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Abstract

In order to separate two sample components during a chromatographic separation, there must be a difference in their retention behavior. All retention parameters are described and explained in this chapter and their effect is illustrated in animations.

Level: Basic

The most important separation parameters are:

Definition

- Retention time: \( t_R \)
- Retention factor: \( k \)
- Selectivity: \( \alpha \), tells us about the difference in retention between the individual components (calculated by the ratio of their retentions).
- Efficiency: \( N \), also referred to as the plate number, shows the relation between retention time and peak width and defines column quality and separation power.

A number of parameters (click here for a simulation of \( R_s \) as a function of three parameters) influence the separation and the resolution. Some are related to the retention time, others cause a peak to broaden. A detailed description of the parameters is described in the van Deemter equation.

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Retention time

Every sample injected onto a column will take a finite time to travel through the column, even if there is no affinity of the sample components for the stationary phase. In this case, the time between applying the sample and its elution from the column will only depend on the speed at which the mobile phase is flowing through the column, and the column dimensions. The time taken for elution is therefore called the 'void', 'hold-up', 'dead time' time (\( t_0 \)) also called 'unretained time' (\( t_M \)) of the column.
Sample components that exhibit some degree of affinity for the stationary phase will spend some time in the stationary phase and are retained on the column longer than the hold-up time.

The time between injection and elution of a retained sample component is called the retention time ($t_R$) for that particular component. The retention time is the sum of time a sample component spends in the mobile phase and the amount of time it spends in the stationary phase. The latter is called the net or adjusted retention time ($t^{\prime}_R$). The fundamental relationship describing retention in chromatography (both gas and liquid) is: $t_R = t^{\prime}_R + t_0$.

**Retention factor**

The retention factor is a variable indicating how much time a component spends in the stationary phase compared to a non-retained inert component. The chromatographic system (mobile and stationary phase) can be regarded as a system of two immiscible phases. When there is sufficient interaction, a sample component will be distributed between the two phases. The term $k$ indicates the ratio of the dissolved component in both phases and is called the retention factor.

The retention factor and the retention time can be used to describe the chromatographic behavior of a sample component in a column. The retention factor is a relative value (dimensionless).

- $k = 0$ means that a sample component spends ‘zero times’ longer in the stationary phase than in the mobile phase. In other words: the component does not spend any time in the stationary phase and it therefore is not retained.
- $k = 1$ means that the component spends just as much time in the stationary phase than in the mobile phase. The retention time is twice the column hold-up time. The peak in the chromatogram appears at 2 times $t_0$.
- $k = 4$ means that the component spends 4 times to in the stationary phase and a mobile phase time of one time to. The total retention time is 5 times $t_0$. The peak in the chromatogram appears at 5 times the distance to.

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**Retention factor**
The advantage of using the retention factor, rather than the retention time is the fact that it is independent of the column length and the flow rate of the mobile phase.

The k-value of a component in a given chromatographic system can be readily estimated from the chromatogram. Comparing the distance between the component peak and the t<sub>0</sub>-peak to the distance between the t<sub>0</sub>-peak and the injection point yields the desired result. The retention factor k depends on:

- **Sample component and stationary phase** Since the k-value describes the retention behavior of sample components, it is determined by both the sample and the type of stationary phase. In fact, it is a measure for the degree of interaction between a sample component and the stationary phase.
- **Amount of stationary phase** The retention time of a sample component that is retained by a given stationary phase is dependent on the amount of stationary phase. A greater amount of stationary phase (thus thicker film) leads to higher retention times and higher the k-values.
- **Temperature** The retention factor for a sample component is directly related to the temperature. It is for this reason that the retention factor is only defined in isothermal GC. It should not be used in temperature-programmed separations, although many chromatographers are not aware of this principle.

**Distribution coefficient**

The distribution coefficient (or physical equilibrium constant) KD describes the distribution equilibrium of sample components in terms of their concentrations:

\[
K_D = \frac{concentration_{component\in stationary\ phase}}{concentration_{component\in mobile\ phase}} = \frac{C_s}{C_m}
\]

The constant KD is a physico chemical constant that only depends on the type of analyte, the type of stationary phase and the temperature. It does not depend on the volumes of the phases.

The retention factor k describes the distribution of sample components in terms of true mass amounts in both phases, rather than in terms of their concentrations. The retention factor can be written as:

\[
k = \frac{total_{component\in stationary\ phase}}{total_{component\in mobile\ phase}} = \frac{C_s V_s}{C_m V_m} = K_D \frac{V_s}{V_m}
\]
where,
\[ V_s = \text{volume of stationary phase in the column} \]
\[ V_m = \text{volume of mobile phase in the column} \]

**Phase ratio**

The phase ratio \( \phi \) is defined as: \( \beta = \frac{V_m}{V_s} \)

Therefore, the retention factor can be simplified to: \( k = \frac{K_D}{\beta} \)

In a capillary GC column, \( \beta \) is equal to the internal column volume (gas volume) in relation to the volume of the (liquid) stationary phase. \( \beta \) can be calculated from the ratio between the internal column diameter and the film thickness \( d_f \) of the capillary column:

\[ \beta = \frac{250 \times \text{I.D. (mm)}}{d_f \text{ (µm)}} \]

The internal diameter I.D. is expressed in mm and the film thickness \( d_f \) in µm. The reduction of the volume of the gas phase by the volume of the stationary phase is neglected here.

**Efficiency and theoretical plates**

The ability to produce narrow peaks is called the efficiency of a column. An efficient column produces narrow peaks. This means that all molecules of the same compound elute within a narrow time window.

The efficiency of a column is expressed by the theoretical plate number. The so-called ‘plate theory’ assumes that a column is divided into a number of equilibria in narrow zones called theoretical plates. This number is the plate number \( N_{th} \).

A sample component present in the mobile phase is transported step by step from one plate to the next. A perfect equilibrium is established between the mobile and the stationary phase within each plate, which causes a slight retention of the sample component. Retention, in turn, depends on the affinity of the sample component for the stationary phase.

**Peak height vs. Plate number**
The width of a peak is not dependent on the peak area or height. This is related to the injected amount of the component. If the same amount of a component is injected onto three columns of similar type but with different efficiency:

- The column with the highest efficiency (highest number of plates) will deliver the highest and narrowest peak (1),
- The column with the lowest efficiency exhibits the widest peak and the lowest peak height (3).
- The total peak area of the three peaks are equal.
- The retention time of the three peaks is the same in all three cases, assuming that the properties of the stationary phase of all three columns are the same.

The maximum of the curve is the retention time of the component concerned; it is a mean value. Peak broadening, or band broadening, is related to the fact that each component represents a large number of identical but individual molecules. Chromatography is, in fact, the separation of groups of molecules.

The separating power (the efficiency) plays a crucial role in the final separation result.

In general the separation becomes better as the number of plates increases. The plate number provides information about the separation power of a column. A column, for example, having a plate number of 60,000 has much more separating power than a column with 15,000 plates.

A high plate number (N_{th}) provides better resolution.

**Theoretical plate number N_{th}**

\[
N_{th} = 5.545 \left( \frac{W_{0.5}}{W_{0.5}} \right)^{-2}
\]

\(W_{0.5}\) = peak width at half height of the peak
\(t_r\) = gross retention time of the compound
The efficiency of a column can also be expressed in the 'height (actually the width) equivalent to one theoretical plate' (HETP), which refers to the length of the column that corresponds to one theoretical plate.

\[ H = \frac{L}{N_{th}} \]

\[ L = \text{column length in mm.} \]

\[ N_{th} = (\text{theoretical}) \text{, plate number} \]

The plate number can be calculated from a chromatogram as follows:

1. **Measure** \( t_R \) in the chromatogram.
2. **Measure or calculate the standard deviation** \( \sigma \), which is half the width of a Gaussian distribution at 0.606 of the height, i.e. the width at 0.606 \( h \) equals twice the standard deviation. Since these widths are difficult to measure in practice, we use the width at half height \( W_{1/2} \).
3. For a Gaussian peak, a fixed relationship exists between \( W_{1/2} \) and \( \sigma \): \( W_{1/2} = 2.354 \sigma \) and \( W_B = 4 \sigma \)
4. **Substitution of these values in the equation** for the plate number gives the following relationships:

\[ N_{th} = \left( \frac{t_r}{\sigma} \right)^2 \]
\[ N_{th} = 5.545 \left( \frac{t_r}{W_{1/2}} \right)^2 \]
\[ N_{th} = 16 \left( \frac{t_r}{W_B} \right)^2 \]

**Tip! to measure** \( W_B \) (peak width at base line)

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**Selectivity (Animation)**

In order to separate two components on a given chromatographic system, it is necessary that those two components exhibit different retention behavior. The term which represents this difference is called selectivity. The selectivity (\( \alpha \)) is defined for peak pairs and is given by the ratio of the retention factors of the more retained component (\( k_2 \)) and the less retained component (\( k_1 \)):

\[ \alpha = \frac{k_2}{k_1} \]
The selectivity is always equal to or greater than one. If the selectivity equals one the two sample components cannot be separated and their peaks will overlap. The higher the selectivity, the larger the difference in retention times of the two peaks. A higher selectivity always implies a better separation. Whether a baseline separation is achieved, however, also depends on the peak width.

**CHROMEDIA PROGRAM**

### Resolution (Animation)

Separation in modern chromatography is defined quantitatively in terms of the relative amount of peak area overlap. As GC peaks are ideally Gaussian in shape, this definition is based on the number of standard deviations between the two peaks. Since most chromatographers however prefer to use more easily recognised chromatographic measurements, we define resolution as the average number of peak widths between the two peak maxima, remembering of course that the peak width is equivalent to four standard deviations.

\[
R_s = \frac{t_r (2) - t_r (1)}{0.85 \left( W_{1/2} (1) + W_{1/2} (2) \right)}
\]

Based on this definition, if the resolution is >1.50 then there will be about <1% mutual interference between the two peaks which is acceptable for most purposes except when there is a large difference between the sizes of the two peaks.

\[
R_s = \frac{1}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k}{k + 1} \cdot \sqrt{N_{th}}
\]

A far more useful equation can be derived from this definition as it tells us the effect of chromatographic variables on the resolution, and hence is an aid to column choice and optimisation.

In which:
- \(R_s\) = separation or resolution factor
- \(\alpha\) = selectivity factor
- \(k\) = retention factor
- \(N_{th}\) = theoretical plate number

In qualitative terms, this equation tells us that:

- The sample components must be retained by the stationary phase in order to become separated (\(k > 0\)).
- Separation also depends on different degrees of retention, i.e. the \(k\) values must be different from each other so that the selectivity \(\alpha\) is greater than unity. The greater the difference, the greater is the chance of separation.
- The column plate number \(N\) must be high enough in order to produce sufficiently narrow peaks that are baseline separated. When \(N\) is not sufficiently high, peaks will overlap and the separation will be incomplete.

The simulation shows how the resolution factor changes when we change the three parameters \(\alpha, k\) and \(N_{th}\).
Effect of retention and selectivity on resolution

Effect of selectivity and efficiency on resolution
Asymmetry factor (Animation)

The resolution value for a baseline separation, as mentioned in the previous section, is only valid if the chromatographic peaks are Gaussian in shape and, consequently, symmetrical.

Due to all kinds of chromatographic and instrumental effects, including column overloading and adsorption, the symmetrical peak shape can become distorted. This is expressed by the asymmetry factor $A_s$. In order to determine this factor, which is arbitrary, comparison is made between the left (shorter side $t_R$) and the right (greater $t_R$) side width of the peak, measured at 10% of the peak height from the baseline of the peak. For a symmetrical peak $A_s$ is equal to 1.0.

CHROMEDIA PROGRAM

Rs and asymmetry

- **(a)** $A_s = 1.0$
- **(b)** $A_s = 1.2$
- **(c)** $A_s = 1.5$
- **(d)** $A_s = 2.0$