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Nondependence of Diffusion-Controlled Peak Dispersion on Diffusion Coefficient and Ionic Mobility in Capillary Zone Electrophoresis without Electroosmotic Flow

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The ratio of the ionic mobility, u , and the diffusion coefficient, D , in the expression for the plate number, N , given by $N = uU/2D$, (U being the applied voltage) for capillary zone electrophoresis without electroosmotic flow was substituted by the Einstein relation between these two parameters. As a consequence, the plate number is dependent exclusively on the charge number of the individual analyte, but not on u and D . The validity of this conclusion was demonstrated experimentally with anions of different charge number. Further, the plate number was found to depend on the charge of weak acids according to the degree of dissociation, as expected from theory.

INTRODUCTION

Peak dispersion in capillary zone electrophoresis can be described by a surprisingly simple theory, when only diffusion in the longitudinal direction, the direction of the electrophoretic migration of the ions, is considered. In this case (when dispersion due to the radial temperature profile within the capillary, and peak broadening caused by electroosmotic or hydrodynamic flow, by extracolumn effects and by the difference in electric conductivity between the analyte zone and the buffering electrolyte are neglected), the variance, σ_z^2 , of the solute peak, based on length, is dependent on the diffusion coefficient, D , and the residence time, t , given by

Einstein:

$$\sigma_z^2 = 2Dt \quad (1)$$

Connecting this expression with the relation of the variance on the migration distance, z gives

$$\sigma_z^2 = Hz \quad (2)$$

which is also valid for elution chromatography. An expression for the plate height, H , can be derived for the case, such that the limitations mentioned above are fulfilled (1, 2):

$$H = 2D/uE \quad (3)$$

where u is the ionic mobility and E is the electric field strength.

The plate number, $N = L/H$, which is decisive for the resolution of two components, is thus

$$N = uL/2D \quad (4)$$

U being the applied voltage along the length, L , of the separation capillary.

It can be seen from eqs 3 and 4, that the dispersion property of the separation system depends on an external parameter, the voltage (or the electric field strength $E = U/L$), and on parameters u and D , being specific for the particular analytes.

However, it must be taken into account that these two parameters are related properties: the ionic mobility describes the transport of ions along the gradient, $d\Psi/dz$ of the electric field, Ψ , whereas the diffusion coefficient stands for the transport of particles along the gradient, $d\mu/dz$, of the chemical potential, μ . Both parameters are connected by a relation given by Einstein (cf. e.g. ref 3):

$$D = \frac{u}{ze_0} kT \quad (5)$$

where z is the charge number of the ion, e_0 is the electronic charge, k is the Boltzmann constant, and T is the absolute temperature. It can be concluded from this equation, that the ratio u/D , occurring in the expression for the plate number, must have a distinct, constant value. This relation was mentioned by Giddings (4) and is experimentally evaluated here.

In this paper, the plate number, a measure for the peak dispersion, will be discussed with regard to the correlation of mobility and diffusion coefficient, and the conclusions of the theoretical findings will be supported experimentally for free capillary zone electrophoresis without electroosmosis. In subsequent papers, electroosmotic flow will be taken into consideration, too (5), and an appropriate explanation for the peak dispersion in zone electrophoresis with gel-filled capillaries will be given (6).

EXPERIMENTAL SECTION

Apparatus. The measurements were carried out with an instrument (P/ACE system 2000, Beckman, Palo Alto, CA), which was equipped with a UV-absorbance detector (254 nm). The fused-silica capillary used for zone electrophoresis had a total length of 57.4 cm (50.7 cm to the detector cell), and an inner diameter of 75 μm (Scientific Glass Engineering, Ringwood, Victoria, Australia). Since the measurements needed to be carried out without electroosmosis, the capillary was coated as described below.

The capillary was thermostated at 25 °C. Electrophoresis was performed at a total voltage of 10 or 5 kV, respectively, leading to currents of less than 25 μA in all buffers. Injection was carried out by pressure.

Reagents. Chemicals used for the preparation of the buffer solutions were either of analytical grade (*o*-phosphoric acid, sodium hydroxide; E. Merck, Darmstadt, Germany) or purissimum (lactic acid; Merck). The test substances were of synthesis grade (Merck),

Table I. Ratios of Ionic Mobility, u , and Diffusion Coefficient, D , for Anions with Different Charge Numbers^a

anion	D , 10^{-6} $\text{cm}^2 \text{ s}^{-1}$	u , 10^{-6} cm^2 $\text{V}^{-1} \text{ s}^{-1}$	$(u/D)^{\text{theor}}$, V^{-1}	$(u/D)^{\text{exp}}$, V^{-1}
benzene-1,3-disulfonate	7.17	57.2	77.9	79.8
1-naphthol-3,6-di-sulfonate	5.95	46.3	77.9	77.8
toluene-4-sulfonate	8.23	33.5	39.0	40.7
naphthalene-2-sulfonate	7.51	31.4	39.0	41.8
pyrocatechol violet ^b	4.83	18.6	39.0	38.5

^a The ionic mobilities and the diffusion coefficients were measured at 25 °C as described in the Experimental Section. $(u/D)^{\text{theor}}$: ratio calculated from eq 6. $(u/D)^{\text{exp}}$: ratio obtained from the measured values. ^b Pyrocatechol violet has the charge number 1 under these experimental conditions.

except 2,3-dihydroxybenzoic acid (purum; Fluka, Buchs, Switzerland) and disodium 1-naphthol-3,6-disulfonate (technical grade, Fluka). Pyrocatechol violet was the common metal indicator (Merck). For the coating procedure methylcellulose (Methocel MC, 3000–5000 mPa·s, Fluka) was used. The cross-linking reagents (formaldehyde and formic acid) were of analysis grade (Merck).

Water used as the solvent was distilled twice from a quartz apparatus.

Procedures. In order to prevent electroosmosis, the capillary surface was coated with methylcellulose at an elevated temperature using formaldehyde and formic acid as cross-linking agents according to the procedure described by Hjerten (7).

The mobilities of the sulfonic acids were calculated in the usual way from their migration times and distance (50.7 cm), in a buffering electrolyte at pH 5.1 (phosphate, 0.05 mol/L) at 25 °C.

The diffusion coefficients were determined by the stopped-flow method under conditions identical with those of the mobilities: the variances (second moments) of the peaks obtained after interrupting the electromigration for 2 h, 5 min after injection, were compared to the variances from the normal electropherogram. From the resulting increase of the variances due to the additional diffusion for 2 h the diffusion coefficients were calculated.

The pK_a values of the benzoic acids were determined by potentiometric titration at 25 °C.

RESULTS AND DISCUSSION

It can be seen from eq 5 that the diffusion coefficient D and the ionic mobility u are related quantities. Thus, the ratio u/D , which occurs in the equations for the plate height (eq 3) and the plate number (eq 4), respectively, can be expressed by

$$u/D = ze_0/kT = zF/RT \quad (6)$$

where F is the Faraday constant and R is the gas constant.

It follows that for a given temperature this ratio depends only on the charge number, z , of the ion and is independent of the nature of the particle. For a temperature of 25 °C, this ratio can therefore be numerically expressed by

$$u/D = 38.96z \quad (7)$$

when u is given in $\text{cm}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$ and D in $\text{cm}^2 \cdot \text{s}^{-1}$.

The proof of this assumption can be seen from the results given in Table I, where the ratio u/D is derived from experimental data for five different test substances with two different charge numbers, 1 and 2. The mobilities and the diffusion coefficients, also given in Table I, were measured with the electrophoretic equipment as described in the Experimental Section. The measuring error, expressed by the relative standard deviation for 10 measurements, is 0.67% for the determination of the mobilities and about 6% for the diffusion coefficients.

It can be seen from Table I that the ratios u/D , obtained from the experimental data agree within a few percents with

Table II. Dependence of the Plate Number, N , on the Electric Charge of Mono- and Divalent Anions at Two Different Voltages

anion	charge no.	plate number N			
		8.84 kV		4.42 kV	
		theor ^a	exp	theor ^a	exp
benzene-1,3-di-sulfonate	2	344 000	233 000	172 000	126 000
1-naphthol-3,6-di-sulfonate	2	344 000	232 000	172 000	141 000
toluene-4-sulfonate	1	172 000	137 000	86 000	69 000
naphthalene-2-sulfonate	1	172 000	134 000	86 000	71 000
pyrocatechol violet	1	172 000	122 000	86 000	66 000

^a According to eq 9.

the data theoretically derived from the physical constants e_0 , k , and T , according to eq 7.

The ratio u/D (eq 6), only depending on the charge number, is substituted in eq 3 for the plate height or in eq 4 for the plate number, thus obtaining the following expressions (4, 8, 9):

$$H_i = 2kT/z_i e_0 E = 2RT/z_i F E \quad (8)$$

$$N_i = z_i e_0 U / 2kT = z_i F U / 2RT \quad (9)$$

An analogous expression was discussed for the case that electroosmosis occurs (5) and for zone electrophoresis with gel-filled capillaries (6).

It can be seen from the equations given above, that the plate height as well as the plate number are not dependent on the individual diffusion coefficients or ionic mobilities of the ionic species but (under given experimental conditions) are dependent exclusively on the charge number z_i of the ions. This is the only individual quantity that is decisive for the peak dispersion, because the voltage (or field strength) and the temperature, occurring in these equations, are external parameters, being equal for all ionic species.

For the given experimental conditions, namely for an applied voltage of 10 kV (which leads to a potential drop of 8.84 kV along the migration distance) and for a temperature within the capillary of 25 °C, the plate number is given by $N_i = 172000z_i$. Thus, a maximum plate number of 172 000 can be expected for all monovalent ions with charge number 1 (independent of the particle), 344 000 plates can be expected for all divalent ions ($z = 2$), etc. The plate number for different ions must linearly increase with the charge, according to eq 9. It must be mentioned that the plate number, given by eq 9, is obviously independent of the sign of the electric charge; for a given charge number, it is equal for anions as for cations. For neutrals, it is zero.

It can be seen from Table II, that the experimental values agree with those predicted by theory. For the sulfonic acids

under investigation with $z = 1$, between 122 000 and 137 000 plates are measured, and over 230 000 plates for substances with $z = 2$.

The deviations from the theoretical values (the values found are about 20–25% lower) can be explained by peak distortion caused by different conductivities of the sample zone and buffering electrolyte on the one hand and by Joule heating on the other hand.

The contribution of extracolumn effects to peak broadening can be neglected, because a very narrow input profile was generated due to injection of the samples from pure water as solvent. Compared to injection from buffer as solvent, a drastic peak sharpening is obtained in this way. Peak dispersion in the detector cell is also negligible, because the detector has an aperture of only 200 μm , thus contributing much less than 1% to the total variance (10).

An even better result is found when the peak dispersion is determined as a function of the charge number at a lower voltage, namely 5 kV (4.42-kV effective voltage) and the same temperature of 25 °C. As the voltage is halved compared with the above given conditions, the plate number is also halved, leading to a theoretical value of 86 000 plates per charge number under this experimental realization. The resulting experimental values for the test substances given in Table II show the agreement with the theoretical values as discussed above.

The charge number must depend on the degree of dissociation, α , which is given by the pK_a value of the substance and the pH of the buffering electrolyte. For this case, eqs 8 and 9 can be rearranged, taking into account that the effective charge number is then equal to α , leading to the expressions

$$H = 2kT/\alpha e_0 E \quad (10)$$

$$N = \alpha e_0 U / 2kT \quad (11)$$

in the case of monofunctional acids and bases.

In order to prove this dependence of N on α , the degree of dissociation of three weak electrolytes (substituted benzoic acids with pK_a values of 2.83, 3.53, and 4.58, respectively) was varied by varying the pH of the buffer in the range between 2.5 and 5.6, and the resulting peak dispersion was measured. The plate number, obtainable for the fully dissociated monobasic acids, has a maximum value of 172 000 under the given conditions. Due to experimental reasons (mainly peak distortion due to conductivity differences and Joule heating, as mentioned), values about 20% lower were found, being in the range of 140 000. Thus, for these monobasic acids it is expected that in practice the plate numbers should depend on the degree of dissociation by $N = 140000\alpha$.

The results of the measurements (obtained at a voltage of 10 kV) are given in Table III. It can be seen, that the degree of dissociation, and therefore the effective charge number, varies by a factor of about 5 from 0.22 to 1. The plate numbers at small α are in fact significantly lower than 140 000, namely

Table III. Dependence of the Plate Number, N , on the Net Charge, Corresponding to the Degree of Dissociation, α , for Substituted Benzoic Acids, Using Buffering Electrolyte Systems of Different pHs

pH	acid (pK_a)								
	2,3-dihydroxybenzoic acid (2.83)			2,3-dimethoxybenzoic acid (3.53)			2,4-dimethoxybenzoic acid (4.58)		
	N	α	N/α	N	α	N/α	N	α	N/α
2.5	39 000	0.30	130 000						
3.0	84 000	0.63	133 000	40 000	0.27	148 000			
3.3	97 000	0.74	132 000	49 000	0.37	132 000			
3.5	111 000	0.82	135 000	67 000	0.48	139 000			
4.0	139 000	0.94	148 000	105 000	0.77	136 000	33 000	0.22	149 000
4.5	142 000	0.98	144 000	121 000	0.92	132 000	66 000	0.47	140 000
5.0	130 000	0.99	131 000	124 000	0.97	128 000	94 000	0.72	130 000
5.6	139 000	1.00	139 000	140 000	1.00	140 000	130 000	0.90	144 000

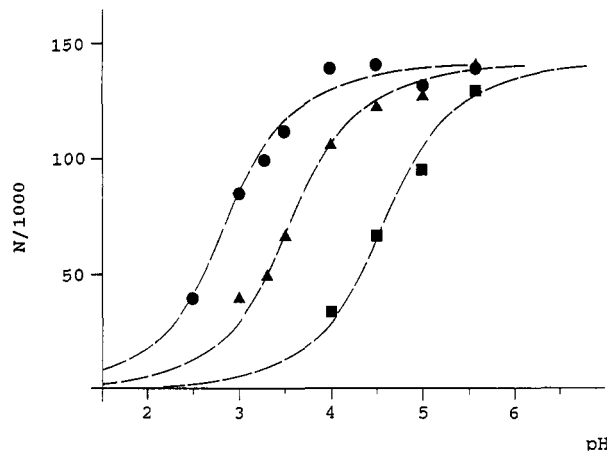


Figure 1. Dependence of the plate number, N , for different anions on the degree of dissociation via the pH of the buffering electrolyte. The net charge of the anions of the weak acids is determined by the degree of dissociation, depending on the pK_a of the acids and the pH of the electrolyte system. The broken curves indicate the theoretical dependence of the plate number on pH (see text). Experimental values for N : (●) 2,3-dihydroxybenzoic acid (pK_a 2.83); (▲) 2,3-dimethoxybenzoic acid (pK_a 3.53); (■) 2,4-dimethoxybenzoic acid (pK_a 4.58).

between 30 000 and 40 000. They increase with increasing pH (and increasing α). At pH values where the acids are fully dissociated, about 140 000 plates are reached.

The experimentally found dependence of the plate number on α , or pH, respectively, can be clearly seen from Figure 1, where the theoretical dependence of N on α as given above, and the function of α on pH, given by $\alpha = 1/(1 + 10^{pK - pH})$, is depicted by the dotted curves. The data points obtained experimentally are in excellent agreement with the theoretical curves, supporting again our basic presumption about the correlation of the plate number on the charge.

The validity of the relation between N and α for the given experimental conditions can also be seen from the ratio N/α , which should be 140 000. In fact, it can be seen from Table III that for all acids and all pH values this requirement is fulfilled.

Therefore it can be concluded, that under the limitations mentioned in the Introduction, the peak dispersion in capillary zone electrophoresis without electroosmotic flow depends

solely on, besides external parameters like temperature and voltage, the effective charge as the single individual property, but not on the diffusion coefficient or ionic mobility, although it is diffusion controlled.

This result is also important for the interpretation of the zone electropherograms, observed from nucleotides or proteins in gel-filled capillaries, where no electroosmosis occurs, too. Oligo- and polynucleotides, e.g., are separated according to their base number and are eluted with increasing charge number. Thus, it can be predicted from eq 9 that the plate number increases within the homologous series of the oligonucleotides. For a 100 mer, plate numbers in the order of 10 millions or more can therefore be reached theoretically.

In fact, it can be seen from published electropherograms (e.g. see refs 11–13) that the peak widths (which are based on time) remain about constant. Since the migration velocity decreases with increasing base number, and therefore the velocity of the components through the detector also decreases, it follows that the peak widths, based on length, are decreasing with increasing base number. This means, that according to eq 2, the plate height also decreases. Therefore, according to our presumption, an "efficiency gradient" can be observed within such an electropherogram, which is caused by the properties of the analytes, due to their increasing charge number, and is at least qualitatively in accordance with eq 9. A quantitative description of this effect is given in ref 6.

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