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RESOLUTION AND COLUMN EFFICIENCY IN CHROMATOGRAPHY

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SUMMARY

Expressions for resolution and column efficiency in chromatography are reviewed. An exact equation relating the resolution to the elution volume and the plate number is derived. It is further shown that the resolution can be expressed in an especially simple form in terms of the partition coefficient, and this relation is used to determine the peak capacity for a chromatographic column.

INTRODUCTION

The efficiency of a chromatographic column is reflected in its ability to separate peaks of solutes having closely related chemical and physical properties. A quantitative measure of the separation of two solute peaks is given by the resolution, R, defined as follows[1,2].

$$R = 2\left(\frac{|E_2 - E_1|}{W_1 + W_2}\right) \tag{1}$$

where E_1 and E_2 denote the elution volumes of the peaks, and W_1 and W_2 their respective widths. Obviously, in eqn. 1 the order in which the peaks are numbered is immaterial.

For a Gaussian peak, the width is usually taken to be four times the standard deviation, σ. Hence,

$$R = \frac{|E_2 - E_1|}{2(\sigma_1 + \sigma_2)} \tag{2}$$

Representing the normalized concentration distribution in a Gaussian peak by

$$c(V) = \frac{1}{\sigma\sqrt{2\pi}} \cdot \exp\left(-\frac{(V - E)^2}{2\sigma^2}\right) \tag{3}$$

where V is the efflux volume, we can calculate the extent of overlapping of the peaks for any value of R. In particular, we find that for two peaks of equal size the

tops are barely separated for $R = 0.6$, whereas virtually complete separation is achieved for $R = 1.5$.

Eqn. 2 can be generalized to include those instances in which the peaks are non-Gaussian, by replacing the standard deviation with the square-root of the variance. However, in this instance, eqn. 2 loses some of its quantitative character, because for non-Gaussian peaks the analytical form of the concentration distribution function is in general unknown, and consequently it is not possible to determine exactly the extent of overlapping of the peaks for a given value of R.

Although R can be evaluated for any two solutes by substituting the respective values of E and σ into eqn. 2, there is a need for a simple equation that relates R to commonly used chromatographic parameters and allows a convenient determination of R over a sufficient range of parameter values.

DERIVATION OF EQUATIONS

Considering two arbitrary peaks and concentrating on one of them, we may write:

$$E_1 = E$$
$$E_2 = E + \Delta E$$
$$\sigma_1 = \sigma$$
$$\sigma_2 = \sigma + \Delta\sigma$$

Hence, eqn. 2 takes the form

$$R = \frac{|\Delta E|}{4\sigma\left(1 + \frac{\Delta\sigma}{2\sigma}\right)} \tag{4}$$

Using the definition of the number of theoretical plates

$$N = \frac{E^2}{\sigma^2} \tag{5}$$

we can re-write eqn. 4 in the form

$$R = \frac{\sqrt{N}\,|\Delta E|}{4E\left(1 + \frac{\Delta\sigma}{2\sigma}\right)} \tag{6}$$

In a first-order approximation, the term $\Delta\sigma/2\sigma$ in the denominator of eqn. 6 can be neglected. This approximation, which implies that both peaks have the same width, has been used in several previous investigations[3–5]. Thus, the resulting equation

$$R = \frac{\sqrt{N}\,|\Delta E|}{4E} \tag{7}$$

is the master equation for both PURNELL's[3] and KNOX's[4] equations in gas chromatography. However, the approximation involved limits the validity of these equations to a rather narrow range of R values, and to avoid this limitation the term $\Delta\sigma/2\sigma$ in eqn. 6 has to be evaluated.

From eqn. 5, we obtain

$$\Delta\sigma = \frac{E + \Delta E}{\sqrt{N} + \Delta\sqrt{N}} - \frac{E}{\sqrt{N}} = \frac{\sqrt{N}\,\Delta E - E\Delta\sqrt{N}}{\sqrt{N}\,(\sqrt{N} + \Delta\sqrt{N})} \tag{8}$$

Hence

$$\frac{\Delta\sigma}{2\sigma} = \frac{\frac{1}{2}\left(\frac{\Delta E}{E} - \frac{\Delta\sqrt{N}}{\sqrt{N}}\right)}{1 + \frac{\Delta\sqrt{N}}{\sqrt{N}}} \tag{9}$$

Substituting eqn. 9 into eqn. 6 and rearranging, we obtain

$$R = \frac{\frac{1}{4}(\sqrt{N} + \Delta\sqrt{N})\frac{|\Delta E|}{E}}{1 + \frac{1}{2}\left(\frac{\Delta E}{E}\right) + \frac{1}{2}\left(\frac{\Delta\sqrt{N}}{\sqrt{N}}\right)} \tag{10}$$

This equation is completely general and can be used over the entire range of R values. In most instances of practical interest, it is sufficient to use a simpler, approximate form of this equation. The denominator in eqn. 10 can be written in the form

$$\left[1 + \frac{1}{2}\left(\frac{\Delta E}{E}\right)\right] \cdot \left[1 + \frac{1}{2}\left(\frac{\Delta\sqrt{N}}{\sqrt{N}}\right)\right] - \frac{1}{4}\left(\frac{\Delta E}{E}\right) \cdot \frac{\Delta\sqrt{N}}{\sqrt{N}} \tag{11}$$

where the last term in general is small and therefore can be neglected. Eqn. 10 then takes the form

$$R = \frac{(\sqrt{N} + \Delta\sqrt{N})\frac{|\Delta E|}{E}}{4\left[1 + \frac{1}{2}\left(\frac{\Delta\sqrt{N}}{\sqrt{N}}\right)\right] \cdot \left[1 + \frac{1}{2}\left(\frac{\Delta E}{E}\right)\right]} \tag{12}$$

Putting

$$\sqrt{N} = \sqrt{N_1}$$

$$\sqrt{N} + \Delta\sqrt{N} = \sqrt{N_2}$$

we have

$$\frac{\sqrt{N} + \Delta\sqrt{N}}{1 + \frac{1}{2}\left(\frac{\Delta\sqrt{N}}{\sqrt{N}}\right)} = \frac{2}{\frac{1}{\sqrt{N_1}} + \frac{1}{\sqrt{N_2}}} = \bar{\bar{\sqrt{N}}} \tag{13}$$

where $\bar{\bar{\sqrt{N}}}$ is the harmonic average of $\sqrt{N_1}$ and $\sqrt{N_2}$. Eqn. 12 can therefore be written in the form

$$R = \frac{\bar{\bar{\sqrt{N}}}\,|\Delta E|}{4(E + \frac{1}{2}\Delta E)} \tag{14}$$

Although eqn. 14 is not exact, it represents a substantial improvement over eqn. 7. It is similar in form to SAID's equation[6], in which a hypothetical average peak was used to take into account the unequal broadening of the two peaks involved. The

advantage of the present treatment is that the magnitude of the error involved in the approximation is clearly indicated.

Eqns. 10 and 14 are based only on the definitions of the resolution, eqn. 2, and the number of theoretical plates, eqn. 5. They are consequently applicable to all kinds of chromatography, independent of the mechanism of the chromatographic process. In linear partition chromatography, including gas–liquid chromatography and gel chromatography, eqn. 14 can be subjected to further analysis. In this instance, the elution volume is a well defined function of the partition coefficient, and we can write[7]

$$E = V_0 \left(1 + \frac{\gamma}{b}\right) \tag{15}$$

where V_0 is the volume of the mobile phase in the column, γ the partition coefficient and b the ratio between the volumes of the mobile and stationary phases. Substituting eqn. 15 into eqn. 14, we obtain

$$R = \tfrac{1}{4} \sqrt{\bar{N}} \cdot \frac{|\Delta\gamma|}{b + \gamma + \tfrac{1}{2}\Delta\gamma} \tag{16}$$

This equation determines the change in the partition coefficient $\Delta\gamma$, which is required to obtain a certain resolution with a given column. It therefore provides a useful tool for studying the performance of a chromatographic column. The role of $\sqrt{N}$ as a measure of column efficiency is also clearly shown. In general, the value of $\sqrt{N}$ for different solutes is not known very accurately, which may give rise to some difficulty in determining the average $\sqrt{\bar{N}}$. From the theoretical expression for N (or its equivalence H)[7], it follows that N is a function of the partition coefficient and the solute diffusion coefficients in the mobile and stationary phases. As a substantial part of the final peak width is due to eddy dispersion and instrumental broadening, both of which are essentially the same for all solutes, we can conclude that the variation of $\sqrt{N}$ is in general small. The harmonic average $\sqrt{\bar{N}}$ can therefore in practice be replaced by any value of $\sqrt{N}$ in the interval investigated.

PEAK CAPACITY

As an illustration of the use of eqn. 16, we have derived an expression for the peak capacity in a chromatographic column, *i.e.*, the number of peaks that can be resolved on a column under specified conditions[8]. We assume $\sqrt{N}$ to be constant over the range of γ-values considered. Allowing γ to increase, $\Delta\gamma$ is positive and eqn. 16 can be written in the form

$$\frac{b + \gamma}{\Delta\gamma} = \frac{\sqrt{N}}{4R} - \tfrac{1}{2} = A \tag{17}$$

where, for a given R, A is a constant.

Starting from $\gamma = 0$, eqn. 17 yields for the $(n + 1)$ th peak

$$\frac{b + \sum_{i=1}^{n} \Delta\gamma_i}{\Delta\gamma_{n+1}} = A \tag{18}$$

This can be rewritten in the form

$$\Delta\gamma_{n+1} = \frac{1}{A}\left(b + \sum_{i=1}^{n-1} \Delta\gamma_i + \Delta\gamma_n\right) \tag{19}$$

As

$$\Delta\gamma_n = \frac{1}{A}\left(b + \sum_{i=1}^{n-1} \Delta\gamma_i\right) \tag{20}$$

we obtain from eqn. 19 for $n \geqslant 1$

$$\Delta\gamma_{n+1} = \Delta\gamma_n\left(1 + \frac{1}{A}\right) \tag{21}$$

Observing that

$$\Delta\gamma_1 = \frac{b}{A} \tag{22}$$

we obtain by successively applying eqn. 21

$$\Delta\gamma_{n+1} = \frac{b}{A}\left(1 + \frac{1}{A}\right)^n \tag{23}$$

Combining eqns. 18 and 23, we finally obtain

$$\left(1 + \frac{1}{A}\right)^n = 1 + \frac{1}{b}\sum_{i=1}^{n} \Delta\gamma_i \tag{24}$$

This equation determines the number of peaks, n (in addition to the peak at $\gamma = 0$), which can be crowded into a given interval of the partition coefficient and have the resolution R.

We can use eqn. 24 to determine the number of resolvable peaks in gel chromatography. Considering two peaks as being resolved when $R = 1$, and assuming that in gel chromatography γ may vary between 0 and 1, we have

$$\sum_{i=1}^{n} \Delta\gamma_i = 1 \tag{25}$$

and

$$A = \frac{\sqrt{N} - 2}{4} \tag{26}$$

Inserting these relations into eqn. 24, taking logarithms and rearranging, we obtain

$$n = \frac{\ln\left(1 + \frac{1}{b}\right)}{\ln\left(\frac{\sqrt{N} + 2}{\sqrt{N} - 2}\right)} \tag{27}$$

It should be noted that eqn. 27 is compatible with eqn. 17 (for $R = 1$ and $\gamma \leqslant 1$) only when

$$\sqrt{N} \geqslant 4b + 2 \tag{28}$$

which also marks the limit for which $n \geqslant 1$ in eqn. 27.

For $b = 0.35$ (column filling consisting of tightly packed spheres of equal size) and $\sqrt{N} \gg 2$, we have

$$n = 0.338\sqrt{N} \tag{29}$$

Therefore, the total number of resolvable peaks in gel chromatography becomes

$$n' = 1 + 0.338\sqrt{N} \tag{30}$$

This equation differs from GIDDINGS' original equation[8] with respect to the value of the numerical factor. This is due to the fact that the conditions for gel chromatography have been specified in somewhat different ways in the two treatments.

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