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Suggested definition of zone resolution and zone capacity in separations of weak electrolytes or ampholytes by steady-state electrophoretic methods

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Abstract

A new definition of zone resolution in the general class of steady-state electrophoretic methods is proposed. Under certain assumptions, the limiting zone capacity can be described by a single explicit expression of wide applicability. The expressions for zone resolution and zone capacity were used to describe the separation of weak electrolytes or ampholytes in ordered series of zones with a Gaussian or square-wave concentration profile or with the profile which is the transition between these shapes.

1. Introduction

Steady-state electrophoretic methods [1–3] include powerful separation tools such as isoelectric focusing (IEF) [4–12], isotachopheresis (ITP) [13–19] and methods that can be considered as a transition between typical IEF and ITP (e.g., IEF with electrophoretic mobilization [20–27], ITP with carrier ampholytes [14,19,28,29]). Some of the transition (intermediate) methods can be regarded also as electrophoretic focusing in a steady-state moving natural pH gradient [30].

The separation power of the method can be reasonably visualized by the number of components that can be resolved into individual zones. For this purpose, the definition of zone breadth is necessary. Several such definitions could be suggested and defended; the most convenient one identifies the breadth of the

Gaussian concentration distribution with the distance between two inflection points, which is twice the standard deviation. When the concentration profiles of the separated zones can be approximated by the Gaussian curves, terms such as zone resolution, R_s , and peak capacity n_c , [3,10,30,31] are generally applicable. A search of the literature shows that a number of approaches have been suggested to describe the degree of zone separation [3,7–10,31–35]. As they describe systems composed of the Gaussian zones or boundaries characterized by a standard deviation, the relationships derived can be transferred to the description based on the R_s value [35].

Unfortunately, the steady-state electrophoretic methods mentioned above often produce zones with nearly square-wave forms of their concentration profiles which are separated by sigmoidal boundaries. Typical examples include well sepa-

rated ITP zones [1,2,13,17,36–39] or IEF zones with the use of a small number of carrier ampholytes and/or a high intensity of the electrical field [1,2,8,11,40,41]. The degree of zone separation was described with the help of the boundary width for strong electrolytes [42]. However, there is a smooth transition between Gaussian and square-wave concentration profiles [1,2,8,43]. The fundamental unity of the electrophoretic separation processes discussed so far was supported by general electrophoretic models [1,2,43–47]. Therefore, it seemed meaningful to seek some characteristics of the separation power of the steady-state electrophoretic methods that would not be sensitive to the shape of the concentration profile of the separated zones. Especially a parameter that would cover the Gaussian, square-wave and intermediate shapes would be valuable. However, a more generally applicable definition of zone resolution leading to great mathematical complexity in the calculation of resolving power would be useless.

In this paper, the resolution between neighbouring zones is considered in the array of the equidistant bands with equal mass of the considered components. The suggested description of zone separation is based on the definition of the term called the effective length of the concentration gradient which generally exists in any system with an array of steady-state zones. The simplified model enables one to define such a width for concentration profiles of various shapes.

2. Theory

2.1. Model definition

Here we discuss an idealized system of monoprotic weak electrolytes or mono-monovalent ampholytes, with equal diffusion coefficients, D_i , with a small degree of ionization, so that the component effective charge is much smaller than unity, $|z_i| \ll 1$. The solution is convection-free and stationary. Separation occurs only due to the differences in pK_a or isoelectric point, pI , dispersion due only to the diffusion. The pK_a or pI

values of compounds are evenly spaced on the pH scale. Since, for the estimation of the limiting peak capacity, a large number of the separated compounds is of interest, the difference between the neighbouring zones amounts to only a fraction of a pH unit, $\Delta(\text{pH})$. For a series of ampholyte zones, it is $\Delta(\text{pH}) = \Delta(pI)$. For all compounds considered, the derivative of the pH dependence of the effective mobility of a compound, $d\mu_i/d(\text{pH})$, is considered to be a constant within the zone and the same for all compounds.

When the zones are sufficiently overlapped, a continuous constant pH gradient, dpH/dx , can be assumed. As D_i values are equal and constant, the conductivity can also be assumed to be constant along the length of the whole series of the zones, L . This assumption includes the neglect of the contribution of the solvent ions to the conductivity, which implies that the zone pH is close to neutral. As the area of the cross-section of the channel, A , is also constant, the electrical field, E , is constant. The model may be considered as artificial but it is often used for modelling the steady state in idealized IEF [1,2,5,7–10,12,30,43,46].

As steady-state IEF can be treated as zero-velocity steady-state ITP [12,17,37], it is reasonable to examine the conditions for the applicability of above IEF model to the idealized ITP of weak electrolytes. For this treatment, the zone existence diagram [18] is useful. It appears that weak electrolytes with the same D_i can generate zones with approximately the same conductivity under steady-state conditions. For example, the series of zones can cover the pH range from 4 to 10 while the conductivity varies by only about 10% over the entire pH range. Applying the above assumptions used for IEF also to ITP of a series of weak electrolytes, we have $\Delta(\text{pH}) = \Delta(pK_a)$. Further, it follows from the model description that the pH difference, $\Delta(\text{pH})$, and the potential difference, ΔU , between the neighbouring zones are independent of whether their shape is Gaussian or square-wave and whether the steady-state zone moves or not.

As mentioned previously, owing to the change

in the field intensity, the shape of the concentration distribution curves of the zones can change from Gaussian to nearly square wave. According to the model described, the influence of this transition on the important variables, including Δx , $\Delta(\text{pH})$, $d\mu/d(\text{pH})$, mean zone mobility and zone conductivity can be neglected for the estimation of the limiting zone capacity.

Generally, a series of zones is studied (see Fig. 1). In a one-dimensional arrangement, the zones are evenly spaced by Δx , which is the distance of zone maxima or between the boundaries. When the array consists of zones of equal mass of components, m_i , equal concentrations in zone maxima, c_{mi} , result.

Common features of the series of concentration profiles of real zones include their maxima, slopes on both zone sides, inflection points at these slopes and asymptotic declination of the concentration toward both sides. At the contacts between the neighbouring zones, boundaries exist at the places of greatest mixing of separated compounds. For a series of equal zones with

arbitrary shape, the tangent at the inflection point, $(dc_i/dx)_{ip}$, and the distance between the zone maxima or the distance between the boundaries, Δx , can be well defined (see Fig. 1).

Let us define the effective length of the concentration gradient, ω , as

$$\omega = \bar{c} / |dc_i/dx|_{ip} \quad (1)$$

where the total average concentration of all considered compounds, \bar{c} , (either carriers or spacers or analytes) is expressed with the help of m_i and Δx as

$$\bar{c} = m_i / A \Delta x \quad (2)$$

According to the model adopted, \bar{c} can be considered as constant within the series of zones. In Fig. 1, the dimensions of x and c variables are bound by Eq. 2 and the zone concentration profiles are shown with $\bar{c} = 1$ and $\Delta x = 1$ arbitrary unit.

It follows from the definition that ω is not concentration dependent and that the narrower the Gaussian peak, the smaller is the ω obtained. These properties are similar to those of the standard deviation of the Gaussian peak, σ . However, the parameter ω can be defined also for other forms of zone concentration profiles, which opens up the possibility for the comparison of zones with different shapes.

The resolution of the separated zones is proportional to the level of purity of the respective zone or inversely proportional to the degree of mixing of the neighbouring zones. For qualitative analysis, it is more practical to relate the resolution to the detectability of zones of the individual components. Mathematically, it can be expressed with the ratio of the distance between the zones to a suitable feature of the concentration profile of the zone. For a Gaussian profile, the curve standard deviation, σ_i , is usually chosen [3,8,10,31–33,35].

Here, the resolution in the array of evenly spaced equal zones, R_z , is defined as the dimensionless quantity by the relationship

$$R_z = (\Delta x / \omega)^{1/2} \quad (3)$$

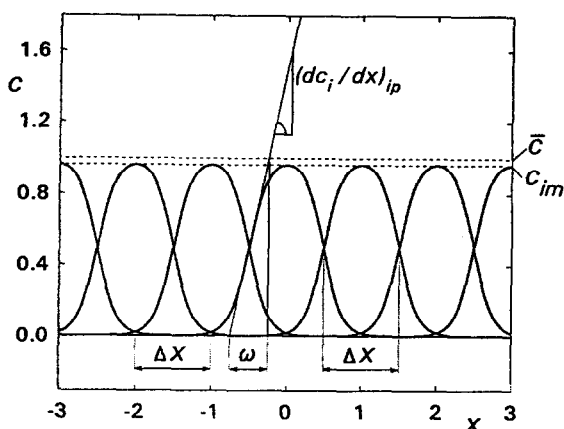


Fig. 1. Definition of the effective length of the concentration gradient. c_i = Component concentration; c_{im} = component concentration in the zone maximum; \bar{c} = average total concentration of the considered components in the zone array; x = zone array length coordinate; Δx = distance between the zone maxima or the distance between the zone boundaries; $(dc_i/dx)_{ip}$ = tangent of the concentration gradient at its inflection point; ω = effective length of the concentration gradient. The dimensions of variables c and x are bound by Eq. 2.

As R_z is based on ω , a comparison of the zone resolution between series of zones of different shape can be made.

The zone capacity, n_z , is defined as the upper limit of the resolvable zones for a given technique under the prescribed conditions [3,31,32]. It is obtained by allowing each hypothetical zone occupy a distance Δx . For the overall length of the series of the zones, L , it is

$$n_z = L/\Delta x \quad (4)$$

The zone capacity is dependent on the quality of the zone separation or, in other words, on the degree of the mutual mixing of the compounds in the neighbouring bands. It is usually expressed in terms of the resolution. As long as ΔU and $\Delta(\text{pH})$ are constant along L , the translation of R_z into information on the total maximum number of resolvable components can be made by combination of Eqs. 3 and 4:

$$n_z = L/(R_z^2 \omega) \quad (5)$$

or, again by virtue of Eq. 3,

$$n_z = L/[R_z(\Delta x \omega)^{1/2}] \quad (6)$$

The use of the above-mentioned definitions can be illustrated by Fig. 2, where some important simplified cases occurring in the steady-state electrophoretic methods are outlined. In Fig. 2a, a series of evenly spaced, overlapping Gaussian zones models the background of carrier ampholytes in IEF and in the focusing in the moving steady-state pH gradient. In Fig. 2b, a series of well separated, evenly spaced Gaussian peaks models the separated analytes in IEF and in some transition methods. In Fig. 2c, a series of equal nearly square-wave zones separated by symmetrical boundaries models the ITP of weak electrolytes with very small differences in their $\text{p}K_a$ values and IEF with a small number of carriers and/or with a large field intensity.

2.2. Explicit expressions for ω

Series of Gaussian zones

From the properties of the Gaussian curve, for the tangent at the inflection point we have

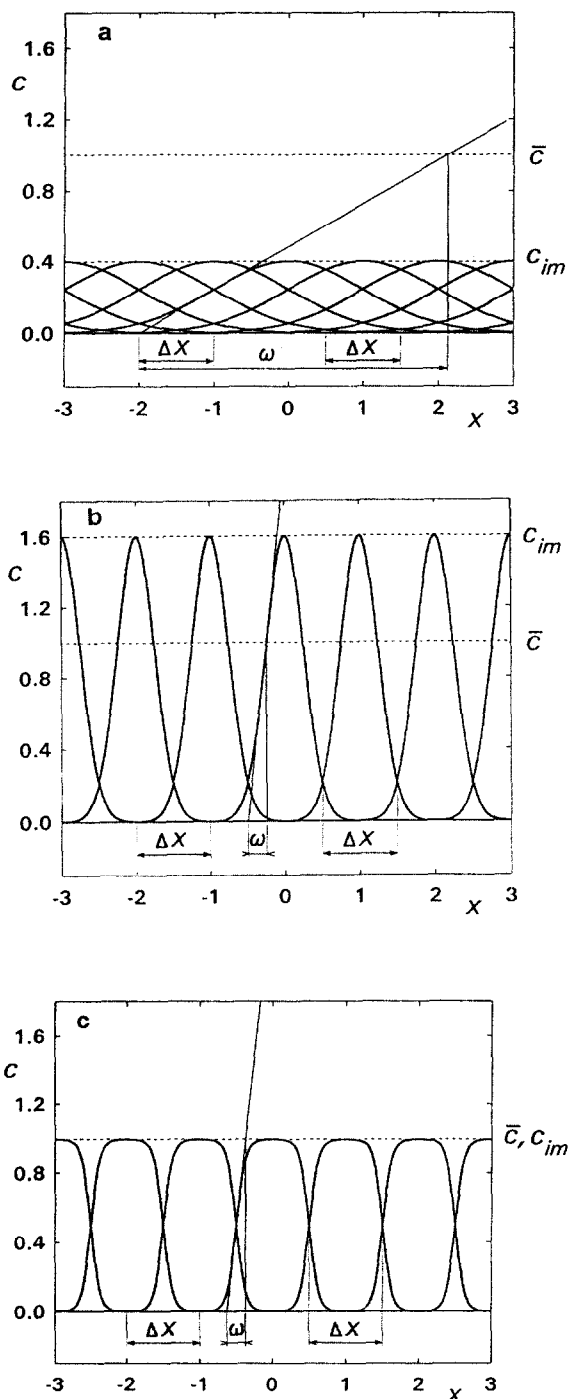


Fig. 2. Typical concentration profiles occurring in the separation of weak electrolytes by steady-state electromigration methods. (a) $\omega = 4\Delta x$; (b), (c) $\omega = 0.25\Delta x$. For explanation of symbols, see Fig. 1.

$$|dc_i/dx|_{ip} = c_{mi}/(e^{1/2}\sigma_i) \quad (7)$$

and m_i within the Gaussian peak can be calculated as

$$m_i = c_{mi}A\sigma_i(2\pi)^{1/2} \quad (8)$$

Eqs. 1, 2, 7 and 8 yield for the effective length of the concentration gradient in the series of equal Gaussian peaks, ω_p ,

$$\omega_p = (2\pi e)^{1/2}\sigma_i^2/\Delta x \quad (9)$$

According to the model adopted, for σ_i^2 in steady-state electrophoretic methods we can write [3,5,7,8,10,30]

$$\sigma_i^2 = RT/\{EF[-dz_i/d(\text{pH})][d(\text{pH})/dx]\} \quad (10)$$

where F is the Faraday charge, R is the gas constant and T is the absolute temperature. Then, by insertion of Eq. 10 in Eq. 9, we obtain for ω_p

$$\omega_p = (2\pi e)^{1/2}RT/\{FE\Delta x[-dz_i/d(\text{pH})][d(\text{pH})/dx]\} \quad (11)$$

The selectivity, or the relative difference in the mobilities of the compounds in the neighbouring zones, p , is introduced [15,18,32,42,48] as

$$p = \Delta\mu/\bar{\mu} \quad (12)$$

where $\Delta\mu$ is the difference in the effective mobilities of the compounds in the neighbouring zones and $\bar{\mu}$ is their mean. Since, in the model considered, $\bar{\mu}$ is also the effective mobility of the whole series of the zones, we have

$$p = [-d\mu_i/d(\text{pH})]\Delta(\text{pH})/\bar{\mu} \quad (13)$$

The effective mobility and effective charge of compound are bound by the Nernst relationship:

$$\mu_i = z_iFD_i/RT \quad (14)$$

Insertion of $\Delta(\text{pH}) = \Delta x[d(\text{pH})/dx]$ and Eq. 14 in Eq. 13 yields for p

$$p = \Delta x[d(\text{pH})/dx][-dz_i/d(\text{pH})]/z \quad (15)$$

Then, by use of Eq. 15, Eq. 11 can be written in the form

$$\omega_p = (2\pi e)^{1/2}RT/zFEp \quad (16)$$

Series of square-wave zones separated by symmetric boundaries

For low dissociation of weak electrolytes, $|z_i| \ll 1$, the ratio of the ion mobilities may be approximated by the ratio of the respective dissociation constants. For example, for weak acids, the selectivity can be then expressed as [15,39,48]

$$p = \Delta K_a/\bar{K}_a \quad (17)$$

where ΔK_a is the difference in the acidic K_a values of the compounds in the neighbouring zones and \bar{K}_a is their geometric mean. Generally, the boundary between the zones of the weak electrolytes in ITP is unsymmetric. For $p \ll 1$, which is of practical interest for examining the separation power, it can be considered as symmetric [15,39].

Let us consider a well developed square-wave zone which is symmetrical around the origin, $x = 0$, and the sigmoidal boundaries are symmetric around the $-\Delta x/2$ and $\Delta x/2$ coordinates, respectively. The concentration profile of the considered component in such a zone (see Fig. 2c) can be expressed as

$$c_i/\bar{c} = 1/\{1 + \exp[-\beta(x + 0.5\Delta x)]\} - 1/\{1 + \exp[-\beta(x - 0.5\Delta x)]\} \quad (18)$$

where β is the reciprocal distance [39]. For both weak anions and cations with $p \ll 1$, in the present notation we have

$$\beta = (zF/RT)(v/\bar{\mu})(\Delta K_a/\bar{K}_a) \quad (19)$$

where v is the linear velocity of the whole series of steady-state zones. The derivatization of Eq. 18 yields the tangent at the inflection point of the concentration profile in the boundary as

$$|dc_i/dx|_{ip} = \bar{c}\beta/4 \quad (20)$$

From Eqs. 1 and 20, the effective length of the concentration gradient in methods giving the symmetrical boundary between the weak electrolytes, ω_b , is

$$\omega_b = 4/\beta \quad (21)$$

Since for the potential field in the considered series of the zones

$$E = v/\mu \quad (22)$$

we obtain by insertion of Eqs. 17, 19 and 22 in Eq. 21

$$\omega_b = 4RT/FEzp \quad (23)$$

By comparison of Eqs. 16 and 23, we can see that the only differences are in the expressions for selectivity (see Table 1) and in the numerical coefficients. For the same E , z and p , the ratio of the respective equations may be summarized as $\omega_p = 1.033\omega_b$. The difference can be neglected and a numerical coefficient of 4 will be adopted in further calculations. The appropriate selectivity parameters used should be consistent with the compounds and method considered (see Table 1).

Transition from Gaussian to square-wave zones

The above-mentioned concentration profiles are certain limiting cases. Computer modelling has shown a smooth transition from one to another, e.g., by a continuous increase in E [1,2,8].

Let us consider the steady state depicted in Fig. 2a, where Gaussian peaks are depicted with $\sigma_i = \Delta x$ and $\omega = 4\Delta x$. Now, on increasing E by, e.g., factor 16, two cases may occur:

(a) When the system contains the background maintaining the continuous pH gradient [5,8,10], the Gaussian shape of the considered zones continues. This situation may be exemplified by

analytes in IEF (see Fig. 2b), where Gaussian peaks are shown with $\omega = 0.25\Delta x$.

(b) Without a background, the bands acquire the square-wave zones [1,2,8,30,43] (see Fig. 2c), where also $\omega = 0.25\Delta x$. Hence, for the small ω , the zone shape appears to be dependent on the presence of the background pH gradient.

For well developed square-wave zones, Δx can be considered as composed of two parts, namely the zone of the respective component with the length, l_i , and the effective length of the concentration gradient, ω :

$$\Delta x = l_i + \omega \quad (24)$$

In Fig. 2c, $l_i = 3\omega$. The limiting case, when $l_i = 0$, or when

$$\Delta x = \omega \quad (25)$$

may also be seen as a transition between square-wave and Gaussian zone shapes. At the transition point, the linear relationship between m_i and the zone height for Gaussian peaks starts to transform into a linear relationship between m_i and the zone length for square waves. Then, ω should be intermediate between the ω of the two typical cases, ω_p and ω_b . Since they are almost identical, ω in the transition point should not be very different from either of them. With the use of Eq. (23), condition (25) for the transition point can be represented as

$$\Delta x = 4RT/EFzp \quad (26)$$

Almgren [8] estimated the transition from Gaussian zones to square-wave zones in IEF from the calculated pH and conductivity profiles. The transition point was considered as the state when the calculated smooth linear pH profile begins to change into a staircase course, where, the steepness of the pH profile at the zone maximum is 15% lower and that at the midpoint between the zone maxima by 15% higher than the average gradient steepness. In the present notation, he found for this state (Eq. 47 in Ref. 8):

$$\Delta pI = \{4RT[d(\text{pH})/dx]/FE[-dz_i/d(\text{pH})]\}^{1/2} \quad (27)$$

which, by insertion of

Table 1
Summary of expressions for the selectivity, p , in steady-state electrophoretic modes for $p \ll 1$ and $|z_i| \ll 1$

System	Expressions for p
Weak acids, bases	$\Delta\mu/\bar{\mu}$, $\Delta z/z$ $\Delta K_a/\bar{K}_a$, $\Delta K_b/\bar{K}_b$ $\Delta(pK_a)[-d\mu_i/d(\text{pH})]/\bar{\mu}$ $\Delta(pK_a)[-dz_i/d(\text{pH})]/z$
Good ampholytes	$\Delta(pI)[-d\mu_i/d(\text{pH})]/\bar{\mu}$ $\Delta(pI)[-dz_i/d(\text{pH})]/z$

$$\Delta pI = \Delta x d(\text{pH})/dx \quad (28)$$

in Eq. 27 and squaring gives, after rearrangement,

$$\Delta x = 4RT/\{FE\Delta x[-dz_i/d(\text{pH})][d(\text{pH})/dx]\} \quad (29)$$

With Eq. 15, this leads finally to an equation that is identical with Eq. 26. It indicates that the expression of ω for the Gaussian or for the square-wave concentration profiles in steady-state electrophoretic methods can be applied also for the transition between these profile forms.

The profile transition may be illustrated by Fig. 3, where the concentration courses of the single zone are shown for ω values of $0.25\Delta x$, $0.5\Delta x$ and Δx . With the background pH gradient, the Gaussian curves 1, 2 and 5, respectively, result. Without the background and the same ω values, curves 3, 4 and 5, respectively, are obtained. Without the presence of the background pH gradient, the square-wave shape starts to develop at $\omega < \Delta x$.

2.3. Zone resolution

The zone resolution, R_z , was defined for the series of zones by Eq. 6. For Gaussian peaks, from Eqs. 6 and 9 we have

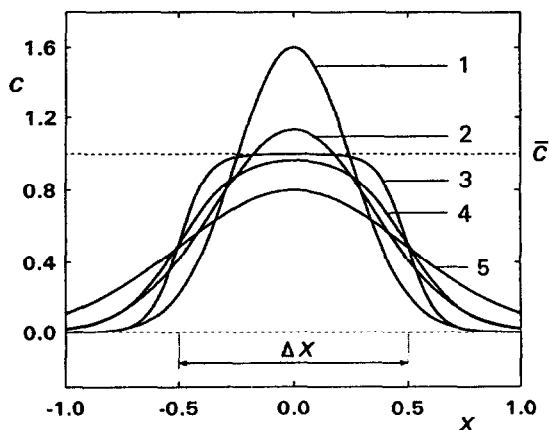


Fig. 3. Dependence of a single zone concentration profile on ω and on the presence of a background continuous pH gradient. Curves: 1, 2, 5 = with background pH gradient; 3, 4, 5 = without background; 1, 3, $\omega = 0.25\Delta x$, 2, 4, $\omega = 0.5\Delta x$; 5, $\omega = \Delta x$. For explanation of symbols, see Fig. 1.

$$R_z = (2\pi e)^{-1/4} \Delta x/\sigma_i = \Delta x/(2.033\sigma_i) \quad (30)$$

The resolution of Gaussian peaks is described in terms of R_s , defined as [3,10,32]

$$R_s = \Delta x/4\sigma_i \quad (31)$$

From Eqs. 30 and 31, the relationship between R_z and R_s for the series of Gaussian peaks is

$$R_z = R_s \cdot 2/1.016 \approx 2R_s \quad (32)$$

The ratio of R_z for peaks to R_z for the square zones is, by virtue of Eqs. 3, 16 and 23, only $(1.033)^{1/2} = 1.016$, which also justifies the common expressions for both zone forms.

For methods giving the zones modelled by Figs. 2 and 3, from Eqs. 3 and 23 we obtain

$$R_z = (FEzp\Delta x/4RT)^{1/2} \quad (33)$$

As Eqs. 15 and 28 yield

$$zp\Delta x = (\Delta pI)^2 \{[-dz_i/d(\text{pH})]/[d(\text{pH})/dx]\} \quad (34)$$

this indicates that Eq. 33 is equivalent to the previous relationships for the resolution of the Gaussian zones in IEF [3,10]. The only difference is in the numerical coefficient that relates R_z to R_s (see Eq. 32). In Fig. 2b and 2c, the zones are separated with the same $R_z = 2$. Both the Gaussian ($\Delta x = 4\sigma$) and square-wave zones ($l_i = 3\omega$, $\Delta x = 4\omega = 16/\beta$) may be considered as detectably resolved. The content of the pure compound in the respective Gaussian zone, Φ_p , is 95.4%. This content in the respective square-wave zone, Φ_b , can conveniently be calculated with the help of the boundary width, w , when the boundary is considered as a 1:1 mixed zone composed of the neighbouring pure compounds. With the help of Eq. 18, we obtain

$$w = 4 \int_{\Delta x/2}^{\infty} dx / \{1 + \exp[-\beta(x - 0.5\Delta x)]\} = 4 \ln 2/\beta \quad (35)$$

Apparently, it follows from Eqs. 21 and 35 that $w = \omega_b \ln 2$. Then, Φ_b is given by

$$\Phi_b = 1 - (w/2)/\Delta x \quad (36)$$

For $R_z = 2$, from Eqs. 3, 21, 35 and 36

$\Phi_b = 1 - \ln 2/8 = 91.3\%$. For ITP of the square-wave zones of strong electrolytes, it was suggested [42] that one can define the required resolution with the help of the same zone purity as that for the series of Gaussian peaks with $R_s = 1$. In the present notation, this means setting $\Phi_p = \Phi_b$ or $\Phi_b = 95.4\%$ of the respective compound. Then Δx should be $\Delta x = (\omega/2)/0.0456 = \omega \ln 2/0.0912 = 7.60\omega$ and, from Eq. 3, $R_z = 7.6^{1/2} = 2.75$.

For the definition of the just-resolved Gaussian zones in IEF, the following criterion is often used [7–9,33]:

$$\Delta x = 3.08\sigma_i \quad (37)$$

According to Eqs. 31, 32 and 37, this corresponds to $R_s = 0.77$ and $R_z = 1.54$. Φ_p is then 87.4%. When $R_z = 1.54$ is applied also for the square-wave zones, $\Phi_b = 85.4\%$. Despite the different approaches to the definition of the zone resolution, the differences in the zone purity can be considered as not critical for estimation of the separation power of the examined methods.

2.4. Zone capacity

For an array of Gaussian peaks, we obtain the known equation for estimation of the limiting peak capacity [3] by insertion of Eqs. 9 and 32 in Eq. 6:

$$n_z = L/(R_s 4\sigma_i) \quad (38)$$

It is usually accepted that $R_s = 1$ or, in the present notation, $R_z = 2$. If this value of R_z is considered adequate also for the square-wave and transition concentration profiles, we obtain by combination of Eqs. 6 and 23 for $R_z = 2$

$$n_z = [(L^2 FEz_p)/(16\Delta x RT)]^{1/2} \quad (39)$$

By insertion of Eq. 15, we can write Eq. 39 in the form known for the estimation of the peak capacity in IEF [3,10]:

$$n_z = \{L^2 FE[-dz_i/d(\text{pH})][d(\text{pH})/dx]/16RT\}^{1/2} \quad (40)$$

According to the model adopted, it is possible to introduce the potential drop across the whole

series of zones, U , as $U = -EL$ and the total pH difference across L , $\delta(\text{pH})$, as $\delta(\text{pH}) = L[d(\text{pH})/dx]$. The zone capacity can then be expressed in the form

$$n_z = \{FU\delta(\text{pH})[dz_i/d(\text{pH})]/16RT\}^{1/2} \quad (41)$$

which indicates that the maximum number of resolvable components should not be dependent on the length of the zone array. This conclusion is important for comparison of n_z in ITP and IEF, respectively. In IEF, L may theoretically be up to the whole length of the channel. In ITP, the length of the zone array is generally only a fraction of the channel length. When applying ITP to weak electrolytes with a small degree of dissociation, $|z_i| \ll 1$, the migration velocity of the steady-state zones is substantially smaller than the velocity of fully dissociated analytes in the adjusted sample zone. On the other hand, the undissociated analytes in the sample remain stationary until they are dissociated. In this way, the series of adjusted zones may occupy a substantial part of the channel length in ITP of the weakly dissociated electrolytes. Hence, according to the last-mentioned equations, the zone capacity of ITP may be close to that of IEF in the discussed cases.

As the final equations for the limiting zone capacity are based on ω , which is applicable to zones of different shapes, it is similarly valid for steady-state electrophoretic methods giving a series of Gaussian and square-wave zones and for zones with a shape that is intermediate between them.

3. Conclusions

Based on the above discussion, a comparison of the limiting zone capacities of the examined steady-state methods with the zone capacities of other electrophoretic methods can be made. Previously [3,10,49,50], the limiting peak capacities of capillary zone electrophoresis (CZE) and IEF were found to be similar. This can also be supported by Eq. 39 when the selectivity for CZE expressed as $p = \Delta x/L$ is

taken into consideration. Further, the zone capacities of ITP and CZE of strong electrolytes were found to be of the same order of magnitude [42]. Together with the relationships derived here, it can be concluded that, for the same total potential over the channel, the limiting zone capacities of IEF, ITP and CZE are comparable. Based on the zone concentration profiles discussed previously [30], similar expressions for n_z can also be expected for intermediate methods such as IEF with electrophoretic mobilization and isotachopheresis with polyampholytic spacers.

The above relationships were derived for symmetrical zones and boundaries. It can be expected that, owing to the applicability to the above-discussed different shapes of the concentration profiles, small deviations from symmetry will not cause serious changes in the estimation of the resolution or of the limiting zone capacity by the final equations.

The suggested description of the separation efficiency is fully compatible with the equations used so far. It shows that under some assumptions, the previous equations for the zone capacity can be used not only for the Gaussian peaks, but also for the square-wave zones of the weak electrolytes and the transition between the peak and the square wave. The similar expressions obtained and the values of the system parameters for the description of the separation efficiency of the different zone shapes give further support to the fundamental unity of the methods discussed.

References

- [1] W. Thormann and R.A. Mosher, in A. Chrambach, M.J. Dunn and B.J. Radola (Editors), *Advances in Electrophoresis*, Vol. 2, VCH, Weinheim, 1988, p. 45.
- [2] R.A. Mosher, D.A. Saville and W. Thormann, *The Dynamics of Electrophoresis*, VCH, Weinheim, 1992.
- [3] J.C. Giddings, *Unified Separation Science*, Wiley-Interscience, New York, 1991.
- [4] E. Schumacher, *Helv. Chim. Acta*, 40 (1957) 2322.
- [5] H. Svensson, *Acta Chem. Scand.*, 15 (1961) 325.
- [6] H. Svensson, *Acta Chem. Scand.*, 16 (1962) 456.
- [7] O. Vesterberg and H. Svensson, *Acta Chem. Scand.*, 20 (1966) 820.
- [8] M. Almgren, *Chem. Scr.*, 1 (1971) 69.
- [9] H. Rilbe, *Ann. N.Y. Acad. Sci.*, 209 (1973) 11.
- [10] J.C. Giddings and H. Dahlgren, *Sep. Sci.*, 6 (1971) 345.
- [11] W. Thorman, R.A. Mosher and M. Bier, *J. Chromatogr.*, 351 (1986) 17.
- [12] K. Shimao, *Electrophoresis*, 8 (1987) 14.
- [13] E. Schumacher and T. Studer, *Helv. Chim. Acta*, 47 (1964) 957.
- [14] F.M. Everaerts, J.L. Beckers and Th.P.E.M. Verheggen, *Isotachopheresis*, Elsevier, Amsterdam, 1976.
- [15] W. Thormann, *Sep. Sci.*, 19 (1984) 455.
- [16] P. Radi and E. Schumacher, *Electrophoresis*, 6 (1985) 195.
- [17] K. Shimao, *Electrophoresis*, 7 (1986) 121.
- [18] P. Boček, M. Deml, P. Gebauer and V. Dolník, *Analytical Isotachopheresis*, VCH, Weinheim, 1988.
- [19] S. Hjertén and M. Kiessling-Johansson, *J. Chromatogr.*, 550 (1991) 811.
- [20] S. Hjertén and M. Zhu, *J. Chromatogr.*, 346 (1985) 265.
- [21] S. Hjertén, J. Liao and K. Yao, *J. Chromatogr.*, 387 (1987) 127.
- [22] S. Hjertén, K. Elenbring, F. Kilár, J.L. Liao, A.J.C. Chen, C.J. Siebert and M.D. Zhu, *J. Chromatogr.*, 403 (1987) 47.
- [23] M.A. Firestone and W. Thormann, *J. Chromatogr.*, 436 (1988) 309.
- [24] F. Kilár and S. Hjertén, *Electrophoresis*, 10 (1989) 23.
- [25] M. Zhu, D.L. Hansen, S. Burd and F. Gannon, *J. Chromatogr.*, 480 (1989) 311.
- [26] B.T. Wehr, M. Zhu, R. Rodriguez, D. Burke and K. Duncan, *Am. Biotechnol. Lab.*, 22 (1990) 23.
- [27] M. Zhu, R. Rodriguez and T. Wehr, *J. Chromatogr.*, 559 (1991) 479.
- [28] F. Acevedo, *J. Chromatogr.*, 470 (1989) 407.
- [29] T. Manabe, H. Yamamoto and T. Okuyama, *Electrophoresis*, 10 (1989) 172.
- [30] K. Šlais, *J. Microcol. Sep.*, 5 (1993) 469.
- [31] J.C. Giddings, *Anal. Chem.*, 39 (1967) 1027.
- [32] J.C. Giddings, *Sep. Sci.*, 4 (1969) 181.
- [33] H. Svensson, *J. Chromatogr.*, 25 (1966) 266.
- [34] E. Glueckauf, *Trans. Faraday Soc.*, 51 (1955) 34.
- [35] A.S. Said, *Sep. Sci. Technol.*, 13 (1978) 647.
- [36] M. Bier, R.M. Cuddeback and A. Kopwille, *J. Chromatogr.*, 132 (1977) 437.
- [37] K. Shimao, *Electrophoresis*, 7 (1986) 297–303.
- [38] R.J. Routs, *Ann. N.Y. Acad. Sci.*, 209 (1973) 445.
- [39] W. Thormann and R.A. Mosher, *J. Trans. Soc. Comput. Simul.*, 1 (1984) 83.
- [40] M. Bier, R.A. Mosher and O.A. Palusinski, *J. Chromatogr.*, 211 (1981) 313.
- [41] L.M. Hjelmeland and A. Chrambach, *Electrophoresis*, 4 (1983) 20.
- [42] P. Gebauer and P. Boček, presented at the 4th International Symposium on High Performance Capillary Electrophoresis, Amsterdam, February 1992, abstracts, p. 90; *Electrophoresis*, in press.

- [43] E.V. Dose and G. Guiochon, *Anal. Chem.*, 63 (1991) 1063.
- [44] M. Bier, O.A. Palusinski, R. Mosher and D.A. Saville, *Science*, 219 (1983) 1281.
- [45] R.A. Mosher, D. Dewey, W. Thormann, D.A. Saville and M. Bier, *Anal. Chem.*, 61 (1989) 362.
- [46] V.G. Babskii, M.Yu. Zhukov and V.I. Yudovich, *Mathematical Theory of Electrophoresis*, Consultants Bureau, New York, 1989.
- [47] W. Thormann, R.A. Mosher and M. Bier, *J. Chromatogr.*, 351 (1986) 17.
- [48] P. Gebauer and P. Boček, *J. Chromatogr.*, 320 (1985) 49.
- [49] J.C. Giddings, *Anal. Chem.*, 53 (1981) 945A.
- [50] J.C. Giddings, *J. Chromatogr.*, 480 (1989) 21.