



Article Study of Efficiency of Capacity Gradient Ion-Exchange Stationary Phases

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Abstract: Highly efficient columns are necessary for the modern analytical applications of liquid chromatography. In this work, the separation efficiency of ion-exchange capacity gradient stationary phases combined with eluent concentration gradient was studied by a theoretical approach. In the course of our work three different scenarios of capacity gradients were used with different shapes (linear, convex and concave). The resolutions of different gradient columns were calculated for each scenario. As a reference, a uniform column was considered, which had the same analysis time as the non-uniform column. In the case of separation of ions with same charges, the gradient column offered only a marginal advantage compared to the uniform column due to the bandwidth compression caused by the capacity gradient. In the case of ions with different charges, however, the advantage of the gradient column was more significant. This was mainly due to the increased retention time difference of solutes. Ion-exchange capacity gradient columns may be a new way to separate ions more efficiently.

Keywords: ion-exchange capacity gradient; resolution; efficiency; gradient elution mode; stationary phase design



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1. Introduction

To improve selectivity, application of stationary phase gradients in high performance liquid chromatography was introduced decades ago [1,2]. Since it is difficult to prepare and pack stationary phase gradients, these applications were carried out by coupling columns with different chemistries. In ion chromatography, however, axial ion-exchange gradient can be achieved using cyclic polyether-modified cross-linked polymers as stationary phases [3]. These phases offer the possibility of complexation with inorganic salts [4,5]forming anion-exchange sites. By altering the cation type of the eluent throughout chromatographic analysis, capacity gradients can be produced due to the influence of the cation on the ion-exchange capacity of the column. This can be accomplished by switching the eluent during the run (e.g., sodium hydroxide to potassium hydroxide), resulting in significantly shorter analysis times due to decreasing capacity [6,7]. Crown ether [8–10] or cryptand-based columns [11,12] are often used to achieve efficient separations of various anions through the use of this type of gradient. Different types of cryptands can be covalently attached to a solid support and are able to bind metal cations due to their three-dimensional cavity [13]. The chromatographic run is conducted by altering the cation component of the hydroxide eluent, similar to how crown ether phases operate to create a capacity gradient [14,15]. Capacity gradients on cryptand columns offer the benefit of being able to separate both non-polarizable and polarizable anions in the same run, a feat that proves to be difficult with latex-agglomerated anion exchangers and aminated grafted polymers [6]. Cryptand phases can also be used as bifunctional packing materials for the separation of both cations and anions [16].

The characteristics of a column suitable for capacity gradient elution based on anionic complex formation between borate compounds and diols were investigated by Yamamoto et al. [17]. The ion-exchange capacity of the column decreased during the separation. The columns were successfully used for the analysis of anions of different charges by a binary gradient elution. The reproducibility of this technique, however, was not satisfying.

Preparation of stationary phases with adjustable selectivity is also possible by controlling different ratios of the co-monomers in hydrophilic interaction/ion-exchange mixedmode chromatography [18]. The development of a mixed phase chromatographic stationary phase with adjustable selectivity is advantageous to meet the needs of complex samples. The ratio of sodium 4-styrenesulfonate to dimethylaminoethyl methacrylate monomer allowed the control of charge and polarity of the stationary phase, as well as separation selectivity. This advantage was demonstrated by the successful separation of various compounds.

Each of the examples listed above can be considered a dynamic capacity gradient. Several authors explored the potential of static stationary phase gradients. There are several examples for preparation of stationary phases with gradient functionalities, including photo grafting techniques for monolithic capillary columns [19] or destructive and direct bonding methods [20,21] for silica columns. The applicability of gradient stationary phases for large solutes with on-off-like elution mechanisms, as well as the combination of mixed-mode stationary phase gradients and mobile phase gradients, was studied by Fekete et al. [22]. These gradient phases have the potential to exhibit an increase in resolution when combined with a mobile phase gradient, as shown for the case of particle size gradients [23,24]. Recently, ion-exchange columns with longitudinal stationary phase gradients were prepared and studied [25]. It was shown that these phases may provide a significant improvement in chromatographic performance in gradient elution mode. The development of these ion-exchangers, however, based on experimental considerations and reasoning. The aim of this work is to study the retention behavior of solutes and the chromatographic performance of static ion-exchange capacity gradient phases under different gradient scenarios using a theoretical approach.

2. Methods

2.1. Calculation of Elution Profiles

The equilibrium-dispersive model (EDM) was solved using the Martin-Synge algorithm [26] to calculate elution profiles. When mass transfer kinetics is rapid and the dispersion coefficient of the solute can be accurately determined in linear conditions, the differential mass balance of the solute [27–29] can be expressed as:

$$\frac{\partial c(z,t)}{\partial t} = -\frac{u}{k} \frac{\partial c(z,t)}{\partial z} + \frac{D_a}{k} \frac{\partial^2 c(z,t)}{\partial z^2}$$
(1)

where *c* is concentration of the compound in the mobile phase (mol/L or g/L), *t* the time (min), *z* the distance along the column (cm), *u* the linear velocity of the eluent (cm/min), and *k* the retention factor of the solute. The dispersion coefficient, D_a (cm²/min) can be estimated as:

D

$$a = u \frac{H}{2} \tag{2}$$

where *H* is the height equivalent to a theoretical plate (cm).

According to the Martin and Synge plate model [30], the column is divided into a series of continuous flow mixers, with the length of each vessel being equal to *H*. In each flow mixers, the following ordinary differential equation was solved accurately:

$$\frac{d c_m[t]}{d t} = -\frac{u}{1+k_m} \frac{c_m[t] - c_{m-1}[t]}{H}$$
(3)

where c_m and c_{m-1} are the concentration profiles of the solute in the *m*th and (m-1)th vessels. Note that position of the *m*th vessel, z_m is m H. The solution for the last vessel gives the elution profile.

 k_m was calculated as:

$$k_m = K_{A/OH} \left(\frac{Q_m}{[OH^-]}\right)^n \tag{4}$$

where $K_{A/OH}$ is the ion-exchange selectivity coefficient that is the product of phase ratio and ion-exchange equilibrium constant, *n* the charge of the solute ion, Q_m the ion-exchange capacity of the column at the position of *m*th vessel (eqiv/L), and [OH⁻] the eluent concentration (mol/L). In the case of gradient elution, eluent concentration at any *z* position of the columns is given as

$$\left[\mathrm{OH}^{-}\right] = \left[\mathrm{OH}^{-}\right]_{0} + \beta \left(t - \frac{z}{u}\right) \tag{5}$$

where $[OH^{-}]_{0}$ is the initial eluent concentration and β the slope of gradient (mol/L/min).

2.2. Ion-Exchange Capacity Gradients

We studied three scenarios with different ion-exchange capacity gradients. For each, we looked at both the positive and negative gradients, and compared the resolutions of the resulting non-uniform columns to that of a uniform column (a column with the same analysis time as the non-uniform column, and a constant ion-exchange capacity, Q_e). In the case of a positive gradient, the velocity and width of the component band decrease during its migration toward the end of the column. For a negative gradient, the opposite is observed: toward the end of the column, both parameters increase. According to our calculations, of the two peak sharpening effect (increasing velocity and band compression), the latter is the more significant, so in the following only positive gradients are discussed. The examined scenarios were the following:

1. Linear gradient

$$Q_z = \frac{q_L - q_0}{L} \, z + q_0 \tag{6}$$

2. Convex gradient

$$Q_{z} = (q_{L} - q_{0}) \left(\frac{z}{L}\right)^{i} + q_{0}$$
⁽⁷⁾

3. Concave gradient

$$Q_z = (q_0 - q_L) \left(\frac{L - z}{L}\right)^i + q_L \tag{8}$$

where q_0 and q_L are capacity values at the beginning and end of the column, respectively, and *i* is a positive integer (*i* goes 2 to 5 in this work). The profiles of the different capacity gradients are illustrated in Figure 1:



Figure 1. Profiles of the different capacity gradients used in this work. Linear (blue) curve is calculated by Equation (6). Parameter *i* of Equation (7) (convex curves): 2—orange, 3—red, 4—brown, 5—gray. Parameter *i* of Equation (8) (concave curves): 2—green, 3—purple, 4—ping, 5—yellow.

In the course of our work, the value of q_0 was changed between 0.1 and 1, and the value of q_L between 1 and 10 mequiv/L.

2.3. Determination of Equivalent Ion-Exchange Capacity

Equivalent ion-exchange capacity, Q_e , can be defined as the capacity of a uniform ionexchange column which provides the same analysis time, as the capacity gradient column. In other words, the retention time of the last eluting ion is the same for both columns.

According to the gradient retention model published by Jandera and Churáček [31], retention time, t_R , in gradient elution mode, can be expressed as:

$$t_{R} = t_{0} + \frac{[OH^{-}]_{0}}{\beta} \left[\left(1 + (n+1) \frac{\beta}{[OH^{-}]_{0}} k_{0} t_{0} \right)^{\frac{1}{n+1}} - 1 \right]$$
(9)

where t_0 is the dead time and k_0 is the retention factor of the solute at the initial eluent concentration, $[OH^-]_0$ (see also Equation (4)). By rearranging Equation (9) to Q_e , the equivalent ion-exchange capacity can be calculated.

The column with the equivalent ion-exchange capacity serves as a benchmark, enabling comparison of its effectiveness to the columns with varying capacity gradients.

2.4. Parameters of Calculations

Using Python version 3.8 from the Anaconda Python Distribution, with NumPy and SciPy packages, numerical calculations were done. The numerical parameters required for these calculations, such as the column and molecular parameters, are listed in Table 1.

Table 1. The numerical parameters required for numerical calculations and their respective values.

Parameter	Value		
column length (<i>L</i>)	25 cm		
mobile phase velocity (<i>u</i>)	25 cm/min		
initial eluent concentration $([OH^-]_0)$	0.001 mol/L		
rate of change in eluent concentration (β)	0.010 mol/L/min		

Model ions and their ion-exchange selectivity coefficients used for the calculations are listed in Table 2.

Table 2. The values of the ion-exchange selectivity coefficients of the anions used in this work.

Anion	K _{A/OH}
monovalent ion 1 (mono1)	1.51
monovalent ion 2 (mono2)	5.04
divalent ion 1 (di1)	29.05
divalent ion 2 (di2)	66.21

Through the example of these anions, we wanted to demonstrate the advantage of a column with a gradient ion-exchange capacity in the case of gradient elution separation of ions of different charges.

3. Results

3.1. Resolution Analysis of Non-Uniform Columns

In this section, we examine whether the capacity gradient column has an advantage over the uniform column with the same analysis time under isocratic elution conditions.

3.1.1. Separation of Ions with the Same Charges

Figure 2a shows the variation in the ratio of the resolutions of the gradient and the uniform column as the function of q_0 and q_L in case of linear gradient for monovalent anions.



Figure 2. Ratio of separation parameters of gradient and uniform columns as the function of q_0 and q_L for linear capacity gradient. (a) Ratio of gradient and uniform column resolutions, (b) Ratio of bandwidths calculated for gradient and uniform columns for mono2.

The marginal advantage of the gradient column is due to the extra bandwidth reducing effect caused by the capacity gradient. Figure 2b shows the ratio of bandwidths calculated for gradient and uniform columns for the *mono2* ion. The slight advantage of the gradient column is manifested only in the less important range from the practical point of view, since in this range the resolutions of the individual columns are minimal and the retention times are close to the dead time.

The same conclusion can be drawn when examining the separation of divalent ions through the example of *di1* and *di2* ions. Figure 3 shows the comparison of the peaks of the *di2* ion calculated for the gradient (g) and for the uniform (u) column. Calculation parameters were the following: $q_0 = 0.4 \text{ meqiv/L}$, $q_L = 2 \text{ meqiv/L}$, $Q_e = 1.29 \text{ meqiv/L}$. With these parameters a ~4% gain in resolution can be observed, when separating *di2* ion from *di1* ion.



Figure 3. Peaks of the *di*2 ion calculated for the gradient column (blue) and for the uniform (orange) column (AU—arbitrary unit).

Figure 3 confirms the validity of Equation (9) and the calculation of Q_e , since the retention times of the two peaks are perfectly match.

3.1.2. Separation of Ions of Different Charges

The separation of ions with different charges on a column with a gradient ion-exchange capacity was modeled using the example of separating *mono1-di1* and *mono2-di2* ions. Figure 4 shows the ratio of resolutions and the ratio of the difference between retention times calculated for the gradient and uniform column, as a function of q_L and q_0 .

Figure 4 clearly shows that the separation of monovalent and divalent ions with capacitive gradient columns resulted in significantly better resolution improvements than that the separation of ions with the same charge. The significant improvement in resolution is mainly due to the increase in the retention time difference of the solute ions. Accordingly, ion-exchange capacity columns provides different selectivities than uniform columns under gradient elution.

As shown in Figure 4a,c, the value of the capacity at the beginning and the end of the column is a crucial factor for the separation efficiency. Each q_0 value has an optimal q_L where the resolution will be maximum. The ion-exchange selectivity constants of the particular ions are also a decisive factor to be considered during separation design.

Figure 5 shows the calculated chromatograms of *mono2* and *di2* ions in case of gradient (g) and uniform (u) columns. Calculation parameters were the following: $q_L = 7$ meqiv/L, $q_0 = 0.5$ meqiv/L, $Q_e = 4.2$ meqiv/L. With these parameters the gradient column has ~24% advantage compared to the uniform column in terms of resolution.



Figure 4. Ratios of resolutions (**a**,**c**) and retention time differences (**b**,**d**) for separations of *mono1-di1* (**a**,**b**) and *mono2-di2* (**c**,**d**) ions.



Figure 5. Calculated chromatograms of *mono2* and *di2* ions in case of gradient (blue) and uniform (orange) columns (AU—arbitrary unit).

3.1.3. Effect of Capacity Gradient Profile on Resolution

The profile of the ion-exchange capacity gradient (Q_z) is also an important factor to be considered in terms of the column efficiency. Table 3 shows the increase in resolution of the gradient column compared to the uniform column in case of different Q_z profiles at given q_0 and q_L values (0.5 and 7 meqiv/L, respectively).

Scenario	i	Q_e , meqiv/L	R _S -u	R _S -g	Improvement, %
Linear	1	0.420	2.12	2.63	24
Convex	2	0.330	1.56	2.45	57
	3	0.282	1.22	2.33	90
	4	0.250	0.99	2.22	125
	5	0.228	0.81	2.12	163
Concave	2	0.520	2.67	3.02	13
	3	0.568	2.91	3.17	9
	4	0.596	3.03	3.25	7
	5	0.614	3.12	3.30	6

Table 3. Improvement in resolution for different ion-exchange capacity-profile scenarios.

As can be seen from the data above, the capacity gradient profile is also a decisive factor in addition to the initial and final capacity values in terms of efficiency. The advantage of a gradient column is more significant in case of convex gradient profiles, while concave profiles offer negligible advantages.

4. Conclusions

The use of ion-exchange capacity gradient in IC columns was studied by a theoretical approach. The capacity gradient was modeled with different functions (linear, convex and concave). Resolutions of these non-uniform columns were calculated for each scenario as a function of the limiting capacity values for separations of monovalent and divalent anions. The results were compared with the resolutions of the reference uniform columns, whose analysis time was the same as for the gradient column. The calculations showed that there is only a minor improvement in separation efficiency for ions with the same charges. This slight advantage is due to the minor extra bandwidth compression caused by the capacity

gradient. As for separation of monovalent and divalent ions, the advantage of the gradient column is more significant, due to the improved selectivity of the columns. By delicately choosing the profile and the limiting values of the ion-exchange capacity, application of these non-uniform columns together with eluent concentration gradients can be a potential improvement to achieve more efficient separations.

It is important to note that, although the results were calculated specifically for ion chromatographic separations, the conclusions drawn are also valid for other retention mechanisms. In reversed-phase chromatography, it is not the different charge but the molecular size that plays an important role in the enhanced selectivity of gradient stationary phases.

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