ELSEVIER

Journal of Chromatography A, 744 (1996) 115–121

JOURNAL OF CHROMATOGRAPHY A

Selectivity, differential mobility and resolution as parameters to optimize capillary electrophoretic separation

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Abstract

Selectivity, differential mobility, and resolution have been tested as the optimization functions to find the optimum pH of operational electrolyte for separation by capillary electrophoresis when organic acids occurring in human serum have been selected as a model mixture for computer simulations. Using tabulated values of ionic mobilities and pK_a values, either selectivity or differential mobility or resolution for the hardest-to-separate pair of separands are calculated and plotted vs. pH. The optimum pH is the pH value, at which the optimization function reaches its maximum.

Keywords: Selectivity; Resolution; Mobility, differential; pH Optimization; Organic acids

1. Introduction

A quantitative description of the separation achieved has been needed since the introduction of separation analytical methods. The first attempt to express the efficiency of the electrophoretic separation was made by Giddings who proposed the term of selectivity as a velocity difference ΔU of two ions divided with the average value of velocity $\bar{U}$ [1]:

$$p = \frac{\Delta U}{\bar{U}} \tag{1}$$

where p is selectivity and U is migration velocity.

Later Gebauer and Boček modified the expression for selectivity to describe separation of ions in isotachophoresis [2–4]: the mobility difference was related to the mobility of the ion with corresponding lower absolute value of electrophoretic mobility

$$p = \frac{\bar{\mu}_1 - \bar{\mu}_2}{\bar{\mu}_2} \tag{2}$$

where $\bar{\mu}$ is effective mobility.

Different terms of selectivity have been used by other authors such as $\Delta\mu$ [5], or as the selectivity coefficient (analogous to relative retention in chromatography) μ_1/μ_2 [6,7] or t_1/t_{ref} [8], or $t_1/\bar{t}$ [9]. In a number of papers, selectivity has been used rather freely without any exact definition.

The expression for resolution R_s in zone electrophoresis has been introduced by Giddings [1]:

$$R_s = \frac{1}{4}\sqrt{\bar{N}}\,\frac{\Delta U}{\bar{U}} \tag{3}$$

where $\bar{N}$ is the mean number of theoretical plates.

Jorgenson and Lukacs rearranged this equation and expressed resolution as [10]

$$R_s = 0.177\,(\bar{\mu}_1 - \bar{\mu}_2)\left[\frac{V}{D\,(\mu_{avg} + \mu_{osm})}\right]^{\frac{1}{2}} \tag{4}$$

where V is voltage, D diffusion coefficient, $\bar{\mu}_1$ and $\bar{\mu}_2$ are effective mobilities of the individual

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0021-9673/96/$15.00
PII S0021-9673(96)00449-9

separands, μ_{avg} is the average mobility, and μ_{osm} is electroosmotic mobility.

In chromatography, resolution generated per unit time has been proposed to express the speed of separation [11].

This paper shows how selectivity, or differential mobility or resolution can be used to optimize the electrophoretic separation. The optimum pH of the operational electrolyte can be predicted if tabulated data of pK_a values and ionic mobilities are available.

2. Results and discussion

2.1. Optimization of separation conditions

For any analysis, two parameters are typically optimized (and minimized): cost of analysis and time of analysis. In the following text under the term of optimization, the analysis time optimization/minimization is meant exclusively. Optimization of capillary zone electrophoresis means to identify separation conditions under which the analysis can be performed in the shortest time at sufficient resolution. Typical parameters to be optimized involve voltage, effective length of capillary, time of sample injection and composition of operational electrolyte. The last parameter involves usually conductivity/ionic strength and pH or any other parameter used to affect and thus distinguish effective mobilities of separands. If it is the acid–base equilibrium by the use of which the separation is optimized, a buffer is used as the operational electrolyte. However, even other factors can be used (and optimized) to achieve separation such as ion-association with multicharged ions [12], sieving effect for separation of polymers [13], complex forming equilibria [14], addition of nonaqueous solvents [15], etc. When optimizing separation conditions, the composition of operational electrolyte should be the first parameter considered, since the other parameters (voltage, capillary effective length, etc.) depend strongly on it.

Manipulation of the pH of the operational electrolyte is the most frequent tool when trying to achieve separation and the only one which can be used routinely for theoretical modelling and predictions, since the determination of effective mobility of analytes requires the data on ionic mobility and pK_a only. Fortunately, there are extensive tables of pK_a values [16–21]. Ionic mobilities (or ionic conductivities) are not tabulated to such an extent as pK_a values [22,23]; however, there are equations enabling the ionic mobilities to be estimated with an acceptable accuracy [24–30].

To demonstrate the optimization strategy, six acids which occur in human serum [31] were selected as the appropriate model mixture (Table 1). Mobility curves of the individual acids are shown in Fig. 1 as calculated by using Excel 5.0. Obviously, at pH values, where the curves cross, the corresponding effective mobilities are equal and a separation cannot be achieved. During optimization, separation of all combinations of pairs to be separated is compared to identify the hardest-to-separate pair under the given condition. This is quantitatively done by comparing the optimization function (selectivity, differential mobility, resolution, etc.) for all combinations of pairs of separands and finding the corresponding minimum value. This minimum value is then plotted against the studied parameter (e.g., pH) and the value of this parameter, at which the optimization function reaches its maximum, is found as the optimum value for the separation of the particular mixture. This optimization strategy is valid generally and, after

Table 1
Components of model mixture and their pK_a values and ionic mobilities

	pK_1	pK_2	pK_3	μ_1 ($\times 10^{-9}$ m^2 V^{-1} s^{-1})	μ_2 ($\times 10^{-9}$ m^2 V^{-1} s^{-1})	μ_3 ($\times 10^{-9}$ m^2 V^{-1} s^{-1})
Pyruvate	2.49			−42.3		
Lactate	3.86			−36.5		
Malate	3.46	5.05		−32.6	−59	
α-Ketoglutarate	2.8	5.006		−33.6	−59	
Citrate	3.13	4.76	6.4	−28.7	−54.7	−74.4
Phosphate	2.21	7.47	12.36	−35.1	−61.5	−71.5

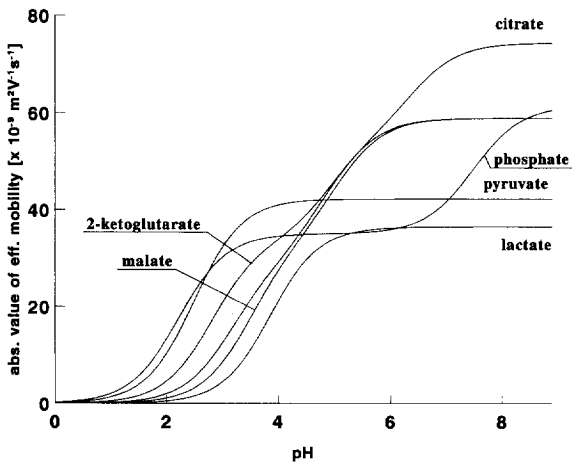

Fig. 1. Mobility curves of pyruvate, phosphate, α-ketoglutarate, citrate, malate and lactate.

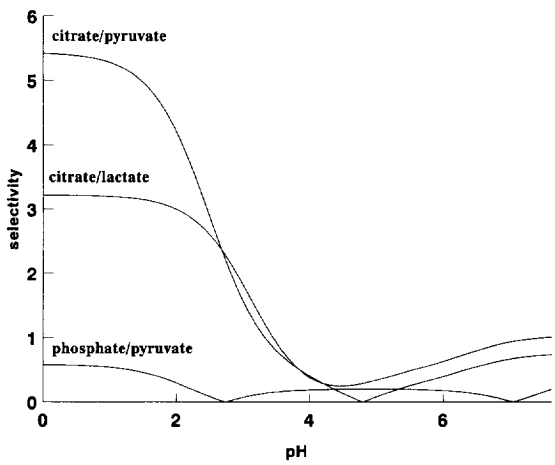

Fig. 2. Selectivity as a function of pH for citrate/pyruvate, citrate/lactate and phosphate/pyruvate.

more data are available on complex-forming equilibria, interaction of proteins and nucleic acids with sieving matrices, etc., it can be used to optimize even other parameters of the electrophoretic separation.

2.2. Selectivity

Selectivity is the separation parameter which is considered in capillary electrophoresis frequently. To calculate selectivity, we prefer the equation by Gebauer and Boček (Eq. 2) [2–4] to that by Giddings (Eq. 1) [1]. The reason is that the former equation has a clear physical meaning: it is proportional to the length of capillary which is needed to achieve a full separation of two analytes. It can be obtained from an equation introduced originally by Everaerts and Martin [32] for separation in isotachophoresis. However, it is valid even in zone electrophoresis if diffusion can be neglected (i.e., when the run time is short). Both equations (by Giddings [1] and by Gebauer and Boček [2–4]) provide similar results namely in the areas of limited separation. Note that in capillary electrophoresis, selectivity is defined in the case only, when both analytes move in the same direction.

Dependence of selectivity on pH when separating the model mixture of organic acids is shown in Fig. 2. (It was calculated as well as all the other plots by using Excel 5.0.) To keep the plot clear, the number of curves was reduced. The citrate/pyruvate pair represent a pair of analytes which do differ by their effective mobilities at any pH and so they can be separated at any pH. The effective mobilities of citrate and lactate are equal at pH 4.80 and so these two compounds cannot be separated at this pH. Mobility curves of phosphate and pyruvate cross twice in Fig. 1, at pH 2.73 and 7.05, which correspond to zero values of selectivity in Fig. 2 and reflects no separation at these pH values. From Eq. 2, it is clear that selectivity reaches the maximum value at the lowest mobilities of the slower moving analyte, i.e., at extreme values of pH (pH 0 for anions, pH 14 for cations). Obviously, the magnitude of selectivity at these extremes depends on the differences of pK_a values for both separands: the lower the pK_a difference, the lower the selectivity value.

As pointed out above, a full separation of a multicomponent mixture is controlled by the separation of the hardest-to-separate pair. That is why the value of selectivity for the separation of this critical couple is needed only. Minimum selectivity needed for the separation of the chosen model mixture is shown in Fig. 3. The curve reaches zero value at several pH values as the mobilities of at least two analytes are equal: phosphate/pyruvate (pH 2.73); phosphate/α-ketoglutarate (pH 4.08); phosphate/citrate (pH 4.38); phosphate/malate (pH 4.44); pyruvate/α-ketoglutarate (pH 4.74); pyruvate/citrate (pH 4.80); pyruvate/malate (4.86); phosphate/lac-

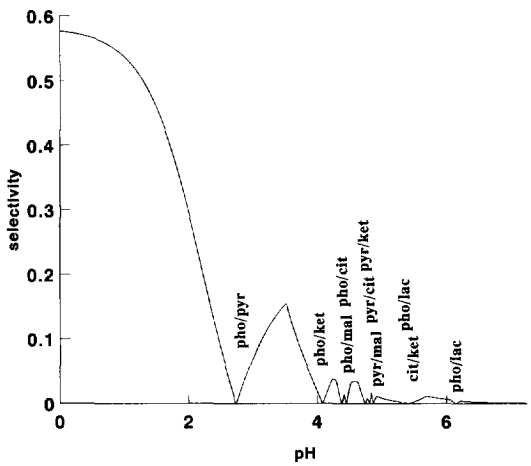

Fig. 3. Minimum selectivity for separation of model mixture of anions from Fig. 1 as a function of pH. Valleys with zero selectivity are shown with the corresponding hardest-to-separate pairs. Abbreviations: pho, phosphate; pyr, pyruvate; cit, citrate; ket, α-ketoglutarate; lac, lactate; mal, malate.

tate (pH 5.31); citrate/α-ketoglutarate (pH 5.40); phosphate/lactate (pH 6.15). When optimizing separation, the pH value to be determined is that at which the minimum selectivity reaches its maximum value. Obviously this is at pH 0, when pyruvate/phosphate is the hardest-to-separate pair. The shortest capillary would be needed to achieve the full separation at this pH; however, very low values of mobilities indicate that this pH is not valuable for real-world analysis. This documents clearly the limitation of the practical use of selectivity. However, for the optimization, when the values of mobility do not drop below reasonable values through the whole interval tested (e.g., when ion-association or sieving matrices are involved), selectivity can be used to optimize the operational electrolyte composition [12,33].

2.3. Selectivity and electroosmotic flow

Electroosmotic flow itself does not contribute to the electrophoretic separations. However, in special cases, when the sample contains separands of opposite charge, or some separands exhibit very low effective mobility, electroosmotic flow can be useful by carrying the analytes to the detector and thus enabling reasonable migration times to be achieved [34,35].

As pointed out by Jorgenson and Lukacs [10], in the presence of electroosmotic flow, electroosmotic mobility has to be included in the denominator of the equation for selectivity. Therefore Eq. 2 has to be rewritten, to reflect the real velocity of analytes in the capillary:

$$p = \frac{\bar{\mu}_1 - \bar{\mu}_2}{\mu_{osm} + \bar{\mu}_2} \tag{5}$$

However, in the presence of electroosmotic flow, the separation conditions are more frequently optimized by using experimental data. Because of this, it is useful to rearrange Eq. 5 by replacing mobilities with migration time t, when t_0 is the migration time of an uncharged marker:

$$p = \frac{t_2 - t_1}{t_1\left[1 + \frac{t_2}{t_0}\right]} \tag{6}$$

2.4. Differential mobility

When the separation speed is the most important factor for the analysis, the difference between effective mobilities is the parameter to be optimized. (In the presence of electroosmotic flow, the use of which is advantageous to separate analytes exhibiting very low effective mobility, the difference between apparent mobilities has to include electroosmotic flow.) The use of differential mobilities stems from the necessity to achieve the maximum velocity difference which is, at constant electric field strength, proportional to maximum mobility difference.

Similar to the optimization strategy when selectivity is used, the pH is sought at which the minimum value of the differential mobility reaches its maximum. The minimum differential mobility for the chosen model mixture is given in Fig. 4. The differential mobility exhibits the zero value at the pH when at least two separands exhibit the same effective mobilities. These pairs and corresponding pH values have been identified in Fig. 3 already. For the optimization purpose, however, the pH value corresponding to the maximum value is of interest. In Fig. 4, the curve of minimum differential mobility exhibits the highest value at pH 3.31, which expresses the status when pairs of citrate/malate and pyruvate/phosphate exhibit the same differential mobility.

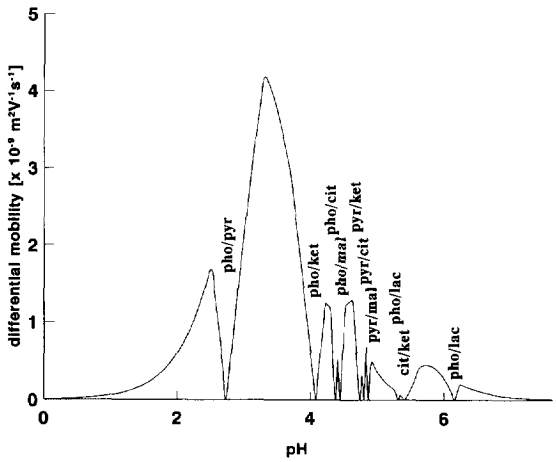

Fig. 4. Minimum differential mobility vs. pH for the model mixture from Fig. 1. Valleys with zero mobility diference are shown with the corresponding hardest-to-separate pairs. For abbreviations see Fig. 3.

This pH determines the optimum pH for the separation of the model mixture. The drawback of the procedure using differential mobility is that it does not take into the account diffusion and thus, at lower ionization, it can predict results better than they are in reality.

For calculation of differential mobility $\bar{\mu}_{\mathrm{dif}}$ from experimental data, the input data are preferred in the form of migration times. By rearranging the simple equation describing the velocity of electromigration v_i of the i-th solute,

$$v_i = \bar{\mu}_i E = \frac{l_{\mathrm{sep}}}{t_i} \tag{7}$$

where $\bar{\mu}_i$, E, l_{sep} and t_i are effective mobility, electric field strength, effective length of capillary and migration time, respectively, an equation useful for such a calculation is obtained

$$\bar{\mu}_{\mathrm{dif}} = \frac{t_2 - t_1}{t_1}\bar{\mu}_2 = \frac{t_2 - t_1}{t_1 t_2}\frac{l_{\mathrm{sep}}}{E} \tag{8}$$

This equation shows a relationship between selectivity and differential mobility: differential mobility is selectivity multiplied by the absolute value of the effective mobility of the slower separand and is proportional to the speed with which selectivity is generated.

2.5. Resolution

Resolution R_s is defined as:

$$R_s = \frac{t_2 - t_1}{\frac{w_1}{2} + \frac{w_2}{2}} \tag{9}$$

where w_1, w_2 are peak widths.

The peak width w_i can be expressed for the peak with the shape of a Gaussian curve as $4\sigma_{t,i}$. The distance, $\sigma_{x,i}$, which an average ion reaches by diffusion in time t_i is given as

$$\sigma_{x,i} = \sqrt{2D_i t_i} \tag{10}$$

where D_i is diffusion coefficient.

The velocity of electromigration v_i relates both forms of variance

$$v_i = \bar{\mu}_i E = \frac{\sigma_{x,i}}{\sigma_{t,i}} \tag{11}$$

The relation between mobility $\bar{\mu}_i$ and diffusion coefficient D_i is given by the Nernst–Einstein equation [36]:

$$\bar{\mu}_i = \frac{z_i F D_i}{RT} \tag{12}$$

where z_i, F, R, T are charge of ion, Faraday constant, gas constant and temperature, respectively. This equation can be used for weak acids and bases if charge z_i is replaced with the ionization degree α_i. However, when using Eq. 12 for multivalent ions, the effect of ion association on the actual mobility should be kept in mind.

The time interval between the passage of an average ion moving by diffusion from the peak center and the peak center itself through the detector $\sigma_{t,i}$, i.e., variance given in time unit, can be expressed by Eq. 13 which is obtained by combining Eqs. 10–12:

$$\sigma_{t,i} = \frac{\sqrt{2D_i t_i}}{\bar{\mu}_i E} = \sqrt{\frac{2RTl_{\mathrm{sep}}}{z_i F \bar{\mu}^2 E^3}} \tag{13}$$

Since the average ion continues in dispersion even after the peak enters the detection cell, the overall variance caused by diffusion $\sigma_{t,i}$ is larger; it can be calculated either using the second moment [37] or as a sum of a convergent sequence. For the majority of

real experiments, however, it is sufficient to express $\sigma_{t,i}$ with Eq. 13.

By combining Eqs. 9–13, resolution can be expressed as:

$$R_s = \sqrt{\frac{FEl_{sep}}{8RT}} \frac{\bar{\mu}_1 - \bar{\mu}_2}{\frac{\bar{\mu}_2}{\sqrt{z_1}} + \frac{\bar{\mu}_1}{\sqrt{z_2}}} \quad (14)$$

If we compare Eqs. 3 and 14, some difference can be seen: Giddings used average number of theoretical plates which he assumed to approach "sufficiently well for similar, close-lying peaks" [1]. If the charges of both separands are equal, Eqs. 3 and 14 become identical as can be shown by calculating the number of theoretical plates using the variance from Eq. 13. However, Eq. 14 is preferred if there are charge differences between the separated analytes.

For monovalent weak acids, Eq. 14 can be rewritten using α_i as dissociation degree

$$R_s = \sqrt{\frac{FEl_{sep}\alpha_2}{8RT}} \frac{1 - \frac{\mu_2}{\mu_1}\frac{\alpha_2}{\alpha_1}}{1 + \frac{\mu_2}{\mu_1}\left(\frac{\alpha_2}{\alpha_1}\right)^{\frac{3}{2}}} \quad (15)$$

where μ_1, μ_2 and α_1, α_2 are ionic mobilities and dissociation degrees of the particular separands.

Eq. 14 or Eq. 15 can be used to calculate resolution for the given pH when ionic mobilities and pK_a values are known. The dependence of minimum resolution on pH for the model mixture is given in Fig. 5. Not surprisingly, resolution reaches zero values at the same pH as selectivity and differential mobility. The maximum resolution for the hardest-to-separate pair of separands is found at pH 3.51.

When optimization is compared by using selectivity, mobility difference and resolution, the results obtained are similar. Selectivity as shown above always reaches the maximum at lower ionization. If this maximum is neglected (which may generate a significant error) the second maximum can be considered. In this particular case it corresponds to pH 3.51, at which selectivity for separation of pairs phosphate/pyruvate and α-ketoglutarate/phosphate is equal. It is just coincidence that this optimum pH is identical with that obtained from the resolution calculations. Minimum differential mobility as the optimization function gave slightly different results: the optimum pH 3.31 corresponds to the pH value when the differential mobilities for pairs of citrate/malate and pyruvate/phosphate are equal. When differential mobility is calculated, the pair of citrate/malate replaces α-ketoglutarate/phosphate as the hardest-to-separate pair in the pH interval 3.31–3.70. (These two pairs provide close curves in the pH interval 3.3–4 for all of the optimization functions used.) If the citrate/malate pair is neglected, the maximum differential mobility for the pairs of phosphate/pyruvate and α-ketoglutarate/phosphate is found at pH 3.46, which is close to the pH value of 3.51 obtained for the resolution using a selectivity optimization.

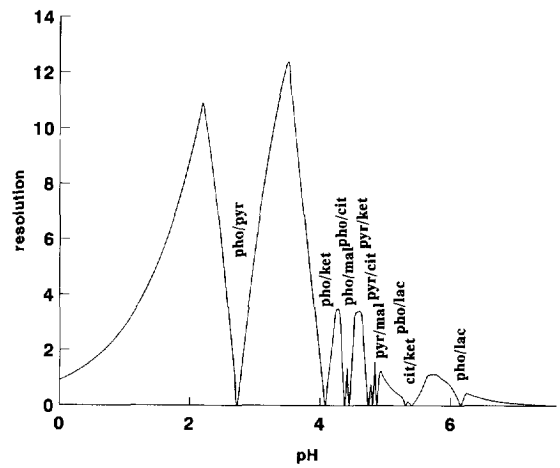

Fig. 5. Minimum resolution vs. pH for the model mixture from Fig. 1. Valleys with zero resolution are shown with the corresponding hardest-to-separate pairs. For abbreviations see Fig. 3.

3. Conclusion

The theoretical approach has been adopted which enables pH of the operational electrolyte to be optimized when ionic mobilities and pK_a values are needed for the calculation. Selectivity, differential mobility and, using the newly derived equation, resolution can be easily calculated for the hardest-to-separate pair of separands at each pH and plotted vs. pH. The pH, at which any of the functions reaches its maximum, corresponds to the optimum pH of the operational electrolyte. To optimize other parameters

than pH, the experimental data are needed because of a lack tabulated data. Equations were derived which enable selectivity and differential mobility to be calculated from experimental data.

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