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CALCULATION OF RETENTION VOLUMES IN ION-EXCHANGE CHROMATOGRAPHY USING THE GRADIENT ELUTION TECHNIQUE

SEPARATIONS ON CATION-EXCHANGE RESINS

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SUMMARY

For the separation of metal ions and for the separation of organic bases on cation-exchange columns, calculation methods are given by which the eluent retention volumes can be predicted if the gradient elution technique is used. For the calculation, equilibrium constants (ion exchange, protonation, complex formation) and data concerning the resin and column are necessary. The methods are recommended if exploratory calculations or optimizations are made.

INTRODUCTION

In the separation of multi-component mixtures, high efficiencies (good resolution, short elution time, narrow peaks, etc.) can be obtained only by using a stepwise or preferably a gradient elution technique^{1,2}. If the gradient elution technique is used, the composition of the eluent and also the distribution coefficient of the solute, which determine its migration rate, change continuously during the elution procedure.

The migration rate of solute A can be expressed by the general equation³

$$\left(\frac{dx}{dv}\right)_A = \frac{1}{D_A} \quad (1)$$

where dv is the infinitesimal eluent volume under the effect of which the solute A moves forward in a column volume dx , and D_A is the volume distribution coefficient of A. In order to obtain the eluent volume necessary to move the band of A to the end of the column, eqn. 1 has to be solved by integration between the limits 0 and X (total volume of the column) and 0 and V (eluent volume necessary to move the midpoint of the band to the end of the column):

$$\int_0^X dx = \int_0^V \frac{1}{D} \cdot dv \quad (2)$$

To obtain the total eluent volume, V_R , the void volume of the column also has to be considered. The integration can be carried out easily if D is constant (in isocratic elution) or if the relationship between D and v is simple. Some examples of such calculations were presented earlier⁴⁻⁶. In many instances, however, the relationship is more complicated and therefore the integration cannot be carried out directly. This problem may be overcome by means of mini-computers and by using numerical integration. By this means, using the equations introduced earlier⁶, the retention volumes of different types of ions can easily be calculated also in those instances where the concentration or pH is changing during the elution and where the relationships are complicated. These calculations can be used advantageously for exploratory purposes when planning new separation procedures or for the optimization of the conditions of a given procedure. In this paper, some typical calculation schemes are presented.

For the calculation of retention times in the gradient elution technique, the numerical integration method was used by Jandera and Churáček⁴ and by Abbott *et al.*⁷, who solved partition chromatographic separation problems by this means.

SEPARATION OF METAL IONS ON A CATION-EXCHANGE COLUMN USING A COMPLEX-FORMING AGENT AND A LINEAR pH GRADIENT

For simplicity, let us consider that bivalent metal ions are to be separated. As the eluent a buffer containing a complex-forming dibasic acid, H_2L , and its sodium salt with a constant total concentration but with a linearly changing pH is used. On increasing the pH, the free ligand concentration and the extent of complex formation of the metal ions increase. On the formation of unchanged or negatively charged complexes, the sorption strength of the metals gradually decreases.

In the system involving a metal ion, M^{2+} , the following chemical reactions are to be considered:

Ion exchange:

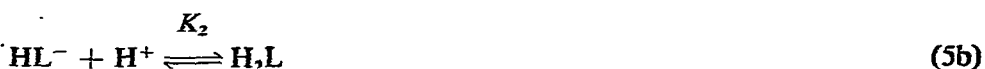
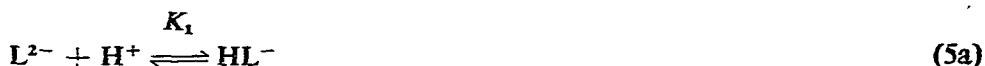


where R denotes the equivalent amount of resin.

Complex formation:



Protonation of the ligand



Taking the complex formation and protonation reactions as side-reactions, and considering that the total concentration of the metal ion in both phases is low compared with that of the eluent or that of the sodium in the resin phase, i.e. $C_L \gg C_M$ and $Q \gg [M]$, the distribution coefficient can be calculated to a good approximation by the following equation⁶:

$$D_M = \frac{K^x}{\alpha_{M(L)}} \cdot \frac{Q^2}{[Na^+]^2} \quad (6)$$

where

$$\alpha_{M(L)} = 1 + \left[\frac{C_L}{\alpha_{L(H)}} \right] \beta_1 + \left[\frac{C_L}{\alpha_{L(H)}} \right]^2 \beta_2 \quad (7)$$

and

$$\alpha_{L(H)} = 1 + [H^+] K_1 + [H^+]^2 K_1 K_2 \quad (8)$$

In these equations, K^x denotes the volume ion-exchange equilibrium constant of reaction 3, Q is the volume capacity of the ion-exchange resin (mequiv./ml), C_L is the total concentration of the complex-forming ligand, β_1 and β_2 are the overall stability constants of the complexes ML and ML_2 , respectively, and $[H^+]$ and $[Na^+]$ are the concentrations of the hydrogen and sodium ions in the eluent solution. $[M]$ denotes the metal ion concentration in the resin phase.

If a pH gradient of the type

$$pH = pH_0 + bv \quad (9)$$

is used, where b is the pH gradient ($\Delta pH/\Delta v$) and v is the eluent volume, the actual pH during the migration is

$$pH = pH_0 + b \left(v - a \sum_1^{i-1} \Delta x \right) \quad (10)$$

The second term in parentheses (which has to be subtracted) is the void volume of the part of the column just passed by the solute band during migration; a is the void fraction of the column ($a \approx 0.4$).

The pH is changed by adding sodium hydroxide to the solution containing the complexing agent or by mixing the complex-forming weak acid and its salt solution in different proportions. By this means the sodium ion concentration in the eluent is also increased. As the relationship between the pH and sodium ion concentration can be approximated with a linear equation during the neutralization of a dibasic acid with sodium hydroxide (in the buffer range only), the sodium ion concentration is

$$[Na^+] \approx [Na^+]_0 + 0.5 C_L b \left(v - a \sum_1^{i-1} \Delta x \right) \quad (11)$$

because

$$\Delta[Na^+] \approx \Delta pH \cdot \frac{C_L}{2}$$

For informatory calculations the second term in parentheses can be neglected or, better, if the alteration in the pH does not exceed more than one or two units during elution, a constant sodium concentration which is the mean of the initial and the final concentrations can be used.

To obtain the necessary eluent volume distribution coefficient, the reciprocal of the distribution coefficient is calculated and integrated according to eqn. 2 until its value approaches the total column volume X .

Thus,

$$\frac{\Delta v}{K^x Q^2} \cdot \sum_1^n \left[1 + \frac{C_L \beta_1}{1 + K_1 10^{-\text{pH}} + K_1 K_2 \cdot 10^{-2\text{pH}}} + \frac{C_L^2 \beta_2}{(1 + K_1 \cdot 10^{-\text{pH}} + K_1 K_2 \cdot 10^{-2\text{pH}})^2} \right] [\text{Na}^+]^2 = \sum_1^n \Delta x \rightarrow X \quad (12)$$

Making use of eqns. 10 and 11, the summation according to eqn. 12 can be carried out until the value of the left-hand side of the equation just reaches the value of X (total volume of the resin column), and the retention volume $V_R = n\Delta v$ can be obtained. As eluent increments $\Delta v = 1$ ml can be used.

It should be noted that eqn. 12 can also be used if the pH is kept constant but the total concentration of the complexant is changed during elution. The calculation will be simpler, because instead of eqns. 10 and 11 the following equations are used:

$$C_L = C_L^0 + e \left(v - a \sum_1^{i-1} \Delta x \right) \quad (13)$$

and

$$[\text{Na}^+] = [\text{Na}^+]_0 + em \left(v - a \sum_1^{i-1} \Delta x \right) \quad (14)$$

where e is the concentration gradient of C_L expressed in $\Delta M/\text{ml}$ and m is the degree of neutralization of the weak acid H_2L , which is kept constant ($m = 0.5-2$). It is preferable, however, to use a constant C_L and changing pH, because if C_L changes the ionic strength also changes rapidly and, by domination of the displacement effect of the sodium ions, the differences between the individual complex-forming behaviour of the metal ions will be suppressed and therefore the selectivity will decrease.

To prove the validity of the above deduced relationships the retention volumes of copper, zinc and cadmium were calculated using oxalate ions as the complex-forming agent and a linear pH gradient of 0.012 pH/ml. The data used for calculation were as follows: $\Delta v = 1$ ml; $\text{pH}_0 = 2$; $a = 0.4$; $b = 0.012$ pH/ml; $[\text{Na}^+]_0 = 0.75 \cdot 10^{-1} M$; $C_L = 10^{-1} M$; $Q = 1.9$ mequiv./ml; $X = 3.08$ ml, $\log K_1 = 3.8$; and $\log K_2 = 1.4$. The ion-exchange and complex formation constants were as follows: for copper, $K^x = 2.6$, $\log \beta_1 = 4.5$ and $\log \beta_2 = 8.9$; for zinc, $K^x = 2.2$, $\log \beta_1 = 3.7$ and $\log \beta_2 = 6.0$; and for cadmium, $K^x = 2.6$, $\log \beta_1 = 2.9$ and $\log \beta_2 = 4.7$.

The calculated $\sum \Delta x$ values were plotted against v (Fig. 1a). The V_R values were found to be 3, 64 and 129 ml, respectively.

For a comparison of the calculated data with those obtained experimentally, in Fig. 1b the chromatogram of the separation of the three metal ions using the above conditions is presented. A cation-exchange column of length 80 mm and diameter 7 mm was prepared from Amberlite CG-120 (150–300 mesh) resin as described previously⁸. The resin column was equilibrated with eluent of pH 2, containing $10^{-1} M$ oxalic acid–sodium oxalate. From the metal salt-containing stock solutions (0.05 M), 0.050-ml portions were introduced on to the top of the column. The eluent was delivered by a mixing apparatus, using a pH gradient of 0.012 pH/ml. During the elution, the effluent was collected in 2-ml fractions, which were analysed photometrically. The calculations were carried out using an EMG-666 desk computer (EMG, Budapest, Hungary).

As can be seen in Fig. 1, the calculated and experimentally obtained data are in fairly good agreement.

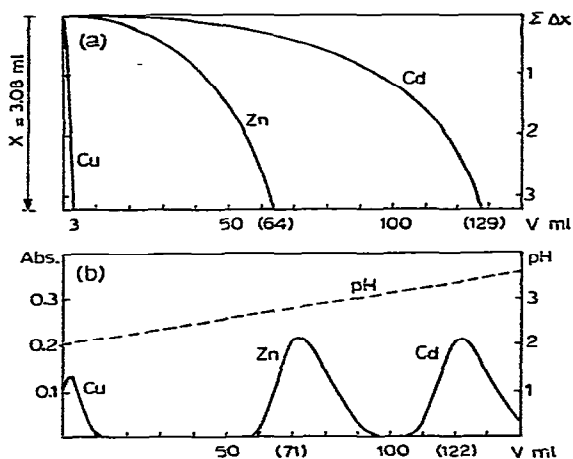


Fig. 1. (a) Calculated $\Sigma \Delta x$ values versus eluent volumes. (b) Separation of copper, zinc and cadmium ions. Eluent: 0.1 M oxalate solution of continuously changing pH. Ion-exchange column: Amberlite CG-120 (150–300 mesh), length 80 mm, diameter 7 mm.

SEPARATION OF ORGANIC BASES ON A CATION-EXCHANGE COLUMN USING A pH GRADIENT

As uncharged organic bases, amines take up protons in aqueous solution to form protonated base cations, which are able to take part in cation-exchange reactions. The reactions are as follows:

Protonation:



Ion exchange:



The distribution coefficient of the base is given by⁹

$$D_B = \frac{K^x Q}{[Na^+]} \cdot \frac{1}{\left(1 + \frac{1}{K [H^+]}\right)} \quad (17)$$

where K is the protonation constant of the base B and K^x is the volume ion-exchange equilibrium constant of the cation BH^+ referred to sodium ion.

Using a buffer solution of constant total concentration, C_{bu} , but of gradually increasing pH, the adsorption strength of the base can be continuously decreased. In that simple case, if a linear pH gradient is used the pH change in the column can be described by

$$pH = pH_0 + b \left(v - a \sum_1^{i-1} \Delta x \right) \quad (18)$$

If the pH gradient is produced by continuous addition of sodium hydroxide to the eluent, consisting of a monobasic weak acid and its sodium salt, or by mixing the solution of a weak acid and its sodium salt solution in different proportions, but of constant total concentration, the change in the sodium ion concentration can be approximated to

$$[Na^+] = [Na^+]_0 + 0.5 C_{bu} b \left(v - a \sum_1^{i-1} \Delta x \right) \quad (19)$$

For calculation of the eluent volume necessary to elute the base B from a column of volume X using the pH gradient, the following equation is used:

$$\frac{\Delta v}{K^x Q} \sum \left(1 + \frac{1}{K \cdot 10^{-pH}} \right) [Na^+] = \sum_1^n \Delta x \rightarrow X \quad (20)$$

The summation of the term on the left-hand side of eqn. 20 should be carried out until the value of X is just achieved.

It should be noted that the pH gradient can also be produced in such a way that a solution of constant sodium concentration is used and the concentration of the weak acid is decreased gradually in the eluent. In this instance the calculation is simpler, because $[Na^+]$ is constant.

It must be remembered, however, that in both of the above examples, the effect of pH on the absorption strength of the eluted species is greater than that of the sodium ion. Therefore, in exploratory calculations a mean but constant sodium ion concentration can be used, so that the calculations are much simpler.

Another important aspect is that during the derivation of the equations it was assumed that in the ion-exchange reaction with the metal ion or in the elution of bases, with the protonated base cation only the sodium ion is involved. This assumption can be made only if the sodium ion concentration is much higher than that of the hydrogen ion, e.g., if the eluent concentration is equal to or higher than $10^{-1} M$ and $pH \geq 2$. On the other hand, if the pH is very low, the ion-exchange reaction with the hydrogen ions must also be taken into consideration, which makes the calculations more complicated.

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