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GRADIENT ELUTION IN LIQUID CHROMATOGRAPHY

I. THE INFLUENCE OF THE COMPOSITION OF THE MOBILE PHASE ON THE CAPACITY RATIO (RETENTION VOLUME, BAND WIDTH, AND RESOLUTION) IN ISOCRATIC ELUTION —THEORETICAL CONSIDERATIONS

P. JANDERA and J. CHURÁČEK
Department of Analytical Chemistry, University of Chemical Technology, Pardubice (Czechoslovakia)

SUMMARY

The influence of the composition of binary mobile phases on the capacity ratios in adsorption, partition and ion-exchange chromatography is treated theoretically. It is shown that the relationship between the capacity ratio and the concentration of the more efficient eluting agent in a binary mobile phase can be expressed by two simple relationships, the first being valid in adsorption and ion-exchange chromatography, and the second in chromatography based on the partition mechanism (liquid–liquid chromatography, salting-out chromatography and solubilization chromatography on ion exchangers in mixed aqueous–organic media). Simplifying assumptions and limitations of the theory are discussed. The equations for retention volumes, retention ratios (separation factors), peak widths and resolution in isocratic elution chromatography are derived as a function of the mobile phase composition. The theory presented forms a sound basis for the quantitative theory describing solvent programmed (gradient elution) chromatography.

INTRODUCTION

The appropriate selection of the mobile phase and its composition in liquid chromatography is important for the achievement of adequate chromatographic separations of samples within a reasonable time. The flexibility and the moderate amounts of time, effort and expense required in comparison with another means of improving the resolution, such as changing the nature of the stationary phase (sorbent) or column dimensions, has made this method particularly attractive.

The nature and composition of the mobile phase affect the capacity ratios, k', of the compounds to be separated. In some instances, the change in the composition of the mobile phase can cause some change in selectivity for the components of the sample, *i.e.*, some variation in the separation factor, α, for two compounds with adjacent bands is to be expected. Both the selectivity and the capacity ratio and, consequently, the nature and composition of the mobile phase, influence the resolution of these two adjacent chromatographic bands[1]:

$$R_s = \underbrace{\frac{1}{4}\sqrt{N}}_{\text{I}} \cdot \underbrace{\frac{\alpha - 1}{\alpha}}_{\text{II}} \cdot \underbrace{\frac{k_2'}{1 + k_2'}}_{\text{III}} \tag{1}$$

where N represents the number of theoretical plates of the column. The terms I, II and III relate to the efficiency, selectivity and capacity of the separation, respectively.

Moreover, discontinuous or continuous changes in the composition of the mobile phase during the elution (stepwise or gradient) represent an efficient solution to the so-called "general elution problem" of poor resolution of the early eluted components of the sample and excessive broadening of the last eluted bands[2]. A considerable improvement in resolution and shortening of the time of analysis has been widely appreciated. Numerous workers have described various concentration gradient generating devices[3–13] and reported equations correlating the mathematical function of the concentration gradient with the experimental parameters of the devices[7,8,14–22].

A correlation between the resolution and the composition of the mobile phase can be expressed only if the mathematical function describing the influence of the composition of the mobile phase on the capacity ratio is known for the compounds to be separated.

The relationship between the composition of the mobile phase and the capacity ratio (or some related characteristic, such as retention volume or distribution coefficient) has been studied for a large number of practical systems of sorbent–mobile phase–sample, but most of the relationships reported were valid for specific cases only. A few quantitative studies on the positions of maxima and the band width in solvent programmed (gradient or stepwise elution) separations by liquid chromatography[15,23–30] suffer from a lack of general applicability and the resulting equations are complex and often graphical or numerical methods have to be used in their solution. The considerations have been limited to a linear or exponential gradient shape and, in most instances, to the calculations of the retention volumes only.

Snyder and co-workers[31–33] derived equations for the retention volume and resolution in gradient and stepwise elution separations in adsorption chromatography. This approach seems to be promising but it remains limited to concentration gradients with the "solvent strength" parameter, ε°, being changed in a linear manner during the elution and also involves graphical methods.

In the present study, an attempt is made to give a more general approach to the problem. Firstly, the relationship between capacity ratios and the composition of the mobile phase is considered theoretically in different modes of liquid chromatography — adsorption, partition and ion-exchange. It is shown that for many practical cases, this relationship can be expressed with certain simplifying assumptions in the form of two simple equations. Based on these two equations, the relationship between the composition of the mobile phase and the retention volume, band width and resolution is derived for isocratic elution chromatography (constant mobile phase composition). The development of the theory for gradient and stepwise elution and practical verification of the equations derived will be presented in later papers in this series.

INFLUENCE OF THE COMPOSITION OF THE MOBILE PHASE ON THE CAPACITY RATIO, k'

The mathematical form of the relationship between the capacity ratio and the composition of the mobile phase will generally depend on the nature of the sorption mechanism and the interaction forces involved. Therefore, theoretical considerations concerning adsorption, partition and ion-exchange chromatography are discussed separately.

Adsorption chromatography

Based on theoretical considerations, an equation for the distribution coefficient, D, in adsorption chromatography using a single-solvent mobile phase has been derived[34]:

$$\log D = \log V_a + \bar{a}\,(S^0 - A_s \cdot \varepsilon^0) \tag{2}$$

where V_a, the adsorbent surface volume, is the volume of an adsorbed solvent monolayer per unit weight of adsorbent; $\bar{a}$ is the adsorbent surface activity function, related to the energy of adsorption of the sample on to an active site on the adsorbent surface (thus expressing the activity of the adsorbent); S^0 is the dimensionless free energy of adsorption of a sample compound on an adsorbent of standard activity ($\bar{a} = 1.00$) from n-pentane as solvent; A_s represents the molecular area of the adsorbed sample molecule on the adsorbent surface (units of 8.5 Å^2); ε^0 is the solvent strength parameter describing the influence of the solvent on the adsorption and is not influenced by the properties of an adsorbent or sample. The values of ε^0 have been tabulated for a number of solvents commonly used in adsorption chromatography[34].

The distribution coefficient in a binary solvent system, a–b, D_{ab}, can be expressed as[34]:

$$\log D_{ab} = \log D_a + \bar{a}A_s(\varepsilon_a^0 - \varepsilon_{ab}^0) \tag{3}$$

where the subscripts a and ab represent the distribution coefficients and solvent strengths in solvent a, and in a mixture containing both solvents a and b, respectively. Solvent b is assumed to be a much stronger eluent than solvent a ($\varepsilon_b^0 > \varepsilon_a^0$).

The solvent strength parameter in such a binary solvent mixture, ε_{ab}^0, is related to the mole fraction of the solvent b in the mobile phase, x_b, by the equation[34]:

$$\varepsilon_{ab}^0 = \varepsilon_a^0 + \frac{\log(x_b \cdot 10^{\bar{a}n_b \cdot (\varepsilon_b^0 - \varepsilon_a^0)} + 1 - x_b)}{\bar{a}n_b} \tag{4}$$

where ε_b^0 refers to the strength of the pure solvent b and n_b is the effective molecular area of an adsorbed solvent molecule b (units of 8.5 Å^2). The values of n_b have been tabulated for a number of solvents[34].

The relationship between the capacity ratio in a binary solvent system, k'_{ab}, and the mole fraction of the solvent b, x_b, can be derived by combining eqns. 3 and 4, considering the following relationship between the distribution coefficients and capacity ratios:

$$\frac{k'_{ab}}{k'_a} = \frac{D_{ab}}{D_a} \tag{5}$$

where k'_a refers to the capacity ratio in the pure solvent a.

This gives a rather complex equation:

$$\log k'_{ab} = \log k'_a - \frac{A_s}{n_b} \cdot \log \left[x_b \left(10^{\bar{a} n_b (\varepsilon_b^0 - \varepsilon_a^0)} - 1 \right) + 1 \right] \tag{6}$$

which give rise to considerable difficulties in further mathematical derivations.

We examined the error brought about by simplifying eqn. 6 to the form

$$\log k'_{ab} \approx \log k'_a - A_s \cdot \bar{a}(\varepsilon_b^0 - \varepsilon_a^0) - \frac{A_s}{n_b} \log x_b \approx \log k'_b + \log x_b^{-\frac{A_s}{n_b}}$$

Using the symbols c for x_b, k' for k'_{ab} and k'_0 for k'_b and introducing $n = \frac{A_s}{n_b}$, we obtain a simple relationship:

$$k' \approx k'_0 \cdot c^{-n} \tag{7}$$

The validity of eqn. 7 is subject to an error that increases with a decrease in the concentration of the solvent b, x_b. This error would not exceed 10% (relative) if

$$\frac{x_b \left(10^{\bar{a} n_b (\varepsilon_b^0 - \varepsilon_a^0)} - 1 \right) + 1 - x_b \cdot 10^{\bar{a} n_b (\varepsilon_b^0 - \varepsilon_a^0)}}{x_b \left(10^{\bar{a} n_b (\varepsilon_b^0 - \varepsilon_a^0)} - 1 \right) + 1} \leqslant 0.1 \tag{8}$$

or

$$x_b \geqslant \frac{0.9}{0.1 \cdot 10^{\bar{a} n_b (\varepsilon_b^0 - \varepsilon_a^0)} + 0.9} \tag{9}$$

Considering 10% (relative) as the maximum tolerable error, it can be shown that the value of the term $\bar{a} n_b (\varepsilon_b^0 - \varepsilon_a^0)$ should not fall below 1.91 for $x_b \geqslant 0.1$ or below 1.56 for $x_b \geqslant 0.2$.

Let us investigate this condition for two of the most useful adsorbents in adsorption chromatography, alumina and silica. The practical values used in the following discussion were taken from Snyder's book[34].

Alumina. For almost all common solvents, $n_b \geqslant 5$ (exceptions: acetone ($\varepsilon^0 = 0.56$; $n_b = 4.2$) and methyl ethyl ketone ($\varepsilon^0 = 0.49$; $n_b = 4.6$)). For activated alumina, $\bar{a} = 1.00$, while for alumina deactivated with 15% water, $\bar{a} = 0.59$. Thus, using solvents with $n_b \geqslant 5$, the minimum difference in solvent strengths between solvents a and b ($\varepsilon_b^0 - \varepsilon_a^0$) should not fall below 0.65 for alumina deactivated with 15% water and 0.38 for activated alumina.

Silica. According to Snyder's data[35], n_b is approximately 10 for aliphatic solvents with $\varepsilon^0 \geqslant 0.35$ in work on silica and $\bar{a} = 1.12$ for activated small pore silica (20–40 Å) and 0.69 for silica deactivated with 10% water. Consequently, the minimum acceptable difference ($\varepsilon_b^0 - \varepsilon_a^0$) is 0.17 for activated silica and 0.28 for small pore silica containing 10% water.

The properties of large pore silica ($\geqslant$ 150 Å) are somewhat different. Here, $\bar{a} = 0.83$ for activated silica and 0.69 for silica deactivated with 10% water. In this instance, the minimum difference ($\varepsilon_b^0 - \varepsilon_a^0$) for the relative error of 10% is 0.23 for activated and 0.28 for deactivated silica.

The examination of the table of solvent properties in Snyder's book[34] shows that the differences in ε^0 values ($\varepsilon_b^0-\varepsilon_a^0$) exceed 0.28 for a large number of practical combinations of solvents used as components of binary solvent mixtures. Consequently, the use of the simplified eqn. 7 has a sound foundation for many practical systems in which $x_b \geqslant 0.1$.

Exceptions to eqn. 7 may be expected owing to the limited validity of eqn. 4 for some practical systems that contain a relatively strong solvent b as one of the components of the binary mobile phase. This may be a consequence of the increasing complexity of interactions in the liquid phase or of the tendency of molecules of b to become localized upon strong adsorption sites. Both phenomena cause deviations in the experimental values of ε^0 in binary mixtures from those calculated with eqn. 4. Some of the deviations due to the localization can be corrected empirically by assuming a value of n_b larger than that calculated from the molecular area of solvent b ($n_b \approx 10$ for aliphatic solvents with $\varepsilon^0 \geqslant 0.35$ and silica as the adsorbent)[35]. In such a case, the validity of eqn. 7 remains unchanged.

Various interactions between solvent and sample molecules and specific interactions between the adsorbent and solvent molecules (secondary solvent effects) were assumed to be negligible in the derivation of eqns. 2–4. As these interactions become stronger in strong solvent systems, eqn. 2 is no longer valid and should be corrected by the addition of a correction term, Δ_{eas} (ref. 34):

$$\log D = \log V_a + \bar{a}\,(S^0 - A_s \cdot \varepsilon^0) + \Delta_{eas} \tag{10}$$

It is difficult to predict theoretically the extent to which the term Δ_{eas} depends on the concentration of the mobile phase and the validity of eqn. 7 in such a system requires experimental examination.

Partition chromatography

The capacity ratio in partition chromatography is given by

$$k' = \frac{[x]_s}{[x]_m} \cdot \frac{V_s}{V_m} \tag{11}$$

where $[x]_s$ and $[x]_m$ refer to the concentration of the sample component in the stationary (s) and mobile (m) phases and V_s and V_m are the total volumes of each phase in the column (ideal behaviour in dilute solutions is assumed and consequently the activity coefficients are neglected).

The distribution of a sample molecule between the stationary and the mobile phase is determined by the polarity of both phases. The Hildebrand solubility parameter, δ, is a useful measure of solvent polarities in partition chromatography. According to solubility parameter theory[36–38], the distribution is related to the solubility parameters of the sample component (δ_x), stationary (δ_s) and mobile (δ_m) phases and the molal volume of the sample component ($\bar{V}_x$) by the equation

$$\log \frac{[x]_s}{[x]_m} = \bar{V}_x \cdot \frac{[(\delta_x - \delta_m)^2 - (\delta_s - \delta_x)^2]}{2.3\,RT} \tag{12}$$

where R is the gas constant and T is the absolute temperature.

Solubility parameters have been tabulated[37] for a number of solvents forming an eluotropic series, as well as the partial contributions to the δ values due to different interaction forces (dispersion, dipole induction, dipole orientation and hydrogen bonding).

The δ values vary linearly with solvent composition for binary solvent mixtures[36]. We expressed this relationship as follows:

$$\delta_{ab} = \delta_m = \delta_a + (\delta_b - \delta_a)\cdot c \tag{13}$$

where δ_a and δ_b relate to the solubility parameters of the two components, a and b, of the binary solvent mixture and c is the concentration (mole fraction) of solvent b in this mixture. Introducing this relationship into eqn. 12 and combining with eqn. 11, we obtain:

$$\log k' = \log \frac{V_s}{V_m} + \frac{\bar{V}_x}{2.3\,RT}\cdot[(\delta_x - \delta_a)^2 - (\delta_s - \delta_x)^2] - \frac{2\,\bar{V}_x}{2.3\,RT}\cdot(\delta_x - \delta_a)\cdot(\delta_b - \delta_a)\cdot c + \frac{\bar{V}_x}{2.3\,RT}(\delta_b - \delta_a)^2\cdot c^2 \tag{14}$$

Assuming that the term with c^2 can be ignored, to a first approximation, we obtain a simple relationship between the capacity ratio and the concentration of the more efficient eluent in the binary mobile phase:

$$k' \approx k_0'\cdot 10^{-n\cdot c} \tag{15}$$

where

$$k_0' = \frac{V_s}{V_m}\cdot 10^{\frac{\bar{V}_x}{2.3\,RT}\cdot[(\delta_x - \delta_a)^2 - (\delta_s - \delta_x)^2]}$$

and

$$n = \frac{2\,\bar{V}_x}{2.3\,RT}(\delta_x - \delta_a)(\delta_b - \delta_a)$$

The deviation introduced by this simplification does not exceed 10% (relative), provided that

$$\frac{k' - k'_{\text{simplif.}}}{k'} \leqslant 0.1 \tag{16}$$

Using eqn. 14, it can be shown that this assumption holds for the difference in solvent parameter values:

$$\delta_b - \delta_a \leqslant \frac{1}{c}\cdot\sqrt{\left(\frac{2.3\,RT\cdot 0.0458}{\bar{V}_x}\right)} \tag{17}$$

Considering the values 100–200 cm³ as being typical molal volumes of common organic compounds, this maximum tolerable difference can be estimated as

$$\delta_b - \delta_a \leqslant 5\cdot\frac{1}{c}\ (\text{for } \bar{V}_x \approx 100\ \text{cm}^3)$$

or

$$\delta_b - \delta_a \leqslant 3.5\cdot\frac{1}{c}\ (\text{for } \bar{V}_x \approx 200\ \text{cm}^3)$$

at normal temperature (293 °K).

This requirement seems to be reasonable for a number of practical binary solvent systems, mainly in the lower concentration region of solvent b. Therefore, eqn. 15 can be expected to be useful in a number of practical systems.

Sometimes, adsorption on the support of the stationary phase may occur in addition to the partition mechanism, and consequently, failure of eqn. 15 is to be expected. Such a separation, however, is to be avoided.

A number of the experimental relationships between k' and the composition of binary mobile phases agree well with eqn. 15. The partition chromatography of carboxylic acids on Celite support with 0.5 N sulphuric acid as the stationary phase and solutions of butanol in chloroform as the mobile phase[39] or the chromatography of chloronaphthalene and anthraquinone on Permaphase ODS chemically bonded reverse phase in binary water–methanol and water–dioxane mixtures[40] can be mentioned as examples of a linear decrease in log k' with increasing concentration of the stronger eluent in the binary mobile phase.

Ion-exchange chromatography

The ordinary ion-exchange reaction in which an ion B carrying a positive or negative charge of value $r+$ $(r-)$ exchanges with an s-valent ion A^{s+} (A^{s-}):

$$r(\mathrm{A}) + s\mathrm{B} \rightleftarrows r\mathrm{A} + s(\mathrm{B}) \tag{18}$$

can be characterized by means of a conventional selectivity constant, $K_{\mathrm{A}}^{\mathrm{B}}$, defined as:

$$K_{\mathrm{A}}^{\mathrm{B}} = \frac{[\mathrm{A}]^r \cdot (\mathrm{B})^s}{(\mathrm{A})^r \cdot [\mathrm{B}]^s} \tag{19}$$

where the parentheses refer to the concentration in the "inner" resinous phase and the square brackets to that in the outer solution. The respective activity coefficients are included in the value of the selectivity constant.

Provided that the mobile phase containing ion A at a concentration [A] is used in the chromatography of trace amounts of the ion B, the relationship between the capacity ratio of the chromatographed ion B and the concentration of the eluting agent in the mobile phase can be derived as:

$$k' = \frac{V_s}{V_m} \cdot \frac{(\mathrm{B})}{[\mathrm{B}]} \approx \frac{V_s}{V_m} \cdot (K_{\mathrm{A}}^{\mathrm{B}})^{\frac{1}{s}} \cdot Q^{\frac{r}{s}} \cdot [\mathrm{A}]^{-\frac{r}{s}} \tag{20}$$

where Q is the total exchange capacity of the exchanger for the ion A (in the presence of trace amounts only of the ion B and other ions of corresponding charge, the concentration of the eluting ion A^{s+} (A^{s-}) in the inner phase can be assumed to be independent of the concentration [A] and to equal approximately the capacity: $Q \approx (\mathrm{A})$ (ref. 41)).

Introducing $c = [\mathrm{A}]$, $n = \dfrac{r}{s}$ and

$$k_0' = \frac{V_s}{V_m} \cdot (K_{\mathrm{A}}^{\mathrm{B}})^{\frac{1}{s}} \cdot Q^{\frac{r}{s}} \tag{21}$$

eqn. 20 can be written in a simple form as eqn. 7. This equation, derived earlier for adsorption chromatography, can be expected to be valid also in ion-exchange systems, in which the activity coefficients do not show great variability with changing concentration, [A]. In most instances, this requirement is reasonable as far as the trace amounts of ion B that occur in elution analytical chromatography are concerned. Therefore, the practical usefulness of eqn. 7 in ion-exchange chromatography is controlled by the extent to which the activity coefficients depend on the concentration of the ion A in the outer solution (mobile phase).

The presence of some specific interactions of the sample compounds with the functional groups of the ion exchanger (ion-pair formation, complexation) or with the skeleton of the exchanger may not necessarily interfere with the validity of eqn. 7, as these interactions often do not depend on the mobile phase composition. Therefore, these interactions may often be included in the selectivity constant.

Eqn. 7 may sometimes be useful, at least over a limited range of mobile phase compositions, even if chemical equilibria take place in the mobile phase, assuming that one ionic form of the sample compound predominates in the mobile phase.

For example, eqn. 7 is valid if a weak acid is subjected to anion-exchange chromatography using a mobile phase that contains an eluting anion in the concentration range where one dissociated form of the acid predominates and assuming a constant pH value. In such a case, the dissociation constant of the acid and the pH will influence the value of k_0' and eqn. 21 will no longer be valid.

Analogous conditions are required for eqn. 7 to be valid if a weak base is chromatographed on a cation exchanger with a mobile phase that contains an eluting cation or if this base is subjected to chromatography on the acidic (H^+) form of the cation exchanger using a strong (mineral) acid as the eluent.

Complex-forming equilibria may be utilized to influence the behaviour of various organic and inorganic compounds in ion-exchange chromatography. The formation of non-ionic complexes, which are not held up by ion exchangers, is often used, *e.g.*, for the cation-exchange chromatography of rare earths in the presence of complex-forming organic anions, such as citrate, lactate and oxalate[42–44].

Anion-exchange chromatographic separations of weak organic acids with a mobile phase that contains acetates of the cations that yield complexes with these acids[45–47] are another example of this type.

On the other hand, complex ions of some non-ionic compounds can be also subjected to chromatography. Very successful separations of alcohols and sugars in borate media[48–52], of carbonyl compounds in hydrogen sulphite-containing mobile phases[53–55] and the chromatography of the ethylenediaminetetraacetic acid complexes of the rare earths on anion exchangers have been described[56].

Even combined acid–base or complex-forming equilibria can occur in ion-exchange chromatography, such as in the chromatography of organic hydroxy acids in borate medium, where complex anions are formed[57–61].

If only one complex form of the compound chromatographed predominates in the mobile phase under the experimental conditions used, eqn. 7 is well suited to describe the relationship between the capacity ratio of the sample and the concentration of the complex-forming ion or that of the co-ion in the mobile phase.

Sargent and Rieman and other workers reported a number of salting-out chromatographic separations of various organic non-polar compounds (very weak organic acids[62,63], alcohols[64], esters[63], ethers[62], ketones[65], aldehydes[65], amines[66] and nitro-compounds[67]) on both cation and anion exchangers. Solutions containing a strong electrolyte (inorganic salt) are used as the mobile phase. An increase in the concentration of the salt in the mobile phase gives rise to an increase in the amount of the non-electrolyte (chromatographed substance) salted-out into the resinous phase. The relationship between the distribution coefficient, D_v, of the sample substance and the concentration of the salt in the mobile phase in the above experiments can be expressed as[64]:

$$\log D_v = \log D_0 + k \cdot c \tag{22}$$

where D_0 denotes the distribution coefficient of the sample substance in the absence of the electrolyte (in water) and k is the salting-out constant.

This empirical equation is, in general, identical with eqn. 15 derived for partition chromatography (the value of n being negative). In fact, the salting-out chromatography of non-polar compounds on ion exchangers can be considered as a special example of partition chromatography.

Ion exchangers in contact with an aqueous–organic solution (aqueous ethanol, methanol, acetone, acetic acid, etc.) offer another possibility for the partition chromatography of organic non-ionized substances. In such a system, an equilibrium takes place, in which the organic solvent is distributed between the mobile and the resinous phase so that the liquid inside the resinous particles contains more water than the outer solution[41,68]. Hence, the solubility of an organic substance is different in both phases. This effect has been utilized for chromatographic separations of various organic non-polar substances (such as higher aliphatic carboxylic acids, esters, alcohols, glycols, phenols and saccharides) on both cation- and anion-exchange columns in mixed aqueous–organic solvents. The experimental values of log D_v show a linear decrease with an increase in the concentration of the organic solvent in the mobile phase over almost the entire concentration range used in practical chromatographic runs, *e.g.*, in the chromatography of alcohols and phenols on both cation- and anion-exchange columns in acetic acid solutions[69] or in the chromatography of ketones on cation exchangers in solutions of acetic acid and various alcohols[70]. This means that eqn. 15 is also suitable for expressing the approximate relationship between k' and the concentration of the organic solvent in the mobile phase in such systems.

If ionic compounds are subjected to chromatography on ion-exchange columns in mixed aqueous–organic media, the influence of the mobile phase on the distribution of these compounds between the two phases is much more complex. In addition to the solubility differences, the dielectric properties of the solutions become important and may influence the distribution in the opposite direction to the solubility effects. Consequently, the distribution of the sample substance between the stationary and the mobile phase is often characterized by the occurrence of a minimum at a certain composition of the mobile phase[71].

Conclusion

The above considerations of the various mechanisms of liquid chromatography have shown that the relationship between the capacity ratio of the compound chromatographed and the concentration of the more efficient eluent in the mobile phase can be expressed as

$$k' \approx k'_0 \cdot c^{-n} \tag{7}$$

in adsorption and ion-exchange chromatography, where the compounds are sorbed on to some active centres of the sorbent surface, and as

$$k' \approx k'_0 \cdot 10^{-n \cdot c} \tag{15}$$

in partition chromatography, including salting-out chromatography on ion-exchange columns and the chromatography of non-ionic substances on ion exchangers in mixed aqueous–organic solvents.

These simplified equations can be assumed to be valid in many practical systems, sometimes even if interfering equilibria (such as acid–base or complex-forming equilibria) are present.

INFLUENCE OF THE COMPOSITION OF THE MOBILE PHASE ON THE RETENTION VOLUME, RETENTION RATIO, PEAK WIDTH AND RESOLUTION

The relationships expressing this influence can be derived by substituting k' from eqns. 7 and 15 into the definition equations in different types of chromatography.

In adsorption and ion-exchange chromatography, where eqn. 7 is assumed to be valid, the following equations can be written.

For the retention volume:

$$V_R = k' \cdot V_m + V_m \approx V_m (k'_0 \cdot c^{-n} + 1) \tag{23}$$

For the retention ratio:

$$\alpha = \frac{k'_2}{k'_1} \approx \frac{k'_{02}}{k'_{01}} \cdot \frac{c^{-n_2}}{c^{-n_1}} \approx \alpha_0 \cdot c^{(n_1 - n_2)} \tag{24}$$

where $\alpha_0 = \dfrac{k'_{02}}{k'_{01}}$.

Provided that $n_1 > n_2$, an increase in the concentration of the more efficient component in the mobile phase will cause an increase in the α values, and for $n_1 < n_2$ an increase in this concentration will decrease the α values.

In practice, neighbouring eluted peaks often correspond to substances that are very similar in structure and properties. Consequently, the values of n_2 are very close to those of n_1, *i.e.* $n_2 \approx n_1$. In this case, the α values are not influenced by the composition of the mobile phase, *i.e.* $\alpha \approx \alpha_0 =$ constant.

Similarly, the relationship for the peak width, w, can be derived as:

$$w = \frac{4\ V_R}{\sqrt{N}} \approx \frac{4\ V_m}{\sqrt{N}} \cdot (k'_0 \cdot c^{-n} + 1) \tag{25}$$

where N is the total number of plates in the column.

The resolution of the two compounds 1 and 2 achieved on a column with a constant number of plates for both chromatographed substances can be expressed as:

$$R_s = \frac{\sqrt{N}}{2} \cdot \frac{k'_2 - k'_1}{k'_2 + k'_1 + 2} \approx \frac{\sqrt{N}}{2} \cdot \frac{k'_{02} \cdot c^{-n_2} - k'_{01} \cdot c^{-n_1}}{k'_{02} \cdot c^{-n_2} + k'_{01} \cdot c^{-n_1} + 2} \tag{26}$$

and, for $n_2 \approx n_1 \approx n$, as:

$$R_s \approx \frac{\sqrt{N}}{2} \cdot \frac{k'_{02} - k'_{01}}{k'_{02} + k'_{01} + 2c^n} \tag{27}$$

The value of α cannot be influenced by a change in the composition of the mobile phase if $n_1 \approx n_2$. Provided that N is independent of the mobile phase composition, any change in R_s values introduced by the change in this composition can be attributed to the changing values of the capacity ratio, k'_2. In this case, the concentration of the efficient component in the mobile phase required for a certain resolution, R_s, can be estimated using the equation

$$c = \left(\frac{k'_{02}}{2}\right)^{\frac{1}{n}} \cdot \left(\frac{\sqrt{N}}{2R_s} \cdot \frac{\alpha_0 - 1}{\alpha_0} - \frac{\alpha_0 + 1}{\alpha_0}\right)^{-\frac{1}{n}} \tag{28}$$

In partition and salting-out chromatography, where eqn. 15 can be applied, an analogous approach leads to the following equations.

For the retention volume:

$$V_R = k' \cdot V_m + V_m \approx V_m (k'_0 \cdot 10^{-nc} + 1) \tag{29}$$

For the retention ratio:

$$\alpha = \frac{k'_2}{k'_1} \approx \frac{k'_{02}}{k'_{01}} \cdot \frac{10^{-n_2 c}}{10^{-n_1 c}} \approx \alpha_0 \cdot 10^{(n_1 - n_2) \cdot c} \tag{30}$$

where

$$\alpha_0 \doteq \frac{k'_{02}}{k'_{01}}.$$

As in adsorption and ion-exchange chromatography, compounds that have similar properties can be expected to have $n_2 \approx n_1$ and, consequently, the retention ratio will not depend on the mobile phase composition, *i.e.* $\alpha \approx \alpha_0 =$ constant.

The elution peak width is then given by:

$$w = \frac{4\,V_R}{\sqrt{N}} \approx \frac{4\,V_m}{\sqrt{N}} (k'_0 \cdot 10^{-nc} + 1) \tag{31}$$

and for the resolution of the two neighbouring peaks 1 and 2, we can write:

$$R_s = \frac{\sqrt{N}}{2} \cdot \frac{k'_2 - k'_1}{k'_2 + k'_1 + 2} \approx \frac{\sqrt{N}}{2} \cdot \frac{k'_{02} \cdot 10^{-n_2 c} - k'_{01} \cdot 10^{-n_1 c}}{k'_{02} \cdot 10^{-n_2 c} + k'_{01} \cdot 10^{-n_1 c} + 2} \tag{32}$$

and, if $n_2 \approx n_1 \approx n$,

$$R_s \approx \frac{\sqrt{N}}{2} \cdot \frac{k'_{02} - k'_{01}}{k'_{02} + k'_{01} + 2 \cdot 10^{nc}} \tag{33}$$

In this case again, the composition of the mobile phase influences the R_s values via the adjustment in k' values only.

The concentration of the efficient component in the mobile phase required for a given resolution can be estimated by means of the equation

$$c \approx \frac{1}{n} \cdot \log \frac{k'_{02}}{2} \left(\frac{\sqrt{N}}{2\,R_s} \cdot \frac{\alpha_0 - 1}{\alpha_0} - \frac{\alpha_0 + 1}{\alpha_0} \right) \tag{34}$$

assuming $n_2 \approx n_1 \approx n$.

SYMBOLS

A_s	sample effective molecular area (units of 8.5 Å^2)
D (D_a, D_{ab})	sample adsorption (weight) distribution coefficient: the ratio of the concentration of sample compound in the stationary phase (in moles per gram of the adsorbent) to the corresponding concentration in the mobile phase (in the solvent a, binary mixture of solvents a and b)
D_0	volume distribution coefficient in salting-out chromatography, in the absence of the electrolyte
D_v	volume distribution coefficient: the ratio of the concentration of sample compound in the stationary phase (in moles per millilitre of the stationary phase or ion-exchange phase) to the corresponding concentration in the mobile phase
K_A^B	selectivity constant for the exchange of ion B for ion A
N	number of theoretical plates in the column
Q	ion-exchange capacity of the exchanger (related to 1 ml of swollen exchanger in the column)
R	gas constant
R_s	chromatographic resolution of two compounds (given by eqn. 1.
S^0	sample adsorption energy in standard chromatographic system (adsorbent of a standard activity, $\bar{\alpha} = 1.00$; *n*-pentane used as the solvent)
T	absolute temperature
V_a	adsorbent surface volume: the volume of a monomolecular layer of the solvent sorbed on unit weight of the adsorbent (millilitres per gram)
V_m	total volume of the mobile phase in the column
V_R	retention volume
$V'_R = V_R - V_m$	reduced retention volume
V_s	total volume of the stationary phase in column
$\bar{V}_x$	molal volume of the compound x
c	concentration of the efficient component in the binary mobile phase
Δ_{eas}	correction term for secondary adsorption effects: interactions between the adsorbent and compound or solvent

	(used in calculations of distribution coefficients in adsorption chromatography)
g_s	total weight of the adsorbent in the column
k	salting-out constant
k' (k'_a, k'_b, k'_{ab})	capacity ratio: the ratio of the total amount of the sample compound in the stationary phase to that in the mobile phase under equilibrium conditions (in solvents a, b and their mixture): $k' = \frac{V_s}{V_m} \cdot D_v;\quad k' = \frac{g_s}{V_m} \cdot D;\quad k' = \frac{V'_R}{V_m}$
k'_0	constant in eqns. 7 and 15: the capacity ratio of the sample compound in the mobile phase where $c = 1$ (eqn. 7) or $c = 0$ (eqn. 15)
n	exponential constant in eqns. 7 and 15
n_b	effective molecular area of an adsorbed solvent molecule b (where b is the efficient component of a binary solvent mixture); units of 8.5 Å^2
r, s	charges of ions: stoichiometric coefficients in ion-exchange equilibria
w	peak width
x_b	mole ratio of solvent b in binary solvent mixture
$\alpha = k'_2/k'_1$	retention ratio (separation factor) of compounds 2 and 1
α_0	retention ratio in the mobile phase where $c = 1$ (eqn. 24) or $c = 0$ (eqn. 30)
$\bar{\alpha}$	adsorbent surface activity function
δ (δ_x; δ_m; δ_s; δ_a; δ_b; δ_{ab})	Hildebrand solubility parameter (relating to compound x, mobile (m) and stationary (s) phase, solvents a and b and their binary mixture, respectively)
ε^0 (ε^0_a, ε^0_b, ε^0_{ab})	solvent strength parameter (relating to solvents a and b and their binary mixture, respectively)
[ ]	refers to concentrations in "outer" solutions
( )	refers to concentrations in the "inner" ion-exchanger phase

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