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THEORETICAL TREATMENT OF RESOLVING POWER IN OPEN TUBULAR COLUMN SUPERCRITICAL FLUID CHROMATOGRAPHY

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SUMMARY

Theoretical considerations of column pressure drop, column diameter, pressure programming, injector and detector volumes, connector tubing, stationary phase film thickness and detector time constant as applied to open tubular supercritical fluid chromatography are treated. These theoretical considerations show that open tubular supercritical fluid chromatography is a viable technique offering its greatest potential in the pressure–temperature region around the critical point of the mobile phase.

INTRODUCTION

Various aspects of supercritical fluid chromatography (SFC) have received theoretical treatments in previously published literature. The main parameters that have been studied in past work are the effects of column pressure drop on column efficiency[1,2], the effect of the density or pressure of the mobile phase on solute retention[3–6], mobile phase density effects on selectivity[7,8], the effect of linear density programming on the resolution of members of homologous series[9] and the effect of capillary column dimensions on the times of analysis required for large numbers of theoretical plates[10]. Conclusions which have resulted from these studies apply, in most cases, equally well to both open tubular and packed columns. The main differences between using packed and open tubular columns in SFC arise because column pressure drops and column volumes are much smaller in open tubular columns than in packed columns.

This paper is primarily concerned with the theoretical aspects of SFC as they relate to open tubular column chromatography. Principle topics that are treated include the effects of column pressure drop (or density change) on selectivity, retention and resolution; the effects of injector and detector volumes, connector tubing, sample size, stationary phase film thickness and pressure drop on efficiency; the speed of analysis; and pressure programming.

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SYMBOLS AND BASIC EQUATIONS

The object of any chromatographic system is to provide a means of separating compounds from each other for reasons of characterization, identification or quantitation. A fundamental mathematical expression of the ability of a chromatographic system to accomplish this aim is:

$$R_s = \tfrac{1}{4}N^{\frac{1}{2}} [(\alpha - 1)/\alpha] [k'/(1 + k')] \quad (1)$$

This expresses the resolution, R_s, between any two compounds as a function of column efficiency, N, selectivity, α, and the capacity factor, k'. The capacity factor, k', is defined as $(t_R - t_0)/t_0$, where t_R is the elution time of a compound of interest and t_0 is the elution time of a theoretically unretained substance. Selectivity, α, is calculated by the expression k'_2/k'_1, where k'_2 and k'_1 are the capacity factors of two components to be separated from each other. These values are always chosen so that k'_2 is always the more retained component. The column efficiency, N, expressed in terms of theoretical plates, represents the number of statistical partitioning steps the column is capable of providing. N can be calculated by the expression $16(t_R/t_w)^2$. Here t_R is the retention time and t_w is the time width of the peak defined as four standard deviations of its concentration profile. Very often N is expressed in terms of effective theoretical plates, N_{eff}. In this case, N_{eff} is equal to $N[k'/(1 + k')]^2$. The advantage of this is that resolution is directly proportional to $N_{eff}^{\frac{1}{2}}$, while this is not the case with N. The number of theoretical plates can also be used to calculate a theoretical plate height, H. This is done by dividing the column length, L, by N.

Applications of various theories of fluid dynamics and mass transfer phenomena have lead to the development of numerous equations describing the effects of different variables on H. For the case being studied here, that of smooth wall open tubes coated with a uniform film of thickness d_f, the resulting expression for H is[11]:

$$H = \frac{2D_m}{v} + \frac{(1 + 6k' + 11k'^2)d_c^2 v}{96(1 + k')^2 D_m} + \frac{2k'd_f^2 v}{3(1 + k')^2 D_s} \quad (2)$$

In this equation D_m is the diffusion coefficient of the solute in the mobile phase, D_s is the diffusion coefficient of the solute in the stationary phase, v is the mobile phase linear velocity, d_c is the column diameter and d_f is the stationary phase film thickness. It is the effect of operating conditions on the variables in these equations for R_s and H, as well as instrumental contributions to the peak width, t_w, which determine the total resolving power of a chromatographic system.

PHYSICOCHEMICAL BASIS

Pressure plays a very important role in SFC. It has a direct effect on retention, selectivity and diffusion. All three of these parameters in turn have important relationships to the resolving power of a chromatographic system. In order to understand the effects of column pressure drop and operating pressures, it is necessary to understand how pressure affects each of these variables.

It is the density of the mobile phase, and not its pressure, that is the controlling physical parameter. All column pressure effects can be related to the density of the mobile phase. If pressure changes are small enough, the relationship of the change in density, to the change in pressure, can be approximated by the relationship:

$$\Delta\rho/\rho = s(\Delta P/P) \tag{3}$$

Here ρ is the density, $\Delta\rho$ is the change in density, P is the pressure, ΔP is the change in pressure and s is the fractional density change per fractional change in pressure. The value of s under operating conditions typically used in SFC varies from 0.2 to 6. In the density and temperature region most often used, s varies in value from about 0.7 to 4.4, with half of this density region having s values greater than 2.0.

The pressure drop across the column with supercritical fluid mobile phases is approximately linear[12–14]. Using a model in which density change with pressure is linear, the density change of the mobile phase along the column length is also linear. This means that the density of the mobile phase in the column can be described by the equation:

$$\rho = D - wl \tag{4}$$

In this expression, D is the density of the mobile phase at the head of the column, l is the length along the column and w is the rate at which the density changes along the column. The total density drop across the column is equal to wL, where L is the total column length.

The effect of mobile phase density on selectivity and retention is calculated from the following two equations[8]:

$$\ln \alpha = B_0 - m\rho \tag{5}$$

$$\ln k' = a - b\rho \tag{6}$$

Values for the constants B_0, m, a and b will vary depending on the compound types being separated, the nature of the mobile phase, the nature of the stationary phase and the temperature of the mobile phase.

Diffusion in the mobile phase is dependent upon both its density and viscosity. Other important variables are the operating temperature and the molecular weights of the mobile phase and solute. In studying the effect of the mobile phase density on diffusion for a specific mobile phase and solute at constant temperature, the following relationship[15] can be used:

$$D_m = z/\eta\rho \tag{7}$$

Here z is a constant and η is the viscosity. Density is used in the above equation instead of the mobile phase molar volume to be consistent with the terminology used in this paper.

PRESSURE DROP EFFECTS

Retention and selectivity

Eqns. 4 and 6 can be combined to obtain an expression for k' as a function of the distance a solute has traveled along the column. This expression can be integrated over the column length and then divided by the column length to obtain an average value for k'. The resulting expression, which can be used to calculate the observed k' value, is:

$$k'_{obs} = e^{(a - bD)} (e^{bwL} - 1)/bwL \tag{8}$$

By combining eqns. 4 and 5 and following the same procedure, a similar equation which solves for the observed selectivity is derived:

$$\alpha_{obs} = e^{(B - mD)}(e^{mwL} - 1)/mwL \tag{9}$$

In both of these equations, wL is simply the density change, $\Delta\rho$, across the column. This leads to the interesting conclusion that changes in observed selectivity and retention values caused by a linear density change along a column are only dependent on the total density drop which occurs. The rate at which the density changes along a column is only important to the extent that it affects the total density drop.

Other conclusions that can be drawn from these equations describe how the observed values of α and k' relate to the operating density and density drop. In order to maintain constant retention or selectivity as the density drop is increased, the density at the head of the column must be increased. Conversely, if the density at the head of the column is held constant while $\Delta\rho$ is allowed to increase, both selectivity and retention will increase. The effects of increasing density drop on retention and selectivity are such that if k'_{obs} is kept constant, selectivity will always decrease, and if selectivity is kept constant, k'_{obs} will always increase. This is because the constant b in eqn. 6 will always be greater than the constant m in eqn. 5. If m could be greater than b, it would be possible for selectivity to go up with density drop at constant k'_{obs}. The reason that density drops affect selectivity at all is that in order to maintain a constant k'_{obs}, the increase in density above that which gives the desired k'_{obs} under zero density drop conditions, is more than 50% of the total density drop across the column. Since the average column density increases, the average selectivity decreases.

Column efficiency

Pressure drop effects on column efficiency are expected to be small[1]. This is because, to a first approximation, the product between the diffusion coefficient in the mobile phase and the mobile phase density is constant. For this reason the mobile phase velocity should be kept inversely proportional to its density in order to maintain the same efficiency. Since in a constant mass flow situation the mobile phase velocity is inversely proportional to its density, this criteria is met, and no efficiency loss results. For these same reasons, if constant mass flow is maintained during pressure programming, no efficiency losses should occur. The mobile phase linear velocity

will gradually decrease as its density increases, exactly compensating for the slower diffusion rates.

More realistically, the relationship given in eqn. 7 is followed. This means that in order to maintain the same efficiency throughout the column, the column mobile phase velocity must be controlled so that the product $v\eta\rho$ remains constant. In a constant mass flow situation, changes in D_m due to viscosity changes will cause losses in efficiency. Evidence that this should indeed be the case is shown in plots of $D_m\rho$, ρ and η *versus* P for supercritical carbon dioxide shown in Fig. 1[16]. The product $D_m\rho$ and the value of η remain almost constant until close to the critical pressure. At this point the product $D_m\rho$ gradually goes down as the value of η goes up. Taking values from the data plotted by Van Wasen *et al.*[16] for CO_2, the product $D_m\rho$ goes down by a factor of six and the viscosity goes up by a factor of seven when the pressure is increased from 50 to 500 bar. The product $\eta D_m\rho$, however, changes only by about 20%. The result of this is that unless a large density drop is experienced, at constant pressure and under constant mass flow conditions, the loss of column efficiency is probably small.

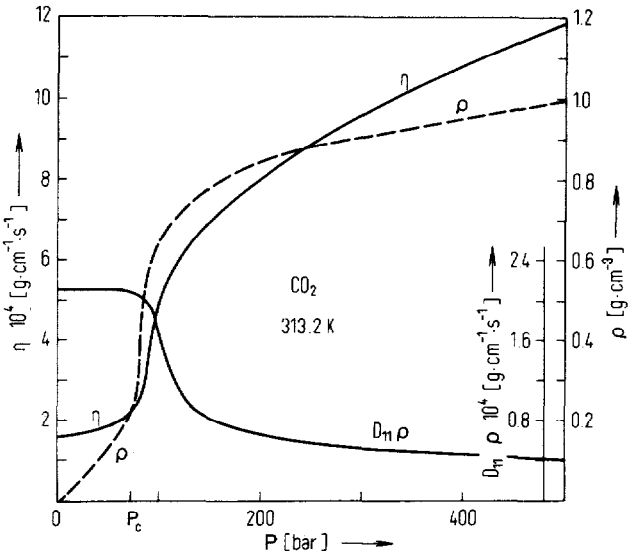

Fig. 1. Density, ρ, viscosity, η, and self-diffusion coefficient, D_{11}, of pure carbon dioxide as a function of pressure at 40°C[16].

Under pressure programming conditions, if one starts out at the optimum mobile phase velocity, one could easily be at several times the optimum mobile phase velocity by the end of a run. This becomes increasingly more likely since higher-molecular-weight species with even lower diffusion coefficients are eluted at higher pressures. In order to maintain column efficiency, the mobile phase mass flow-rate must be decreased at higher pressures. The magnitude of this decrease will depend on the molecular weights of the compounds being eluted, and on the change in viscosity of the mobile phase.

Resolution

In order to study the effect of density change along the length of a column on resolution, data were taken from material published by Sie and co-workers[3,17] on the behavior of *n*-alkanes in carbon dioxide, and on the behavior of dialkyl phthalates in *n*-pentane. Using this data, constants for eqns. 5 and 6 were calculated for use in eqns. 8 and 9. Tables I and II demonstrate the effects of density drop across a column for each of these two cases. The subscripts i, o and obs represent, respectively, conditions at the inlet of the column, conditions at the outlet of the column and the observed value which would be seen on a chromatogram. Resolution was set equal to one for cases where the density drop was zero, and was normalized to the zero density drop case for all other situations.

Careful study of the data presented leads to the following conclusions:

(a) On the average, α_{obs} changes by 0.001 unit per 10% density drop up to a 30% density drop.

TABLE I

DENSITY DROP EFFECTS ON THE RESOLUTION OF ALKANES IN CARBON DIOXIDE

Calculations based on: $\ln \alpha = 1.140 - 2.040\,\rho$; $\ln k' = 2.731 - 8.165\,\rho$.

$\Delta\rho/D$ (%)	k'_{obs}	α_{obs}	R_s	k'_i	k'_o	α_i	α_o
0	5.0	2.363	1.000	5.00	5.00	2.363	2.363
10	5.0	2.362	1.000	4.71	5.30	2.328	2.397
20	5.0	2.361	0.999	4.40	5.65	2.288	2.436
30	5.0	2.360	0.999	4.07	6.06	2.244	2.479
40	5.0	2.357	0.998	3.71	6.55	2.193	2.527
50	5.0	2.352	0.997	3.33	7.15	2.135	2.583
0	2.0	1.879	1.000	2.00	2.00	1.879	1.879
10	2.0	1.879	1.000	1.79	2.22	1.829	1.930
20	2.0	1.876	0.998	1.58	2.49	1.772	1.985
40	2.0	1.864	0.991	1.14	3.22	1.632	2.116

TABLE II

DENSITY DROP EFFECTS ON THE RESOLUTION OF DIALKYL PHTHALATES IN PENTANE

Calculations based on: $\ln \alpha = 0.251 - 0.617\,\rho$; $\ln k' = 6.84 - 25.86\,\rho$.

$\Delta\rho/D$ (%)	k'_{obs}	α_{obs}	R_s	k'_i	k'_o	α_i	α_0
0	10.0	1.153	1.000	10.0	10.0	1.153	1.153
10	10.0	1.153	1.000	7.8	12.6	1.147	1.160
20	10.0	1.152	0.994	5.8	16.0	1.138	1.166
30	10.0	1.150	0.982	3.9	20.3	1.128	1.173
40	10.0	1.147	0.966	2.4	26.3	1.115	1.180
50	10.0	1.142	0.937	1.3	34.3	1.098	1.188
0	5.0	1.135	1.000	5.0	5.0	1.135	1.135
10	5.0	1.134	0.993	3.7	6.5	1.127	1.141
20	5.0	1.133	0.987	2.7	8.5	1.117	1.149
40	5.0	1.127	0.947	0.9	14.8	1.090	1.164

(b) The sensitivity of α_{obs} to density drop increases at lower k' values.
(c) When α_{obs} is large, density drops have little effect on resolution.
(d) Selectivity decreases more rapidly as density drops become larger.
(e) Resolution losses occur because of losses in selectivity.
(f) Selectivity increases at larger k' values where the mobile phase density is lower.

Roughly, doubling the value of either b or m in eqns. 5 and 6 will double the sensitivity of α_{obs} to density drops. Doubling both b and m will result in a four-fold increase in the change of α_{obs} with pressure drop. The value of b is easily doubled by going to higher members of a homologous series. This is because, in most cases, b is directly proportional to the carbon number of the compound in the series eluted. It is not unlikely then for changes in α_{obs} to be twice those shown in Tables I and II. If it is assumed that most compounds will be approximately twice as sensitive to density changes as demonstrated in Tables I and II, and it is desired to maintain resolution where the density change with pressure is at its greatest ($s \approx 4$), the minimum density and pressure drop can be calculated for small α. The percentage density and pressure drop giving 5% resolution losses are shown in Table III at various α as well as the percentage of resolution lost with 10% pressure drop.

TABLE III

PRESSURE DROP EFFECTS ON RESOLUTION

α	$\Delta R_s = -5\%$		$\Delta P/P = 10\%$ Loss in R_s (%)
	$\Delta\rho/D$ (%)	$\Delta P/P$ (%)	
1.005	1.3	0.3	100
1.01	2.5	0.6	80
1.02	5.1	1.3	40
1.03	7.7	1.9	27
1.04	10.4	2.6	19
1.05	13.1	3.3	15

The loss in selectivity becomes quite dramatic at larger pressure drops. When the constants are chosen for eqns. 5 and 6 to be consistent with the assumptions in the previous paragraph, the plot shown in Fig. 2 can be obtained. For most situations the changes in α can be expected to lie close to those shown. However, the dependency of α_{obs} on the variables m and b as outlined will probably make the $\Delta\alpha_{obs}$ vary in extreme cases from one-fourth to four times that shown on the graph. It is this loss in selectivity under large density drop conditions which makes small particle packed columns almost useless when one desires to work in the pressure–temperature region where the change in density with pressure is greatest. Based on pressure drops at mobile phase linear velocities of 1 cm/sec experienced on 10 cm long columns packed with 10-μm, 5-μm and 3-μm particles[18], losses in selectivity in this pressure–temperature region can be expected to lie in the ranges shown in Table IV.

If higher pressures are used where the density change is small along the column, the loss in selectivity due to density drop becomes negligible. High pressure drops

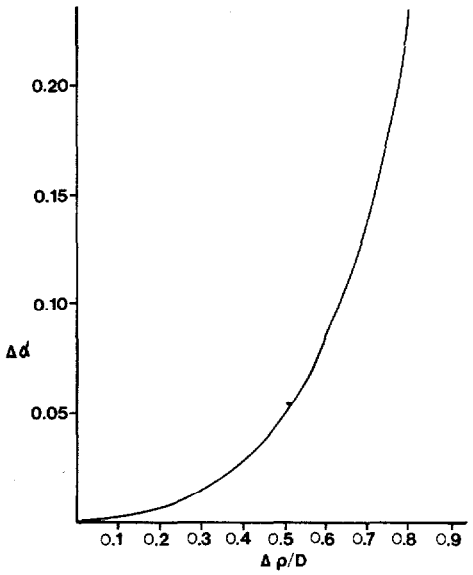

Fig. 2. Density drop effects on α at constant k'.

can be tolerated if one does not work in the pressure regions where density changes rapidly with pressure. Unfortunately, this restricts the flexibility of the analytical system in handling wide molecular weight range samples.

MAXIMUM RESOLUTION LIMITS

A preliminary treatment of resolution limits in SFC has already appeared in the literature[10]. The maximum resolution limit can be defined as the smallest α a system is capable of resolving. In SFC there are two main limiting factors on the value of α capable of being resolved. These two factors are the number of theoretical plates which can be produced, and the loss in selectivity caused by column pressure drop. The limiting column length occurs when the loss in selectivity due to pressure drop, $\Delta\alpha$, is equal to $1 - \alpha$ for the α which would have been resolved. At this point the minimum α resolved has a value of between $1\Delta\alpha$ and $2\Delta\alpha$ based on an α value measured under hypothetical zero density drop conditions.

On the basis of the previous section, the loss in selectivity at low density drop is approximately equal to $-0.02\ \Delta\rho/D$. If it is desired to maintain this resolving

TABLE IV

PARTICLE SIZE EFFECTS ON SELECTIVITY

Particle size (μm)	$\Delta\alpha_{obs}$ *range expected*
10	0.01–0.07
5	0.03–0.22
3	0.09–0.29

power where the density of the mobile phase changes most rapidly with pressure, s must assume the value of four in eqn. 3. Solving for $\Delta\alpha$ then gives it a value equal to $-0.08\ \Delta P/P$. Poiseuille's law can be used to calculate values for ΔP, and eqn. 1 can be solved for $1 - \alpha$. Combining these expressions results in the equation:

$$0 = \left[1 - \frac{4\ R_s}{N^{\frac{1}{2}}}\left(\frac{1 + k'}{k'}\right)\right]^{-1} - 2.56\ \frac{\eta\ vNH}{d_c^2\ P} - 1 \qquad (10)$$

Substitution of appropriate values in place of variables in eqn. 10 allows the calculation of values for N by successive iterations using a programmable calculator. The results of these calculations are shown in Table V. A resolution value of 1 was arbitrarily chosen as the minimum useful separation. Observed k' values of 1 and 5 were chosen since they represent the most useful retention range for actual separations. Data in the upper half of Table V are based on calculated optimum mobile phase velocities and resulting theoretical plate heights. Likewise, results in the lower half of the table represent conditions at 10 times the optimum mobile phase velocity. The mobile phase flow-rates and theoretical plate heights are based on a mobile phase diffusion coefficient of $2 \cdot 10^{-4}$ cm^2/sec, a k' of 1, a stationary phase film thickness equal to 1% of the column diameter and a diffusion coefficient in the stationary phase of $1 \cdot 10^{-6}$ cm^2/sec. No efforts were made to compensate for the effect of increasing k' on calculated optimum mobile phase velocities because the uncertainty in published values for diffusion coefficients in the mobile and stationary phases represents a larger variance in the optimum mobile phase velocity than would be seen in changing k' from 1 to 5. The data in Table V should only be regarded as representative of the effects of different column diameters on resolution limits and maximum useful column lengths. Values for the other variables used in eqn. 10 are a viscosity of $5 \cdot 10^{-4}$ g/cm · sec[16] and a pressure of 40 atm. This pressure was chosen because it is where the maximum change in the density of *n*-pentane with pressure occurs at temperatures commonly used.

TABLE V

MAXIMUM PERFORMANCE FOR VARIOUS COLUMN DIAMETERS

$d_c(mm)$	α_{min}	$L(m)$	$v(cm/sec)$	k'	N_{eff}	$H(mm)$	$\Delta P/P$	$t_R(days)$
0.20	1.003	3243	0.06	1	$6.8 \cdot 10^6$	0.12	0.02	125
0.10	1.005	644	0.12	1	$2.7 \cdot 10^6$	0.06	0.03	12.4
0.05	1.008	128	0.24	1	$1.1 \cdot 10^6$	0.03	0.05	1.2
0.20	1.002	2306	0.06	5	$1.3 \cdot 10^7$	0.12	0.01	267
0.10	1.003	458	0.12	5	$5.3 \cdot 10^6$	0.06	0.02	26.4
0.05	1.006	91	0.24	5	$2.1 \cdot 10^6$	0.03	0.03	2.6
0.20	1.011	1205	0.60	1	$4.9 \cdot 10^5$	0.61	0.07	4.6
0.10	1.018	238	1.20	1	$2.0 \cdot 10^5$	0.30	0.11	0.46
0.05	1.029	47	2.40	1	$7.9 \cdot 10^4$	0.15	0.18	(1.1 h)
0.20	1.008	856	0.60	5	$9.7 \cdot 10^5$	0.61	0.05	9.9
0.10	1.013	169	1.20	5	$3.9 \cdot 10^5$	0.30	0.08	0.98
0.05	1.020	34	2.40	5	$1.6 \cdot 10^5$	0.15	0.13	(2.3 h)

In order to obtain reasonable analysis times at the resolution limits, only 50 μm I.D. columns (or smaller) are practical. The use of 50 μm I.D. columns allows the achievement of over a million effective theoretical plates in slightly over a day, and more than 10^5 effective theoretical plates in less than 2 h. The maximum column lengths for the 50 μm I.D. columns are achievable with present technology.

An interesting observation occurs when one notices how the maximum column length changes with increasing retention. The maximum length decreases with increasing retention, but the resolving ability of the system increases. This is a result of the effective number of theoretical plates increasing with retention, which in turn causes the minimum α resolvable to decrease. To compensate for this increased resolving power, the column must be shorter so that the loss in resolution due to any density drop does not exceed the resolution gained from the increased number of effective theoretical plates. One should also realize that the resolving power at higher k' values is much better than that indicated in Table V because of the increase in selectivity which occurs at lower mobile phase densities. The magnitude of this increase in resolving power will depend on the rate at which the selectivity changes with changes in the mobile phase density.

SPEED OF ANALYSIS

The time of analysis can be mathematically expressed as:

$$t_R = NH(1 + k')/v \tag{11}$$

When fast analysis times are sought, higher than optimum mobile phase linear velocities are used. As the value of v is increased, the ratio H/v approaches the value $H_{min}/2v_{opt}$. Here H_{min} and v_{opt} are the column plate height and mobile phase velocities where H has its smallest value. The value of H_{min}/v_{opt} is approximately 0.6 $d_c^2/6D_m$ for capillary columns when k' is between one and five. Good packed columns have values around $2d_p^2/3D_m$ under these same conditions.

Using the same mobile phase, equal speeds of analysis for packed and capillary columns occur when the values of H_{min}/v_{opt} for the two systems are equal. This occurs when $d_c = 2.6\ d_p$[19]. However, changes in observed selectivity due to density drops across columns add the requirement of identical pressure drops across the packed and capillary columns. Packed columns have much lower permeabilities than capillary columns. This difference in permeability makes it physically impossible for this criteria to be met. Under high enough operating pressures where density changes are small with pressure, and resulting selectivity losses become insignificant, packed columns will always give the fastest analysis speeds because of the ability to use very small particle diameters. At lower pressures where density drops can be injurious to selectivity, capillary columns will give better speeds of analysis. This is because the low pressure drop, and therefore greater selectivity, available from capillary columns reduces considerably the number of theoretical plates which are required.

Capillary SFC can be competitive with high-pressure liquid chromatography (HPLC). If a diffusion coefficient of $2 \cdot 10^{-6}$ cm^2/sec is assumed for liquid solutes, and $2 \cdot 10^{-4}$ cm^2/sec for supercritical fluid solutes, identical speeds of analysis can be achieved when $d_c/d_p = 26$. In the case of 5-μm and 3-μm particles, this requirement

is met when 0.13 mm I.D. and 0.08 mm I.D. open tubular columns are used, respectively. In order to achieve superior speeds of analysis over HPLC, smaller than 0.08 mm I.D. columns are required.

Capillary gas chromatography (GC) will always be faster than capillary SFC, and usually faster than SFC with packed columns. Using 0.1 cm^2/sec as a diffusion coefficient in gases and $2 \cdot 10^{-4}$ cm^2/sec in supercritical fluids, identical speeds of analysis are achieved when $d_c(SFC)/d_c(GC) = 0.045$ and $d_p(SFC)/d_c(GC) = 0.017$. For 0.20 mm I.D. columns in GC, this would require 8 μm I.D. columns for capillary SFC, and 3-μm particles for packed columns. Capillary columns 8 μm I.D. are too small to be practical. Based on the change in selectivity which occurs for homologous series on using denser mobile phases, the selectivity for similar compounds will generally be better in GC than SFC. While the use of 3-μm particles is feasible in packed columns, this reduced selectivity makes it necessary to use more theoretical plates than in GC. The higher operating fluid densities which are required in packed columns (because of selectivity losses at lower pressures) make diffusion coefficients in packed columns more likely to be closer to $1 \cdot 10^{-4}$ cm^2/sec. Therefore, smaller than 2-μm particles are needed to give faster analysis times than available in GC.

PRESSURE PROGRAMMING

Ideal pressure programming rates in terms of the mobile phase density can be predicted[8] based on the following equation:

$$\ln k' = A + B_0 n - mn\rho \tag{12}$$

If it is desired to have members of homologous series elute at regular time intervals, the carbon number, n, becomes equal to some constant, j, times the sum of the time, t, and a reference elution time, t'. Letting all members of the homologous series be eluted at the same k', and solving for ρ, the following equation results:

$$\rho = (A - \ln k')/[mj(t + t')] + B_0/m \tag{13}$$

Combining variables to simplify this equation results in the following:

$$\rho = \rho_A - K/(t + t') \tag{14}$$

The constants K and t' determine the spacing and k' of components as they elute, and ρ_A represents the threshold density at which all components in the homologous series would coelute. Eqn. 5 provides a convenient means for calculating ρ_A. By making a few constant pressure chromatographic runs and plotting $\ln \alpha$ against ρ, the intercept at $\ln \alpha = 0$ gives ρ_A.

Actual use of a density program of this sort should maximize selectivity in a pressure programmed run. Nevertheless, the reduction in selectivity as density is increased will still cause decreasing resolution of compounds as ρ_A is approached. This is because the derived equation calculates a density which would give the desired k' value for component n at time t. Since k' values for eluted components all approach the same value as ρ approaches ρ_A, decreasing resolution is observed, but some finite value of resolution is always present since ρ_A is never reached.

INJECTOR AND DETECTOR VOLUMES

Very small injector and detector volumes are required for capillary chromatography systems[20,21]. Not only are the volumes involved important, but the laminar and non-laminar flow characteristics also have important effects. It is beyond the scope of this paper to discuss these flow effects, and the reader is referred to recently published literature to gain a greater understanding of this subject[22,23]. In well-designed systems, flow characteristics of the detector and injector will have minimal effects on band broadening. The sample load and volume will have the greatest effects on efficiency. A general equation[24] relating the effects of a plug of sample entering or leaving a column on observed peak widths is:

$$\frac{\tau_i}{\tau_c} = \frac{N_i}{N}\left(\frac{N}{12}\right)^{\frac{1}{2}} \tag{15}$$

Here τ_i and τ_c are the peak width standard deviations due to the injector or detector and the column, respectively. The values for N and N_i represent the number of theoretical plates provided by the column and the number of those plates filled by the injector or detector volume, respectively. Using this equation in conjunction with equations for N, an equation predicting the effect of different injector and detector volumes can be derived:

$$V_i = 0.866\pi d_c^2 (LH)^{\frac{1}{2}} [1/(1 - \Delta R_s)^2 - 1]^{\frac{1}{2}} (1 + k') \tag{16}$$

In this equation, V_i is the injector or detector volume and ΔR_s is the fractional loss in resolution. The column plate height, H, is a function of the column diameter. This makes injector and detector volumes directly proportional to d_c to the 5/2 power. Column length has to be increased by a factor of four to double permissible volumes. The larger elution volumes of retained compounds have a considerable effect on the value of V_i, causing it to be proportional to $1 + k'$.

Examples of permissible volumes causing only a 1% loss in resolution are shown in Table VI. These numbers are based on a column length of 20 m, a plate height of 0.6 d_c and a k' value of 1. The length of the column which could be used for on-column detection is shown under the symbol L_i. In most practical circumstances, sample application and detection takes place using the mobile phase in the liquid state. This results in an expansion of the injection volume on application to the column, and a contraction in peak volume before entering the detector. Where this is the case, the values for V_i and L_i in Table VI need to be divided by the ratio of this expansion from the liquid to the supercritical state.

TABLE VI

DETECTOR VOLUMES CAUSING 1% RESOLUTION LOSSES

V_i (μl)	L_i (cm)	d_c (μm)
1.5	4.8	200
0.27	3.4	100
0.05	2.4	50

The achievement of these small detector and injector volumes is within the range of present technology. With injectors, this can be done with very small volume valves or split injection. On-column detection has already been demonstrated to be feasible with 50 μm I.D. columns[25].

CONNECTOR TUBING

For capillary SFC instrumentation, it is most convenient to let the column act as its own connector tubing. Because of this, the advantages recently published for very short lengths of connector tubing[23,26–28] do not apply to this technique since the real tubing length in question then is equivalent to the entire column length. No other tubing is present in the injector or detector that could contribute to band broadening. Retention of compounds in sections regarded as connecting tubing is usually minimal since lower temperatures are present to help insure the transfer of compounds to and from the rest of the column. The variance, σ^2, in peak width which occurs by flow through connector tubing of length l_c, which is of the same diameter as the column, is given by:

$$\sigma^2 = \left(\frac{2D_m}{v} + \frac{d_c^2\, v}{96D_m}\right) l_c \tag{17}$$

An equation for the length of connector tubing which can be used as a function of the loss in resolution that it would cause can be derived using the above expression:

$$l_c = [(1 - \Delta R_s)^{-2} - 1]LH\,(1 + k')\,(2D_m/v + d_c^2\, v/96D_m)^{-1} \tag{18}$$

Connector tubing length is independent of column diameter when H and v are expressed as functions of D_m and d_c. Permissible connector lengths are, however, directly proportional to column length and solute retention.

If the same mobile phase conditions present in the column are maintained, connector tubing lengths can be up to 6% of the column length with resolution losses of less than 1%. This would allow the use of 123 cm of connector tubing at a k' of 1 for 20-m columns. Cooling the mobile phase to the liquid state drastically changes this situation. This is a result of the lower diffusion rates in the liquid state and the band compression due to the smaller volume occupied by the liquid phase. A 20-m column now suffers a 1% loss in resolution with only 4 cm of connecting tubing below the critical temperature. This emphasizes the importance of short lengths of connecting tubing when the mobile phase is allowed to liquefy.

STATIONARY PHASE FILM THICKNESS

Examination of the Golay equation for H as a function of various column parameters shows that the contribution of stationary phase film thickness to plate

height is the greatest when $k' = 1$. The fraction, F, of the plate height due to the stationary phase for $k' = 1$ and at the optimum linear velocity is:

$$F = \frac{96(d_f/D_c)^2 \ (D_m/D_s)}{35 + 96(d_f/d_c)^2 \ (D_m/D_s)} \tag{19}$$

Technically, d_c in this equation should be $(d_c - 2d_f)$. When the ratio of d_f/d_c is small, this correction becomes insignificant. This equation was used to calculate the information given in Fig. 3. A ratio of D_m/D_s equal to 100 was assumed in this example. The expected result of being able to use thicker films than in GC, but thinner than in liquid chromatography is shown. At a k' of 1, film thicknesses equal to 1% of the column diameter would cause plate heights to increase by a factor of 2.7 for GC, 0.027 for SFC and $2.7 \cdot 10^{-4}$ for liquid chromatography. The resulting loss in resolution in SFC due to this 4% phase ratio would only be 1.4% at a k' of 1, and less than that at all other k' values.

There is a good reason for using thicker films in SFC as compared to GC. The weakest point in the instrumentation for SFC is the detector. To improve detector sensitivity, the column has to be able to handle larger sample sizes. A 100 μm I.D. column coated with a 0.3% phase ratio as in GC can probably expect a maximum sample capacity of approximately 40 ng without causing more than 1% resolution loss. Using a 3% phase ratio increases this figure by an order of magnitude, making maximum sample loads of 400 ng feasible. This ten-fold increase in sample capacity can also serve to decrease detector sensitivity requirements by the same factor.

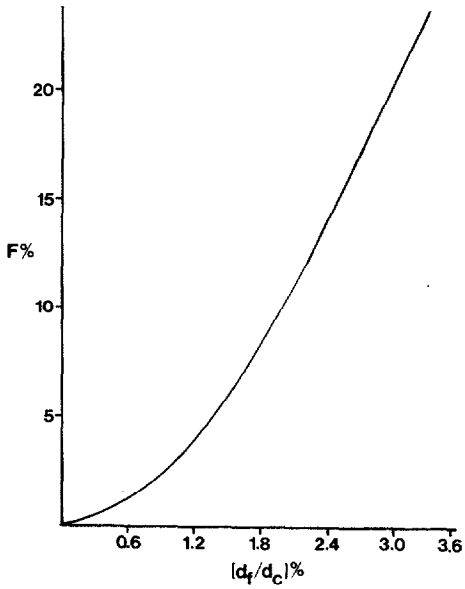

Fig. 3. Fraction of the theoretical plate height due to the stationary phase as a function of d_f/d_c at $k' = 1$.

DETECTOR TIME CONSTANT

The loss in column efficiency due to the detector time constant, τ_d, is[21]:

$$E = \tau_d N^{\frac{1}{2}}/t_R \tag{20}$$

where E is the fractional loss in column efficiency. If it is desired to have little loss in the resolution of poorly retained peaks ($k' < 1$), or for high speed chromatography where $v \gg v_{opt}$, the above equation can be rewritten in terms of column variables as:

$$\tau_d = 100\ EN^{\frac{1}{2}}\ d_c^2(1 + k') \tag{21}$$

The coefficient of 100 is a result of giving D_m a value of 2×10^{-4} cm^2/sec and giving H/v a value for unretained peaks.

Allowing an efficiency loss of 1%, and calculating the detector time constant compatible with a 50 μm I.D. column delivering 30,000 theoretical plates, gives τ_d a value of 4 msec. For peaks eluting with k' values of 1–5, 20 msec time constants should be adequate.

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