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## ISOTACHOPHORESIS

## THE QUALITATIVE SEPARATION OF CATION MIXTURES

J. L. BECKERS AND F. M. EVERAERTS

*Department of Instrumental Analysis, Eindhoven University of Technology, Eindhoven  
(The Netherlands)*

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SUMMARY

The formulae needed for computations on buffered systems are given. Using these formulae, a computer program was evaluated and calculations were correlated with the results of some experiments.

The possibility of the simultaneous separation of cations depends on the differences between the effective mobilities, which can be affected by the choice of pH, counter-ion and solvent.

The qualitative simultaneous separation of cations was studied in eight electrolyte systems using methanol and water as solvents. In each system, simultaneous separations of 7-10 cations were possible, which could be increased by combining electrolyte systems.

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## INTRODUCTION

Several techniques are available for the quantitative and qualitative analyses of ions, and each has its own disadvantages and limitations. Because of mutual interactions of the ions, simultaneous separations are sometimes troublesome. Great differences in concentrations may be a source of difficulties, and sometimes complicated pre-treatments are necessary.

Isotachophoresis is a technique with many advantages in comparison with other well known techniques. Simultaneous separations of ions are possible<sup>1-3</sup>, but until now the possibilities of analysing ions have not been fully studied. In forthcoming papers we shall describe some results of experiments carried out on the qualitative and quantitative separations of cations and anions, including the theory and some phenomena characteristic of the systems chosen.

The theory of buffered systems and some qualitative aspects of the separation of cations are discussed in this paper, and some operational systems are presented.

The principle of this technique is that ionogenic compounds from the sample are separated in consecutive zones, which are ordered according to the effective mobilities of the ionic species in these zones. The concentrations are adjusted to the concentration of the leading electrolyte chosen. The effective mobility of the leading ion is larger than any of the sample ions. The velocities of all the zones are equal and

constant if a stabilised current is applied. The last zone is formed by the terminator, the effective mobility of which is lower than that of any of the sample ions.

#### THEORETICAL

The ability to separate ions by electrophoretic techniques depends on differences between the effective mobilities, and therefore features that influence the mobility are important. Some of these are discussed here.

The absolute mobility is proportional to the equivalent conductance at zero concentration:

$$\Lambda_0^* = (m_0^+ + m_0^-) \cdot F \quad (1)$$

The effective mobility is correlated with the absolute mobility.

The most important phenomena that influence the effective mobility are: relaxation and electrophoretic effects; partial dissociation; and solvation.

#### *Relaxation and electrophoretic effects*

ONSAGER derived for the relaxation and electrophoretic effects<sup>4</sup> the following expression:

$$\Lambda^* = \Lambda_0^* - \alpha^* \sqrt{c^*} (|n^+| + |n^-|) \quad (2)$$

where:

$$\alpha^* = \frac{0.985 \cdot 10^6}{(DT)^{\frac{3}{2}}} \cdot \frac{2q}{1 + \sqrt{q}} \cdot (|n^+| \cdot |n^-|) \Lambda_0^* + \frac{29(|n^+| + |n^-|)}{(DT)^{\frac{3}{2}} \eta_0} \quad (3)$$

$$q = \frac{|n^+| \cdot |n^-|}{|n^+| + |n^-|} \cdot \frac{\lambda_0^+ + \lambda_0^-}{|n^+| \lambda_0^- + |n^-| \lambda_0^+} \quad (4)$$

For methanol as solvent:

$$\alpha^* = 1.15 \cdot \frac{2q}{1 + \sqrt{q}} \cdot |n^+ \cdot n^-| \Lambda_0^* + 55.3(|n^+| + |n^-|) \text{ (at } 25^\circ \text{C)} \quad (5)$$

For water as solvent:

$$\alpha^* = 0.274 \cdot \frac{2q}{1 + \sqrt{q}} \cdot |n^+ \cdot n^-| \Lambda_0^* + 21.14(|n^+| + |n^-|) \text{ (at } 25^\circ \text{C)} \quad (6)$$

To compare the effects in different solvents of different charges on the cations, we calculated the effective mobility according to this expression for monovalent and divalent cations in water and methanol, from a hypothetical absolute mobility of  $50 \cdot 10^{-5}$ , at a concentration of 0.01 N. The results are shown in Table I.

These effects are even greater for smaller dielectric constants and for cations with higher charges.

TABLE I

COMPUTED EFFECTIVE MOBILITIES OF MONOVALENT AND DIVALENT CATIONS IN WATER AND METHANOL

	Water		Methanol	
	$m_0 \cdot 10^5$	$m_{eff} \cdot 10^5$	$m_0 \cdot 10^5$	$m_{eff} \cdot 10^5$
1-1	50	46	50	37.5
2-1	50	43	50	25

### Partial dissociation

If the dissociation is complete, the effective mobility is equal to the absolute mobility, neglecting the influences of other effects. If substances are partially dissociated, the effective mobility can be defined as:

$$m_{eff} = \sum_{i=1}^n \alpha_i m_i \quad (7)$$

Two main types of dissociation can be distinguished, protonic dissociation and complex formation.

*Protonic dissociation.* A proton takes part in the dissociation reaction. The degree of dissociation depends on the pH and the equilibrium constant, e.g.:

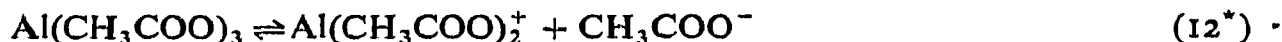


$$K = \frac{c_{\text{H}^+} \cdot c_{\text{CH}_3\text{COO}^-}}{c_{\text{CH}_3\text{COOH}}} \quad (\text{p}K = 4.75) \quad (9)$$

*Complex formation.* Now a particle other than a proton takes part in the dissociation reaction, e.g.:



The degree of complex formation depends mainly on the partial concentrations. Sometimes, however, both types affect the mobility, such as the cation  $\text{Al}^{3+}$ :



If the value of the dielectric constant decreases, the inter-ionic forces increase. This results, especially for cations with higher charges, in a stronger complex formation and the pK values of the dissociations depend on the dielectric constant.

### Solvation

To describe the exact effect of the solvation is difficult. In general, cations with large radii and low charges have a small degree of solvation, whereas highly charged cations and cations with small radii have a large degree of solvation. In general, cations with a large degree of solvation have small mobilities.

\* Not all the steps of these dissociations are given here.

In water and in methanol, the mobilities of the alkali metal ions decrease in the sequence  $Cs > Rb > K > Na > Li$ , *i.e.* in the order of their decreasing radii. The differences between the mobilities, however, seem to be favoured in methanol. In water, Cs, Rb and K ions are very difficult to separate, while in methanol the differences in mobility are such that these cations can be separated easily. Also, the mobilities of Ba, Sr, Ca and Mg ions diminish in the order of their decreasing radii.

Organic cations often have high mobilities in methanol. Even the large cation Tba has a rather high mobility, about the same as that of the very small cation Li. This indicates that the Tba ion is hardly solvated, probably due to the screening effect of the groups surrounding the charge. In water, however, the Tba ion has a rather low mobility. The cation Tma has the highest mobility in methanol, except for  $H^+$ .

#### *Computations on buffered systems*

EVERAERTS AND ROUTS<sup>5</sup> described a theory of buffered systems for the separation of anions in isotachopheresis. The results of the computations agreed with the experimental results. However, they did not take into account the effects according to the ONSAGER expression and no corrections were made for the temperature, which differs from zone to zone.

For the derivation of the formulae needed for computations in buffered systems, the following assumptions are made: the electrical current is constant; the diffusion, hydrostatic flow and electroendosmosis are negligible; the cross-section of the capillary is constant; the activity coefficients on the concentrations are negligible; and the influence of the temperature on the relaxation and electrophoretic effects is negligible.

For the computations, the following equations have to be considered: the equilibrium equations; the isotachopheretical conditions; the mass balance of the buffer; the principle of electroneutrality; and the modified Ohm's law.

We took into account the possible need for corrections for temperature, protonic dissociations, relaxations and electrophoretic effects.

*The equilibrium equations.* If we consider:



the following equilibrium constants are valid:

$$K_{B,i} = \frac{c_H \cdot c_{B,i}}{c_{B,i-1}} \quad (15)$$

$$K_{P,i} = \frac{c_H \cdot c_{P,i-1}}{c_{P,i}} \quad (16)$$

Therefore,

$$c_{B,i} = K_{B,i} \cdot c_{B,i-1} / c_H \quad (17)$$

$$c_{P,i} = c_H \cdot c_{P,i-1} / K_{P,i} \quad (18)$$

Substituting the expressions for  $c_{B,i-1}$  and  $c_{P,i-1}$  etc., up to  $c_{B,0}$  and  $c_{P,0}$  in eqns. 17 and 18:

$$c_{B,i} = c_{B,0} \cdot \frac{\prod_{j=1}^i K_{B,j}}{c_H^i} \tag{19}$$

$$c_{P,i} = c_{P,0} \cdot \frac{c_H^i}{\prod_{j=1}^i K_{P,j}} \tag{20}$$

for

$$M_i = \frac{\prod_{j=1}^i K_{B,j}}{c_H^i}$$

and

$$N_i = \frac{c_H^i}{\prod_{j=1}^i K_{P,j}}$$

these expressions will be:

$$c_{B,i} = c_{B,0} \cdot M_i \tag{21}$$

$$c_{P,i} = c_{P,0} \cdot N_i \tag{22}$$

The total concentrations of the buffer and the cation present are:

$$c_B^i = c_{B,0} \left[ 1 + \sum_{i=1}^{n_B} \left( \frac{\prod_{j=1}^i K_{B,j}}{c_H^i} \right) \right] = c_{B,0} \left( 1 + \sum_{i=1}^{n_B} M_i \right) \tag{23}$$

$$c_P^i = c_{P,0} \left[ 1 + \sum_{i=1}^{n_P} \left( \frac{c_H^i}{\prod_{j=1}^i K_{P,j}} \right) \right] = c_{P,0} \left( 1 + \sum_{i=1}^{n_P} N_i \right) \tag{24}$$

*Isotachophoretical condition.* All the zones move with the same speed, and therefore:

$$E_U \cdot m_{PU} = E_V \cdot m_{PV} \tag{25}$$

where  $m_{PU}$  and  $m_{PV}$  are the effective mobilities of the cation species in zones U and V, respectively.

The effective mobility of an ion species can be defined as:

$$m_{eff} = \sum_{i=1}^n \alpha_i m_i \tag{26}$$

This means:

$$m_{PU} = \left( \sum_{i=1}^{n_{PU}} c_{PU,i} m_{PU,i} \right) / c'_{PU} = \sum_{i=1}^{n_{PU}} N_{U_i} m_{PU,i} / \left( I + \sum_{i=1}^{n_{PU}} N_{U_i} \right) \quad (27)$$

$$m_{BU} = \left( \sum_{i=1}^{n_{BU}} c_{BU,i} m_{BU,i} \right) / c'_{BU} = \sum_{i=1}^{n_{BU}} M_{U_i} m_{BU,i} / \left( I + \sum_{i=1}^{n_{BU}} M_{U_i} \right) \quad (28)$$

*The mass balance of the buffer.* For the mass balance of the buffer, we can write:

$$E_U(m_{BU}c'_{BU} + m_{PU}c'_{BU}) = E_V(m_{BV}c'_{BV} + m_{PV}c'_{BV}) \quad (29)$$

In combination with eqn. 25:

$$c'_{BU} \left( I + \frac{m_{BU}}{m_{PU}} \right) = c'_{BV} \left( I + \frac{m_{BV}}{m_{PV}} \right) \quad (30)$$

In the steady state, the quantity of the buffer entering a zone is equal to the quantity leaving the zone.

*Electroneutrality.* For electroneutrality, we can write:

$$c_{OHU} + \sum_{i=1}^{n_{BU}} i c_{BU,i} = c_{HU} + \sum_{i=1}^{n_{PU}} i c_{PU,i} \quad (31)$$

or:

$$c_{OHU} + c'_{BU} \cdot \frac{\sum_{i=1}^{n_{BU}} i M_{U_i}}{I + \sum_{i=1}^{n_{BU}} M_{U_i}} = c_{HU} + c'_{PU} \cdot \frac{\sum_{i=1}^{n_{PU}} i N_{U_i}}{I + \sum_{i=1}^{n_{PU}} N_{U_i}} \quad (32)$$

*Modified Ohm's law.* For an equal current density:

$$I/K = \text{Constant} = E_U \lambda_U \quad (33)$$

Therefore:

$$I/K = E_U (c_{OHU} m_{OHU} + c_{HU} m_{HU} + \sum_{i=1}^{n_{PU}} i c_{PU,i} m_{PU,i} + \sum_{i=1}^{n_{BU}} i c_{BU,i} m_{BU,i}) \quad (34)$$

Assuming in the right-hand term of eqn. 34,  $Q_1$  and  $Q_U$  for the first and  $U$ th zone, respectively, the function:

$$QU = (Q_1/Q_U) - I \quad (35)$$

must be zero.

#### *Procedure of computation*

With the given formulae a computer program can be developed and if the composition of the first zone, all  $pK$  values and mobilities of the ion species present are known, all quantities can be computed. The procedure of computation is given below.

Assuming a certain pHV in the following zones,  $M_i$ ,  $N_i$ ,  $m_{PV}$  and  $m_{BV}$  can be directly computed. Next,  $c'_{PV}$  and  $c'_{BV}$  can be computed using eqns. 30 and 32.

Substituting all quantities in eqn. 35, the value of QU at this pHV is obtained and for the correct pHV value this function must be zero.

The relationship between the function QU and pHV is shown in Fig. 1a for the system chosen. Where Fig. 1a shows two zero-points, the second zero-value is the correct one (at the incorrect zero-point, negative concentrations are obtained).

In the computer program, one searches for a pHV value, starting from pHL, at which QU is positive, and next for a pHV value at which QU is negative. The correct pHV value is obtained by iterating between these two values until the function QU is zero, within a certain deviation. This procedure is shown in Fig. 1b.

For the correct pH value, all relevant quantities are computed and printed. An example of the output is shown in Fig. 1c. The computations were made with a time-sharing Honeywell-Bull computer, and the language used was FORTRAN 4.

### Some computations

Computations were made for the cations  $Ba^{2+}$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Fe^{2+}$ ,  $K^+$ ,  $Ag^+$  and  $Na^+$ . These cations were chosen because the slope of the function  $\Lambda^*_0 = f(\sqrt{c^*})$  agreed with the expected slope derived from the ONSAGER relationship. If other influences that affect the mobility are present, the decreasing effect should be greater and computations will be impossible, because our computer program does not deal with complex formation.

For the above cations, the electrical resistance and the other quantities were computed (see Table II, (a)). In Fig. 2a these resistances are plotted as a function of the step-heights measured. Although one must expect one continuous relationship, two distinguishable curves are obtained, one for the monovalent and one for the divalent cations. This can be ascribed to the influence of the difference in temperature between the zones and the effects according to the ONSAGER relationship.

The results of the calculations, including these effects, are given in Table II, (b)

TABLE II

RESULTS OF SOME CALCULATIONS WITH AND WITHOUT CORRECTIONS

Cation	Step height (mm)	Calculated values								
		(a) Without corrections		(b) With temperature correction		(c) With corrections for the temperature and Onsager effects				
		$1/\lambda \cdot 10^3$	pH	Concn.	$1/\lambda \cdot 10^3$	pH	Concn.	$1/\lambda \cdot 10^3$	pH	Concn.
K <sup>+</sup>	220	0.874	5.390	0.0100	0.828	5.390	0.0100	0.8930	5.390	0.0100
Ag <sup>+</sup>	260	1.029	5.362	0.0094	0.966	5.360	0.0094	1.0440	5.362	0.0094
Na <sup>+</sup>	302	1.272	5.321	0.0086	1.183	5.320	0.0086	1.2825	5.320	0.0086
Ba <sup>2+</sup>	264	1.013	5.365	0.0048	0.950	5.365	0.0048	1.1152	5.364	0.0048
Ca <sup>2+</sup>	284	1.077	5.354	0.0046	1.006	5.354	0.0046	1.1818	5.353	0.0046
Mg <sup>2+</sup>	314	1.210	5.331	0.0044	1.122	5.331	0.0044	1.3215	5.331	0.0044
Fe <sup>2+</sup>	312	1.188	5.335	0.0045	1.102	5.335	0.0045	1.2969	5.334	0.0045





PHL =	5.00				
	0.01	1.00000E-13	0.01	0.00	0.00
	0.0156	0.0056	0.01	0.00	0.00
Stofzone =	1				
	4.9718	2.60770E-08	62.36		
	0.0094	8.80030E-14	0.0094	0.00	0.00
	0.015	0.0056	0.0094	0.00	0.00
	1.0269				
Stofzone =	2				
	4.9308	7.82311E-08	50.43		
	0.0086	7.30952E-14	0.0086	0.00	0.00
	0.0142	0.0057	0.0086	0.00	0.00
	1.2699				
Output:	pHV	Deviation pHV		$m_{eff}$	
	$d_P$	$c_{P,0}$	$c_{P,1}$	$c_{P,2}$	$c_{P,3}$
	$d_B$	$c_{B,0}$	$c_{B,1}$	$c_{B,2}$	$c_{B,3}$
	Resistance of the zone				

Fig. 1c. Example of the output of the computer program.

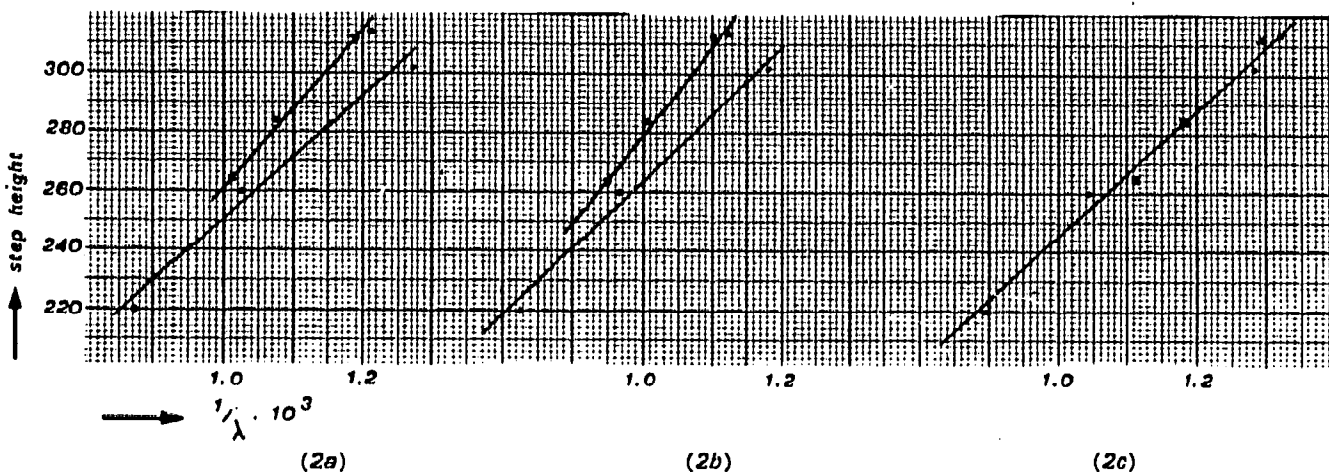


Fig. 2. Relationship between the resistances of the zones as calculated and the step heights of some cations found experimentally.

and (c), while Fig. 2, (b) and (c), shows the relationships between the computed resistances and the step heights measured.

From these results it can be concluded that for relative computations the influence of the temperature is not important, but corrections to allow for the ON-SAGER relationship are necessary.

Although corrections to allow for complex formation will be necessary, these are rather difficult to make, as the pK values of the equilibria and the mobilities of the complexes are not always known.

EXPERIMENTAL

Apparatus

The experiments were carried out with an instrument that has been described elsewhere<sup>1</sup>. The sample was introduced by a four-way sample tap. A constant direct



current power source with a maximum potential of 20 kV was used. We used the Micrograph BD 5 recorder (Kipp & Zonen), which is especially useful because of its automatic zero suppression module.

The effective mobilities of some cations were sometimes very low. The increment in electrical resistance during the analyses, due to the movement of the zones with small conductivities, required the use of higher potentials than were available. In this paper, the term "too slow" is used for these cations.

Sometimes, the length of the capillary was too short for complete separation of the cations. Sometimes also, the zones, although separated, cannot be detected because the resolving power of the thermometric detector is too small to detect small lengths of zones or small differences in temperature between two zones. In these instances the term "not separated" is used in this paper.

The sample tap volume (about 20  $\mu$ l) is rather large and corresponds to the contents of about a 14-cm length of the capillary tube.

If the concentrations of the sample ions are chosen to be too high, complete separation, according to the isotachopheretical principle, cannot be expected.

The average time for analyses was about 45 min.

### *Experiments*

The effective mobilities of cations can easily be influenced. Sometimes this can be an advantage, especially if the cations have the same or almost the same effective mobilities in an electrolyte system and cannot be separated because of this factor. By changing the system, it may be possible to separate such cations.

The use of different solvents\* or other buffers\*\* allows numerous possibilities. To investigate some of these possibilities, experiments in both water and methanol were carried out, using buffered and non-buffered systems. The conditions of these systems are summarized in Table III.

Detailed description of these systems are given later. In Tables IV and V the step heights \*\*\* found in the electropherograms of the experiments in water and methanol, respectively, are given.

For some systems, a scheme can be given to show which series of cations can be separated simultaneously. The interpretation is as follows. Ions placed in one circle and ions placed in circles directly connected by lines, cannot be separated simultaneously, *e.g.*, in Fig. 3 (system WHCl), Ba<sup>2+</sup> and Pb<sup>2+</sup> cannot be separated because they are placed in the same circle, and Ca<sup>2+</sup> and Al<sup>3+</sup> cannot be separated because they are connected directly by a line, whereas Ba<sup>2+</sup> and Al<sup>3+</sup> can be separated because they are not directly connected by a line. If Ba<sup>2+</sup>, Ca<sup>2+</sup> and Al<sup>3+</sup> are present, they form a mixed zone together. Li<sup>+</sup> and Tea<sup>+</sup> can be separated because they are not connected by a line.

### AQUEOUS SYSTEMS

#### *The system WHCl*

The leading electrolyte used is HCl in water, and Tris in water is used as the terminator. Many mono-, di- and trivalent ions have about the same step heights.

\* The influence of the dielectric constant and solvation.

\*\* The change of pH and the influence of complex formation.

\*\*\* The step height in an isotachopherogram is a qualitative measure for the ion species, where the distance between two successive peaks gives all necessary quantitative information.

TABLE IV

STEP HEIGHTS (mm) OF THE CATIONS FOR THE AQUEOUS SYSTEMS

Cation	System				
	WHCl	WHIO <sub>3</sub>	WKAC	WKAC	WKDIT
H <sup>+</sup>	60	72	—	—	—
K <sup>+</sup>	216	290	220	280	300
Na <sup>+</sup>	292	400	302	384	410
Li <sup>+</sup>	352	492	378	476	504
NH <sub>4</sub> <sup>+</sup>	215	292	220	282	303
Ag <sup>+</sup>	—	—	260	338	too slow
Tl <sup>+</sup>	217	295	—	—	—
Tma <sup>+</sup>	302	437	340	430	442
Tea <sup>+</sup>	400	560	432	540	568
Tba <sup>+</sup>	too slow	too slow	too slow	808	—
Tris <sup>+</sup>	434	625	490	610	680
Im <sup>+</sup>	306	412	310	432 <sup>c</sup>	551
Cs <sup>+</sup>	208	—	—	—	—
Rb <sup>+</sup>	213	—	—	—	—
Guan <sup>+</sup>	285	391	294	372	399
S.C. <sup>+</sup>	324	450	343	434	477
Co <sup>2+</sup>	290	416	318	407	—
Ni <sup>2+</sup>	291	415	318	403	too slow
Mg <sup>2+</sup>	294	408	314	396	430
Cu <sup>2+</sup>	290	404	387	442	too slow
Ca <sup>2+</sup>	266	372	284	362	388
Mn <sup>2+</sup>	285	412	320	420	440
Cd <sup>2+</sup>	318	420	341	446	too slow
Fe <sup>2+</sup>	294	410	312	508	too slow
Sn <sup>2+</sup>	248–270	—	1276 <sup>b</sup>	too slow	—
Pb <sup>2+</sup>	250	too slow	371	486	too slow
Ba <sup>2+</sup>	255	352	264	338	368
Zn <sup>2+</sup>	294	404	320	415	too slow
Fe <sup>3+</sup>	too slow	too slow	1128 <sup>b</sup>	too slow	too slow
La <sup>3+</sup>	241	366	322	402	634
Ce <sup>3+</sup>	246	368	325	416	834
Cr <sup>3+</sup>	312 <sup>a</sup>	498	390	too slow	too slow
Al <sup>3+</sup>	272	380	360	too slow	—

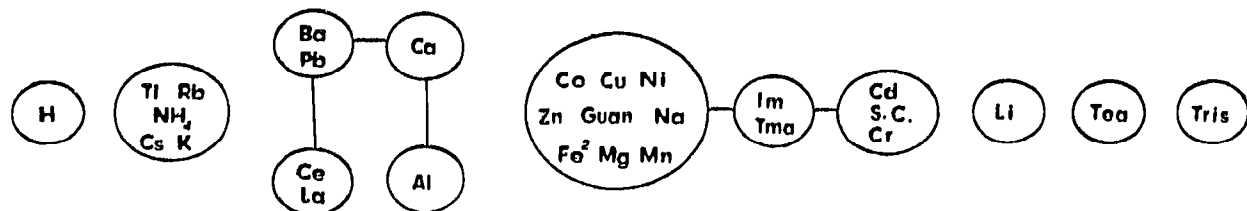
<sup>a</sup> Double step.<sup>b</sup> Estimated value from an experiment at lower current density.<sup>c</sup> The step height for imidazole at pH 6.53 is 472.

Fig. 3. Simultaneous separation of cations in the system WHCl.

Their effective mobilities are nearly equal, so that separations are impossible with the apparatus available.

Ions can be separated if they differ in step height by about 20 mm in this system (Table IV).

TABLE V

STEP HEIGHTS (mm) OF THE CATIONS FOR THE METHANOLIC SYSTEMS

Cation	System			Cation	System		
	MHCl	MKAC	MTMAAC		MHCl	MKAC	MTMAAC
H <sup>+</sup>	124	—	—	Ni <sup>2+</sup>	262	510	560
K <sup>+</sup>	195	195	198	Mg <sup>2+</sup>	240	397	436
Na <sup>+</sup>	222	230	230	Zn <sup>2+</sup>	too slow	821	960
Li <sup>+</sup>	257	270	260	Pb <sup>2+</sup>	too slow	946	1080
Rb <sup>+</sup>	180	—	188	Ba <sup>2+</sup>	332	335	350
Cs <sup>+</sup>	168	—	173	Ca <sup>2+</sup>	241	425	456
Ag <sup>+</sup>	—	1031	—	Cd <sup>2+</sup>	628	1025	—
NH <sub>4</sub> <sup>+</sup>	179	—	193	Co <sup>2+</sup>	272	497	540
Tris <sup>+</sup>	292	321	317	Cu <sup>2+</sup>	383	too slow	—
Tl <sup>+</sup>	—	218	—	Mn <sup>2+</sup>	296	483	520
Tma <sup>+</sup>	154	151	150	Fe <sup>2+</sup>	390	too slow	—
Tca <sup>+</sup>	170	177	186	Fe <sup>3+</sup>	340	too slow	—
Tba <sup>+</sup>	265	260	250	Al <sup>3+</sup>	256	too slow	—
Guan <sup>+</sup>	192	203	198	Cr <sup>3+</sup>	290	too slow	—
S.C. <sup>+</sup>	209	191	184	Ce <sup>3+</sup>	310	too slow	—
Im <sup>+</sup>	176	599	—	La <sup>3+</sup>	330	too slow	—

Separations of mixtures containing cations with low pK values are difficult; this will be explained in a later paper.

Fig. 3 shows which cations can be separated simultaneously in this system. Fig. 4 shows the electropherogram for the separation of a mixture consisting of Tl<sup>+</sup>, La<sup>3+</sup>, Ca<sup>2+</sup>, Fe<sup>2+</sup>, Cd<sup>2+</sup> and Li<sup>+</sup>. H<sup>+</sup> is the leading ion and Tris<sup>+</sup> the terminating ion.

#### The system WHIO<sub>3</sub>

In this system, the leading electrolyte is HIO<sub>3</sub> in water and the buffering effect is small. The sequence of the step heights of the cations is similar to the sequence in the system WHCl.

The most important shifts\* in step heights are those of Cr<sup>3+</sup>, Ce<sup>3+</sup> and La<sup>3+</sup>. The Pb<sup>2+</sup> ion does not migrate noticeably.

#### The system WKAC

The leading electrolyte is a solution of potassium acetate in water, adjusted to pH 5.39 by adding acetic acid. This pH is chosen because in the following zones the pH of the zone decreases almost to the pK value of acetic acid, producing a maximum buffering effect.

The differences in step heights of the cations, for complete separation, must be about 20 mm in this system.

Fig. 5 shows which ions can be separated simultaneously.

Comparing the step heights in this system with those of the other systems, some shifts in the step heights must be explained.

The most important ones are those of Pb<sup>2+</sup>, Ce<sup>3+</sup>, La<sup>3+</sup>, Al<sup>3+</sup> and Cr<sup>3+</sup>, which

\* See under DISCUSSION.

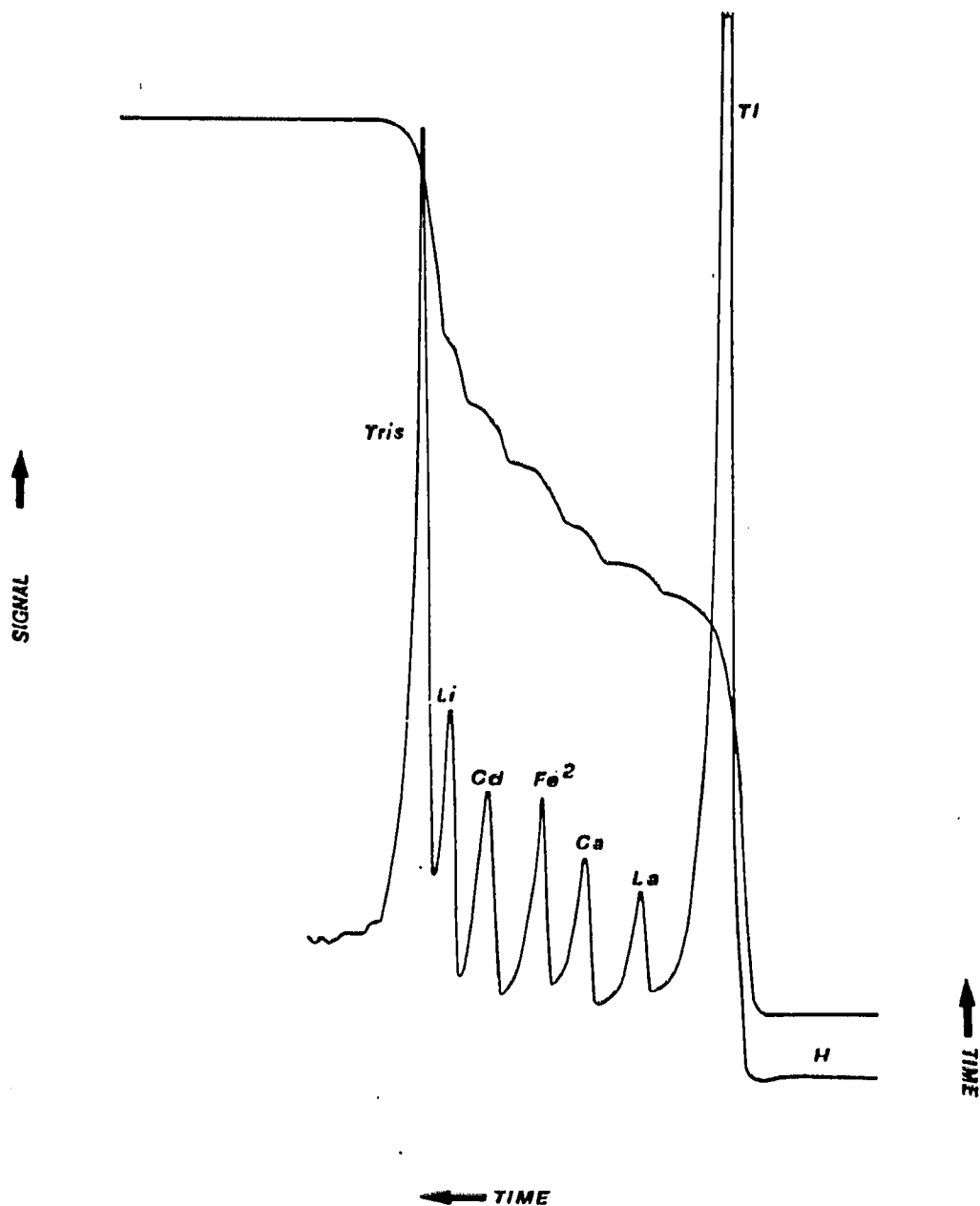


Fig. 4. Electropherogram for the separation of cations in the system WHCl.

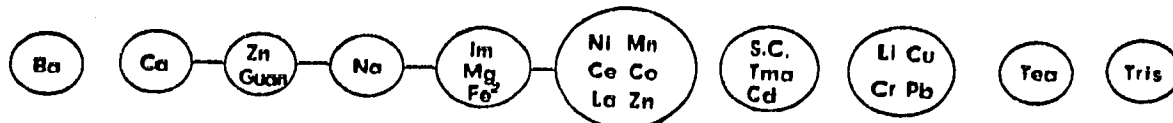


Fig. 5. Simultaneous separation of cations in the system WKAC.

are all polyvalent ions. The reason for the shifts can be found in the higher pH of the system and a stronger complex formation.

Fig. 6 shows the electropherogram for the separation of a mixture of  $Ba^{2+}$ ,  $Ca^{2+}$ ,  $Na^+$ ,  $Ni^{2+}$ ,  $Cd^{2+}$ ,  $Pb^{2+}$  and  $Tea^+$ .  $K^+$  is the leading ion and  $Tris^+$  the terminating ion.

*The system WKAC*

The leading electrolyte is KOH in water, adjusted to pH 6.37 by adding cac-

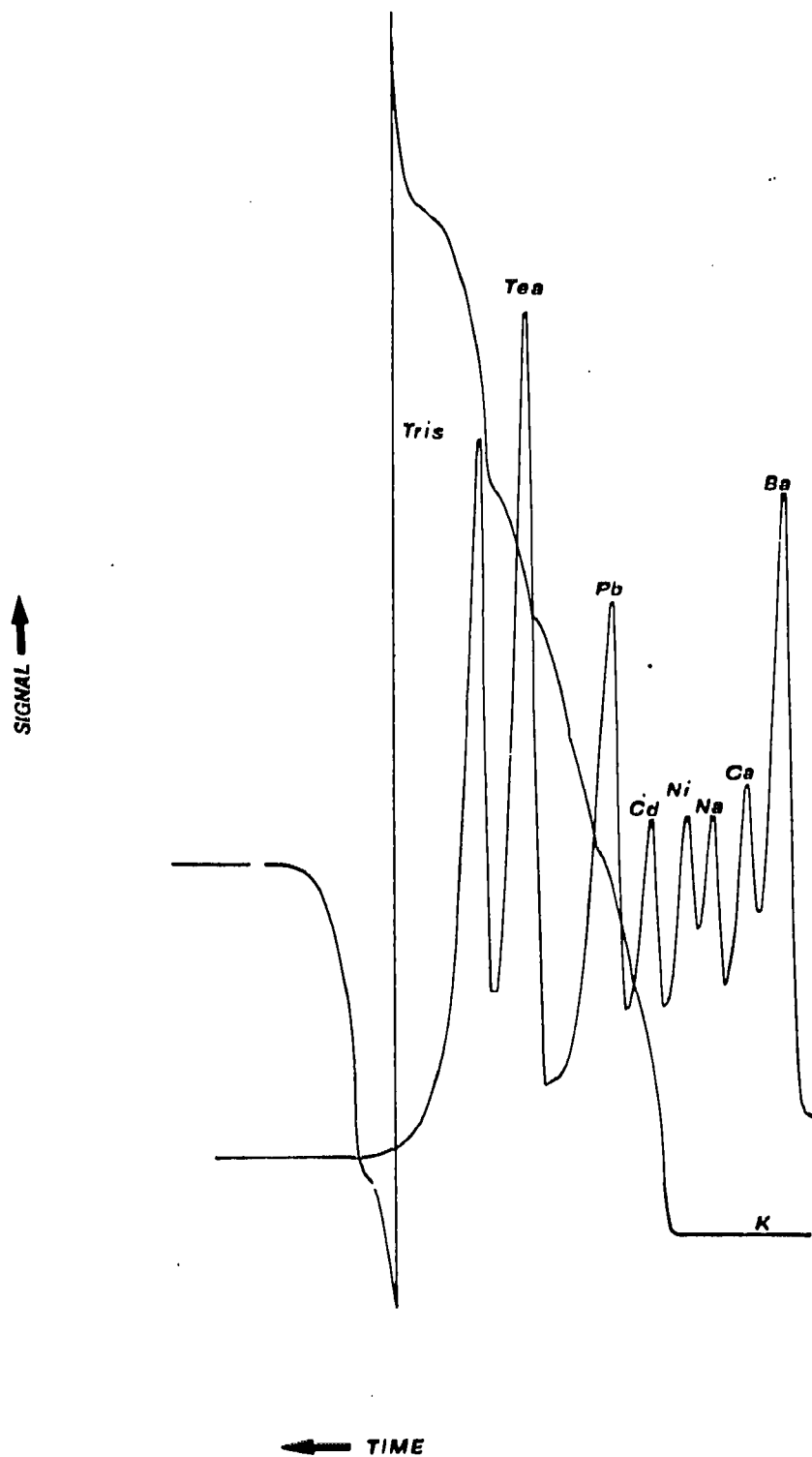


Fig. 6. Electropherogram for the separation of cations in the system WKAC.

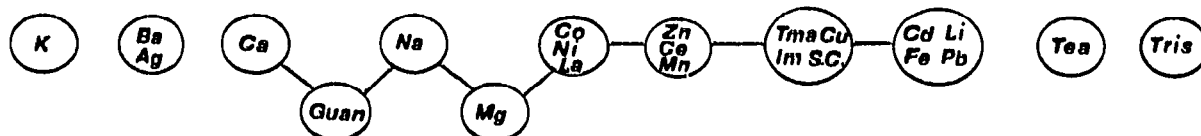


Fig. 7. Simultaneous separation of cations in the system WKAC.

dylic acid. Tris<sup>+</sup> is used as terminator. This higher pH was chosen so as to investigate the effect of increased pH.

In the WKCAC system, all ions have lower effective mobilities, in general, and it is not clear if this is due to the higher pH of the system, to complex formation or to a combination of these effects.

The effective mobilities of Al<sup>3+</sup>, Cr<sup>3+</sup> and Fe<sup>3+</sup> are too low. Imidazole shows a typical shift in step height. It has a p*K* value of 6.95 and at higher pHs its effective mobility will decrease (eqn. 7). To check this effect, some experiments were carried

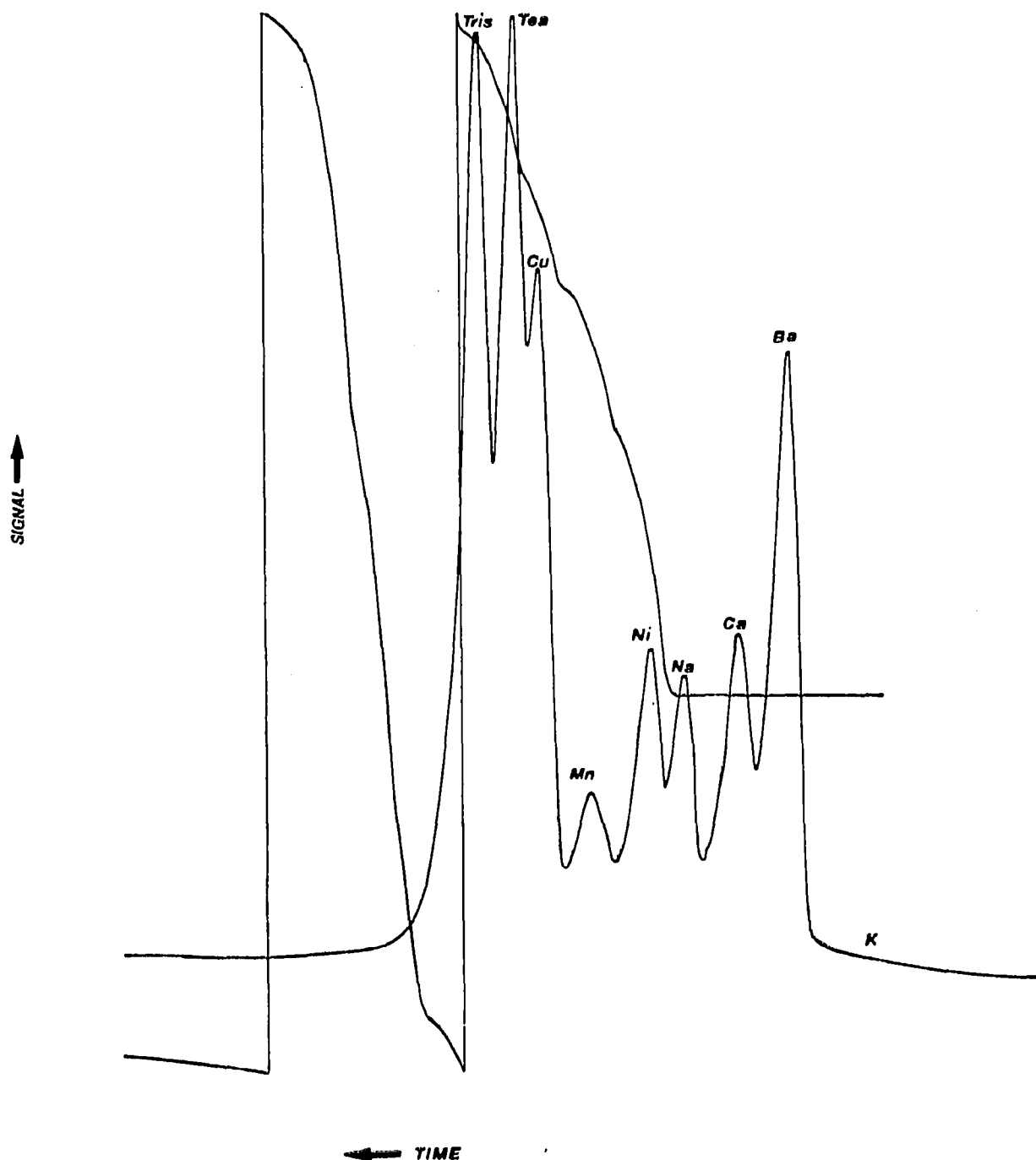


Fig. 8. Electropherogram for the separation of cations in the system WKCAC.



out with the same buffer at a pH of 6.53. For some cations of strong electrolytes, the step heights were identical, while the step height of imidazole increased.

Fig. 7 shows which cations can be separated simultaneously. In Fig. 8, the electropherogram is given for the separation of the cations Ba<sup>2+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup>, Ni<sup>2+</sup>, Mn<sup>2+</sup>, Cu<sup>2+</sup> and Tea<sup>+</sup>. The leading ion is K<sup>+</sup> and the terminating ion is Tris<sup>+</sup>.

*The system WKDIT*

The leading electrolyte is KOH in water, adjusted to a pH of 7.39 by adding diiodotyrosine. The terminator is Tris<sup>+</sup>.

At this pH, many ions do not migrate at all (Table IV), and sometimes precipitates are formed.

While all possible step heights were measured, the above effects were such that no separations in this system could be achieved. For some special purposes, however, this system, could be useful, e.g., in combinations with other systems.

*Combinations of systems*

Some systems suitable for separations of cations are given, and in each system about 7 to 9 cations can be separated simultaneously. Because shifts in step height appear in the different systems, a suitable system can be chosen for a specific problem. However, mixtures can be found with such a composition that total separation in one system is impossible. Combining a set of systems may solve this problem.

An example of this is given below.

Suppose the mixture to be separated consists of K<sup>+</sup>, Na<sup>+</sup>, Li<sup>+</sup>, Tea<sup>+</sup>, Ba<sup>2+</sup>, Pb<sup>2+</sup>, Ni<sup>2+</sup>, Ca<sup>2+</sup>, Fe<sup>2+</sup>, Cd<sup>2+</sup>, Cu<sup>2+</sup> and Cr<sup>3+</sup>. It is clear that separation in one system is impossible, the electropherograms showing many mixed zones, as follows:

In the system WHCl:	H	<i>K</i>	Ca	Fe	Ba	Ni	Cd	<i>Li</i>	<i>Tea</i>	Tris
			Pb	Na	Cr					
				Cu						
								Pb		
In the system WKAC:	K	<i>Ba</i>	<i>Ca</i>	Fe	Ni	<i>Cd</i>	<i>Li</i>	<i>Tea</i>	Tris	
				Na			Cu			
								Cd		
								Cu		
In the system WKCAC*:	K	<i>Ba</i>	<i>Ca</i>	<i>Na</i>	<i>Ni</i>	<i>Li</i>	<i>Tea</i>	Tris		
								Fe		
								Pb		

The quantities of the cations given in italic are known directly from the electropherograms, viz., K, Ba, Li, Tea, Ca, Cd, Na and Ni.

Further, we can compute the following:

$$Cr = (Cd + Cr)_{WHCl} - Cd_{WKAC}$$

$$Pb = (Ba + Ca + Pb)_{WHCl} - Ba_{WKAC} - Ca_{WKAC}$$

$$Cu = (Cu + Li + Pb + Cr)_{WKAC} - Pb_{com} - Cr_{com} - Li_{WHCl}$$

$$Fe = (Cd + Cu + Li + Fe + Pb)_{WKCAC} - Cd_{WKAC} - Cu_{com} - Pb_{com} - Li_{WHCl}$$

\* In this system, Cr<sup>3+</sup> is too slow.

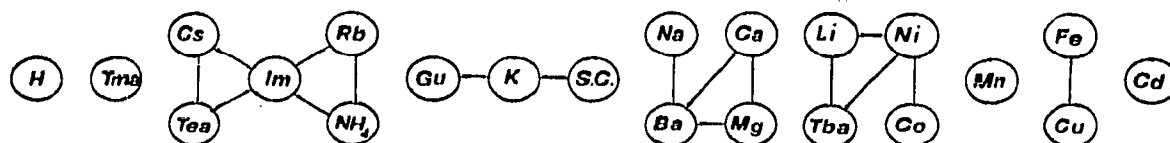


Fig. 9. Simultaneous separation of cations in the system MHCl.

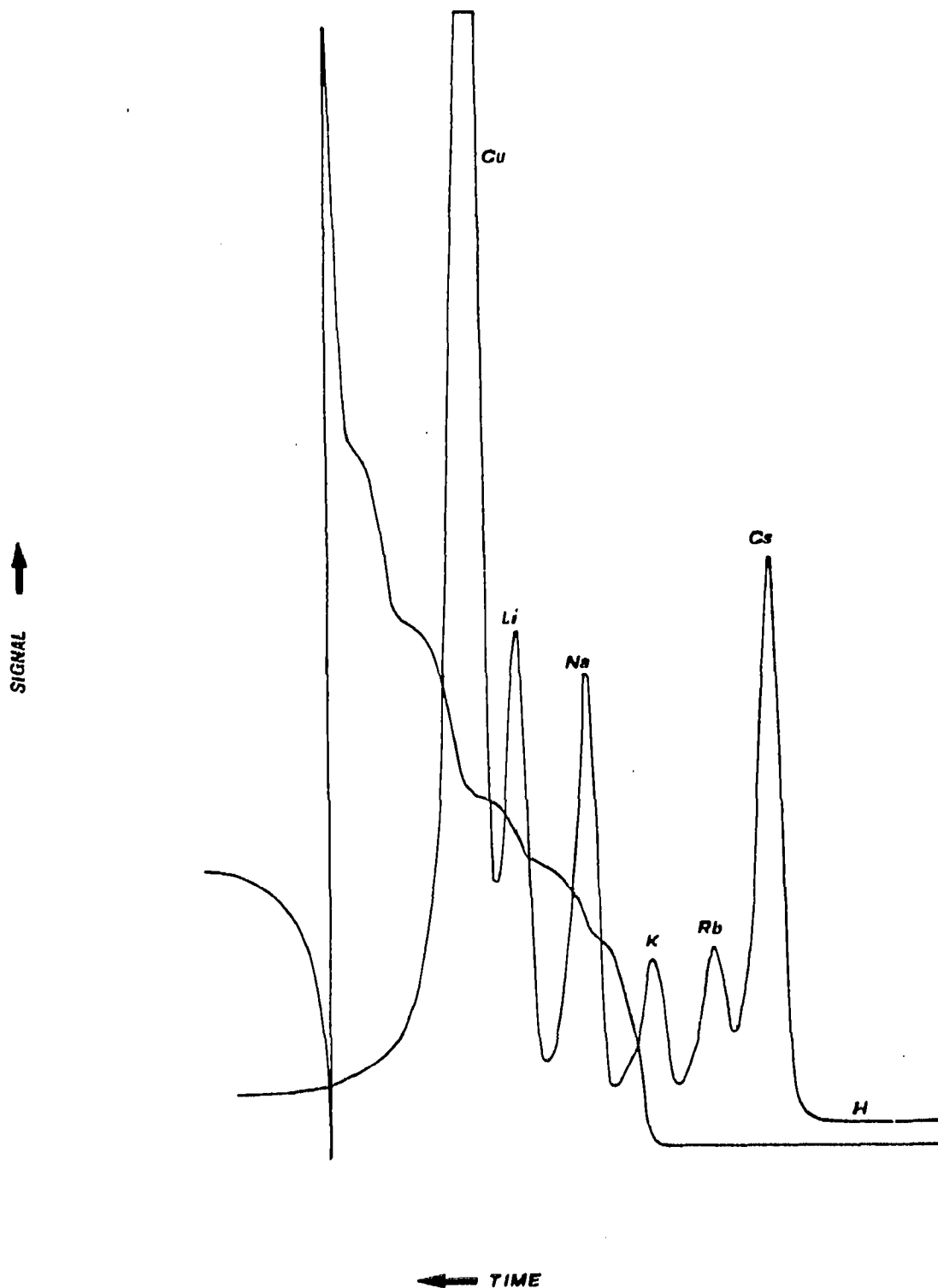


Fig. 10. Electropherogram for the separation of the alkali metals in the system MHCl.

Complex mixtures can be analysed by combining the systems in an appropriate manner. In this paper, only the qualitative aspects are described. Quantitative analyses are being studied at present and will be described in a later paper.

#### METHANOLIC SYSTEMS

The methanol (96 %) was prepared by running it over a column filled with a mixed-bed ion exchanger (Merck V).

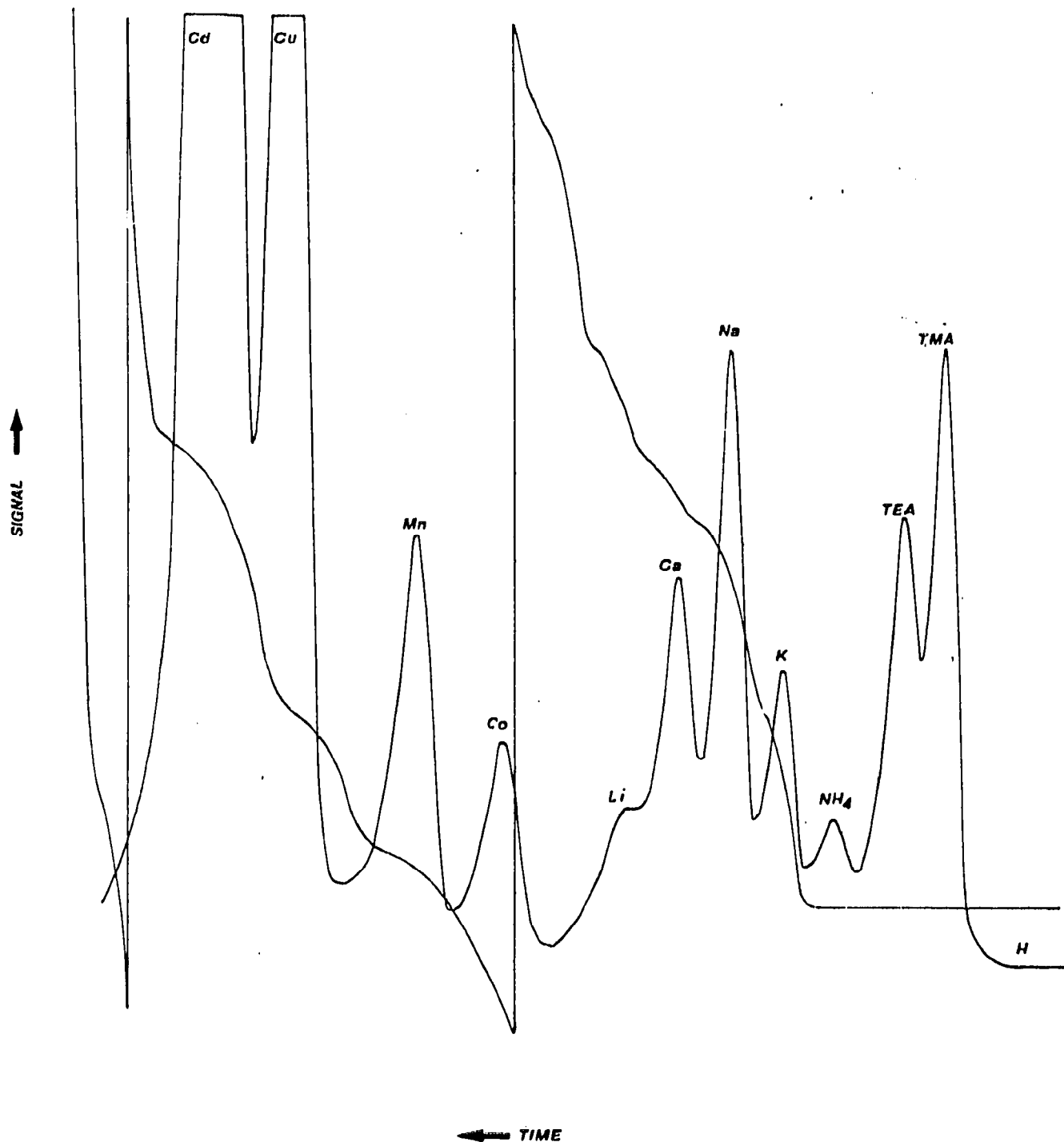


Fig. 11. Electropherogram for the separation of cations in the system MHCl.

### The system MHCl

The leading electrolyte is HCl in methanol and the terminating electrolyte is CdCl<sub>2</sub> in methanol. For further conditions, see Table III. The step heights of the cations in the methanolic systems are given in Table V. The differences in step heights required for a complete separation must be about 8–10 mm.

In comparison with the aqueous systems, especially for monovalent cations, separations can be achieved much more successfully in methanol.

Trivalent cations are difficult to separate. Their electropherograms show very wide, sometimes double, steps because clusters are formed. For this reason, the separations of monovalent and divalent cations only were investigated.

Fig. 9 shows which cations can be separated simultaneously. In Fig. 10, the electropherogram for the separation of the alkali metal ions Cs<sup>+</sup>, Rb<sup>+</sup>, K<sup>+</sup>, Na<sup>+</sup> and Li<sup>+</sup> is given. The leading ion is H<sup>+</sup> and the terminating ion is Cu<sup>2+</sup>. In Fig. 11, the electropherogram is given for a mixture consisting of the cations Tma<sup>+</sup>, Tea<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, Li<sup>+</sup>, Co<sup>2+</sup>, Mn<sup>2+</sup> and Cu<sup>2+</sup>. The leading ion is H<sup>+</sup> and the terminating ion is Cd<sup>2+</sup>. The concentrations of all the cations were about 0.01 *M*, except for Li<sup>+</sup> (0.002 *M*).

### The system MKAC

In this system, the leading electrolyte is potassium acetate in methanol adjusted to a pH of 6.35 by adding acetic acid. The pH is measured with a glass electrode and a calomel electrode, filled with an aqueous saturated solution of KCl as a reference electrode. The terminator used is CdCl<sub>2</sub> in methanol. There are great differences in comparison with the step heights of the cations in the system MHCl. Divalent cations in particular show great shifts in step heights. The trivalent cations have such low effective mobilities that they do not migrate in an appropriate manner.

Fig. 12 shows which cations are simultaneously separated in this system. Fig. 13 shows the electropherogram for the separation of a mixture consisting of Guan<sup>+</sup>, Na<sup>+</sup>, Li<sup>+</sup>, Ba<sup>2+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup> and Ni<sup>2+</sup>. The leading ion is K<sup>+</sup> and the terminating ion is Zn<sup>2+</sup>.



Fig. 12. Simultaneous separation of cations in the system MKAC.

The most important metals in blood are easily separated in this system. Quantitative separations, however, can be more difficult because of the great differences in concentrations. For this reason, other types of detectors with higher resolution powers must be used.

### The system MTMAAC

In the preceding system, the leading ion is K<sup>+</sup> and cations with mobilities higher than that of K<sup>+</sup> cannot be determined. Because many ions are quicker than K<sup>+</sup>, we carried out some experiments with the leading electrolyte tetramethylammonium acetate. TMA is the quickest cation of those considered. In Table V it can be seen that most step heights agree with those of the system MKAC.

All divalent cations are a little slower, possibly due to the higher pH.

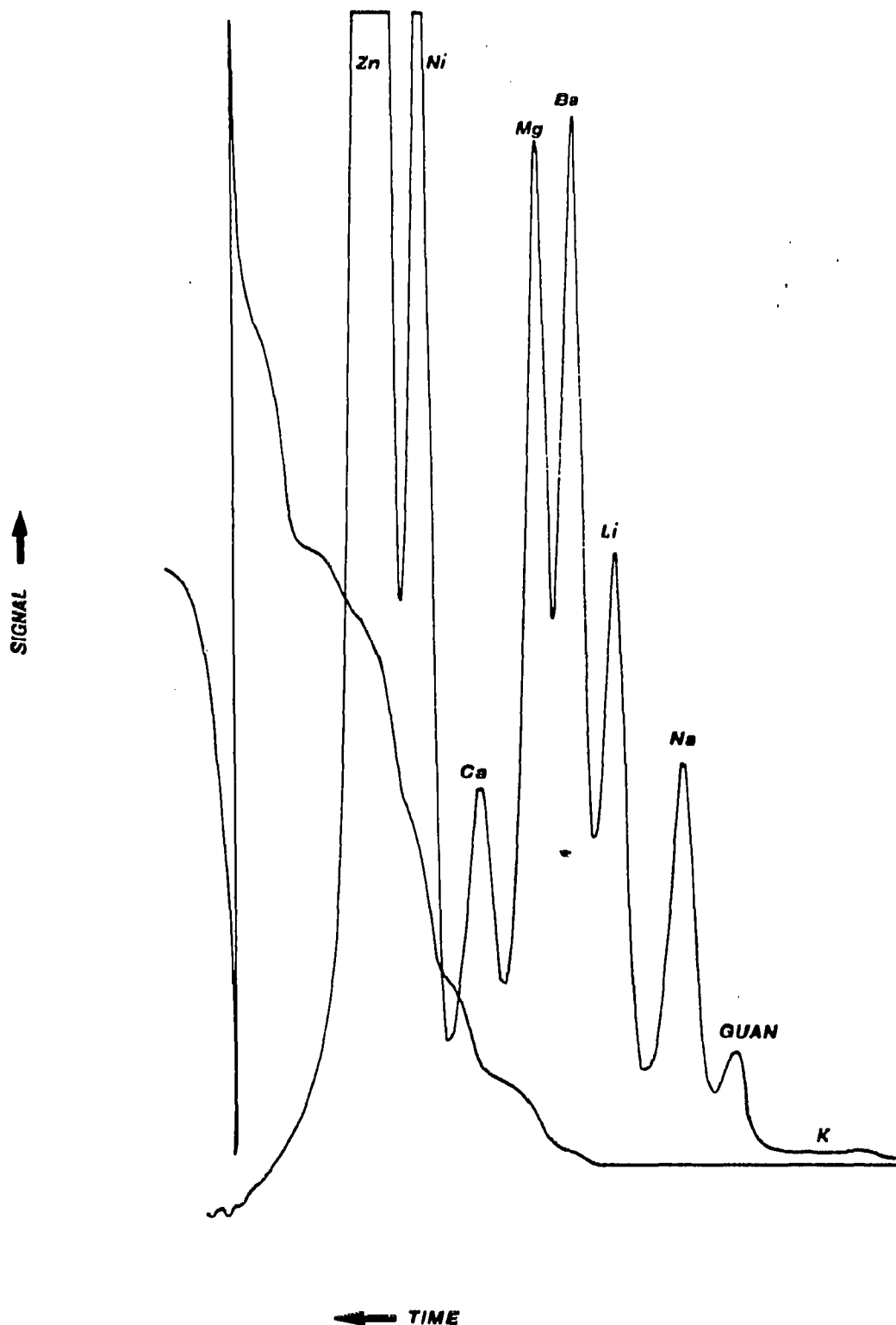


Fig. 13. Electropherogram for the separation of cations in the system MKAC.

DISCUSSION

In this paper, we have described the qualitative aspects of some cationic experiments carried out on isotachopheresis. The possibility of separating cations,

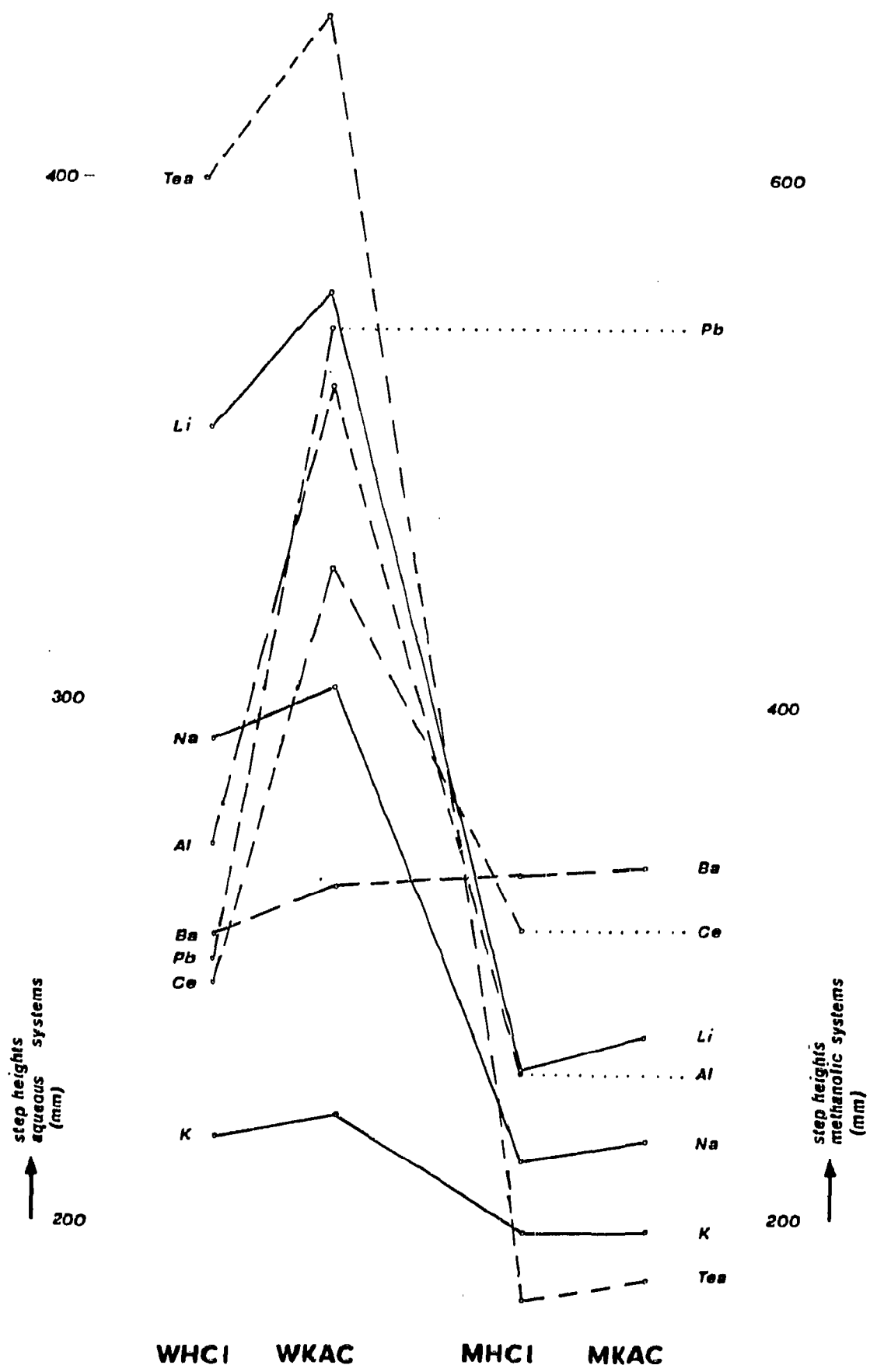


Fig. 14 Step height differences of some cations for the different systems.

as shown here, depends on the type of detection and injection system used. In using a tap, the sample ions are introduced separately from the terminating and leading electrolytes. This can be seen as an advantage, as the length of the capillary can be used for the separations of the sample ions according to the concentrations and effective mobilities of the various ions.

If the sample is introduced by injection, for instance with a Hamilton syringe, the sample ions are mixed with the terminating and/or leading ions. In this instance the length of the capillary tube, available for the separation, must be used for the separation of the sample ions from the leading and/or terminating ions also. Sometimes this can be difficult, especially if the effective mobilities of the sample ions do not differ much from those of the terminating and/or leading ions. The change in pH of the sample caused by the terminating electrolyte can also be a source of difficulties.

The larger volume of the tap is a disadvantage, but smaller concentrations of the sample ions can be handled.

In combining systems, only the combination of aqueous systems has been considered. The possibilities are much greater if methanolic systems can be applied as in our case, where the apparatus is fully lined with PTFE.

Differences in step heights as found in the various systems must be satisfactorily interpreted. The influence of counter-ion and pH always produces changes in the step heights of the cations for the different systems. Complex formation and solvation effects will shift the step heights in relation to other cations. An example of this is given below.

Fig. 14 shows clearly the influence of the various systems on the step heights of the strong cations  $K^+$ ,  $Na^+$  and  $Li^+$ . The behaviours of these cations in the various systems are similar. Highly charged cations such as  $Ce^{3+}$ ,  $Al^{3+}$  and  $Pb^{2+}$  show shifts in step heights, due to the effects of pH and complex formation. Large shifts are also shown for  $Ba^{2+}$  and  $Tea^+$  cations for the aqueous and methanolic systems, due to the effect of solvation and change of dielectric constant, and in methanolic systems the influence on the divalent cations of a change in pH is remarkable.

#### SYMBOLS

$B^{i-}$	Buffer ion with $i$ negative charges
$c^*$	Equivalent concentration (g-equiv./l)
$c^t$	Total concentration (mole/l)
$c$	Concentration (mole/l)
$D$	Dielectric constant of the solvent
$E$	Voltage gradient (V/cm)
$F$	Faraday constant (C/g-equiv.)
$K$	Equilibrium constant
$M_i$	Computation constant
$m$	Mobility ( $cm^2/V$ -sec)
$m_0$	Absolute mobility at $c=0$ ( $cm^2/V$ -sec)
$m_{eff}$	Effective mobility ( $cm^2/V$ -sec)
$N_i$	Computation constant
$n$	Valence of an ion species
$P^{i+}$	Cation with $i$ positive charges

$q$	Electrolyte constant
$T$	Absolute temperature ( $^{\circ}\text{K}$ )
$\alpha$	Degree of dissociation
$\alpha^*$	Real degree of dissociation, as used in the ONSAGER relation
$\Lambda_o^*$	Equivalent conductance of an electrolyte at $c=0$ ( $\text{cm}^2/\text{ohm}\cdot\text{g-equiv.}$ )
$\Lambda^*$	Equivalent conductance of an electrolyte ( $\text{cm}^2/\text{ohm}\cdot\text{g-equiv.}$ )
$\lambda$	Electrical conductivity of a zone ( $\text{r}/\text{ohm}\cdot\text{cm}$ )
$\lambda_o^+$	Ionic equivalent conductivity of a cation at $c=0$ ( $\text{r}/\text{ohm}\cdot\text{cm}$ )
$\eta_o$	Viscosity of the solvent.

*Indices*

B	Ion species B or buffer
P	Ion species P or cation
H	Ion species $\text{H}^+$
OH	Ion species $\text{OH}^-$
U	The $U^{\text{th}}$ zone
V	The $V^{\text{th}}$ zone
$i$	The $i^{\text{th}}$ step of dissociation
com	Computed.

*Examples*

$c_{\text{PU}}^{\text{t}}$	The total concentration of the ion-species P in the $U^{\text{th}}$ zone.
$K_{\text{B},i}$	The equilibrium constant of the ion species B for the $i^{\text{th}}$ step of dissociation.
$c_{\text{PU},i}$	The concentration of the ion-species B, with $i$ positive charges, in the $U^{\text{th}}$ zone.

*Abbreviations*

Guan	Guanidine
Im	Imidazole
pHV	The pH of the $V^{\text{th}}$ zone
pHU	The pH of the $U^{\text{th}}$ zone
pHL	The pH of the leading zone
Tma	Tetramethylammonium
Tba	Tetrabutylammonium
Tea	Tetraethylammonium
Tris	Trishydroxymethylaminomethane
S.C.	Succinyl choline.

## ACKNOWLEDGEMENT

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