased linearly to 300 atm over 30 min.

pounds[28–32]. Several salts with low melting points and liquid ranges greater than 100°C are known. These salts are excellent column deactivating agents for diatomaceous supports. For use in SFC, the liquid organic salts must be non-extractable at the highest density of the mobile phase to be used. If this condition could be met, liquid organic salts would provide a simple means of stationary-phase modification and support deactivation. One such useful salt is Ethoquad 18/25, stearylmethyldipolyoxyethylammonium chloride of average molecular weight 994 ($x + y = 15$), shown below.



Ethoquad 18/25 is a liquid at room temperature and has been used in GC for the separation of essential oils and other polar compounds at temperatures up to 300°C, where it shows a chromatographic selectivity similar to Carbowax 20M[33]. Fig.

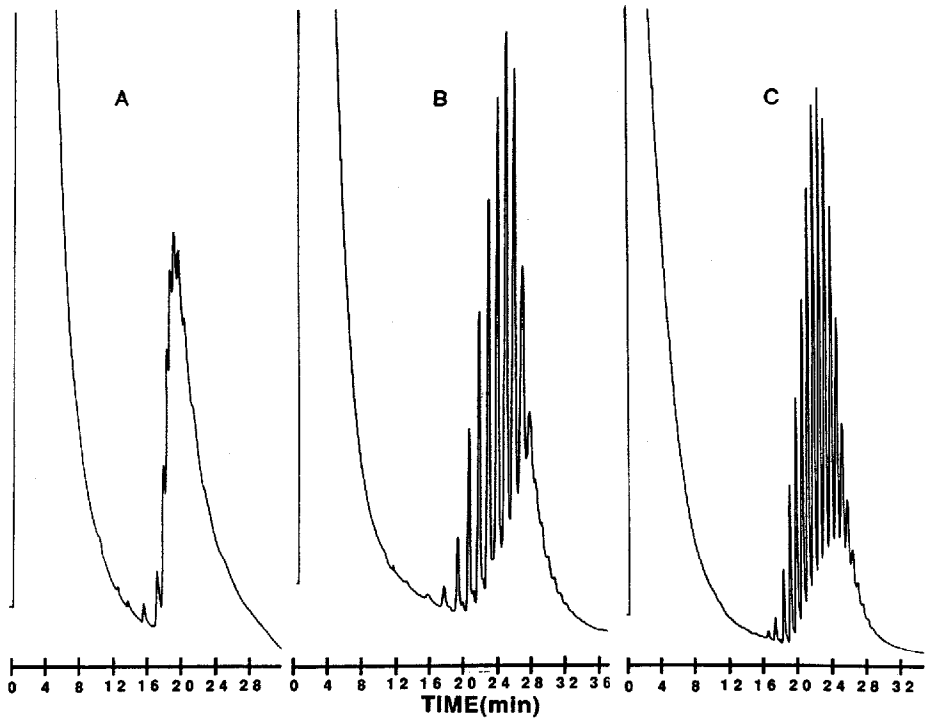


Fig. 10. Separation of Triton X-100 on a 15 cm × 1 mm I.D. column of (A) Alltech PC-18; (B) a 15 cm × 1 mm I.D. column of Alltech PC18 coated with 0.4% (w/w) Ethoquad 18/25; and (C) a 7 cm × 1 mm I.D. column of Vydac octadecylsilanized silica of 10-μm particle diameter and 330 Å nominal pore diameter coated with 3.6% (w/w) Ethoquad 18/25. Columns A and B were prepared by tap-and-fill, and column C by the displacement method. The mobile phase was carbon dioxide, temperature, 80°C, and pressure program, 10 min at 100 atm increased linearly to 400 atm over 30 min.

10 shows a comparison of the separation of Triton X-100 on a pellicular octadecylsilanized packing with and without coating with 0.4% (w/w) Ethoquad 18/25 and on a wide-pore octadecylsilanized silica packing coated with 3.6% (w/w) Ethoquad 18/25. Without Ethoquad, Triton X-100 (which has an approximate average molecular weight of 600) is unresolved on the uncoated pellicular packing but well resolved, as far as the early fraction of the oligomers is concerned, on the Ethoquad 18/25-coated packing. On the wide-pore material, better resolution of the higher-molecular-weight oligomers is achieved, in part due to the use of smaller-diameter particles. In both cases, the separation benefits from the combined deactivation and selectivity modification of the stationary phase, brought about by the use of the liquid organic salt.

Liquid organic salts at high phase loadings are useful stationary phases, which can provide true partitioning interactions. Fig. 11 shows the separation of Triton X-100 on a silica-based packing, heavily loaded with Ethoquad 18/25. Compared to Fig. 10, the resolution is improved, particularly for the heavy oligomers. Fig. 12 shows a separation of cholestane, caffeine, and cholesterol on the same column. Caffeine and cholesterol are difficult to elute at these low concentrations from bond-

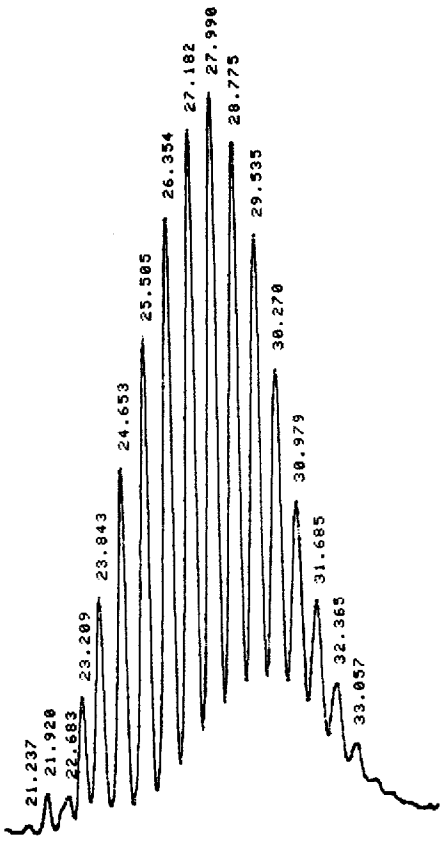


Fig. 11. Separation of Triton X-100 on a 10 cm × 1.0 mm I.D. column of Nucleosil 500, 10 μm particle size, coated with a heavy loading of Ethoquad 18/25 (*ca* 20% w/w). The mobile phase was carbon dioxide, temperature, 80°C, and pressure program, 10 min at 80 atm, increased linearly to 450 atm over 30 min.

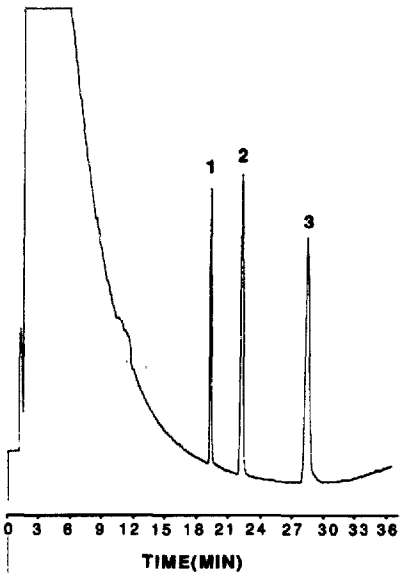


Fig. 12. Separation of 1 = cholestane, 2 = caffeine, and 3 = cholesterol on the same column and the same pressure program as that used for Fig. 11.

ed-phase columns without significant tailing. The heavily loaded Ethoquad 18/25 column shows excellent efficiency and good peak shape. The liquid organic salts may soon find a significant role in the development of partition-based columns of variable selectivity for packed-column SFC.

The problems associated with the chemical and adsorptive activity of silica- and alumina-based packings under supercritical-fluid conditions could be circumvented by using materials with a different structure. Graphitized forms of carbon and polystyrene divinylbenzene macroporous polymers were considered likely candidates. Columns prepared from Carbopack B showed poor mechanical stability, lower-than-expected efficiencies, and short column lifetimes. Stronger forms of porous graphitic carbon, such as those described by Knox *et al.*[34] might be more suitable. Porous polymers, based on polystyrene divinylbenzene, (Porapak Q, PRP-3, and Seragen) were also found to be physically unstable to density changes of the mobile phase. Usually, if the column is opened after pressure programming, the packing will exude from the column like toothpaste from a squeezed tube. As it reaches equilibrium, it will then shrink back into the column. The swelling of cross-linked polymers in supercritical fluids is not unprecedented, as this behavior is noted for cross-linked siloxane polymers used as stationary phases in open-tubular-column SFC[35,36]. There may be a threshold density below which a particular macroporous polymer may not swell and could be used for SFC. However, this would certainly significantly reduce the molecular weight range of analytes that could be separated. The Hamilton PRP-3 material has good LC properties and is at least stable to aqueous organic solvents[37]. This points to the fact that the properties of packings for SFC are not necessarily identical to those observed in LC.

CONCLUSIONS

It is clear from the above discussion that high-performance column packings developed for analytical LC are not ideal for SFC. Prepacked LC columns that have been used in SFC typically have lengths of 10–30 cm, internal diameters of 1.0–5.0 mm, particle sizes of 5–10 μm, pore sizes of 60–150 Å, and surface areas from 100–500 m^2/g. The available column lengths seem reasonable for SFC and the smaller internal diameters of 1.0–2.0 mm are a reasonable compromise between sample loadability and experimental difficulties related to the convenience of instrument design and flame stability (when the flame ionization detector is used). Particle sizes of between 5 and 10 μm are also reasonable in terms of efficiency, although we suspect that the optimum particle size may be closer to 10 than 5 μm. Here, there is a very complex relationship between the column pressure drop, the mobile phase velocity profile along the column, the length of the column, the analyte diffusion coefficients, column temperature, and particle size. To make the most economic use of the available column inlet pressure (density) and column length, the column permeability should be no lower than is dictated by the need to maintain the average column efficiency. This dictates that columns should be less densely packed than is current practice for LC columns. Fortunately, this can be achieved by using the supercritical fluid itself as the packing solvent. This method is simple, has a very low column failure rate, and is by no means as difficult to master as procedures for packing LC columns. There should be little deterrent to adopting the described packing procedure in those laboratories that do not pack their own LC columns.

Greater consideration should be given to the use of wide-pore packings in SFC as a means of lowering the surface area of macroporous packings. No commercially available column packing we have investigated is sufficiently inert for the analysis of all types of polar molecules. Even the most highly silanized and chemically bonded silica supports still contain unreacted silanol groups in appreciable numbers. These groups can condense with proton donor solutes with the elimination of water (chemical reaction) and behave as proton donor solutes to analytes with proton acceptor groups, *e.g.* amines. The difficulty of deactivating diatomaceous supports, which typically have surface areas of 0.5–4.0 m^2/g, to reduce tailing and chemical interactions in GC is well known. Even so-called wide-pore packings used in LC with pore diameters of 500–1000 Å will typically have surface areas between 25–35 m^2/g –already an order of magnitude greater than typical supports used in GC. The adequate deactivation of these column packings will be difficult, but it will only be more difficult for column packings of higher surface area. One approach we have introduced in this paper is the use of liquid organic salts as support masking agents and stationary phase modifiers to adjust selectivity. Another avenue is to seek out less active materials than silica- or alumina-based packings that can withstand the mechanical and physical properties of supercritical fluids. Commercially available silica- and alumina-based packings in current use may not only mar the aesthetic aspects of chromatograms of polar molecules in high concentration, but they may also preclude attempts at trace analyses for the same compounds due to a low recovery of injected material. Derivatization has been very successful in GC in solving problems of this kind and should be seriously considered when developing separations by SFC. At the present state of column technology it may be far less challenging to change the char-

acter of the analyte than the column packing. There may be additional benefits in this approach to enhancing the solubility of polar analytes in supercritical-fluid carbon dioxide.

We have found two kinds of column packings most useful in our studies. Pellicular packings are relatively inexpensive, easily packed into columns by tap-and-fill methods, and have low surface areas and moderate activity. They are convenient for screening unknown samples, because they can be quickly prepared and the packing can be discarded if changed in character by the sample at only a very small fraction of the cost of small-particle columns. Their efficiency is not so low as might be anticipated from a LC point of view. For optimum chromatographic performance, small particle, (10 μm or thereabouts) spherical particles, packed by the supercritical-fluid displacement method and having a wide-pore/low surface area are preferred.

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