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Relationship between zone length and step height in isotachophoresis

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ABSTRACT

The relationship between response factor (*RF*) and specific zone resistance at 25°C (SZR_{25}) in isotachophoresis was evaluated and a linear relationship between *RF* and SZR_{25} values could be established for strong ionic components. Working at a constant electric current, the *RF* value is proportional to the zone length (seconds) per mole of component. Because the SZR_{25} values are linearly related to the step heights in the isotachopherograms, the zone lengths per mole of sample component must be linearly related to the step heights of the sample components. If this relationship is measured for a standard mixture with an equimolar composition, all other unknown sample components can be quantified from the step heights in a single experiment. This principle was verified experimentally for several anionic and cationic leading electrolyte systems at different pH values.

INTRODUCTION

Isotachophoresis (ITP) is a well known separation technique for the qualitative and quantitative analysis of ionic components using differences in effective electrophoretic mobilities. The separation mechanism is well defined and can be described by mathematical models [1–3] and over a wide range applications have been reported [4–8]. Further, the method can be used for the determination of several physico-chemical parameters such as absolute mobilities and p*K* values [9,10].

When applying a separation technique in the analysis of an unknown sample, quantitative information cannot be easily obtained. Generally, the sample composition has to be determined first, after which calibration graphs have to be set up, before quantitative analyses are possible. A unique feature of ITP is that, in contrast to most other separation techniques, the step heights in the isotachopherograms contain both qualitative and quantitative information. In this paper, the relationship between response factor (*RF*) and step height is discussed.

THEORY

When the steady state in ITP is reached, all sample zones migrate with equal velocity, v_{ITP} (cm/s):

$$v_{\mathrm{ITP}} = m_{\mathrm{L}}E_{\mathrm{L}} = m_i E_i$$

where m_i, m_{L}, E_i and E_{L} are the mobilities (cm^2/V · s) of a sample ion *i* and the leading ion L and the electric field strengths (V/cm) in the corresponding zones, respectively.

For a not too high applied electric current density, a linear relationship between step height and the specific zone resistance at 25°C (SZR_{25}, Ω m) is obtained, applying an a.c. detector [11]. Hence if for two standard components, with known p*K* values and mobilities at infinite dilution, the SZR_{25} values are calculated using a mathematical model for the steady state in ITP, a linear relationship between step height and SZR_{25} values can be set up. Applying this relationship, the SZR_{25} value of an unknown sample component can be calculated from its step height. If the SZR_{25} values for an unknown sample component are determined for two different electrolyte systems, its mobility and p*K* value can be calculated with the concept of the isoconductor [10]. For a known pK value, *e.g.*, for strong ionic species, the mobility can be obtained from a single experiment.

For quantification of sample components, the response factor RF (C/mol) can be used, representing the slope of a calibration graph of the product of the zone length ZL (s) and the applied current I (A) *versus* the amount of the sample component Q (mol):

$$RF = \frac{ZL \cdot I}{Q}$$

Once the mobilities and pK values of sample components are known, the RF values can easily be calculated [11]. This means that if for a given electrolyte system the relationship between step height and SZR_{25} value is established from a single experiment, both quantitative and qualitative parameters can be obtained for strong, monovalent ionic species.

TABLE I

MOBILITIES AT INFINITE DILUTION, m (cm^2/V · s), AND pK VALUES FOR IONIC SPECIES USED IN THE CALCULATIONS AND COMPOSITIONS OF SEVERAL ELECTROLYTE SYSTEMS

Ionic species	$m \cdot 10^5$	pK
Acetic acid	−42.4	4.76
β-Alanine	36.7	3.552
ε-Aminocaproic acid	28.8	4.37
Creatinine	37.2	4.828
Histidine	29.6	6.04
Hydrochloric acid	−79.1	−2.0
Imidazole	52.0	7.15
MES[a]	−28.0	6.1
Potassium	76.2	14.0
Tris[b]	29.5	8.1

Anionic electrolyte systems (electrolyte: 0.01 M HCl)

pH	Counter ion	Terminator
3.2	β-Alanine	Propionic acid
4.0	ε-Aminocaproic acid	Pivalic acid
5.0	Creatinine	Pivalic acid
6.0	Histidine	MES[a]
7.0	Imidazole	−

Cationic electrolyte systems (electrolyte: 0.01 M KOH)

pH	Counter ion	Terminator
4.3	Acetic acid	Acetic acid
5.0	Acetic acid	Acetic acid
6.0	MES[a]	Histidine

[a] MES = 2-(N-Morpholino)ethanesulphonic acid.

[b] Tris = Tris(hydroxymethyl)aminomethane.

Relationship between RF and SZR_{25}

In Table I the mobilities at infinite dilution and the pK values of several ionic species and the compositions of several electrolyte systems, used in the calculations and experiments, are given. In order to study the relationship between SZR_{25} values and quantitative parameters, we calculated for monovalent ions with an SZR_{25} value of 50 Ω m, assuming a certain pK value, the mobilities at infinite dilution, the total concentration, the ionic concentration, the zone pH and RF value for a leading electrolyte system of 0.01 M HCl adjusted to pH = 3.2 by adding β-alanine. In Table II, all calculated values are given, and it can be concluded that for components with equal SZR_{25} values (*i.e.*, identical step heights and effective electrophoretic mobilities), the mobility at infinite dilution, the total concentration and the pH increase with increasing pK values, whereas the ionic concentration and the RF values decrease. This means that without further information no quantitative information can be obtained from a single step height in this way.

For strong ionic species, we calculated for the same electrolyte system as used for Table II, for different mobilities at infinite dilution, the SZR_{25} values, the ionic concentration (equal to the c_{tot}),

TABLE II

CALCULATED MOBILITY AT INFINITE DILUTION, m (cm^2/V · s), TOTAL CONCENTRATION, c_{tot} (mol/l), IONIC CONCENTRATION, c (mol/l), ZONE pH AND RF VALUES (10^5 C/mol) FOR A COMPONENT WITH A SZR_{25} VALUE OF 50 Ωm IN A LEADING ELECTROLYTE OF 0.01 M HCl AT pH 3.2 FOR DIFFERENT pK VALUES OF THE COMPONENT

pK	$m \cdot 10^5$	c_{tot}	c	pH	RF
−1.0	−13.78	0.0033	0.0033	3.79	5.12
2.0	−13.96	0.0033	0.0033	3.79	5.05
2.5	−14.37	0.0034	0.0033	3.80	4.91
3.0	−15.50	0.0037	0.0032	3.81	4.56
3.5	−18.73	0.0044	0.0031	3.86	3.82
4.0	−26.42	0.0059	0.0029	3.95	2.85
4.5	−41.69	0.0081	0.0024	4.11	2.07
5.0	−69.07	0.0105	0.0019	4.31	1.60

TABLE III

CALCULATED SZR_{25} VALUES (Ω m), IONIC CONCENTRATION, *c* (mol/l), pH AND *RF* VALUES (10^5 C/mol) FOR STRONG MONOVALENT IONIC SPECIES WITH DIFFERENT MOBILITIES AT INFINITE DILUTION, *m* ($cm^2/V \cdot s$), IN A LEADING ELECTROLYTE OF 0.01 *M* HCl AT pH 3.2

$m \cdot 10^5$	SZR_{25}	*c*	pH	*RF*
−70	9.08	0.0095	3.23	1.77
−60	10.64	0.0089	3.28	1.89
−50	12.85	0.0081	3.33	2.08
−40	16.20	0.0071	3.40	2.35
−30	21.89	0.0060	3.50	2.81
−20	33.57	0.0044	3.65	3.77
−10	70.71	0.0025	3.92	6.83

pH and *RF* values. All calculated values are given in Table III, and indicate that there is a linear relationship between SZR_{25} and *RF* values. In order to check whether such a linear relationship is valid for all electrolyte systems, we calculated the SZR_{25} and *RF* values for strong ionic species for several anionic and cationic electrolyte systems at different pHs. The compositions of the electrolyte systems are given in Table I. In Table IV all calculated values are given and in Fig. 1 the relationships between *RF* and SZR_{25} values are shown for (A) anionic and (B) cationic systems. For cationic systems at a low pH, the contribution of the hydrogen ions to the zone conductivity is large, resulting in an elongation of the zones and higher *RF* values, especially for components with low effective mobilities. In

TABLE IV

CALCULATED VALUES FOR SZR_{25} (Ω m) AND *RF* (10^5 C/mol) FOR STRONG IONIC SPECIES IN SEVERAL ELECTROLYTE SYSTEMS AT DIFFERENT pH

$m \cdot 10^5$ ($cm^2/V \cdot s$)	Anions									
	pH 3.2		pH 4		pH 5		pH 6		pH 7	
	SZR_{25}	*RF*	SZR_{25}	*RF*	SZR_{25}	*RF*	SZR_{25}	*RF*	SZR_{25}	*RF*
−70	9.08	1.77	11.41	1.39	10.88	1.46	11.72	1.35	9.75	1.65
−60	10.64	1.89	13.38	1.46	12.77	1.55	13.75	1.42	11.43	1.77
−50	12.85	2.08	16.18	1.57	15.43	1.68	16.62	1.52	13.80	1.94
−40	16.20	2.35	20.44	1.73	19.48	1.87	21.00	1.66	17.41	2.20
−30	21.89	2.81	27.68	2.00	26.36	2.19	28.46	1.92	23.52	2.64
−20	33.58	3.77	42.67	2.58	40.55	2.88	43.90	2.46	36.08	3.56
−15	45.65	4.74	58.22	3.18	55.24	3.59	59.94	3.02	49.02	4.52
−10	70.71	6.83	90.77	4.45	85.88	5.09	93.55	4.22	75.91	6.52

	Cations					
	pH 4.3		pH 5		pH 6	
	SZR_{25}	*RF*	SZR_{25}	*RF*	SZR_{25}	*RF*
70	10.10	1.57	10.26	1.54	11.80	1.33
60	11.87	1.68	12.01	1.64	13.83	1.39
50	14.29	1.84	14.52	1.79	16.70	1.48
40	17.98	2.10	18.36	2.01	21.10	1.62
30	24.32	2.59	24.74	2.40	28.73	1.86
25	29.42	3.06	30.03	2.73	34.85	2.06
20	37.06	3.96	38.07	3.25	44.27	2.37
15	–	–	51.64	4.21	60.52	2.91
10	–	–	79.03	7.14	94.53	4.07

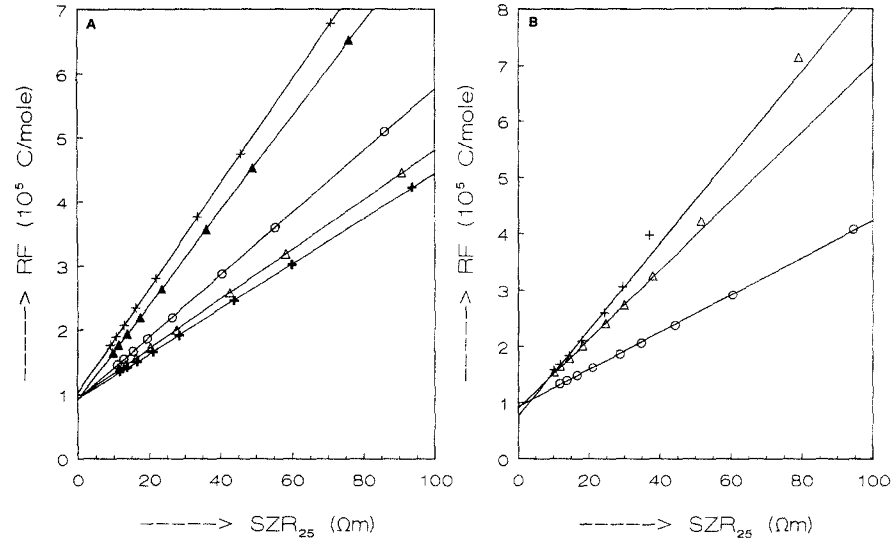

Fig. 1. Calculated relationship between response factor (RF, C/mol) and specific zone resistance at 25°C (SZR_{25}, Ω m) for several strong monovalent ions in (A) anionic electrolyte systems at pH (+) 3.2, (△) 4, (○) 5, (+) 6 and (▲) 7 and (B) cationic electrolyte systems at pH (+) 4.3, (△) 5 and (○) 6.

fact, the mathematical model for ITP is not valid because a moving boundary system is present. For the regression lines in Fig. 1, the deviating RF values are not taken into account.

From Fig. 1, it is clear that in all electrolyte systems linear relationships are obtained between SZR_{25} and RF values. If the mobility of the sample component is very high, the influence of the counter ions on the SZR value is almost zero and hence the y-intercept is about $1 \cdot 10^5$ C/mol, the value of the Faraday constant.

Working at a constant electric current, the RF value for a sample component will be proportional to the ratio of the zone length (s) to the injected amount of the sample component. Because the RF value is linearly related to the SZR_{25} and this parameter is linearly related to the step height, the zone lengths must be linearly related to the step heights for equimolar sample compositions. If this is true in practice, it is a unique advantage of ITP that if the relationship between zone length and step height is measured with a sample of equimolar composition, for all unknown samples (independent of the kind of ionic species) the quantitative composition can be determined directly. For strong divalent ions the same relationship between zone length and step height holds, although the RF value and zone length are twice as large because the concentration in its zone is half the concentration of monovalent ions. Under Results and Discussion these relationships are established for different anionic and cationic sample compositions in different leading electrolyte systems.

EXPERIMENTAL

Instrumentation

For all ITP experiments a laboratory-built apparatus [1] with conductivity and UV detectors (254 nm) was used. In this apparatus a closed system is obtained by shielding the separation capillary from the open electrode compartments with semipermeable membranes. A PTFE capillary tube (0.2 mm I.D.) was used. The sample was introduced with a syringe.

Chemicals

All chemicals were of analytical-reagent grade. Before preparing the sample solutions, all chemicals were dried at 105°C.

RESULTS AND DISCUSSION

To check experimentally whether a linear rela-

TABLE V

MEASURED STEP HEIGHTS, H, AND FROM THESE STEP HEIGHTS CALCULATED MOBILITIES AT INFINITE DILUTION, m (cm^2(V · s), SPECIFIC ZONE RESISTANCES AT 25°C, SZR_{25} (Ω m), CALCULATED RESPONSE FACTORS, RF (10^5 C/mol), EXPERIMENTALLY DETERMINED RF VALUES (10^5 C/mol) AND ZONE LENGTHS, ZL (s), FOR SEVERAL COMPONENTS IN AN EQUIMOLAR SAMPLE COMPOSITION IN DIFFERENT ELECTROLYTE SYSTEMS

Component	H	$m \cdot 10^5$	SZR_{25}	RF		ZL
				Calc.	Exptl.	
Leading electrolyte potassium acetate at pH 4.3, electric current 2 μA, injection volume 1 μl for given zone length						
Silver	18.0	61.54	11.55	1.66	1.64	127.6
Barium	19.7	65.01	12.08	3.32	3.34	134.0
Sodium	24.4	52.50	13.56	1.80	1.74	201.0
TMA[a]	32.8	44.37	16.21	1.97	1.91	152.0
TEA[b]	51.8	32.68	22.20	2.43	2.31	179.0
TRIS	59.9	29.50	24.74	2.63	2.49	195.0
TBA[c]	97.4	–	35.42	–	3.27	253.0
Leading electrolyte potassium acetate at pH 5.0, electric current 2 μA, injection volume 1 μl for given zone length						
Silver	14.6	62.24	11.57	1.62	1.59	117.0
Sodium	18.7	53.99	13.43	1.72	1.68	199.0
Lithium	30.3	39.20	18.70	2.04	1.96	150.0
TEA	39.0	32.65	22.65	2.27	2.16	169.0
TRIS	44.7	29.50	25.32	2.43	2.40	182.5
TBA	68.5	21.04	36.03	3.12	3.08	234.0
Leading electrolyte histidine chloride at pH 6.0, electric current 20 μA, injection volume 3 μl for given zone length						
Chlorate	23.0	67.16	12.23	1.37	1.44	35.6
Fluoride	27.8	57.99	14.26	1.43	1.45	36.5
Sulfamate	32.0	51.50	16.12	1.50	1.55	38.8
Chloroacetate	41.2	41.82	20.05	1.63	1.65	43.3
Benzoate	53.2	34.03	25.15	1.80	1.86	47.0
Octylsulfonate	66.1	27.97	30.70	2.00	2.06	52.3

[a] TMA = tetramethyl ammonium bromide.
[b] TEA = tetraethyl ammonium bromide.
[c] TBA = tetrabutyl ammonium bromide.

tionship can be established between RF values and step heights, we determined the RF values by measuring the zone lengths for different amounts of monovalent strong cationic and anionic sample mixtures at an equimolar sample composition for different electrolyte systems. From the step heights we further calculated the mobilities at infinite dilution and the theoretical RF values. In Table V the measured step heights, the calculated mobilities at infinite dilution, the calculated SZR_{25} values, the calculated and measured RF values and the zone lengths of the components in the sample of equimolar composition are given. The concentrations of all monovalent sample components were 0.00154 M for the cations and 0.00167 M for the anions. The RF values were determined by measuring the zone lengths for different injection volumes. In the cationic mixture, the barium concentration was 0.00077 M and the measured zone length fits those of the monovalent cations.

In Fig. 2, the experimentally determined relationships between (A) step heigth H and specific zone resistance SZR_{25}, (B) response factor RF and SZR_{25}, (C) zone length ZL of the equimolar sample composition and the step height H and (D) response factor RF and step height H for cations in a leading electrolyte system at pH 4.3 and 5 and anions at pH 6 are given (see Table V for further conditions). It can be concluded that all relationships are linear. The y-intercepts in Fig. 2B approximate the expected value of the Faraday constant. When choosing the sample composition, special care must be taken to avoid zone elongation due to impurities originating from the electrolyte system.

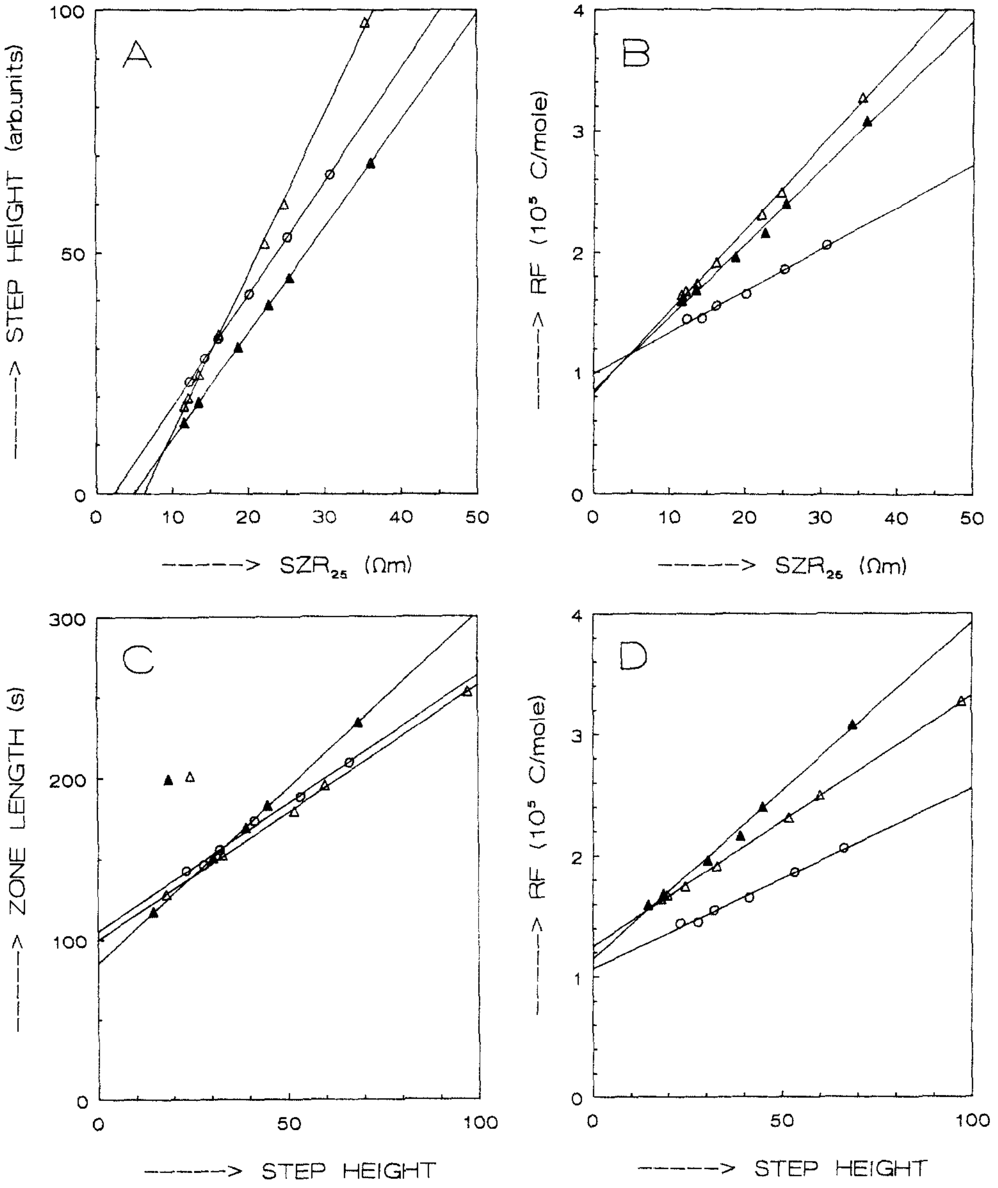

Fig. 2. Experimentally determined relationship between (A) step height H and specific zone resistance SZR_{25}, (B) response factor RF and SZR_{25}, (C) zone length ZL for an equimolar sample composition and H and (D) RF and H for cations in a leading electrolyte system at pH (△) 4.3 and (▲) 5 and anions at pH (○) 6. For further information, see Table V.

For this reason, different sample components are chosen for the different electrolyte systems. Often impurities present in the solvent and the electrolyte systems show a constant zone length, *i.e.*, the component present in the sample mixture with the same step height will have a constant elongation of its zone length. In that case, the zone length of that component does not fit the relationship between zone length and step height, whereas it does fit the relationship between RF value and step height, as the slopes of the calibration graph with and without this impurity are identical. This can be seen in Fig.

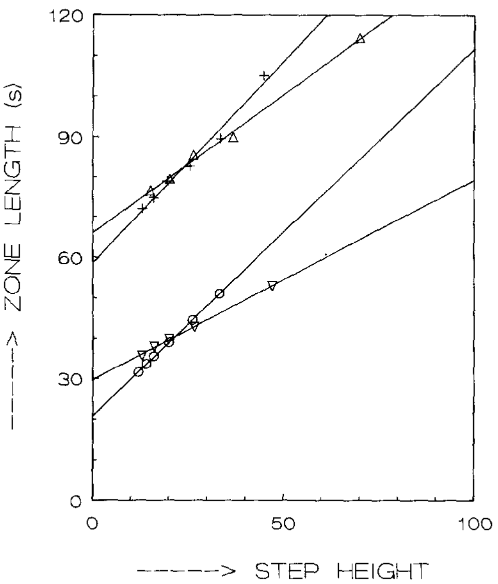

Fig. 3. Experimentally determined relationship between zone length and step height for anionic electrolyte systems at pH (○) 3.2 (10 μA), (+) 4 (10 μA), (△) 5 (10 μA) and (▽) 6 (20 μA). For further information, see text.

2C, where the zone length of sodium (present in the electrolyte system) is much too long, whereas in Fig. 2D the measured *RF* values fit the linear relationship between *RF* and step height.

As a last example, the zone lengths of a mixture with an equimolar composition of 0.00167 *M* chlorate, bromate, sulphamate, iodate, phosphate (only present at pH 3.2 and 4) and octylsulphonate were measured in anionic leading electrolytes at pH 3.2 (concentration of the leading ion 0.005 *M*), 4, 5 and 6. In Fig. 3, the relationships between zone length and step height are given. For all electrolyte systems linear relationships can be observed.

We also repeated several separations at different electric currents. For high electric currents, lower step heights and smaller zone lengths were observed, owing to the higher temperature of the system resulting in higher electrophoretic mobilities. Nevertheless, for all these systems, when applying different electric currents linear relationships were obtained.

CONCLUSIONS

A linear relationship between *RF* and SZR_{25} values was established theoretically for strong anions and cations. The zone lengths per mole of component are proportional to the *RF* values, applying a constant direct current, and the step heights are proportional to the SZR_{25} values. Hence a linear relationship can be expected between the zone lengths for an equimolar sample composition and the step heights of the components. This principle was checked for several anionic and cationic components in different electrolyte systems at different pH values. The results demonstrate that isotachophoresis has the unique advantage that, if the sample components are unknown, for strong ionic species quantitative information can be obtained in a single experiment. This can be of interest in, *e.g.*, the study the kinetics and reaction mechanisms of unknown intermediates.

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