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Michel Martin^a

^a Laboratoire de Chimie Analytique Physique, Ecole Polytechnique, Palaiseau, France

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ON THE FUNDAMENTAL RETENTION EQUATION IN GRADIENT ELUTION LIQUID CHROMATOGRAPHY

Michel Martin

*Laboratoire de Chimie Analytique Physique
Ecole Polytechnique
91128 Palaiseau, France*

SUMMARY

Basic integral equations used to predict the solute retention times or volumes in gradient elution liquid chromatography are carefully examined. In order to simplify the integration procedure, one strongly suggests to select a convention independent of the solvent composition for defining the solute capacity factor. Most of these equations make use of the volume V' of mobile phase which has passed through the peak center (or band maximum) as the integration variable. It is shown that, instead of V' , one can use the volume V flowing from the column in these equations as the integration variable in combination with retention conditions prevailing at the column inlet rather than actually within the column, provided that the gradient is displaced without modification through the column, i.e. without retention of the mobile phase components. The general retention equation is derived for a binary gradient where the strong component of the mobile phase is retained in such conditions that its distribution isotherm is linear, i.e. it has a constant capacity factor, k'_D . This general equation is solved in the specific case of a linear solvent strength gradient. It is shown that the retention of the strong component of the mobile phase leads to an increase of the solute retention time approximately equal to $k'_D t_0 / 2$, where t_0 is the elution time of an inert solute.

INTRODUCTION

The technique of gradient elution liquid chromatography is widely practised today in analytical laboratories. In this technique, the composition of the mobile phase entering the column is modified during the course of the separation of a sample. The main objective pursued when performing such a technique is to provide adequate resolution over most of the length of a chromatogram of a relatively complex sample (1). Indeed, in constant mobile phase composition (i.e., isocratic) liquid chromatography, the resolution of the early eluting compounds may be impaired by lack of adequate retention while the retention time of the most retained compounds may be practically prohibitive and, at the same time, the concentration of these solutes when they elute from the column may be too low for adequate detection. Modifying the composition of the mobile phase during a run allows to select an initial eluent composition of sufficiently low solvent strength to provide a satisfying retention of the first eluting compounds, then to increase the elutropic strength in order to maintain the retention of the last eluting solutes within a reasonable range. Gradient elution analysis may be performed for reaching other goals, such as scouting the polarity range of an unknown sample or selecting the optimal mobile phase for an isocratic separation (2).

It is obviously more complicated to perform a gradient elution analysis than an isocratic separation. The chromatographer must indeed carefully select additional parameters which influence the retention of the solutes as well as their separation, such as the initial and final compositions of the mobile phase, the duration of the gradient run, the shape and rate of variation of the mobile phase composition as a function of time and the mobile phase flow-rate. In order to take full profit of the gradient elution capability of an instrument, one has to select optimal values of these interrelated parameters for the sample at hand. This implies that one is able to derive the functional dependence of the separation performances, and especially of the retention, on these parameters. Because the solute migration in gradient elution does not occur in steady-state conditions, the expression of the retention is more complex than in isocratic elution. In spite of this increased complication, the

fundamental equation for the calculation of the retention volume (or, of the retention time) in gradient elution has been claimed to be derived "without difficulty" (3) or, even, "easily" (4). However, when one looks at the various equations of retention published in the literature, one is stricken by the fact that, firstly, different basic expressions are used, and, secondly, that when similar equations are used, the variables entering these equations do not always have the same meaning.

The purpose of this note is to describe in which conditions these different equations are equivalent and emphasize the approximations done at each step of their derivation.

THEORY

Fundamental retention equation in gradient elution liquid chromatography

The derivation of the retention equation of a solute in gradient elution proceeds from integration of the elementary migration steps of this solute in an environment of a fixed composition. In the following, as usually done in analytical chromatography, a very small amount of solute is supposed to be injected at the inlet of the column during a very short time, at time 0. After a time t , the solute has migrated a distance z along the column axis. Here, z refers to the location of the center of gravity of the solute zone at time t . In this note focusing on solute retention in gradient elution, one neglects the variation of eluent composition within the solute zone, that is, one assumes that the column is of sufficiently high efficiency for these variations to be negligibly small. In these conditions, the solute, at position z at time t , is surrounded by a mobile phase of a given composition $c(z, t)$. During the time increment dt , the solute is displaced a distance dz which depends on its migration velocity, $u [c(z, t)]$, in this mobile phase composition :

$$(1) \quad dz = v [c(z, t)] dt$$

According to the classical chromatographic theory, the solute velocity is related to the mobile phase chromatographic velocity, u , through the solute capacity factor, k' :

$$(2) \quad v = u / (1 + k')$$

In the following, one assumes that the solute concentration will be small enough for its distribution isotherm between the mobile and stationary phases to be linear, so that k' is a constant. Furthermore, one will assume it is so whatever the mobile phase composition. From equations 1 and 2, one obtains the expression of the basic elementary migration step in gradient elution liquid chromatography:

$$(3) \quad dz = \frac{u dt}{1 + k'[c(z, t)]}$$

It has to be noted that, although commonly used, the concept of capacity factor is not in liquid chromatography, where multicomponent mobile phases are used, as straightforward as it is in gas chromatography. Indeed, basically, k' represents the ratio of the number of moles of solute in the stationary phase to the number of moles of solute in the mobile phase. Because with multicomponent phases, a composition gradient generally is developed at the vicinity of the interface, an arbitrary convention has to be defined about the position of the surface dividing the two phases in order to be able to count the number of moles of solutes in each side of the interface. Therefore, the value of the capacity factor is associated to the selected convention. Various conventions can possibly be selected in liquid chromatography (5). They lead to various possible values of the volume of the mobile phase, V_m , contained in the column. Some of the possible conventions depend on the composition of the

mobile phase, and so do the corresponding V_m values. One imagines easily that such a situation poses a considerable problem when trying to compute the retention volume or retention time of a solute in gradient elution conditions, where the composition of the mobile phase is constantly changing. The necessity of simplification required for solving the gradient elution retention equation provides a strong argument for selecting a convention which gives a V_m value independent on the mobile phase composition. Such is the case for the mobile phase volume determined by the weighing method using two solvents of different densities (6).

One will assume in the following that such a convention is adopted. As V_m is unambiguously defined and is constant whatever the mobile phase present in the column, so are the mobile phase velocity, u , provided that the flow-rate, F , is constant, and the elution time, t_0 , of an inert solute, i.e. a solute which is displaced along the column at the velocity of the mobile phase, since we have :

$$(4) \quad u = FL / V_m$$

and :

$$(5) \quad t_0 = V_m / F = L / u$$

where L is the column length. Using equations 4 and 5, one obtains a reduced form of the differential equation 3 :

$$(6) \quad d(z/L) = \frac{d(t/t_0)}{1 + k'[c(z/L, t/t_0)]}$$

which represents the incremental fractional displacement distance along the column during the time

interval dt relative to t_0 which serves as a scale for measuring the time. The solute retention time, t_r , which is the time at which the solute has been displaced a length L , is then obtained from the upper limit of integration of the right-hand side of this equation :

$$(7) \quad \int_0^1 d(z/L) = 1 = \int_0^{t_r/t_0} \frac{d(t/t_0)}{1 + k'[c(z/L, t/t_0)]}$$

The variation of the mobile phase composition c with the position and time has to be known as well as the dependence of k' on c in order to solve this equation. A very similar equation can be written to express the retention volume of a solute, V_r , instead of its retention time. As V_r is equal to $F t_r$, one easily obtains using equation 5 :

$$(8) \quad 1 = \int_0^{V_r/V_m} \frac{d(V/V_m)}{1 + k'[c(z/L, V/V_m)]}$$

where V is the volume of mobile phase which has flown from the column since the solute injection. Here $k'[c(z/L, V/V_m)]$ represents the solute capacity factor in the mobile phase composition present at distance z from the column when a volume V of mobile phase has flown from the column.

Alternative fundamental retention equation for gradient elution

Although expressions similar to equations 7 or 8 are found in the literature (7), most of the basic retention equations are written in different forms by considering not the incremental volume of eluent which has flown from the column but the incremental volume of eluent which has passed through the solute zone maximum. It is quite important to correctly distinguish these two volumes. Let us consider first the solute migration in an isocratic analysis. If a solute is unretained,

it is displaced along the column by the flow of incoming eluent, ahead of this fresh eluent. Its elution volume is equal to V_m , the volume of mobile phase contained in the column. If, now, the solute is retained, it spends some time in the stationary phase and a larger volume of mobile phase, V_r , is required to elute it from the column. This volume is made of the volume V_m necessary to sweep the mobile phase already contained in the column before the solute injection, and of an additional part, V'_r , the corrected retention volume, required to "extract" the solute from the stationary phase. Hence, V'_r , which is equal to $k' V_m$, is the volume of mobile phase which passes through the solute peak maximum during the time spent by the solute in the column (rigorously, V'_r should be the volume of eluent which passes through the center-of-gravity of the solute zone during the time it is in the column, but, as the solute zone has been assumed to be very narrow, the peak maximum and the peak center-of-gravity are supposed to be identical). If a volume V'_r must pass through the peak maximum before the peak is eluted, i. e. before it migrates a distance equal to the length L of the column, the incremental volume dV' which has to pass through the peak to displace it by a distance dz is then such that :

$$(9) \quad dV' / V'_r = dz / L$$

which gives :

$$(10) \quad dV' = k' V_m dz / L$$

This equation serves as the basis of various retention equations in gradient elution chromatography (1, 8-17). By integration, it gives :

$$(11) \quad \int_0^1 d(z/L) = 1 = \int_0^{V_r/V_m} \frac{d(V'/V_m)}{k'[c(z/L, V'/V_m)]}$$

where $k'[c(z/L, V'/V_m)]$ represents the instantaneous value of the capacity factor of the solute in the mobile phase composition present at distance z from the column inlet when a volume V' of mobile phase has passed through the peak maximum. Equations 8 and 11 are equally correct and can both be used to describe the retention characteristics in gradient elution. However, it is interesting to emphasize their difference: 1) their denominators represent the spatio-temporal dependence of k' and $1 + k'$, respectively; 2) they provide as the integration limits, the retention volume and the corrected retention volume, which like in isocratic elution are related by:

$$(12) \quad V_r = V'_r + V_m$$

and 3) the integration variable is, in one case, the volume which of eluent *which flows from the column* (or, which is equivalent, into the column) and, in the other case, the volume of eluent *which passes through the peak maximum*. It is important to correctly distinguish these two variables. Indeed, from above equations, one can easily write:

$$(13) \quad dV' = dV \frac{k'}{1 + k'}$$

In fact, one finds in the literature, retention equations similar to equation 11 in which the integration variable is mistakenly written to be the volume V of eluent entering the column.

Practical basic retention equations

In order to use equations 8 (or 7) and 11, for computing the retention characteristics, it

is necessary to know 1) the dependence of k' on the mobile phase composition, 2) the gradient profile delivered by the gradient generator, i. e., the curve of variation of the mobile phase composition entering the column as a function of time (in this treatment, one neglects the time delay introduced by the connecting tubes between the gradient delivering unit and the column inlet, but taking into account this time is relatively straightforward, see Refs. 1 and 15), and 3) the rate of transportation of the different mobile phase compositions within the column. The dependence of k' on c depends on the type of chromatographic system (ion-exchange, adsorption, reversed-phase, ...) and can be known either using an appropriate retention model or by experimental measurement. The composition dependence on time at the column inlet is generally selected by the chromatographer and, thus, known. Much less accessible to the analyst is the rate of transportation of a given composition within the column and much more complicated are the solutions of equations 8 or 11 in the case of a complex mode of transportation of the gradient.

This is one of the reasons for which all modern theoretical treatments of gradient elution assume that *the gradient is transported unmodified within the column*, i. e. they do not change their shape in the column and they are displaced at the constant velocity of the eluent. In this condition, it is relatively easy to relate the composition at a given point in the column to the composition at the column inlet. Indeed, at time t , the composition at distance z from the column inlet is equal to the composition which entered the column some time, z/u , earlier :

$$(14) \quad c(z, t) = c(0, t - z/u)$$

or, in reduced coordinates, since $L = u t_0$:

$$(15) \quad c(z/L, t/t_0) = c(0, t/t_0 - z/L)$$

Equation 7 becomes immediately :

$$(16) \quad \int_0^{t_r/t_0} \frac{d(t/t_0)}{1 + k'[c(0, t/t_0 - z/L)]} = 1$$

Similarly, one has :

$$(17) \quad \int_0^{V_r/V_m} \frac{d(V/V_m)}{1 + k'[c(0, V/V_m - z/L)]} = 1$$

These equations can be solved if one knows the functions $k'(c)$ and $c(t$ or $V)$ at the column entrance.

Let replace in equation 16 the variable t/t_0 by the new variable X such that :

$$(18) \quad X = t/t_0 - z/L$$

One has :

$$(19) \quad \frac{dX}{d(z/L)} = \frac{d(t/t_0)}{d(z/L)} - 1$$

According to equations 6 and 15, one has :

$$(20) \quad \frac{d(t/t_0)}{d(z/L)} = 1 + k'[c(0, t/t_0 - z/L)]$$

Combining equations 19 and 20 gives :

$$(21) \quad \frac{dX}{d(z/L)} = k'[c(0, X)]$$

This equation can be integrated by noting that, for $z/L=0, X=0$ and that, for $z/L=1, X=t_r/t_0 - 1$:

$$(22) \quad \int_0^1 d(z/L) = 1 = \int_0^{t_r/t_0 - 1} \frac{dX}{k'[c(0, X)]}$$

It is seen in this equation, where the reduced time variable X serves as a dummy integration variable, that the integrand only depends on the composition of the mobile phase at the column inlet. Since the second integral in equation 22 represents a time function of the mobile phase composition integrated between times 0 and $t_r/t_0 - 1$, it can be written as:

$$(23) \quad \int_0^{t_r/t_0} \frac{d(t/t_0)}{k'[c(0, t/t_0)]} = 1$$

where the upper integration limit is the corrected retention time, t'_r , scaled to t_0 . As t'_r is equal to V'_r / F , one can write equation 23 in terms of volume, which gives, using the same correspondence as that between equations 7 and 8:

$$(24) \quad \int_0^{V'_r/V_m} \frac{d(V/V_m)}{k'[c(0, V/V_m)]} = 1$$

Equation 24 has been used frequently to compute the corrected retention volumes in various gradient elution situations (1, 14, 15). It closely resembles equation 11 as it contains only k' in the denominator and gives the corrected retention volume (not the retention volume) in the upper integration limit. However, there are two major differences between these two equations: 1) the

integration variable is now V , the eluent volume flowing from (or into) the column (not the volume passing through the solute zone center), 2) k' refers to the value in the mobile phase composition at the column inlet, not at the actual location of the peak maximum (or center) in the column. This considerably simplifies the computation of the retention volume. However, it has not always been perceived that this simplification is made possible *because of the assumption that the gradient is displaced unperturbed along the column*, as demonstrated above.

Such an assumption implies that there is no distortion of the gradient profile by dispersion as well as no uptake of the stronger component of the mobile phase by the stationary phase (3, 16). This is usually a situation encountered in reversed-phase liquid chromatography with gradient of methanol, acetonitrile or tetrahydrofuran in water, these compounds being generally only weakly retained by the initial mobile phase and frequently much less retained than the sample solutes. In spite of their low intrinsic retention, these solvents can be very effective in reducing the retention of other compounds. This is because of the paramount role of mobile phase interactions in this chromatographic mode. The situation may be quite different in other types of chromatographic systems where interactions in the stationary phase play a major role on retention, such as in ion-exchange chromatography or in adsorption liquid chromatography. In these cases, if the two mobile components differ greatly in retention properties, the adsorption of the strongest component on the stationary phase leads to the so-called demixing effect.

General retention equation in the case of a binary gradient with a linear distribution isotherm of the strongest mobile phase component

In the following, one considers the case of a binary (A-B) gradient where the component (B) with the largest eluotropic strength, i.e. the component whose concentration is increasing with time, is retained by the stationary phase and where the distribution isotherm of B is linear over all the range of concentrations encountered during the gradient. Admittedly, such a situation may not be very frequent as usually isotherms are linear only in a limited concentration range, but it is likely

to offer a first-order approximation to cases where solvent demixing occurs. In these conditions, one can define capacity factor of the mobile phase component B, k'_b , which does not depend on the component B concentration in the mobile phase.

If, as assumed above, the column efficiency is sufficiently large for the dispersion of the gradient to be negligible, a given mobile phase composition is displaced in the column at a velocity v_b given by :

$$(25) \quad v_b = u / (1 + k'_b)$$

Accordingly, the mobile phase composition present at time t at position z along the column is that which entered the column some time, z/v_b , earlier. Then, the solute capacity factor is equal to :

$$(26) \quad k'[c(z, t)] = k'[c(0, t - (1+k'_b)z/u)]$$

or :

$$(27) \quad k'[c(z/L, t/t_0)] = k'[c(0, t/t_0 - (1+k'_b)z/L)]$$

By similarity with the introduction of equation 18 in the precedent case, one defines a reduced time function Y as :

$$(28) \quad Y = t/t_0 - (1 + k'_b) z/L$$

which gives :

$$(29) \quad \frac{dY}{d(z/L)} = \frac{d(t/t_0)}{d(z/L)} - (1 + k'_b)$$

Combining equations 6, 27, 28 and 29, one gets :

$$(30) \quad \frac{dY}{d(z/L)} = k'[c(0, Y)] - k'_b$$

In practical situations, it is obvious that the right-hand side of equation 29 is positive, which means that the solute capacity factor is larger than the capacity factor of the strong component of the mobile phase. If this is not the case, the solute is eluted in isocratic conditions by a mobile phase composition of initial composition. It may happen that during the course of the gradient run, with increasing concentration of B, the capacity factor of the solute becomes smaller than k'_b . It is then moved by displacement chromatography at the velocity v_b . Equation 30 can be integrated by noting that, when $z/L = 0, Y = 0$ and that, when $z/L = 1, Y = t_r/t_0 - (1 + k'_b)$:

$$(31) \quad \int_0^1 d(z/L) = 1 = \int_0^{t_r/t_0 - (1 + k'_b)} \frac{dY}{k'[c(0, Y)] - k'_b}$$

i.e., since Y has here the role of a dummy integration variable :

$$(32) \quad \int_0^{t_r/t_0 - k'_b} \frac{d(t/t_0)}{k'[c(0, t/t_0)] - k'_b} = 1$$

As noted above this equation can be integrated up to the upper integrated limit only in the case where

the final solute k' , i.e., $k'[c(0, t'_r/t_0 - k'_b)]$, is larger than k'_b . If it not the case, the integration has to be stopped when $k'[c(0, t/t_0)]$ becomes equal to k'_b , then the solute is displaced at the velocity v_b . If k'_b is equal to zero, equation 32 obviously becomes identical to equation 23 obtained for a gradient displaced without modification. Equation 32 has a form very similar to that established by Drake in the first paper on the theory of gradient elution (Ref. 7, equation 17) for a linear distribution isotherm of the strong mobile phase component.

The corrected retention time t'_r obtained from equation 32 clearly depends on the value of k'_b as well as on the shape of variation of the solute capacity factor in the eluent present at the column inlet as a function of time. One can integrate equation 32 in the specific case of a linear solvent strength gradient, strongly advocated by Snyder et al. (1, 17). Such a gradient is defined by :

$$(33) \quad \log k'[c(0, t)] = \log k'[c(0, 0)] - S t/t_0$$

where S is a parameter called gradient steepness (1, 17). By noting k'_0 the solute capacity factor, $k'[c(0, 0)]$, at the column inlet at the beginning of the gradient, one obtains when equation 33 is inserted in equation 32 :

$$(34) \quad \int_0^{t'_r/t_0 - k'_b} \frac{d(t/t_0)}{k'_0 e^{-2.3 S t/t_0} - k'_b} = 1$$

The integration of this equation is relatively straightforward and gives :

$$(35) \quad \frac{t'_r}{t_0} = \frac{1}{S} \log \left[\frac{k'_0 - k'_b}{k'_0 e^{-2.3 S (t'_r/t_0 - k'_b)} - k'_b} \right]$$

After development, one gets the expression of the corrected retention time :

$$(36) \quad \frac{t_r}{t_0} = \frac{1}{S} \log \left[1 + \frac{k'_0}{k'_b} (e^{2.3 S k'_b} - 1) \right]$$

It is easy to show that, in the limiting case of vanishing k'_b , this equation becomes :

$$(37) \quad \frac{t_r}{t_0} = \frac{1}{S} \log (1 + 2.3 S k'_0)$$

As expected, this is the equation obtained by Snyder et al. for a linear solvent strength gradient without any gradient deformation within the column (1, 17). These two equations give obviously different results for the computation of the retention time. In the limiting, but practical, cases of relatively small k'_b and large k'_0 values, one can show that the difference between the reduced corrected retention times, which is also the difference between the reduced retention times, given by equations 36 and 37 is simply equal to :

$$(38) \quad \left(\frac{t_r}{t_0} \right)_{k'_b \neq 0} - \left(\frac{t_r}{t_0} \right)_{k'_b = 0} = \left(\frac{t_r}{t_0} \right)_{k'_b \neq 0} - \left(\frac{t_r}{t_0} \right)_{k'_b = 0} = \frac{k'_b}{2}$$

This remarkably simple result, which does not depend on S or k'_0 , holds approximately for k'_b values up to about 5. This means that in such a case, the true retention time will exceed by $2.5 t_0$ the time calculated from equation 37. Equations 36 to 38 can be used to extend the theoretical study of gradient elution to cases where the strong component of the mobile phase is retained by the stationary phase. It must be recalled here that they were obtained with the assumption that the distribution isotherm of this component is linear, which may not be true, in practical situations, for a wide range of variation of the concentration of this component.

SYMBOLS

| | |
|--------|--|
| c | composition of the mobile phase |
| k' | solute capacity factor |
| t | time elapsed since the solute injection |
| t_0 | elution time of an inert solute |
| t_r | solute retention time |
| t'_r | solute corrected retention time |
| u | mobile phase chromatographic velocity |
| v_b | migration velocity of the gradient |
| w | solute migration velocity. |
| z | position along the column axis |
| F | mobile phase flow-rate |
| V | volume of eluent which has flown from the column since the solute injection |
| V' | volume of eluent which has passed through the solute zone maximum since the solute injection |
| V_m | volume of mobile phase contained in the column |
| V_r | solute retention volume |
| V'_r | solute corrected retention volume |
| X | reduced time variable defined by equation 18 |
| Y | reduced time variable defined by equation 28 |

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