ELSEVIER

Fingerprint of anions involving pK_a, mobility and concentration established by using capillary isotachophoresis

Mieczysław Wroński

Department of Chemical Technology and Environmental Protection, University of Łódź, Pomorska 163, 90-236 Łódź, Poland

Abstract

The suggested fingerprint of anions is constructed in a square, two sides of which form the pK_a and absolute mobility, u_0, axis, the other two the concentrations axis in log scale. Every acid is demonstrated by a pK_a-u_0 point and by a dash to the point indicating concentration. The absorption in UV is marked by a small triangle. The suggested experimental procedure involves the isotachophoretic examination of the sample in four buffers of pH 3.25, 4.50, 6.25 and 6.25. The last one prepared from bis-tris propane is of use for discrimination between mono-, di- and trivalent acids from the decrease of mobility. The pK_a values of monovalent acids are found from the established relationship between mobility ratio at different pH and pK_a. The pK_2 values of divalent acids are calculated by assuming that $pK_2 = pH$ at $u = 0.75\ u_{02}$. The quantity of an acid injected on the column n, nanomol, can be calculated from the equation,

$$n = tiu_0(96.5)^{-1}(u + u_{ap})^{-1}$$

where t = zone length (s), i = current (μA), u and u_0 = effective and absolute mobility, u_{ap} = apparent mobility of counter ion. Based on the established equation,

$$\Delta u_{max} = 0.35 u_0 \Delta pK_a$$

where Δu_{max} = maximum attainable difference between effective mobilities of two anions of the same absolute mobility as function of pH_L, ΔpK_a = difference of pK_a values. A new approach to the problem of resolution in isotachophoresis is demonstrated by marking a sphere of inseparability round the point in fingerprint system.

Keywords: Isotachophoresis; Ionization constants; Organic acids; Organic anions

1. Introduction

The enormous variety of known chemical compounds in the environment including natural substances, pollutants and additives are determined using procedures described in analytical standards. On the other hand the problem of new appearing pollutants not reported anywhere is often overlooked. The aim of this paper is to demonstrate the possibility of the determination of the characteristics of unknown compounds in the environment as the first step for identification. Based on isotachophoretic measurements an approach to calculation of absolute mobilities and pK_a values is presented. Results can be shown by plotting them in a mobility–pK_a–concentration scheme, which is very suitable for comparison of different samples.

The problems involved in the determination of pK_a, absolute mobility and concentration of unknown substances is discussed in various books [1,2] and papers, e.g., Refs. [3,4]. However, the derived equations are rather complicated and hardly applicable in

analytical practice. The idea followed in this paper is a continuation of the previously reported one [5,6]. When the explicit relationship between two variables cannot be satisfactory solved, it is replaced by an empirical representation using a set of standards. For example: the pK_a value of monovalent acid in buffers x and y can be given by

$$pK_a = pH_x + \log\frac{u_0 - u_x}{u_x} = pH_y + \log\frac{u_0 - u_y}{u_y} \qquad (1)$$

where pH_x and $pH_y = pH$ of the buffers, u_0, u_x and u_y = absolute and effective mobilities. The rearrangement of Eq. (1) gives

$$\frac{u_x}{u_y} = \frac{1 + 10^{(pK_a - pH_y)}}{1 + 10^{(pK_a - pH_x)}} \qquad (2)$$

As pH_x = constant, pH_y = constant or dependent on migrating zones of pK_a, the pK_a must be explicitly a function of the ratio of mobilities. But the real values of pH_x and pH_y in migrating zones are not known and the solution of Eq. (2) for pK_a is rather complicated. Taking this into account it has been suggested, based on the standards, to use empirical equations relating the mobility ratio and pK_a. Of course the validity of the equations is limited to the procedure involving buffers and mobility calculation.

The effective mobility of an ion u_x can be calculated from isotachophoretic results using an equation suggested by Boček et al.:

$$\frac{1}{u_x} = \frac{1}{u_L} + \left(\frac{1}{u_s} - \frac{1}{u_L}\right)\frac{h_x}{h_s} \qquad (3)$$

where u_L = mobility of the leading ion, usually chloride, $u_L = 79 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}, u_s = mobility of the standard, in this paper trichloroacetate, $u_s = 36.2 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}, h_x and h_s = step heights of the ion X and of the standard ion, respectively.

Inserting the mobilities, Eq. (3) can be written in the form

$$u_x = \frac{79}{1 + 1.18h} \qquad (4)$$

where $h = h_x/h_s$.

Eq. (4) was used for calculation of mobility.

In the course of identification of acids it is often important to discriminate between mono- and polyacids. It is well known [2] that divalent counter ions

decrease the effective mobility of divalent ions much stronger than monovalent ones. For example, the calcium ion and diamines as bis-trispropane [7] decrease the mobility of sulphate without essentially influencing the mobility of monovalent ions. It has been investigated in this paper whether the ratio of mobilities in buffers containing and not containing bistrispropane can be evaluated for discrimination between mono- and polyacids.

In the present calculations the results obtained in this laboratory in addition to results published by Hirokawa et al. [8] were used.

2. Experimental

The measurements were carried out using an isotachophoretic analyser ZK 2 from Labeco (Spišská Nová Ves, Slovak Republic), with preseparation and analytical columns of 160 mm×0.8 mm I.D., 0.3 mm. The driving current was 250 μA and 50 μA, respectively.

The essential set of buffers is summarized in Table 1. The buffers A, B and C were used for determination of mobility and step lengths at three pH values. The ratio of mobilities obtained using buffers C and D was evaluated for discrimination between mono-, di- and trivalent acids.

All chemicals were of analytical-reagent grade. Stock standard solutions of carboxylic acids were prepared at a concentration of 10 mM in three times distilled water and stored at 4°C.

Table 1
Operational electrolyte system used in isotachophoresis

	Leading electrolyte		Terminating electrolyte
	Counter-ion	pH	
A	β-Ala	3.25	0.01 M capronic acid
B	EACA	4.50	0.01 M nicotinic acid
C	Hist	6.25	0.01 M MES
D	BTP	6.25	0.01 M MES

Leading ion = 0.01 M chloride, additive = 0.05% MHEC.
β-Ala = β-alanine, EACA = ε-aminocaproic acid, MES = 2-(N-morpholino)ethanesulfonic acid, BTP = 1,3-bis(tris)hydroxymethyl(methylamino)propane, MHEC = methylhydroxyethylcellulose.
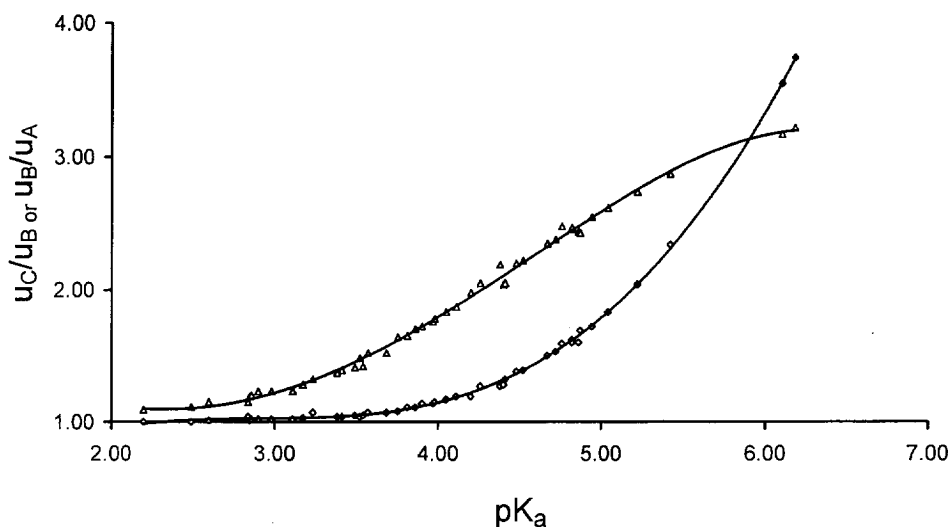
Fig. 1. Relationship between the ratio of mobilities and pK_a values of monovalent acids. $\triangle\triangle\triangle$ u_B/u_A, $\Diamond\Diamond\Diamond$ u_C/u_B.

3. Results and discussion

3.1. Calculation of pK_a values

Fig. 1 and Table 2 demonstrate the ratios of mobilities and the pK_a values of monovalent acids including aspartic and glutamic acids which behave like monovalent acids. The best approximation of the evident relationship can be expressed by the following equations,

$$pK_a = \left[\left(\frac{u_B}{u_A} - 1 \right) \cdot 330 \right]^{0.25},$$

RV $= 1.1 - 2.4\, u_B/u_A$, RSD $= 0.06$ \hfill (5)

$$pK_a = \left[\left(\frac{u_B}{u_A} - 1 \right) \cdot 8.5 \right]^{0.625},$$

RV $= 2.3 - 3.2\, u_B/u_A$, RSD $= 0.13$ \hfill (6)

$$pK_a = \left[\left(\frac{u_C}{u_B} - 1 \right) \cdot 1.1 \cdot 10^5 \right]^{0.142},$$

RV $= 1.1 - 4.0\, u_C/u_B$, RSD $= 0.10$ \hfill (7)

where RV = range of validity of the equation, RSD = residual standard deviation calculated from equation,

$$\text{RSD} = \sqrt{\frac{\sum(y_i - y)^2}{N - 2}}$$ \hfill (8)

where y_i and y = observed and calculated values of pK_a, N = number of acids taken into account.

The pK_a values calculated from Eqs. (2–4) provide almost the same values for monovalent acids but differ in the case of divalent acids. The difference decreases with decreasing difference between first and second pK values of divalent acids. For eaxmple, acetic acid, $pK_a = 4.76$, Eq. (6): 4.87, Eq. (7): 4.82, glutaric acid, $pK_1 = 4.34$, $pK_2 = 5.27$, Eq. (6): 4.70, Eq. (7): 4.90. In the case of high difference between pK_2 and pK_1 of divalent acids, the pK_1 values can be calculated using Eq. (5), as demonstrated in Table 3. The error is rather high because the u_B/u_A values are placed outside of the validity range.

Another approach to the problem of discrimination between mono- and polyvalent acids may be based on different retardation of mobility by divalent cations. This effect may be demonstrated as the ratio of mobilities determined at the same pH, 6.25, using either histidine or BTP as counter ion as in buffer C and D.

As can be derived from Table 4, the ratios for monovalent acids vary between 1.00 and 1.06 whereas for divalent acids between 0.93–0.97 and 0.83 for citric acid, which is sufficient for discrimination.

The calculation of pK_2 values of divalent acids was performed assuming that at $pH_L = pK_2$ the

Table 2
The ratio of mobilities of monovalent acids determined in buffers A, B and C arranged according to increasing value of pK_a

No.	Acid	pK_a	u_B/u_A	u_C/u_B
1	2,3-Dibromopropionic	2.20	1.09	1.00
2	Pyruvic	2.49	1.11	1.00
3	2-Chloro-3-hydroxybutyric	2.60	1.15	1.01
4	3,5-Dinitrobenzoic	2.84	1.15	1.04
5	o-Brombenzoic	2.85	1.19	1.01
6	Chloroacetic	2.86	1.20	1.02
7	Bromoacetic	2.90	1.23	1.02
8	2-Bromopropionic	2.98	1.23	1.02
9	Salicylic	3.11	1.23	1.02
10	Phenoxyacetic	3.17	1.28	1.03
11	Sulfanilic	3.23	1.32	1.07
12	3-Nitro-4-bromobenzoic	3.38	1.37	1.04
13	Mandelic	3.41	1.39	1.04
14	Aspirin	3.49	1.41	1.05
15	p-Nitrobenzoic	3.52	1.48	1.04
16	o-tert.-Butylbenzoic	3.54	1.42	1.05
17	Metoxyacetic	3.57	1.52	1.07
18	Glucuronic	3.68	1.52	1.07
19	Formic	3.75	1.64	1.08
20	m-Bromobenzoic	3.81	1.65	1.11
21	Lactic	3.86	1.70	1.11
22	Aspartic	3.90	1.72	1.14
23	p-Bromobenzoic	3.97	1.76	1.14
24	p-Chlorobenzoic	3.98	1.78	1.15
25	o-Bromophenyloacetic	4.05	1.83	1.17
26	2,4-Dinitrophenol	4.11	1.87	1.19
27	Benzoic	4.20	1.98	1.19
28	Acrylic	4.26	2.05	1.27
29	Glutamic	4.38	2.19	1.27
30	p-tert.-Butylbenzoic	4.40	2.04	1.28
31	Phenylacetic	4.41	2.05	1.32
32	Metacrylic	4.48	2.20	1.38
33	3-Hydroxybutyric	4.52	2.22	1.39
34	Allylacetic	4.67	2.35	1.50
35	5-Bromovaleric	4.72	2.38	1.53
36	Acetic	4.76	2.48	1.59
37	Butyric	4.82	2.46	1.62
38	Nicotinic	4.82	2.47	1.62
39	Benzylaspartic	4.86	2.44	1.60
40	Propionic	4.87	2.43	1.69
41	o-Aminobenzoic	4.94	2.55	1.72
42	Trimethylacetic	5.04	2.62	1.83
43	2,4,-Dinitrophenol	5.22	2.74	2.04
44	3,4-Dinitrophenol	5.42	2.87	2.34
45	MES	6.10	3.17	3.55
46	Cacodylic	6.18	3.22	3.74

effective mobility is equal to 0.75 u_{02}. Assuming the linear relationship between mobility and pH_L in the ranges 4.50–6.25 and 3.25–4.50, the following equation can be derived,

Table 3
Calculation of pK_1 values of some divalent acids using Eq. (5)

No.	Acid	u_B/u_A	Literature		pK_1, calc.
			pK_1	pK_2	
1	Hippuric	1.10	2.70	7.27	2.40
2	Phosphoric	1.06	2.12	7.47	2.11
3	Arsenic	1.05	2.19	6.94	2.02
4	Citraconic	1.10	2.46	6.15	2.40
5	Diethylmalonic	1.03	2.21	7.29	1.77
6	Ethylpropylmalonic	1.05	2.15	7.43	2.02

$$pK_2 = \frac{1.75}{u_C - u_B}(0.75u_{02} - u_B) + 4.50 \qquad (9)$$

In the case when 0.75 $u_{02} < u_B$ the following equation has to be used,

$$pK_2 = \frac{1.25}{u_B - u_A}(0.75u_{02} - u_A) + 3.25 \qquad (10)$$

Table 4
The ratios of mobilities in buffers C and D as the basis for discrimination between mono-, di- and trivalent acids

No.	Acid	u_C/u_D
1	Acetic	1.00
2	Propionic	1.03
3	Chloroacetic	1.04
4	Lactic	1.03
5	Pyruvic	1.00
6	Aspartic	1.00
7	Glutamic	1.00
8	Sulfanilic	1.01
9	p-Nitrobenzoic	1.04
10	Benzoic	1.02
11	Phenylacetic	1.00
12	3,5-Dinitrobenzoic	1.05
13	Salicylic	1.00
14	3-Chloropropionic	1.03
15	Oxalic	0.90
16	Malonic	0.88
17	Succinic	0.95
18	Adipic	0.97
19	Malic	0.94
20	Fumaric	0.93
21	Ketoglutaric	0.93
22	Tartaric	0.94
23	Phthalic	0.95
24	Terephthalic	0.95
25	Hippuric	0.97
26	Phosphoric	0.97
27	Citric	0.83

Table 5
Calculation of absolute mobilities, u_0, (10^{-9} m^2 s^{-1} V^{-1}) using values determined in buffer B and C and Eq. (11)

No.	Acid	Literature		u_B	u_C	u_0 calc.
		u_0	pK_a			
1	Acetic	42.4	4.76	26.3	41.7	42.6
2	Propionic	37.1	4.87	21.4	36.1	37.3
3	2,5-Dinitrophenol	31.3	5.22	14.0	28.6	30.9
4	3,4-Dinitrophenol	31.3	5.42	11.9	27.8	31.2
5	MES	28.0	6.10	5.86	20.8	27.7
6	Cacodylic	29.9	6.18	5.69	21.3	29.3

The results obtained are demonstrated in Table 6. The standard deviation (S.D.) amounts to 0.23.

3.2. Calculation of absolute mobilities

For monovalent acids with $pK_a < 4.5$, the absolute mobilities and effective mobilities calculated for buffer C, pH 6.25, using Eq. (4) are practically the same, $u_0 = u_C$. At $pK_a > 4.5$ the following empirical equation can be recommended,

$$u_0 = \frac{8.6 u_B u_C}{10 u_B - u_C} \tag{11}$$

The utility of Eq. (11) is demonstrated in Table 5.
For calculation of second absolute mobility of divalent acids the following equation was derived,

$$u_{02} = \frac{9.8 u_B u_C}{10 u_B - u_C} \tag{12}$$

The results obtained using Eq. (12) are summarized in Table 6.

3.3. Calculation of concentration of unknown compounds

In order to calculate the concentration of an acid in the sample the number of moles and volume applied on column must be known. The number of injected moles can be calculated by using the equation,

$$n = \frac{t i u}{z F (u + u_{ap})} \tag{13}$$

where t = step length, i = driving current, u = effective mobility, z = relative charge, F = Faraday constant, u_{ap} = apparent counter ion mobility inserted instead of u_k (counter ion mobility) because the value is not constant. By insertion of the ratio u/u_0 for z Eq. (13) becomes,

$$n = \frac{t i u_0}{F (u + u_{ap})} \tag{14}$$

It must be emphasized that Eq. (14) provides

Table 6
Calculation of second absolute mobility of divalent acids, u_{02}, (10^{-9} m^2 s^{-1} V^{-1}) using Eq. (12) and calculation of pK_a values using Eqs. (9,10) on the basis of mobilities determined in buffers A, B and C

No.	Acid	u_A	u_B	u_C	u_{02}, lit.	u_{02}, calc.	pK_2, lit.	pK_2, calc.
1	Oxalic	47.0	64.9	70.3	77.7	77.3	4.27	4.00
2	Malonic	34.8	42.0	57.6	67.0	65.4	5.70	5.43
3	Succinic	14.2	29.3	51.3	60.9	60.9	5.64	5.80
4	Glutaric	11.5	28.5	48.3	55.6	57.0	5.27	5.67
5	Adipic	10.6	26.2	44.7	52.4	52.8	5.28	5.74
6	Malic	22.8	40.4	54.3	59.0	61.5	5.05	4.98
7	Fumaric	29.6	49.2	57.3	61.2	63.6	4.38	4.29
8	Ketoglutaric	32.3	39.0	53.7	59.0	61.0	5.01	5.13
9	Tartaric	29.6	50.8	54.9	60.7	60.3	4.37	4.19
10	Phthalic	25.6	31.1	45.5	52.9	52.2	5.41	5.54
11	Terephthalic	22.1	40.0	47.0	53.0	52.2	4.46	4.48
12	3-Ethylglutaric	11.2	26.0	43.0	50.0	50.5	5.30	5.68
13	Ethylmalonic	21.6	26.7	41.7	51.0	48.4	5.83	5.93
14	Glutaconic	17.7	34.6	49.2	56.0	56.2	5.08	5.39
15	Itaconic	16.5	30.3	48.2	56.0	56.2	5.45	5.64

reliable results only when the value of u_{ap} is found by calibration for every buffer,

$$u_{ap} = \frac{tiu_0}{Fn} - u \tag{15}$$

For divalent acids $z = 2u/u_{02}$, then,

$$n - \frac{tiu_{02}}{2F(u + u_{ap})} \tag{16}$$

and

$$u_{ap} = \frac{tiu_{02}}{2Fn} - u \tag{17}$$

The examination of u_{ap} values calculated for 32 monovalent acids revealed a clear relationship with pK_a of the acids. After passing a range of constant values, the u_{ap} increases with increasing pK_a, and can be expressed by the following equations,

Buffer A $u_{ap} = 54 + 6(pK_a - 2)$, RSD = 3.0   (18)

Buffer B $u_{ap} = 31 + 8.5(pK_a - 3.4)$, RSD = 1.5

$$\tag{19}$$

Buffer C $u_{ap} = 30 + 6.7(pK_a - 5)$, RSD = 0.90

$$\tag{20}$$

In the case of divalent acids no relationship between the u_{ap} and pK_a values could be found and only mean values were calculated, viz. A: $u_{ap} = 54$, S.D. = 8, B: $u_{ap} = 37$, S.D. = 5, C: $u_{ap} = 37$, S.D. = 1.

When the values of u_{ap}, u_0 and u_{02} are known, there is no problem in calculating the concentration of any acids revealed by isotachophoretic examination using Eqs. (14,16).

3.4. Evaluation of separability of anions

The possibility of isotachophoretic separation of ions depends essentially on the difference of their mobilities. Theoretically such differences can be expressed as a function of pK_a and pH, but practical application of derived equations is hardly possible. The approach followed in this paper is based on the established relationship between the difference in mobilities of two acids at the point of maximum, Δu_{max}, difference of pK_a values, ΔpK_a, and absolute

mobility which is the same for both acids. The examination of 25 pairs of monovalent acids revealed an interesting relationship expressed in the equation,

$$\frac{\Delta u_{max}}{u_0 \Delta pK_a} = 0.35, \text{ S.D.} = 0.012 \tag{21}$$

A few results are demonstrated as examples in Table 7.

The pH_L corresponding to Δu_{max}, $pK_{L,max}$ is given by,

$$pH_{L,max} = 0.5(pK_x + pK_y) - 0.47, \text{ S.D.} = 0.03$$

$$\tag{22}$$

where x and y = two acids having the same absolute mobility.

The established relationship can be utilized for construction of a sphere of inseparability round the point in the u_0, pK_a system as demonstrated in Fig. 2. Assuming the minimum difference of mobilities necessary for separation to 0.035 u_0 what corresponds to $\Delta pK_a = 0.1$, a cross can be designed of each arm corresponding to the same value. In fields

Table 7
Relationship between maximum difference of mobilities, Δu_{max}, 10^{-9} m^2 s^{-1} V^{-1}, difference of pK_a and absolute mobility u_0

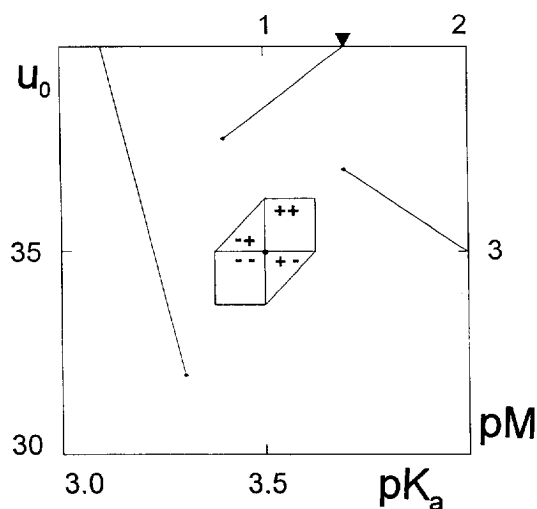| No.	Pairs of acids	pK_a	u_0	Δu_{max}	$\dfrac{\Delta u_{max}}{\Delta pK_a u_0}$
1	o-Isopropylbenzoic	3.635	24.7	6.13	0.345
	p-Isopropylbenzoic	4.354	24.7		
2	o-Isopropylbenzoic	3.635	24.7	9.44	0.341
	4-Phenylbutyric	4.757	24.7		
3	2,3-Dimethylbenzoic	3.738	27.1	4.24	0.352
	2,4-Dimethylbenzoic	4.182	27.1		
4	o-Aminobenzoic	4.939	31.6	0.965	0.355
	p-Aminobenzoic	4.853	31.6		
5	Acetic	4.756	42.4	13.3	0.359
	Glycolic	3.886	42.4		

Fig. 2. The suggested fingerprint design and the sphere of inseparability round a point, $u_0 = 35 \cdot 10^{-9}$ m^2 s^{-1} V^{-1}, p$K_a = 3.5$. Small triangle means UV absorption.

$+ +$ and $- -$ the contribution of Δu_0 and ΔpK_a weaken and in fields $+ -$ and $- +$ strengthen each other. Consequently the spheres of inseparability in fields $+ +$ and $- -$ are larger as compared to fields $+ -$ and $- +$. Δu in fields $+ +$ and $- -$ as a function of pH, increases to a maximum, decreases to zero, changes sign and finally attains the value Δu_0. In fields $+ -$ and $- +$ Δu increases to a maximum and decreases to u_0 without passing zero. The rule of separation can be expressed as follows: two acids can be separated if they do not belong to the same sphere of inseparability.

The concentration scale on fingerprint, Fig. 2, is given in pM values, where p$M = -\log M$, $M =$ molar concentration.

4. Conclusion

The presented methods of examination based on isotachophoresis have been established in order to identify and determine unknown acids found in the environment or formed in the course of chemical reactions. The contribution to identification may also be based on the relationship between structure and mobility [9]. The final proof of identity must be based on examination of suggested compounds. This approach may well be feasible when only a few compounds are present in the sample. In complicated mixtures the formation of mixed zones and the unknown succession of compounds on the isotachopherogram may be difficult to overcome. In such cases the preliminary separation of the mixture into a few groups, e.g., by preparative isotachophoresis [10], may be recommended.

The suggested fingerprint design may be very useful for rapid comparison of different samples and for evaluation of separability.

References

[1] F.M. Everaerts, J.L. Beckers and Th.P.E.M. Verhegen, Isotachophoresis—Theory, Instrumentation and Applications, Elsevier, Amsterdam, 1976.
[2] P. Boček, M. Deml, P. Gebauer and V. Dolnik, Analytical Isotachophoresis, VCH, Weinheim, 1988.
[3] J. Pospichal, P. Gebauer and P. Boček, Chem. Rev., 89 (1989) 419.
[4] T. Hirokawa and Y. Kiso, Anal. Sci., 8 (1992) 737.
[5] M. Wroński, J. Chromatogr. A, 672 (1994) 273.
[6] M. Wroński, Chem. Anal., Warsaw, 40 (1995) 911.
[7] I. Zelensky, V. Madajová, D. Kaniansky, P. Havaši and V. Lednárová, J. Chromatogr., 294 (1984) 317.
[8] T. Hirokawa, M. Nishino, N. Aoki, Y. Kiso, Y. Sawamoto, T. Yagi and J. Akiyama, J. Chromatogr., 271 (1983) D$_1$.
[9] M. Wroński, J. Chromatogr. A, 657 (1993) 165.
[10] T. Hirokawa and Y. Kiso, J. Chromatogr. A, 658 (1994) 343.