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Design of liquid chromatography capillary columns

RAYMOND P. W. SCOTT

Chemistry Department, Georgetown University, Washington, DC 2001 (U.S.A.)

ABSTRACT

The theory of open tubular columns is extended to liquid chromatography without regard to the limitations of the associated chromatographic equipment. Methods are given to permit the optimum column length, radius and film thickness to be calculated to provide the minimum analysis time for any given separation. The basic data required are the separation ratio of the critical pair, the distribution coefficient between the stationary and mobile phase of the first eluted solute of the critical pair, its diffusion coefficient in the mobile phase, the separation ratio of the last eluted peak to the first eluted solute of the critical pair, the viscosity of the mobile phase and the maximum available inlet pressure. It is shown that, with the phase system considered, if the separation is to be achieved in the minimum time then the first solute of the critical pair should be eluted at a capacity factor (k') of 2.7 irrespective of the separation ratio of the critical pair. Consequently the optimum film thickness depends solely on the optimum radius and the distribution coefficient of the solute between the two phases. Due to an optimum value for k' being identified the equations for analysis time and column length are greatly simplified.

INTRODUCTION

Liquid chromatography (LC) column theory is now well established and equations have been developed for packed columns that allow the column length, radius and particle diameter to be calculated, that provides the minimum analysis time for any given separation¹. These equations were derived employing the Van Deemter equation² to describe the dispersion taking place in a packed column. The Van Deemter equation has been shown to accurately predict the plate height at, and around, the optimum mobile phase velocity from basic chromatographic and physical data of the solute–solvent system employed³. The equations necessary to design capillary columns have not, so far, been developed to the same extent for LC and it is the purpose of this paper to examine capillary column design and to develop procedures to calculate the column length, radius and stationary phase film thickness that will achieve a specific separation in the minimum time.

To develop these procedures, the reduced chromatogram will be employed that contains the closest eluted pair of solutes (the critical pair) and the last eluted solute of

any given complex mixture. If the closest eluted solute pair is separated and the last peak of the mixture eluted then it is assumed that the mixture has been completely resolved. There may be rare exceptions where this may not be the case, possibly if the two solutes of the closest eluted pair differ widely in molecular weight⁴, but in the vast majority of separations the assumption will be correct. The basic data that will be employed will be the separation ratio of the critical pair, the distribution coefficient of the first solute of the critical pair between the stationary phase and the mobile phase, its diffusion coefficient in the mobile phase, the separation ratio of the last eluted peak to that of the first solute of the critical pair, the viscosity of the mobile phase and the maximum inlet pressure.

Although capillary, or open tubular columns are the most efficient for chromatographic separations, the demands they make on the associated chromatographic equipment are so stringent that, at this time, their effective use is largely confined to *gas* chromatographic separations. Although much research is being carried out in this area, at this time, there are few, if any, LC capillary column chromatographs commercially available. Small volume sample valves have been developed and the detector cell volumes have been much reduced, but despite this, the column diameters must be made much above the optimum size for effective LC separations to be successfully carried out. In due course, instrumentation will no doubt meet the demands of the optimized, very small diameter, LC capillary columns. As a result, in this paper the shortcomings of contemporary apparatus are largely ignored and thus, by developing LC capillary column design, very fast and highly efficient columns will be ready and available when the appropriate instrumentation is developed.

THEORY

The basic equation describing the dispersion that takes place in an open tubular column was developed by Golay^{5,6} and takes the following form:

$$H = 2D_M/u + (1 + 6k' + 11k'^2)r^2u/24(1 + k')^2D_M + k'^3r^2u/6(1 + k')^2K^2D_S \quad (1)$$

where H is the variance per unit length of the column for the given solute, k' is the capacity factor of the eluted solute, K is the distribution coefficient of the solute between the two phases, D_M is the diffusivity of the solute in the mobile phase, D_S is the diffusivity of the solute in the stationary phase, r is the radius of the column, and u is the linear velocity of the mobile phase. The Golay equation can be put in the reduced form:

$$H = B/u + Cu \quad (2)$$

where $B = 2D_M/u$ and $C = (1 + 6k' + 11k'^2)r^2/24(1 + k')^2D_M + k'^3r^2/6(1 + k')^2K^2D_S$. Differentiating eqn. 2 with respect to u :

$$dH/du = -B/u^2 + C$$

Thus, when $H = H_{\min}$:

$$-B/u^2 + C = 0$$

and

$$u_{\text{opt}} = (B/C)^{0.5} \quad (3)$$

Furthermore,

$$\begin{aligned} H_{\min} &= B/u_{\text{opt}} + Cu_{\text{opt}} \\ &= B/(B/C)^{0.5} + C(B/C)^{0.5} \end{aligned}$$

or

$$H_{\min} = 2(BC)^{0.5} \quad (4)$$

Eqns. 1, 3 and 4 are all well established and important in column design.

The equation that allows the number of theoretical plates required to effect a given separation was derived from the plate theory by Purnell⁷ and is as follows:

$$n = [4(1 + k')/k'(\alpha - 1)]^2 \quad (5)$$

where α is the separation factor of the critical pair, n is the number of theoretical plates, and k' is as previously defined. It should be noted that k' refers to the *first* eluted solute not the second which is also often used and results in a slightly different equation for n .

Now the time required to elute the first of the critical pair, which will be simply related to the total analysis time, is given by:

$$t = l(1 + k')/u_{\text{opt}} \quad (6)$$

where t , is the elution time for the first solute of the critical pair and l , is the length of the column. Now, $l = nH_{\min}$, thus,

$$t = nH_{\min}(1 + k')/u_{\text{opt}} \quad (7)$$

$$= n2(BC)^{0.5}(1 + k')/(B/C)^{0.5}$$

$$= 2nC(1 + k') \quad (8)$$

Substituting for C and n from eqns. 1 and 5,

$$t = [32(1 + k')^3/k'^2(\alpha - 1)^2][(1 + 6k' + 11k'^2)r^2/24(1 + k')^2D_M + k'^3r^2/6(1 + k')^2K^2D_s]$$

which can be simplified to,

$$t = 4(r^2/k'^2 + 7r^2/k' + 17r^2 + 11k'r^2 + 4k'r^2/\beta K^2 + 4k'^2r^2/\beta K^2)/3D_M(\alpha - 1)^2 \quad (9)$$

where $\beta = D_s/D_M$. Now it would appear at first sight, that by differentiating eqn. 9 with respect to r and equating to zero, an expression would be obtained that would give an optimum value r for minimum analysis time. However k' is a function of both d_f , the film thickness of stationary phase and r .

It is, therefore, necessary to obtain an expression for t that does not include either k' or d_f . Now $k' = K/a$, where a is the phase ratio of the column. Furthermore, the volume of mobile phase in the column is $\pi r^2 l$ and the volume of stationary phase is $2\pi r l d_f$. Consequently a is given by, $\pi r^2 l / 2\pi r l d_f = r / 2d_f$ and

$$k' = 2Kd_f/r \quad (10a)$$

Substituting for k' in eqn. 9 from eqn. 10a and simplifying,

$$t = [4/3D_M(\alpha - 1)^2] [r^4/(2K^2d_f^2) + 7r^3/(2Kd_f) + 17r^2 + (11 + 4/\beta K^2)2Krd_f + 16d_f^2/\beta] \quad (10b)$$

Now in order to solve for r or d_f for a minimum value of the analysis time (t) then a further relationship must be obtained between r and d_f . From Poiseuille's equation $l = Pr^2/(8\eta u_{opt})$, thus $l = nH_{min} = 2n(BC)^{0.5} = Pr^2/[8\eta(B/C)^{0.5}]$; thus, $2nB = Pr^2/(8\eta)$, where P is the inlet pressure and η is the solvent viscosity.

Now from the Golay equation $B = 2D_M$, thus,

$$n = Pr^2/(32\eta D_M) \quad (11)$$

It is interesting to note from eqn. 11 that when a column is run at its optimum velocity, the efficiency attainable from a capillary column is directly proportional to the inlet pressure and the square of the radius and inversely proportional to the solvent viscosity and *the diffusivity of the solute* in the mobile phase.

Substituting for n in eqn. 11 from eqn. 5,

$$[4(1 + k')/k'(\alpha - 1)]^2 = Pr^2/(32\eta D_M)$$

Substituting $2Kd_f/r$ for k' and simplifying,

$$d_f = r/(2K(r\psi - 1)) \quad (12)$$

where,

$$\psi = [(\alpha - 1)^2 P / 512\eta D_M]^{0.5} \quad (13)$$

Eqn. 12 gives the required independent relationship between r and d_f . Thus, substituting in eqn. 10 for d_f from eqn. 12,

$$t = [4/3D_M(\alpha - 1)^2] \{ r^4\psi^2 + 5r^3\psi + 11r^2 + (11 + 4/\beta K^2)r^2/(r\psi - 1) + 4r^2/[\beta K^2(r\psi - 1)^2] \} \quad (14)$$

In order to determine the optimum value of the column radius r for the minimum value of analysis time t eqn. 14 should be differentiated and equated to zero and solved for r . However, the algebra becomes extremely clumsy and the solution difficult if not impossible to identify. A simple and practical alternative is to employ a computer with an iterative program that will calculate t for a range of values of r and identify that value of r that gives the minimum value of t . Thus, by employing the appropriate equations the optimum values of d_f and k' can also be calculated.

After identifying the optimum values of r , d_f and consequently k' the column length can be determined as follows:

$$l = nH_{\min}$$

Substituting for n and H_{\min} from eqns. 5 and 4,

$$\begin{aligned} l &= \{[4(1 + k')/k'(\alpha - 1)]^2\}2(BC)^{0.5} \\ &= \{[4(1 + k')/k'(\alpha - 1)]^2\}2\{(2D_M)[(1 + 6k' + 11k'^2)r^2/24(1 + k')^2D_M + \\ &\quad + k'^3r^2/6(1 + k')^2K^2D_S]\}^{0.5} \quad (15) \end{aligned}$$

DISCUSSION OF THEORY

Eqns. 1–8 are well established and although essential for the subsequent development of the equations necessary to calculate optimum column parameters, do not in themselves deserve comment. Eqns. 9 and 10 are similar to those developed by Scott and Hazeldine⁸ for gas chromatographic separations on nylon capillary columns. The treatment given here, however, avoids the assumption of a given column radius and then the optimum film thickness calculated. In this case the optimum radius is *directly determined*. Consequently, the optimum film thickness *appropriate for the optimum column radius* can be calculated. In effect, both column radius and stationary phase film thickness are considered variables.

Eqn. 11 demonstrates that the number of theoretical plates available from a capillary column depends on its radius, inlet pressure, viscosity of the mobile phase and, perhaps a little surprisingly, the diffusivity of the solute in the stationary phase. Taking a viscosity value of 0.003967 P (the viscosity of *n*-heptane at 25°C), a diffusivity value of $2.5 \cdot 10^{-5}$ (the diffusivity of benzyl acetate in *n*-heptane) the efficiency obtainable from columns of different diameter can be calculated for an inlet pressure of 1000 p.s.i. The results are shown in Table I.

It is seen that changing the radius of the column from 1 to 100 μm results in an efficiency change from about two hundred thousand theoretical plates to two thousand million plates. It is also seen that *the smaller the column radius the lower the maximum number of theoretical plates* obtainable from the column. This is contrary to the general impression of many workers in the field who consider that the maximum efficiency is always obtained using columns with the smallest radius. A similar situation has been shown to be true for a packed column where it has been demonstrated¹ that the smaller the particle diameter of the packing the less the maximum number of theoretical plates obtainable for a given inlet pressure. This is

TABLE I
MAXIMUM EFFICIENCIES ATTAINABLE FROM COLUMNS OF DIFFERENT RADIUS

<i>Column radius</i> (μm)	<i>Column efficiency</i> (<i>theoretical plates</i>)
1	$2.17 \cdot 10^5$
10	$2.17 \cdot 10^7$
100	$2.17 \cdot 10^9$
Inlet pressure	1000 p.s.i.
Viscosity of mobile phase	0.00397 P
Diffusivity of solute in the mobile phase	$2.5 \cdot 10^{-5} \text{ cm}^2/\text{s}$

a result of a limited pressure being available and if the fastest separation is obtained by operating at the optimum velocity on a particular column, then to increase the number of theoretical plates the column must be made longer. It follows, that a longer column will have too high an impedance to permit the optimum velocity to be achieved and thus the radius must be increased. In summary, narrow capillary columns should be used for simple separations (large α) and wide columns for difficult separations (small α).

Employing eqn. 14 the analysis time was calculated using a simple iterative computer program that identified both the optimum radius and the optimum film thickness that provided the minimum analysis time. The physical values of the system stated previously were employed with an inlet pressure of 1000 p.s.i. Calculations were carried out for a range of separation ratios for the critical pair extending from 1.01 (a difficult separation) to 1.1 (a moderately simple separation). Three different values 50, 250 and 500 were taken for the distribution coefficient of the first solute of the eluted pair. The value of the capacity factor (k') was also calculated for each separation. The results obtained are shown in Table II.

It is seen that the optimum column radius depends only on the *separation ratio* of the critical pair and is independent of the distribution coefficient. It is also seen that the optimum separation ratio is constant at 2.7 and is independent of both the separation ratio of the critical pair and the distribution coefficient of the first eluted solute. As a consequence the optimum film thickness is defined by the optimum radius and the distribution coefficient.

The value of 2.7 agrees well with the values for the optimum mobile phase velocity predicted by Grushka and Cooke⁹. In the hypothetical design of a capillary column there would be two approaches that would provide the optimum film thickness. One alternative could be to adjust the phase system until the first solute of the critical pair was eluted at a k' of 2.7 and then calculate the optimum radius that would provide the minimum analysis time by the iterative procedure. A second alternative, could be to measure or estimate the magnitude of the distribution coefficient of the first solute and calculate the optimum film thickness after determining the optimum radius. The former method appears more practical, as it employs the column with whatever film thickness it happens to have, and adjusts the phase system to ensure that, that particular film thickness, is optimum for the separation.

TABLE II

OPTIMUM RADIUS AND FILM THICKNESS FOR CAPILLARY COLUMNS CHROMATOGRAPHING SOLUTES OF DIFFERENT SEPARATION RATIOS AND DIFFERENT DISTRIBUTION COEFFICIENTS

Inlet pressure 1000 p.s.i. Viscosity of mobile phase 0.003967 P. Diffusivity of solute in mobile phase $2.5 \cdot 10^{-5}$ cm²/s. K is the distribution coefficient of the first solute of the critical pair between the mobile and stationary phase.

Separation ratios	Optimum radius (μm)	Optimum k'	Optimum film thickness (10^{-6} cm)		
			$K = 50$	$K = 250$	$K = 500$
1.01	1.175	2.7	3.175	0.635	0.318
1.02	0.588	2.7	1.588	0.318	0.159
1.03	0.392	2.7	1.058	0.217	0.106
1.04	0.294	2.7	0.794	0.159	0.079
1.05	0.235	2.7	0.635	0.126	0.064
1.06	0.196	2.7	0.529	0.106	0.053
1.07	0.167	2.7	0.454	0.091	0.045
1.08	0.147	2.7	0.397	0.079	0.040
1.09	0.131	2.7	0.353	0.071	0.035
1.10	0.118	2.7	0.318	0.064	0.032

It also follows, that as k' is a constant, with an optimum value of 2.7 for the physical conditions assumed, then the equations for the analysis time and the column length for those conditions can be greatly simplified. Now,

$$t = [32(1 + k')^3/k'^2(\alpha - 1)]2[(1 + 6k' + 11k'^2)r^2/24(1 + k')^2D_M + k'^3r^2/6(1 + k')^2K^2D_S]$$

Substituting 2.7 for k' in the equation for t , then t_{\min} is given by:

$$t_{\min} = 222(0.296r_{\text{opt}}^2/D_M + 0.239r_{\text{opt}}^2/K^2D_S)/(\alpha - 1)^2 \quad (16)$$

where r_{opt}^2 is determined by the iterative program described above.

Now as stated before, $l = nH_{\min}$. Thus substituting for n and H_{\min} from eqns. 4 and 5,

$$l = 85[(0.296r_{\text{opt}}^2 + 0.239r_{\text{opt}}^2/\beta K^2)^{0.5}/(\alpha - 1)]^2$$

where, again, r_{opt}^2 is determined by the iterative program described above.

It is clear that the column properties determined in this report, depend on the physical constants associated with the phases employed. The effect of solvents of different viscosities, solutes of different diffusivities and columns with different inlet pressures on the optimum column and chromatographic parameters must, in due course, also be identified. It may then be possible, to completely define the fully optimized capillary column and, perhaps, to identify those types of separation that may be amenable to analysis by capillary column LC, employing equipment that is presently available.

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