Static coating of 5 to 50 μm I.D. capillary columns for open tubular column chromatography

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ABSTRACT

Dichlorofluoromethane, trichlorofluoromethane and tetramethylsilane were used in the static coating of small diameter capillary columns (5 to 50 μm I.D.) in order to obtain highly efficient columns for gas and supercritical fluid chromatography. Capillary columns of 5-, 10-, 25-, and 50-μm I.D. were coated with stationary phase films of SE-33, SE-54, OV-215, 50% *n*-octyl, 45% phenoxypolyethyl ether, 50% liquid crystal, 25% biphenyl, 50% pentafluorophenyl and 50% cyanopropyl polysiloxane stationary phases. Resultant evaluations of these columns in gas chromatography gave approximately 9000, 66 000, 45 000, and 19 000 plates m^{-1}, respectively, for the different internal diameters. Important parameters which affect coating efficiency are identified and discussed in detail.

INTRODUCTION

Several advantages are realized from the use of small diameter ($\leqslant 100$ μm I.D.) open tubular columns in gas chromatography (GC) and supercritical fluid chromatography (SFC). The Golay equation indicates that small internal diameter columns should yield higher numbers of theoretical plates than larger I.D. columns. Fields *et al.*[1] showed that experimental height equivalent to a theoretical plate (HETP) data for a 50 μm I.D. column closely matched the theoretical values, and that the experimental HETP data for a 25 μm I.D. column were about 1.5 times higher than the theoretical values in SFC. These authors also showed experimental and theoretical plots of plates m^{-1} *vs.* the capacity ratio, k, for 25 to 100 μm I.D. columns. Results revealed that the practical efficiencies for the 25 μm I.D. columns were significantly better than those for the larger diameter columns.

In SFC, open tubular columns can be operated with relatively small pressure

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drops along the length of the column. Pressure drop is important in SFC because a change in pressure not only changes the mobile phase density and solvating power, but also the selectivity and resolution in a separation. Roth and Ansorgova[2] calculated pressure drops with a CO_2 mobile phase at 107°C for 10, 25, and 50 μm I.D. columns in SFC. Using inlet pressures of 74 and 148 atm, and linear velocities of 2 and 5 cm s^{-1}, the greatest pressure drop was 16.2 atm for a 10 m × 25 μm I.D. column.

The use of small diameter capillary columns in GC is restricted by the low sample capacities of these columns. In addition, conventional GC inlet systems are not designed for the high pressures required for small I.D. columns (up to 27 atm for 25 μm I.D. columns). SFC, on the other hand, is performed under high-pressure conditions (from 60–400 atm) and requires the use of 100 μm I.D. and smaller columns[3,4]. Extremely small diameter columns must be used in open tubular column liquid chromatography (LC) because of the slow diffusion rates of solutes in the mobile phase. Such columns must have internal diameters in the range of 2–15 μm[5–7]. The preparation of efficient columns with such small internal diameters is extremely difficult.

Several methods have been used to prepare small I.D. columns for open tubular column chromatography. First, the dynamic coating method[8] is the oldest coating method, and was originally used to coat 200 μm to 1 mm I.D. columns. Desty *et al.*[9] and Gaspar *et al.*[10] used this method to coat 35 and 67 μm I.D. columns, respectively. The problem associated with the dynamic coating of small I.D. tubing is that the solutions required for reasonably thick films (>0.25 μm) are extremely viscous and require high filling pressures.

Second, the chemical bonding method of coating involves the bonding of monomeric reagents to the column wall. Alkyl groups are covalently bonded by reacting alkylchlorosilanes with surface silanol groups. The problem associated with this method is that the column sample capacity is low, and the tubing must first be etched to produce a greater surface area for the stationary phase films. Currently, fused-silica etching processes are not well developed, and there are an insufficient number of silanol groups on the column wall for the bonding of alkyl groups. Therefore, borosilicate or soda-lime glass columns are more often used. St. Claire and Jorgenson[11] have prepared a 126 cm long, 8 μm I.D. borosilicate glass column yielding 175 000 theoretical plates in LC using this method.

Third, a precipitation coating method developed by Dluzneski and Jorgenson[12] is based on the variable solubilities of the stationary phase polymer in selected solvent systems at various temperatures. Using this method, OV-17 was dissolved in heptane–cyclohexane (70:30) at 70°C. Then the fused-silica tubing was filled with this solution. After the column was filled, the temperature was reduced to room temperature, and the polymer was precipitated onto the column wall. Finally, the solvent was purged out of the column, and the stationary phase was dried and cross-linked. The process was reportedly quite simple and was used to coat columns down to 5 μm I.D. and 4 m long. This coating procedure was reported for LC, but it could be easily applied in the preparation of SFC and GC capillary columns. The main disadvantage is that there is presently no satisfactory method to control the film thickness in this coating process. The stationary phase film thickness is adjusted by changing the temperature used during the precipitation process.

Fourth, the static coating process[13] also requires viscous stationary phase

solutions for coating adequate film thicknesses on the inner walls of small diameter columns. These solutions require high pressures to fill the tubing, and once the tubing is filled, evacuation of the solvent is troublesome. Even with these problems, the static coating process is widely used because the method generally produces the most efficient capillary columns, and film thicknesses are easy to control. Rutten and Luyten[14] compared the static and dynamic coating methods. They found that the static coating method consistently produced higher efficiencies. Later, Alexander *et al.*[15] confirmed these results.

Various techniques have been applied to improve the static coating procedure for small diameter columns. Coating at temperatures much higher than the normal boiling point of the solvent, and under vacuum conditions, often produces sudden expansion in the stationary phase solution in the column. This expansion leaves sections of solution and vapor in the column, which destroys the process. Xu and Vermeulen[16] developed a free release static coating procedure which is done at high temperature (75–85°C), at relatively high pressure (atmospheric), and with a buffer column at the free end of the column (the end not sealed during the evaporation process). They reported that "bumping" was subdued, and that coating speeds were faster than conventional coating speeds.

EXPERIMENTAL

Column material

Fused-silica capillary tubing (5, 10, 25, 50 and 200 μm I.D.) was obtained from Polymicro Technologies (Phoenix, AZ, U.S.A.).

Polysiloxane stationary phases

All stationary phases used in this study have previously been reported. The 25% biphenyl (poly-25-biphenyl)[17], 50% liquid crystal (poly-50-LC)[18], 50% *n*-octyl (poly-50-C$_8$)[19], 50% pentafluorophenyl (poly-50-F$_5$)[20], 45% phenoxypolyethyl ether (poly-45-PPEE)[21] and 50% cyanopropyl (poly-50-cyano)[22] stationary phases were synthesized as reported. Commercially available OV-215 (50% trifluoropropyl, SE-33 (1% vinyl, 99% methyl), and SE-54 (1% vinyl, 5% phenyl) were obtained from Alltech (Deerfield, IL, U.S.A.).

Coating solvents

The following coating solvents were used: dichlorofluoromethane (CHCl$_2$F, Freon 21®), 99.9% pure, Alphagaz (Denver, CO, U.S.A.); trichlorofluoromethane (CCl$_3$F, Freon 11®), 99.9% pure, MG Industries (Valley Forge, PA, U.S.A.); and tetramethylsilane (TMS), 99.9% pure, Petrarch Systems, (Bristol, PA, U.S.A.). The dichlorofluoromethane and trichlorofluoromethane were both filtered into 1 dram vials with PTFE seals in the caps before use. The CHCl$_2$F was maintained at $-15°C$ before use due to its low boiling point.

Preparation of stationary phase solutions

The concentration of polymer needed for a desired film thickness was calculated from the following equations[23]:

$$C = 1000 \left[(\rho r^2)/(r - d_f)^2 - \rho \right] \tag{1}$$

where C is the concentration (mg ml^{-1}) and ρ is the specific density (g ml^{-1}) of the stationary phase, r is the column internal radius (μm), and d_f is the desired film thickness (μm). When the desired film thickness was insignificant compared to the column internal diameter (200 μm I.D. columns with 0.25-μm films), the following equation was used to calculate the concentration of the coating solutions:

$$C = 2000 \ (\rho d_f)/r \tag{2}$$

The stationary phase solutions were prepared by weighing out an appropriate amount of polymer into a crimp-seal vial (Supelco, Bellefonte, PA, U.S.A.). An aliquot of 1 ml of solvent was added, and the vial was sealed. The solutions were sonicated for about 10 min to dissolve the polymers.

Column pretreatment and deactivation

Before deactivation, the fused-silica tubing required a hydrothermal treatment followed by dehydration. However, several of the columns ($\leqslant$ 25 μm I.D.) were not pretreated, but were coated as received. Distilled water was passed through the tubing, and the tubing was flame sealed at both ends before being heated to 250°C for 4 h. Dehydration at 250°C for 2 h was performed by purging a stream of dry nitrogen through the tubing after removing the seals. Deactivation of the pretreated tubing with 50% phenyl polyhydrosiloxane at 350°C for 10 h[24] or 50% cyanopropyl poly-methylhydrosiloxane at 250°C for 10 h[25] was performed as decribed in the literature. The polymers were dissolved in dichloromethane in concentrations of about 1% (w/v) prior to being coated by the dynamic method.

Filling and coating of columns

Fused-silica tubing that was to be filled with a stationary phase solution was wrapped on a column basket, so that one end could unwind freely, after the tubing was on the basket. This end is herein defined as the front of the tubing (or column). The tubing to be filled was placed in a constant temperature bath filled with propylene glycol (for coating temperatures greater than 40°C) or water (when coating temperatures were close to room temperature). The coating bath temperature was maintained using a thermoregulating pump. The front end of the tubing was pushed into the crimp-seal vial containing the coating solution, which was then placed in a stainless steel filling device. Pressure to the filling apparatus was controlled by a high-pressure regulator on a nitrogen gas tank. Pressures ranging from 10–51 atm were used to push the coating solution into the fused-silica tubing. When the solution emerged from the end of the tubing, the end of the tubing was sealed by pushing it into a septum, or forceps were used to push the end into a tube of Apiezon (M or N) grease which was kept cold in a Dewar flask filled with water and ice.

After the tubing was filled with the coating solution, the front end was pulled out of the filling apparatus and connected to a vacuum apparatus equipped with a vacuum meter and gauge tube (DV-6M type; Teledyne Hastings-Raydist, Hampton, VA, U.S.A.), which were used to monitor the vacuum pressure. The minimum observable pressure was 8 μm Hg. As the solvent evaporated, the illuminated tubing changed from the dark, dull color to a shiny, light color. The column baskets had a circumference of 50 cm and nine equally spaced posts, 5.6 cm apart. One post was designated as the

observation point, and each time the solvent front reached it, the time and pressure were recorded. When the entire column was coated, the front was pulled free of the vacuum, and the end was pulled out of the septum or Apiezon grease. After a column was coated, the front was connected to a nitrogen line and the column was purged for 1 to 2 h at about 10–20 atm to ensure that the solvent was removed.

Stationary phase immobilization

Stationary phases were cross-linked using an azo-*tert.*-butane–argon purge[26] for 1 to 4 h. The purging pressures ranged from 2 atm for 50 μm I.D. columns to 4 atm for 10 and 25 μm I.D. columns. Both ends of the column were flame sealed, and the column was heated from 40 to 220°C at 4°C min^{-1}, and held at 220°C for 1 h. Then, the columns to be used in SFC were rinsed overnight with 3 ml of dichloromethane or *n*-pentane.

Column evaluation

A Carlo Erba Fractovap 4160 gas chromatograph was modified by replacing the split valve with a high-pressure regulating valve. The helium head pressure was adjusted using a high-pressure regulator at the helium tank to accommodate high split flows (500–600 ml min^{-1}) and high head pressure (up to 100 atm). Before evaluation, the columns were purged with helium at about 7 atm during a temperature program from 40 to 225°C at 4°C min^{-1}. The evaluation was started after a stable baseline was obtained at 200°C. Carrier flow was adjusted to 24 cm s^{-1}, and 1 μl of an *n*-alkane test mixture (about 10 mg ml^{-1} each of C_{15}–C_{24}) was injected (split injection) with a 10 μl gas-tight syringe. The flame ionization detector sensitivity was 1 to 2 $\cdot$ 10^{-11} A full scale. Injector and detector temperatures were 275 and 300°C, respectively. Peak widths at half height and retention times were recorded using a Hewlett-Packard 3388A integrator.

Two 50 μm I.D. columns were cut into short pieces (1.5-m segments) to evaluate the efficiencies and retention characteristics of the various sections of the column. The front and the end of the columns (1.5 meters each) were evaluated using a Hewlett-Packard 5890 GC (flame ionization detector) equipped with a Hewlett-Packard 3392A integrator which was used to record peak widths, heights, areas and retention times. Helium carrier gas linear velocities were between 23.1 and 24.6 cm s^{-1} with split flows of 245 to 351 ml min^{-1}. The temperatures of the oven, injector and detector were 200, 275 and 300°C, respectively. Efficiencies were calculated from the following equation:

$$n = 5.545 \ (t_r/W_{1/2})^2 \tag{3}$$

where n is the total number of theoretical plates, t_r is the retention time of the test solute and $W_{1/2}$ is the peak width at half height. Theoretical plate heights, h_{theor}, were calculated using the simplified Golay–Giddings equation[27]:

$$h_{theor} = r[(11k^2 + 6k + 1)/3(1 + k)^2]^{1/2} \tag{4}$$

where r is the column radius, and k is the capacity ratio.

A Lee Scientific (Salt Lake City, UT, U.S.A.) Model 501 supercritical fluid chromatograph equipped with a flame ionization detector was used in this study for

SFC measurements. The injection port consisted of a Valco C14W (Valco, Houston, TX, U.S.A.) high-pressure 4-port injector with a 0.2-μl sample loop rotor and a standard SGE (Austin, TX, U.S.A.) stainless steel splitter. Split ratios were adjusted to about 30:1 by varying the length of either a 25 μm I.D. or a 50 μm I.D. fused-silica tube connected to the split line after an on/off valve. The SFC operating conditions are cited in the text.

Samples were obtained from the following: alcohol ethoxylate was obtained from Shell Development (Houston, TX, U.S.A.); tricaproin, tricaprylin, tricaprin, trilaurin, trimyristin, tripalmitin, tristearin, triarachidin, tribehenin and n-alkanes were obtained from Sigma (St. Louis, MO, U.S.A.). All samples were used as received.

RESULTS AND DISCUSSION

There are several problems, both theoretically and practically, with the preparation of small-diameter open tubular columns. Bartle et al.[28] described a phenomenon called Rayleigh instability as one of the driving forces behind stationary phase rearrangement and loss of efficiency. This rearrangement results from infinitesimal perturbations in the stationary phase. Ultimately, stationary phase droplets can be formed in the column from the growth in amplitude of a standing wave in the stationary phase. The rate of growth of this standing wave is given by the following equation:

$$\ln(b/b_0) = (\gamma d_f^3 t)/(12\eta r^4) \tag{5}$$

where b_0 and b are the wave amplitudes at times zero and t, respectively, r is the radius of the column (cm), and γ, d_f and η are the surface tension (mN m^{-1}), film thickness (cm) and viscosity (P) of the stationary phase, respectively. Typical values for η range from 1–10^7 P; values for r, for small I.D. columns, range from 1.5–50 μm; values for γ range from 20–50 mN m^{-1}; and values for d_f range from 0.05–1 μm.

During evacuation of the solvent, in the column coating process, the stationary phase begins to rearrange, and efficiency decreases. Efficiency half-life is defined as the point when one half of the original column efficiency is lost; at this point, $t = t_{1/2}$. Woolley[23] studied the change in efficiency with time at 100°C for a 200 μm I.D. column coated with OV-17. The efficiency decreased by one half after 2.7 h, indicating that a 7% increase in the standing wave amplitude in the stationary phase had occurred. Rearranging eqn. 5 and solving for t at $t_{1/2}$ with a 7% amplitude increase gives:

$$t_{1/2} = 12 \, (\ln 1.07)(\eta a^4/\gamma d_f^3) \tag{6}$$

Efficiency half-life values for 5 to 50 μm I.D. columns are found in Table I. These values indicate that the stationary phase films in small internal diameter open tubular columns are very susceptible to Rayleigh instability. Theoretically, rearrangement occurs so quickly that these columns are impractical, unless a highly viscous stationary phase is coated and immobilized (cross-linked) in a short period of time. Eqn. 5 shows that there are several parameters which can be adjusted (some easier than others) to produce a more stable stationary phase on a column of given radius: the surface tension of the stationary phase, the viscosity of the stationary phase, the time required to coat a column, and the film thickness of the stationary phase.

TABLE I

THEORETICAL EFFICIENCY HALF-LIVES OF SMALL DIAMETER COLUMNS COATED WITH OV-17 STATIONARY PHASE AT 22°C

The $t_{1/2}$ values were calculated using eqn. 6. Values for η and γ were 12.20 P and 33 mN m^{-1}, respectively. These values for η and γ were determined experimentally[23].

Column I.D. (μm)	$t_{1/2}$ (s)
5	0.07
10	1
25	50
50	700

The critical surface tension, γ_c, of the surface to be coated, must be greater than or equal to the surface tension of the stationary phase, γ, or the surface is not wetted. There are no wettability problems associated with untreated fused silica due to its high γ_c of 50–70 mN m^{-1}, and the stationary phases used in this study all had surface tensions less than the critical surface tensions of the deactivated surfaces. Therefore, poor wettability could not be blamed for any lower than theoretical coating efficiencies that were achieved.

Increased coating bath temperatures enhance the movement of solvent molecules toward the vacuum region, thereby increasing coating velocities and reducing the tendency for column plugging. The utilization of higher temperatures in the static coating of small-diameter tubing was pioneered by Kong and Lee[29]. The static coating of SE-54 in a 50 μm I.D. column, using n-pentane as the coating solvent at 50°C, provided high efficiency (16 700 plates m^{-1}) in GC. However, lower column efficiencies (1000–6000 plates m^{-1}) were reported for 50% phenyl[30], 25% biphenyl[31] and 50% polymethylsiloxanes under the same high temperature static coating conditions. Woolley et al.[31] proposed that the major factor leading to such poor column efficiencies was linked to the drastically low viscosities of these stationary phases and their solutions at the higher coating temperatures. They suggested that the viscosities of alkylsubstituted (methyl and n-octyl) polysiloxanes are only marginally reduced with increasing temperature, while phenyl and biphenyl polymethylsiloxanes undergo dramatic viscosity losses (from about 10^9 to 10^3 cP for a temperature change of 20 to 50°C, respectively). In spite of the improved coating velocities at high temperatures, the increase in Rayleigh instability more than offsets this advantage.

Woolley et al.[31] found that the solution to this coating efficiency problem was solved by addressing the variable $(1/\eta)$ in eqn. 5. A highly volatile coating solvent (trichlorofluoromethane, b.p. = 23.7°C), capable of dissolving the polarizable phenyl or biphenyl polysiloxanes, could be used at low coating temperatures. Utilizing highly viscous polysiloxane gums and higher polysiloxane solution viscosities at lower coating temperatures proved to increase the stability of the stationary phase film during coating. The static coating of 50 μm I.D. columns at 22–38°C[31] with 50% phenyl or 25% biphenyl polysiloxanes yielded higher numbers of theoretical plates (12 000–15 000 plates m^{-1} in GC) than those coated at higher temperatures. These results provided evidence that appropriate conditions for the static coating of

TABLE II

PROPERTIES OF COATING SOLVENTS

Data taken from ref. 40.

Coating solvent	Chemical formula	ΔH_{vap} (cal mol^{-1})	B.p. (°C at 1 atm)	Dipole moment (debye)	Density at 25°C (g ml^{-1})
Dichlorofluoromethane (Freon 21)	CHCl$_2$F	6,287	8.9	1.29	1.366
Trichlorofluoromethane (Freon 11)	CCl$_3$F	6,424	23.8	0.46	1.467
Tetramethylsilane (TMS)	SiC$_4$H$_{12}$	6,439	26.5	0.00	0.648
n-Pentane	C$_5$H$_{12}$	6,595	36.1	≤0.05	0.626^a
Dichloromethane	CH$_2$Cl$_2$	7,572	40.0	1.60	1.327

^{a} Density at 20°C.

small-diameter columns with selective stationary phases include low bath temperatures, highly volatile solvents and highly viscous polysiloxanes.

Thus, a most important parameter in the static coating process is the type of solvent used during the coating process. In the coating solution, the solvent facilitates the dispersion of the highly interwoven polymer strands and decreases the viscosity of the solution. The higher the solvent to polymer ratio, the less viscous is the solution, and the easier is the filling and coating of a capillary tube. A good universal coating solvent is one which would dissolve a wide range of polymers to give a non-viscous solution. Many studies have been done to find polymer–solvent systems that will both make a less viscous coating solution, and facilitate the evacuation of the solvent. Several authors[29,32–37] have used single solvents that are volatile and that yield efficient coatings. Janák et al.[36] reported that the use of volatile coating solvents increased the coating speed of a column and decreased the viscosity of the coating solution. The tubing is filled easier with a less viscous coating solution and the solvent is easier to evacuate. Other authors[38,39] have tried using mixed solvents with varying degrees of success.

Table II lists some of the physical properties of two commonly used coating solvents, n-pentane and dichloromethane, as well as the physical properties of the three volatile coating solvents used in this study. The most notable difference is in the boiling points of the solvents. Dichlorofluoromethane has the lowest boiling point (8.9°C) of all of the solvents listed. The dipole moment of the solvent also has an effect on the viscosity of the coating solution. Table II lists the dipole moments of the solvents used in this study. Dichlorofluoromethane clearly has the highest dipole moment, 1.29 debye, of the three solvents examined in this study, while TMS has the lowest, 0.00 debye, due to its symmetry. All of the polymeric stationary phases used in this study dissolved readily into CHCl$_2$F, fewer dissolved into CCl$_3$F, and only the non-polar phases dissolved into TMS, and then only slowly. The use of CHCl$_2$F and TMS in the coating of open tubular columns has not been reported before in the literature.

As shown previously in eqn. 1, decreasing the column radius, r, increases the

TABLE III

COATING VELOCITIES FOR 50 μm I.D. COLUMNS

Stationary phase	Solvent	Coating temperature (°C)	Coating velocities (cm min⁻¹)				Coating pressure (mmHg)			Length (m)	Film thickness (μm)
			Start	Middle	End	Avg.ᵃ	Start	Middle	End		
Poly-50-LC	$CHCl_2F$	35.6	22.2	24.6	61.2	29.8	30	15	10	12	0.10
SE-33	CCl_3F	54.0	22.2	6.8	17.1	9.5	35	14	12	10	0.10
SE-33	$CHCl_2F$	22.6	12.9	12.1	46.3	13.5	19	12	10	7	0.10
Poly-4S-PPEE	$CHCl_2F$	38.4	30.7	17.7	136	27.0	28	14	12	15	0.11
Poly-50-C₈	TMS	38.2	27.3	5.7	52.6	9.9	32	16	12	13	0.25
Poly-25-biphenyl	CCl_3F	60.0	31.2	23.6	292	28.6	28	13	10	20	0.25

ᵃ Avg. = Average coating velocity. This is the observed length of tubing (in cm) that is coated, divided by the time required for it to coat (in min).

required polymer concentration in the coating solution . For example, a 0.15 μm film thickness in a 25 μm I.D. column required a 29 mg ml^{-1} solution for the poly-5-LC stationary phase. The solution was viscous and cloudy at 30.2°C, while it was less viscous and clear at 70°C. A 10 μm I.D. column with a 0.1 μm film thickness required 83 mg ml^{-1} of an SE-54 coating solution. This solution was extremely viscous. Clearly, as the diameter decreases, and even when using a thinner stationary phase film, the coating solution concentration must increase. At film thicknesses greater than 0.25 μm, the poly-25-biphenyl stationary phase formed polymer aggregates at the required concentration.

From eqn. 5, the relationship of coating speed to stationary phase stability is readily observed. Since coating speed is one of the parameters that can be manipulated to minimize Rayleigh instability, coating solvents that provide fast coating speeds should be used. The three solvents used in this study had relatively fast average coating velocities in 50 μm I.D. columns (10–30 cm min^{-1}). This led to the following observation: the coating velocity decreased from the starting rate, and then increased dramatically in the last few meters of the column. Table III lists coating speed data for 50 μm I.D. columns and several selective stationary phases. The starting, middle and ending coating velocities are reported. These range from 12.9 to 30.7 cm min^{-1} at the start, 5.7 to 24.6 cm min^{-1} in the middle and 17.1 to 292 cm min^{-1} at the end of the column. Coating pressures in the vacuum line between the vacuum pump and column are also listed for the start, middle and end sections of the columns. This same change in coating velocity was observed for 25 μm I.D. columns. However, a dramatic change in coating velocity was not observed in the coating of 200 μm I.D. columns. Table IV shows the observed starting, middle and ending coating velocities for several 200 μm I.D. columns. Not only are the average coating velocities slower for the 200 μm I.D. columns (5 to 12 times slower), but the coating velocity steadily decreases to the end of the column. The average coating velocities of 200 μm I.D. columns with *n*-pentane and dichloromethane are 2.5 to 5 times slower than the average coating velocity of the column coated with the more volatile TMS.

To understand why coating velocities changed for these 50 μm I.D. columns, a 14 m × 50 μm I.D. column coated with a 0.25 μm film thickness of poly (50% *n*-octyl) methyl siloxane stationary phase was cut into 1.5-m sections and then evaluated in GC.

TABLE IV

COATING VELOCITIES FOR 200 μm I.D. COLUMNS

Stationary phase	*Solvent*	*Coating temperature (°C)*	*Coating velocities (cm min^{-1})*				*Length (m)*	*Film thickness (μm)*
			Start	*Middle*	*End*	*Avg.[a]*		
Poly-50-cyano	CH$_2$Cl$_2$	22.3	4.3	2.6	1.9	2.5	7	0.25
OV-17v	CH$_2$Cl$_2$	24.1	4.8	2.2	1.6	2.3	10	0.25
Poly-50-LC	CH$_2$Cl$_2$	23.5	3.3	2.8	1.2	1.3	9	0.25
OV-17v	*n*-Pentane	21.8	4.7	3.4	1.5	2.1	8	0.10
SE-33	TMS	21.9	9.4	8.6	5.4	6.4	11	0.25

[a] Avg. = Average coating velocity. This is the observed length of tubing (in cm) that is coated, divided by the time required for it to coat (in min).

TABLE V

MEASURED k VALUES FOR SEGMENTS OF 50 μm I.D. COLUMNS

Segment location (in m)[a]	Measured k
Column 1[b]	
0.0–1.5	23.9
3.0–4.5	20.4
6.0–7.5	15.0
9.0–10.5	7.3
12.5–14.0	2.6
Column 2[c]	
0.0–1.5	21.7
10.5–12.0	2.4

[a] The 1.5-m column segments are reported by their location in the original column before being cut out, with 0.0 m being the front of the original column.

[b] Column 1 was a 14.0 m × 50 μm I.D. column coated with poly-50-C_8 stationary phase ($d_f = 0.25$ μm).

[c] Column 2 was a 12.0 m × 50 μm I.D. column coated with poly-25-biphenyl stationary phase ($d_f = 0.25$ μm).

Table V shows that the values of k were found to fall between 23.9 at the front of the column to 2.6 at the end of the column. Table V also gives the k values for two, 1.5 m sections cut from a 12.0 m × 50 μm I.D. column coated with poly-25-biphenyl ($d_f = 0.25 \mu$m) as evaluated by GC. Again, the same decrease in k values was observed; the k values at the front and end of the column were 21.7 and 2.4, respectively. Both columns produced k values at the front of the column which were 9 times higher than k values at the end of the column. These results indicate that the static coating of 50 μm I.D. columns does not yield a uniform film, but rather, a decreasing film thickness with length.

One explanation for the formation of this phase ratio gradient is that as the polymer solution is pushed through the front of the column when it is being filled, the solution becomes less and less concentrated as it nears the end of the column because the polymer precipitates onto the tubing surface. Fresh solution entering the front of the column continues to deposit stationary phase, becoming less concentrated, as it progresses toward the end of the column. Thus, a phase ratio gradient is developed. A less concentrated solution at the end of the column would also lead to faster coating velocities, since solution viscosity is decreased, and there is less resistance to the escaping solvent. The surface area to volume ratio is four times higher for a 50 μm I.D. column than for a 200 μm I.D. column, so there is less relative polymer-surface contact in 200 μm I.D. tubing. Thus, phase ratio gradients in 200 μm I.D. columns are not expected to be as significant. The consistently decreasing coating velocities in 200 μm I.D. columns tend to support this explanation.

The presence of phase ratio gradients in small I.D. capillary columns present two important, practical considerations. First, if two or more 50 μm I.D. columns are to be made from one long column after it is coated, the front piece of the column will always produce higher k values than the next piece of column, and the end of the column will

TABLE VI

CALCULATED RATIOS OF STATIONARY PHASE FILM THICKNESS RESULTING FROM
SLIGHT VARIATIONS IN COLUMN DIAMETER

Column I.D. (μm)	d_f/d_f'
5	6.8
10	5.4
25	4.0
50	3.2
100	2.6

give the lowest k values of all. Second, phase ratio focusing of solutes may be accomplished by putting the lowest k value end of a column at the injector and the highest k value end at the detector. If the column is connected opposite to this, band broadening will take place over the length of the column.

Other influences on coating of capillaries, described by Giddings[41], are capillary forces. These forces are one of the principal means of retaining the stationary phase on the column surface. Giddings explained that stationary phase accumulates in the column where slight negative deviations in the radius of curvature occur. In a capillary column that has a fractional difference in radius of curvature r' and r, where $r' > r$, and assuming $r \gg d_f$, the stationary phase film thickness, the following equation was derived:

$$d_f^3/d_f'^3 = 1 + (d_f^3 \, \Delta r/J^3 r^2) \tag{7}$$

where J was approximated by Giddings to be nearly the same value for all liquids and to be in the order of 10^{-5}; $\Delta r/r$ is the fractional variation in tube size; and r is the column radius (cm). Differences in starting and ending diameters on rolls of fused-silica tubing are commonly 1 μm, so $\Delta r/r$ ranges from 0.20 to 0.02 for 5 to 50 μm I.D. columns, respectively. Choosing $\Delta r/r$ as 0.01 and $d_f = 2.5 \cdot 10^{-5}$ cm, calculated values for the ratio of stationary phase film thicknesses at the two sites (d_f/d_f') are found in Table VI. Clearly, column diameter plays a significant role in the calculated ratios. The smaller the column internal diameter, the higher the ratio. Just a slight variation in the radius of curvature is enough to cause stationary phase voids on some parts of the surface and thick films of phase on other parts of the surface, especially when 5 to 25 μm I.D. columns are considered. Rayleigh instability would have a more pronounced effect on the thick film areas in the column, and column efficiency would be diminished. These thick film areas, combined with Rayleigh instability in these regions, partially explain the low efficiency observed for the 5 μm I.D. column in Table IX. Giddings predicted that $\Delta r/r$ must be kept to within several parts in 10^5 to have a film thickness within a 10% tolerance. Thus column efficiency and coating capacity are directly related to the inner surface of the tubing.

Although there are several problems associated with the successful preparation of highly efficient small I.D. columns, optimization of the coating parameters has led to some successful results. Tables VII, VIII and IX show coating data and GC

TABLE VII

GC EFFICIENCIES FOR 50 μm I.D. FUSED-SILICA COLUMNS COATED WITH POLYSI-LOXANE STATIONARY PHASES (0.25 μm FILM THICKNESS)

Stationary phase	Coating temperature (°C)	Coating solvent	Column length (m)	Capacity factor (k)	Efficiency[a] (plates m⁻¹) (× 10³)
SE-33	56.7	TMS	1.5	33.9	19.2 ± 5.6
SE-33	54.0	CCl₃F	10.0	9.2	13.6 ± 3.2
SE-33	67.7	CCl₃F	2.0	34.8	10.8 ± 1.5
Poly-50-C₈	38.3	TMS	12.5	20.0	14.7 ± 0.5
Poly-50-C₈	37.7	CCl₃F	14.0	15.2	12.6 ± 0.6
Poly-50-C₈	37.0	TMS	12.0	7.6	12.5 ± 0.2
OV-215	24.4	CHCl₂F	17.0	9.6	15.8 ± 3.6
OV-215	23.5	CHCl₂F	10.4	3.7	10.0 ± 3.5
OV-215	23.0	CHCl₂F	10.0	4.5	8.9 ± 1.1
				4.2	10.2 ± 3.8[b]
Poly-45-PPEE	37.5	CHCl₂F	15.0	5.2	12.2 ± 2.7
Poly-45-PPEE	38.4	CHCl₂F	15.0	3.8	11.5 ± 0.3
Poly-45-PPEE	24.5	CHCl₂F	10.0	7.3	10.3 ± 1.6
Poly-50-LC	38.1	CHCl₂F	19.5	10.5	16.7 ± 3.5
Poly-50-LC	35.6	CHCl₂F	15.5	4.9	13.3 ± 2.7
Poly-50-LC	38.5	CHCl₂F	17.0	9.3	11.8 ± 1.6
			11.5	13.1	12.8 ± 2.2[c]

[a] Column efficiencies are an average of three values. The standard deviation is also shown.
[b] After 5 days of use in SFC-CO₂, uncross-linked.
[c] After 18 days of use in SFC-CO₂, uncross-linked; the end 5.50 m was cut off.

TABLE VIII

GC EFFICIENCIES FOR 25 μm I.D. FUSED-SILICA COLUMNS COATED WITH POLYSI-LOXANE STATIONARY PHASES

Stationary phase	Film thickness (μm)	Coating solvent	Coating temperature (°C)	Column length (m)	Efficiency[a] (plates m⁻¹) (× 10³)
SE-33	0.25	CCl₃F	78	4.0	25.8 ± 5.1
SE-33	0.25	CCl₃F	56	1.8	19.7 ± 2.4
SE-33	0.25	TMS	56	1.8	18.2 ± 1.7
SE-33	0.50	CHCl₂F	28	3.5	28.4 ± 3.1
SE-54	0.50	CHCl₂F	25	4.0	45.2 ± 0.1
Poly-50-C₈	0.50	CHCl₂F	25	4.0	26.1 ± 0.8
Poly-25-biphenyl	0.12	CCl₃F	38	6.5	19.0 ± 4.1
Poly-50-LC	0.20	CHCl₂F	19	8.0	11.9 ± 0.3
Poly-50-LC	0.15	CHCl₂F	25	4.0	21.2 ± 0.6

[a] The k values ranged between 12 and 20 for the above columns. The only exception was the column coated with poly-50-C₈ which had a k of 33.0 using n-hexadecane as the test solute. Normal alkane test solutes (C₁₄–C₂₁) were used to determine k and efficiency data for all of the columns except the liquid crystal columns where phenanthrene was used. These reported values are the average and standard deviation of two to three values.

TABLE IX

GC EFFICIENCIES FOR 5 AND 10 μm I.D. FUSED-SILICA COLUMNS COATED WITH
POLYSILOXANE STATIONARY PHASES

Stationary phase	Film thickness (μm)	Coating solvent	Coating temperature (°C)	Column length (m)	Capacity factor (k)	Efficiency[a] (plates m^{-1}) ($\times 10^3$)
10 μm I.D. columns						
Poly-25-biphenyl	0.05	$CHCl_2F$	26.6	2.0	3.7	13.3 ± 1.3
Poly-50-F$_5$	0.05	$CHCl_2F$	26.4	2.0	14.2	62.9 ± 7.5
Poly-50-F$_5$	0.05	$CHCl_2F$	27.2	2.1	17.3	53.0 ± 9.4
5 μm I.D. column						
Poly-50-F$_5$	0.15	$CHCl_2F$	27.8	0.9	3.3	9.2 ± 6.4

[a] Columns were evaluated at 175°C or 200°C. The average and standard deviation of three measurements are shown.

efficiencies of 50 , 25, 10 and 5 μm I.D. columns, respectively. The static coating of 50 μm I.D. columns with solute-selective stationary phases at lower temperatures with any of the three selected volatile coating solvents (TMS, CCl_3F or $CHCl_2F$) yielded highly efficient columns. Stationary phases, such as poly-25-biphenyl or poly-50-LC polymethylsiloxanes, that have been traditionally difficult to coat on small-diameter capillaries with dichloromethane as the coating solvent, presented no problems when coated at low-to-moderate temperatures with CCl_3F or $CHCl_2F$ (13 000–16 000 and 12 000–17 000 plates m^{-1}, respectively, which is up to 75% of the theoretical efficiency). The poly-45-PPEE polysiloxane stationary phase that contains polar polyethyl ether side groups dissolved well in $CHCl_2F$ and coated almost as well (10 000–12 000 plates m^{-1}) as the more easily-coated poly-50-C_8 (13 000–15 000 plates m^{-1}), or SE-33 (19 000 plates m^{-1}, up to 90% of the theoretical efficiency) stationary phases. In addition, OV-215, a commercially available stationary phase that is generally quite difficult to coat with more conventional solvents (ethyl acetate, b.p. = 77.2°C, and acetone, b.p. = 56.2°C), was readily dissolved in $CHCl_2F$ and uniformly coated on 50 μm I.D. columns to yield high numbers of theoretical plates (9000–16 000 plates m^{-1} in GC, up to 75% of the theoretical efficiency). By coating these solute-selective polysiloxanes at low bath temperatures and at rapid coating speeds, highly efficient 50 μm I.D. capillary columns were prepared for GC and SFC.

Several 25 μm I.D. columns were successfully coated using the low boiling solvents. Film thicknesses, coating temperatures, column lengths, k values, and GC efficiencies are listed in Table VIII. The column coated with SE-33 using TMS had about the same efficiency as the column coated using CCl_3F at the same temperature and with the same film thickness. The 56–78°C coating temperatures for three of the SE-33 columns appeared to have little effect on coating efficiency. The high efficiencies of these columns (18 000–25 000 plates m^{-1}) can be attributed to the high viscosity of this stationary phase, even at the higher temperatures. Fig. 1 is a chromatogram of an ethoxylated alcohol sample (ethoxylated C_{12} and C_{13} alcohols) which shows the high efficiency separation possible on a 25 μm I.D. column. The number of ethylene oxide groups in the starting alcohols ranged from 0 to 27. This chromatogram shows that the
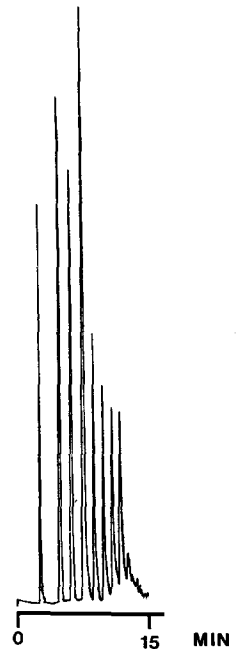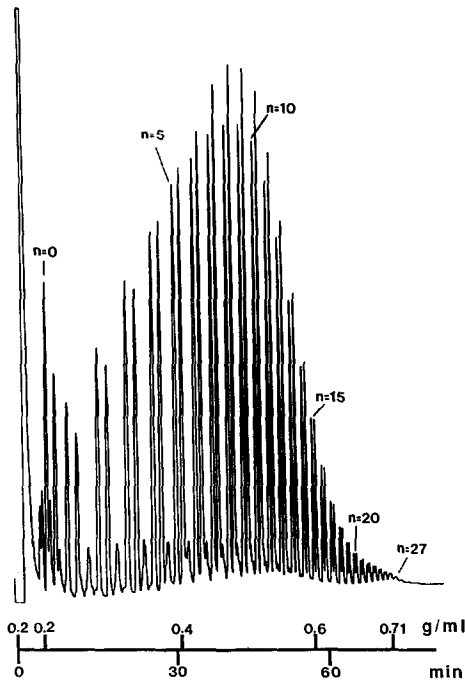
Fig. 1. SFC chromatogram of ethoxylated C_{12} and C_{13} alcohols. Column: 5 m × 25 μm I.D. fused-silica column coated with a poly-25-biphenyl stationary phase (d_f = 0.25 μm). Conditions: CO_2 at 120°C; and linear density program from 0.2 g ml^{-1} to 0.71 g ml^{-1} at 0.0075 g ml^{-1} min^{-1} after an initial 5-min isopycnic period.

Fig. 2. SFC chromatogram of triglycerides (T_{18-60}). Column: 0.7 m × 10 μm I.D. fused-silica column coated with a poly-50-cyano stationary phase (d_f = 0.1 μm). Conditions: solvent backflush injection; inject at 0.3 g ml^{-1}, ramp down to 0.1 g ml^{-1} at 0.096 g ml^{-1} min^{-1}, then, ramp from 0.1 g ml^{-1} to 0.3 g ml^{-1} at 0.068 g ml^{-1} min^{-1}; the chromatographic analysis was started at 0.3 g ml^{-1} and ramped at 0.01 g ml^{-1} min^{-1} to 0.7 g ml^{-1}.

25 μm I.D. column has adequate sample capacity for this sample when coated with a 0.25 μm film thickness.

An important parameter in Rayleigh instability and column efficiency is the film thickness of the stationary phase. Although the coating of thick films in 25 μm I.D. tubing is difficult, a film thickness of 0.5 μm was coated on short pieces of 25 μm I.D. tubing. Table IX shows that three columns were successfully coated with thick films of non-polar stationary phases, and yielded high efficiencies (up to 103% of the theoretical efficiency). Columns longer than 4 m were difficult to coat with a film thickness of 0.5 μm because the coating velocity decreased to zero. Often, only 2 m lengths coated successfully. The effects of increased polymer concentration and column length on coating efficiency is seen in the two columns coated with the liquid crystal stationary phase. Increasing the film thickness (from 0.15 to 0.20 μm) and column length (4 to 8 m) resulted in decreased coating efficiency.

The 10 μm I.D. columns were coated with poly-50-C_8, poly-25-biphenyl, poly-50-cyano, poly-50-F_5, and SE-54 stationary phases. Table IX lists the coating

data for the 10 μm I.D. columns that were evaluated by GC. The poly-50-F_5 columns coated well and resulted in efficiencies up to 59% of the theoretical efficiency. The 10 μm I.D. columns coated with poly-50-cyano and SE-54 were not successfully evaluated by GC. For 10 and 5 μm I.D. columns that were 1 to 2 m long, the problem was not in coating the columns, but in evaluating these columns. Extremely high pressures were required for the GC carrier gas (100 atm), and the GC injector proved to be totally inadequate; the carrier gas leaked around the septum and a seal on the split valve failed. The poly-50-F_5 polysiloxane statically coated on a 5 μm I.D. column quite easily. The chromatogram in Fig. 2 shows a separation of a triglyceride mixture in SFC on a 0.7 m × 10 μm I.D. column. The solvent backflush injection method described by Lee *et al.*[42] was used to inject the sample onto the column. Although the injection volume diminished the resolution of this separation, the analysis time was less than 15 min, and 6 of the 9 components had baseline resolution. Injection and detection methods presently limit what can be done with these small I.D. columns in GC and SFC and must be improved before use of these columns becomes practical.

ACKNOWLEDGEMENTS

REFERENCES

1 S. M. Fields, R. C. Kong, J. C. Fjeldsted, M. L. Lee and P. A. Peaden, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 7 (1984) 312.
2 M. Roth and A. Ansorgova, *J. Chromatogr.*, 465 (1989) 176.
3 C. P. M. Schutjes, E. A. Vermeer, J. A. Rijks and C. A. Cramers, *J. Chromatogr.*, 253 (1982) 1.
4 P. A. Peaden and M. L. Lee, *J. Chromatogr.*, 259 (1983) 1.
5 F. J. Yang, *J. Chromatogr. Sci.*, 20 (1982) 241.
6 J. H. Knox and M. T. Gilbert, *J. Chromatogr.*, 186 (1979) 405.
7 G. Guiochon, *Anal. Chem.*, 53 (1981) 1318.
8 G. Dijkstra and J. de Goey, in D. H. Desty (Editor), *Gas Chromatography*, Academic Press, New York, 1958, p. 56.
9 D. H. Desty, A. Goldup and W. T. Swanton, in N. Brenner, J. E. Callen and M. D. Weiss (Editors), *Gas Chromatography*, Academic Press, New York, 1962, p. 105.
10 G. Gaspar, P. Arpino and G. Guiochon, *J. Chromatogr.*, 15 (1977) 256.
11 R. L. St. Claire and J. W. Jorgenson, *J. Chromatogr. Sci.*, 23 (1985) 186.
12 P. R. Dluzneski and J. W. Jorgenson, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 332.
13 J. Bouche and M. Verzele, *J. Gas Chromatogr.*, 6 (1968) 501.
14 G. A. F. M. Rutten and J. A. Luyten, *J. Chromatogr.*, 74 (1972) 177.
15 G. Alexander, G. Garzo and G. Palyi, *J. Chromatogr.*, 91 (1974) 25.
16 B. Xu and N. P. E. Vermeulen, *Chromatographia*, 18 (1984) 520.
17 J. C. Kuei, J. I. Shelton, L. W. Castle, R. C. Kong, B. E. Richter, J. S. Bradshaw and M. L. Lee, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 7 (1984) 13.
18 J. S. Bradshaw, L. Schregenberger, H-C. K. Chang, K. E. Markides and M. L. Lee, *J. Chromatogr.*, 358 (1986) 95.
19 J. C. Kuei, B. J. Tarbet, W. P. Jackson, J. S. Bradshaw, K. E. Markides and M. L. Lee, *Chromatographia*, 20 (1985) 25.
20 J. S. Bradshaw, R. S. Johnson, N. W. Adams, M. A. Pulsipher, K. E. Markides and M. L. Lee, *J. Chromatogr.*, 357 (1986) 69.
21 C. A. Rouse, A. C. Finlinson, B. J. Tarbet, J. C. Pixton, N. M. Djordjevic, K. E. Markides, J. S. Bradshaw and M. L. Lee, *Anal. Chem.*, 60 (1988) 901.

22 B. E. Richter, J. C. Kuei, L. W. Castle, B. A. Jones, J. S. Bradshaw and M. L. Lee, *Chromatographia*, 17 (1983) 570.

23 C. L. Woolley, *Ph. D. Dissertation*, Brigham Young University, Provo, UT, 1987.

24 K. E. Markides, B. J. Tarbet, C. L. Woolley, C. M. Schregenberger, J. S. Bradshaw and M. L. Lee, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 378.

25 K. E. Markides, B. J. Tarbet, C. M. Schregenberger, J. S. Bradshaw, M. L. Lee and K. D. Bartle, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 741.

26 R. C. Kong, S. M. Fields, W. P. Jackson and M. L. Lee, *J. Chromatogr.*, 289 (1984) 105.

27 J. C. Giddings, *Anal. Chem.*, 36 (1964) 741.

28 K. D. Bartle, C. L. Woolley, K. E. Markides, M. L. Lee and R. S. Hansen, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10 (1987) 128.

29 R. C. Kong and M. L. Lee, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 319.

30 C. L. Woolley, K. E. Markides, M. L. Lee and K. D. Bartle, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1986) 506.

31 C. L. Woolley, B. J. Tarbet, K. E. Markides, J. S. Bradshaw, M. L. Lee and K. D. Bartle, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 113.

32 K. Grob and B. Grob, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 856.

33 K. Grob, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 1 (1978) 93.

34 T. Wännman, L. Blomberg and S. Schmidt, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 32.

35 B. L. Goodwin, *J. Chromatogr.*, 172 (1979) 31.

36 K. Janák, V. Kahle, K. Tesarik and M. Horká, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 843.

37 B. Xu and N. P. E. Vermeulen, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 181.

38 R. Kong and M. L. Lee, *Chromatographia*, 17 (1983) 451.

39 B. Xu and N. P. E. Vermeulen, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 713.

40 R. L. Weast (Editor), *Handbook of Chemistry and Physics,* CRC Press, Boca Raton, FL, 67th ed., 1986.

41 J. C. Giddings, *Anal. Chem.*, 4 (1962) 458.

42 M. L. Lee, B. Xu, E. C. Huang, N. M. Djordjevic, H-C. K. Chang and K. E. Markides, *J. Microcolumn Sep.*, 1 (1989) 7.