9.2.3.7 Retention Parameters in Column Chromatography

Retention parameters may be measured in terms of chart distances or times, as well as mobile phase volumes; e.g., t_R' (time) is analogous to V_R' (volume). If recorder speed is constant, the chart distances are directly proportional to the times; similarly if the flow rate is constant, the volumes are directly proportional to the times.

Note: In gas chromatography, or in any chromatography where the mobile phase expands in the column, V_M, V_R and V_R' represent volumes under column outlet pressure. If F_c, the carrier gas flow rate at the column outlet and corrected to column temperature (see *Flow Rate*), is used in calculating the retention volumes from the retention time values, these correspond to volumes at column temperatures.

The various conditions under which retention volumes (times) are expressed are indicated by superscripts: thus, a prime (′; as in V_R') refers to correction for the hold-up volume (and time) while a circle (°; as in $V_R°$) refers to correction for mobile-phase compression. In the case of the net retention volume (time) both corrections should be applied: however, in order not to confuse the symbol by the use of a double superscript, a new symbol (V_N, t_N) is used for the net retention volume (time).

Hold-up Volume (Time) (V_M, t_M)

The volume of the mobile phase (or the corresponding time) required to elute a component the concentration of which in the stationary phase is negligible compared to that in the mobile phase. In other words, this component is not retained at all by the stationary phase. Thus, the hold-up volume (time) is equal to the *Retention Volume (Time) of an Unretained Compound*. The hold-up volume (time) corresponds to the distance OA in Fig. 9.2.1.A and it includes any volumes contributed by the sample injector, the detector, and connectors.

$$t_M = V_M / F_c$$

In gas chromatography this term is also called the *Gas Hold-up Volume (Time).*

Corrected Gas Hold-up Volume (V_M^o)

The gas hold-up volume multiplied by the compression (compressibility) correction factor (j):

$$V_M^o = V_M j$$

Assuming that the influence of extracolumn volume on V_M is negligible,

$$V_M^o = V_G$$

(see *Interparticle Volume of the Column*).

Total Retention Volume (Time) (V_R, t_R)

The volume of mobile phase entering the column between sample injection and the emergence of the peak maximum of the sample component of interest (OB in Fig. 9.2.1.A), or the corresponding time. It includes the hold-up volume (time):

$$t_R = V_R / F_c$$

Peak Elution Volume (Time) ($\bar{V}_R$, $\bar{t}_R$)

The volume of mobile phase entering the column between the start of the elution and the emergence of the peak maximum, or the corresponding time. In most of the cases, this is equal to the total retention volume (time). There are, however, cases when the elution process does not start immediately at sample introduction. For example, in liquid chromatography, sometimes the column is washed with a liquid after the application of the sample to displace certain components which are of no interest and during this treatment the sample does not move along the column. In gas chromatography, there are also cases when a liquid sample is applied to the top of the column but its elution starts only after a given period. This term is useful in such cases.

Adjusted Retention Volume (Time) (V_R', t_R')

The total elution volume (time) minus the hold-up volume (time). It corresponds to the distance AB in Fig. 9.2.1.A:

$V_R' = V_R - V_M$
$t_R' = t_R - t_M = (V_R - V_M)/F_c = V_R'/F_c$

Corrected Retention Volume (Time) (V_R^o, t_R^o)

The total retention volume (time) multiplied by the compression correction factor (j):

$V_R^o = V_R j$
$t_R^o = V_R j/F_c = V_R^o/F_c$

Net Retention Volume (Time) (V_N, t_N)

The adjusted retention volume (time) multiplied by the compression correction factor (j):

$V_N = V_R' j$
$t_N = V_R' j/ F_c = V_N /F_c$

In liquid chromatography, the compression of the mobile phase is negligible and thus, the compression correction factor does not apply. For this reason, the total and corrected

retention volumes (times) are identical ($V_R = V_R^o$; $t_R = t_N$) and so are the adjusted and net retention volumes (times) ($V_R' = V_N$; $t_R' = t_N$).

The specific retention volume at column temperature (V_g^Θ)

The net retention volume per gram of stationary phase (stationary liquid, active solid or solvent-free gel (W_S):

$$V_g^\Theta = V_N/W_S$$

Specific retention volume at 0°C (V_g)

The value of V_g^θ corrected to 0°C:

$$V_g = V_g^\theta \frac{273.15K}{T_c} = \frac{v_N}{W_S}\frac{273.15K}{T_c}$$

where T_c is the column temperature (in kelvin).

Retention Factor (k)

The retention factor is a measure of the time the sample component resides in the stationary phase relative to the time it resides in the mobile phase: it expresses how much longer a sample component is retarded by the stationary phase than it would take to travel through the column with the velocity of the mobile phase. Mathematically, it is the ratio of the adjusted retention volume (time) and the hold-up volume (time):

$$k = V_R'/V_M = t_R'/t_M$$

If the distribution constant is independent of sample component concentration, then the retention factor is also equal to the ratio of the amounts of a sample component in the stationary and mobile phases respectively, at equilibrium:

$$k = \frac{\text{amount of component in the stationary phase}}{\text{amount of component in the mobile phase}}$$

If the fraction of the sample component in the mobile phase is R (see *Retardation Factor*), then the fraction in the stationary phase is (1 - R); thus

$$k = (1 - R)/R$$

Note: In former nomenclatures and in the literature one may find the expressions *Partition Ratio*, *Capacity Ratio*, *Capacity Factor* or *Mass Distribution Ratio* to describe this term.

In the literature the symbol k' is often used for the retention factor, particularly in liquid chromatography. The original reason for this was to clearly distinguish it from the partition coefficient (distribution constant) for which the symbol K had been utilized. Since, however, the distribution constants are all identified with a subscript, there is no reason to add the prime sign to this symbol. It should be emphasized that all the recognized nomenclatures (IUPAC, BS, ASTM) have always clearly identified the capacity factor with the symbol k and not k'.

Logarithm of the Retention Factor

This term is equivalent to the R_M value used in planar chromatography (see R_M *value*). The symbol κ is suggested to express log k:

$$\kappa = \log k = \log [(1 - R)/R]$$

Retardation Factor (R)

The fraction of the sample component in the mobile phase at equilibrium; it is related to the retention factor and other fundamental chromatography terms:

$$R = 1/(k + 1)$$

Relative Retention (r)

The ratio of the adjusted or net retention volume (time) or retention factor of a component relative to that of a standard, obtained under identical conditions:

$$r = V_{Ri}'/V_{R(st)}' = V_{Ni}/V_{N(st)} = t_{Ri}'/t_{R(st)}' = k_i/k_{st}$$

Depending on the relative position of the peak corresponding to the standard compound in the chromatogram, the value of r may be smaller, larger or identical to unity.

Separation Factor (α)

The relative retention value calculated for two adjacent peaks ($V_{R2}' > V_{R1}'$):

$$\alpha = V_{R2}'/V_{R1}' = V_{N2}/V_{N1} = t_{R2}'/t_{R1}' = k_2/k_1$$

By definition, the value of the separation factor is always greater than unity.
The separation factor is also identical to the ratio of the corresponding distribution constants.

Note: The separation factor is sometimes also called the "selectivity". The use of this expression is discouraged.

Unadjusted Relative Retention (r_G or α_G)

Relative retention calculated by using the total retention volumes (times) instead of the adjusted or net retention volumes (times):

$$r_G = V_{Ri} / V_{R(st)} = t_{Ri} / t_{R(st)} = \frac{k_i + 1}{k_{st} + 1}$$

Subscript G commemorates E. Glueckauf, who first used this expression.

Relative retention (r) and separation factor (α) values must always be measured under isothermal conditions. On the other hand, the unadjusted relative retention (r_G or α_G) values may also be obtained in programmed-temperature or gradient-elution conditions. Under such conditions, the symbol RRT (for *Relative Retention Time*) has also been used to describe the unadjusted relative retention values.

Using the same stationary and mobile phases and temperature, the relative retention and separation factor values are reproducible between chromatographic systems. On the other hand, the unadjusted relative retention (and "relative retention time") values are only reproducible within a single chromatographic system.

Retention Index; Kováts (Retention) Index (I)

The retention index of a sample component is a number, obtained by interpolation (usually logarithmic), relating the adjusted retention volume (time) or the retention factor of the sample component to the adjusted retention volumes (times) of two standards eluted before and after the peak of the sample component.

In the *Kováts Index* or *Kováts Retention Index* used in gas chromatography, *n*-alkanes serve as the standards and logarithmic interpolation is utilized:

$$I = 100\left[\frac{\log X_i - \log X_z}{\log X_{(z+1)} - \log X_z} + z\right]$$

where X refers to the adjusted retention volumes or times, z is the number of carbon atoms of the *n*-alkane eluting before, and (z +1) is the number of carbon atoms of the *n*-alkane eluting after the peak of interest:

$$V_{Rz}' < V_{Ri}' < V_{R(z+1)}'$$

The *Kováts (Retention) Index* expresses the number of carbon atoms (multiplied by 100) of a hypothetical normal alkane which would have an adjusted retention volume (time) identical to that of the peak of interest when analyzed under identical conditions.

The *Kováts Retention Index* is always measured under isothermal conditions. In the case of *temperature-programmed gas chromatography* a similar value can be calculated utilizing direct numbers instead of their logarithm. Since both the numerator and denominator contain the difference of two values, here we can use the total retention volumes (times).

Sometimes this value is called the *Linear Retention Index*:

$$I^{\mathrm{T}} = 100\left[\frac{t_{\mathrm{Ri}}^{\mathrm{T}} - t_{\mathrm{Rz}}^{\mathrm{T}}}{t_{\mathrm{R(z-1)}}^{\mathrm{T}} - t_{\mathrm{Rz}}^{\mathrm{T}}} + z\right]$$

where $t_{\mathrm{R}}{}^{\mathrm{T}}$ refers to the total retention times (chart distances) measured under the conditions of temperature programming. The value of I^{T} will usually differ from the value of I measured for the same compound under isothermal conditions, using the same two phases.