CHREV 182

ISOTACHOPHORESIS IN INORGANIC CHEMISTRY

PETR BOČEK* and FRANTIŠEK FORET

Institute of Analytical Chemistry, Czechoslovak Academy of Sciences CS-611 42 Brno (Czechoslovakia)

CONTENTS

1 INTRODUCTION

The purpose of this article is to review the current status of isotachophoresis in inorganic chemistry and especially the application of isotachophoresis to inorganic analytical chemistry It is not intended as a complete survey of the technique, its history and inorganic applications. which would require more space and would duplicate already published monographs[1-4] reviewing the relevant literature till 1979–1980 The examples chosen were selected so as to illustrate the present state of art of the technique, and results obtained with modern instrumentation are described where possible For priorities. the chronological bibliography[5] may be consulted

The primary contribution of any analytical method to the development of other chemical disciplines is that it gives characteristic information on the composition of the object (sample) in question at a given time Since isotachophoresis has specific analytical aspects, which are not currently encountered with other techniques and which modify in a characteristic way the analytical information provided by this technique. the following chapter is devoted to these aspects

2 ANALYTICAL ASPECTS OF ISOTACHOPHORESIS

2 1   Principles

Isotachophoresis is an electromigrational separation method, and thus may be applied to ionogenic substances in solution In this analytical method, substances are separated according to their effective mobilities The concept of the substance and of its effective mobility is of critical importance in the interpretation of isotachophoretic separations

To explain the concept of effective mobility, the description given by Tiselius[6] may serve as a basis a substance, present in a solution in forms whose molar fractions are $x_0, x_1, \quad , x_n$ and mobilities $u_0, u_1, \quad , u_n$ and which are in rapid dynamic equilibrium with one another, migrates under the influence of an electric field as the only substance with a certain effective mobility, $\bar{u}$, defined by the relationship

$$\bar{u} = x_0u_0 + x_1u_1 + \quad + x_nu_n \tag{1}$$

From a macroscopic point of view, this mixture of different forms (ions or neutral molecules) thus appears during electromigration as an uniform substance with defined mobility and charge For such a mixture the concept of the "ion-constituent" was introduced[7] This is regarded as an ionic component of an electrolyte, being both in the form of already existing ions and a potential source of ions, created by different complexes and neutral molecules (cf, ref 8) As an example, the "phosphate" constituent PO_4 in solution may be in the form of the ions PO_4^{3-}, HPO_4^{2-} and $H_2PO_4^{-}$ and the neutral molecule H_3PO_4 In isotachophoresis, the constituent can also comprise particles formed by an association (complex-forming) equilibrium with a counter ion For example[1], in a zone of sulphate with Cd^{2+} as the counter ion, the sulphate constituent comprises the particles H_2SO_4, HSO_4^{-}, SO_4^{2-} and $CdSO_4$

In current isotachophoretic practice the term substance is used instead of "ion-constituent" Frequently, when speaking about zones, shorter terms are used, e g , instead of "the zone of the sulphate ion-constituent" the term "sulphate zone" is employed

The separation of substances in isotachophoresis does not proceed in a background electrolyte, but in a discontinuous electrolyte system Therefore, a prerequisite of any isotachophoretic analysis is a knowledge of the range of mobilities of the ions to be separated in order to be able to choose suitable leading and terminating electrolytes, this is not necessary, e g , in zone electrophoresis

A basic feature which differentiates isotachophoresis from other electrophoretic techniques is that zones migrate at the same velocity from the moment equilibrium is established, hence the name iso-tacho From this the specific nature of both qualitative and quantitative isotachophoretic analysis follows

Unlike zone electrophoresis, e g , on paper and/or in other planar or column arrangements, isotachophoresis cannot furnish data for the identification of the separated zones, such as distances migrated The source of qualitative information in isotachophoresis is the magnitude of the detector response, which is recorded as the step height on the isotachopherogram There is no universal procedure for the qualitative evaluation of the steps on the record, the interpretation is based mainly on the comparison of the analysis of standard substances and of the sample under the same working conditions

The principle of quantitative analysis is simple, being based on a calibration with standard substances, the quantitative parameter in the isotachopherogram is the step length This simple approach is possible because the zone boundaries are very sharp due to the self-sharpening effect and the concentrations in the respective zones are fixed —adjusted to the composition of the leading electrolyte This principle of adjusting the concentrations of the substances in their zones makes isotachophoresis a very effective method for concentrating minor components from a mixture, for their detection and/or identification and for preparative purposes

The contribution of isotachophoresis to inorganic chemistry stems from its separation possibilities and is thus based on the chemistry of solutions The separation of substances in isotachophoresis proceeds according to their effective mobilities which are strongly related to the chemical equilibria existing in solutions The main possibilities of selectively influencing the effective mobilities follow from relationship 1, where the molar fractions x_i depend on acid-base and complex formation equilibria and the ionic mobilities u_i depend on solvation and the ionic strength of the solution

Of key importance is the possibility of separating the various ionic species involved in chemical reactions The question arises as to whether the given ionic species migrate as an uniform substance and cannot thus be separated one from another, or whether they migrate independently as individual substances and can be separated In this problem an important factor is the reversibility of the chemical system involving the ionic species which is determined by the nature of the ions in the solution and by the arrangement of the electrolyte system during the isotachophoretic analysis

2 2   Isotachophoresis of species involved in reversible chemical equilibria

When dissolving even a simple compound in water, very complicated mixtures can be formed For example in a solution of a salt of Cd^{2+} in a medium containing chloride ions, cadmium is present in a number of species, namely Cd^{2+}, $CdCl^+$, $CdCl_2$, $CdCl_3^-$, $CdCl_4^{2-}$ If the chemical equilibria among these species are reversible, then in a solution containing chloride ions cadmium may migrate electrophoretically as an ion-constituent formed by the element Cd

The reversibility of a chemical reaction in electrophoresis means that the chemical equilibrium is established much more rapidly than the electromigration proceeds For the correct migration of isotachophoretic zones, i e , for their analytical stability[9], however, not only the reversibility of the reactions but also a steady state between the chemical reactions and the electromigration in the isotachophoretic zone must be ensured This means that in a complex-forming equilibrium, where a cation M^+ reacts with a ligand Y^- to a complex MY^-

$$M^+ + Y^- \rightleftharpoons MY^- \tag{2}$$

(the signs of the charges have symbolic meaning only and do not indicate the number of charges on the ions), the cation M^+ must be in a steady state with ligand Y^- in the isotachophoretic zone, although they migrate in opposite directions in the electric field This necessary permanent contact is ensured by selecting a suitable electrolyte arrangement

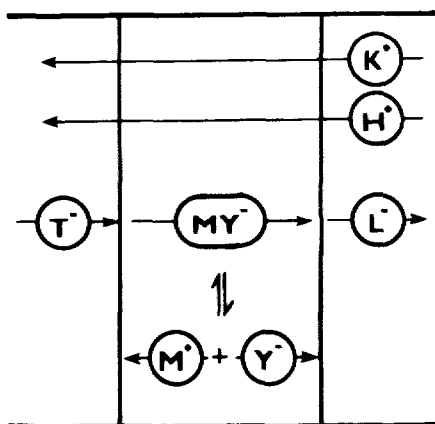The basic arrangement used for fast complex formation equilibria in isotach-

Fig 1 Isotachophoretic migration of a kinetically labile complex K^+ and H^+ are common counter ions, L^- and T^- are the leading and terminating anions, respectively After ref 1

ophoresis is represented by the complexing counter ion method[10], separating anions with a complexing counter cation and *vice versa* As an example of the use of this method, the anionic separation of halides and sulphates with the use of Cd^{2+} as the complexing counter cation, and the cationic separation of lanthanides using α -HIBA (α -hydroxyisobutyric acid) as the complexing counter anion[11], may be mentioned

In the case of fast complex formation equilibria where the electrolyte configuration does not ensure permanent contact between the cation and the ligand, the sample zone may be analytically unstable[9], i e , the appropriate substance is lost from the zone during its migration However, even such systems may be used for very selective analyses as has already been shown for anionic metal complexes M^{II} -EDTA (EDTA = ethylenediaminetetraacetate), this has been called the "bleeding zone technique"[12 13]

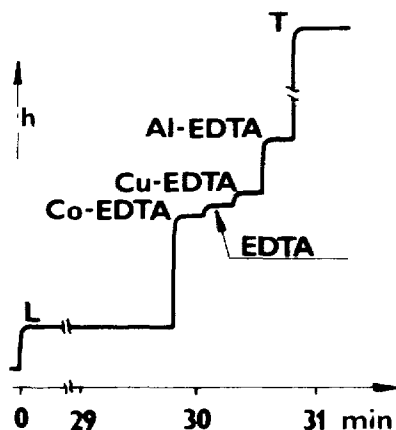A detailed study of bleeding zones has been performed in anionic systems for

Fig 2 Separation of a model mixture of Cu(II)–EDTA, Co(II)–EDTA, Mn(II)–EDTA, Al(III)–EDTA and EDTA at pH = 2 2 L = 0 015 *M* Cl⁻ (HCl + KCl), pH = 2 2, T = 0 01 *M* glutamic acid h = Step height, the signal of the potential gradient detector After ref 14

kinetically labile metal–EDTA complexes[13] The migrating zone of the complex is shown in Fig 1 from which it is seen that cation M continually leaves the zone and the complex decomposes The amount of the complex decomposed depends on the conditional stability constant of the complex and further on the parameters of the equipment and electrolytes used, it is independent of the sampled amount of the separand and of the driving current, i e , of the analysis time From an analytical point of view, two cases are of interest (i) the complex is decomposed completely during the analysis, (ii) the complex decomposition is negligible (the complex is detected practically quantitatively) The migrational stability of the bleeding zones of complexes may be influenced by appropriate selection of the pH which has a strong influence on the values of the conditional stability constants and thus on the decomposition of the zones (for details, see ref 13)

The zones of kinetically labile complexes having small stability constants decompose completely during the analysis, whereas complexes with high stability constants exhibit negligible bleeding and thus migrate practically quantitatively under the usual working conditions and analysis times

A special case is represented by the so-called enforced stability of zones of complexes[13] of the MY^- type in anionic isotachophoresis This stability is caused by the behaviour of the free ligand ion, which migrates behind the zone of the complex in question The complex MY^- decomposes during the migration of its zone and cation M^+ leaves the zone through its rear boundary However, when entering the free ligand zone, M^+ recombines with ligand, reforming MY^- If the recombination is a sufficiently fast reversible process reaching equilibrium within the region of the zone boundary, then the zone of the complex migrates quantitatively —its stability is enforced

An example of this migration behaviour is found in the analysis of a mixture containing Al, Cu, Mn and Co in the form of their EDTA complexes[1 14], using an acidic leading electrolyte (pH = 2 2), see Fig 2 The steps on the record illustrate various forms of migration behaviour

the zone of the kinetically inert Al–EDTA complex migrates quantitatively

the zone of the kinetically labile Cu–EDTA complex has sufficient isotachophoretic stability to migrate practically quantitatively

the zone of free EDTA formed by complete decomposition of the very unstable Mn–EDTA complex

the enforced stable zone of the Co–EDTA complex which migrates quantitatively owing to the following EDTA zone

2 3 Isotachophoresis of species involved in equilibria that are not instantly reversible

A number of inorganic species in solution are not involved in instantly reversible chemical equilibria Some metal ions notably those of Rh, Ru, Cr, etc , form complexes at rather slow rates, so that when a solution of such a cation is prepared we have in fact a mixture of complexes For example[4], $RhCl_6$ in aqueous HCl will hydrolyze and slowly form a mixture of chloroaquo complexes which under zone electrophoretic analysis yield a number of bands

If the lifetime of the species involved in equilibria is greater or comparable with the isotachophoretic analysis time, then the individual species may be observed
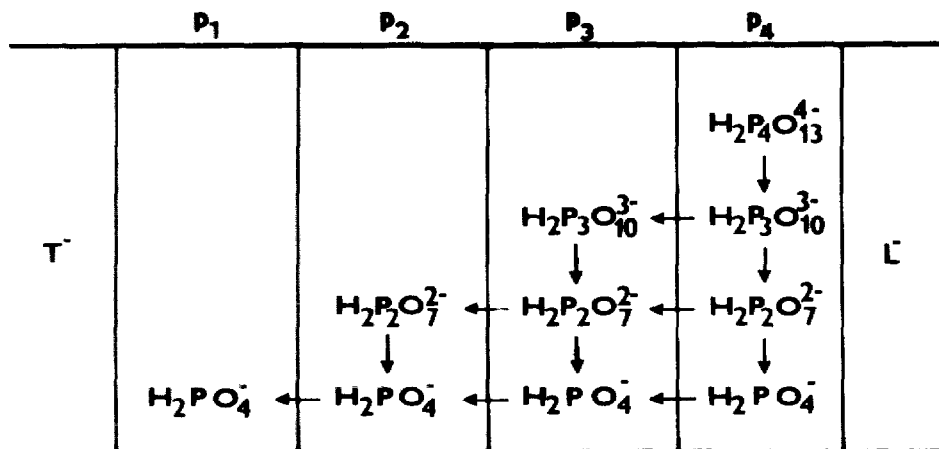
Fig 3 Quasi-steady state isotachophoretic migration of condensed phosphates After ref 15, with permission

as individual zones One must, however, bear in mind that such systems are strictly only quasi-isotachophoretic since the zone lengths change with time in a way dependent on the rate of interconversion the individual species In these cases the analysis record represents the composition of the solution each step on the record corresponds to the amount of the given species in the solution at the time of detection A correct interpretation of the record requires a more detailed treatment based on the knowledge of the kinetics of the chemical equilibria involved

Not instantly reversible equilibria occur not only with a number of complexes of transition metals, but also with various condensed compounds, e g , polyphosphates The situation is analogous, i e , the zones migrate in a quasi-steady state configuration where the zone lengths change with time Such a system is illustrated in Fig 3 where the tetraphosphate $P_4O_{13}^{6-}$ in aqueous solution degrades step by step (also during the migration) and time-dependent concentrations of the species $P_3O_{10}^{5-}$, $P_2O_7^{4-}$ and PO_4^{3-} are observable[15]

The quantitative analysis of such systems necessitates a detailed knowledge of the rate constants of the reactions involved, calibration with standards having a
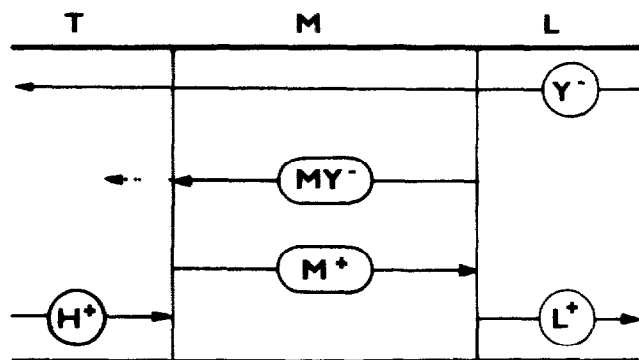

Fig 4 The cationic isotachophoretic system with complexing counter ion After ref 16

composition very similar to that of the analyzed samples and the differences in analysis times should be minimized[15]

Not instantly reversible equilibria between the substances to be separated and the counter ion have a considerable influence on the isotachophoretic migration As has already been shown[9], the zones of the separated substances are analytically unstable in such cases

In order to illustrate these effects Fig 4 shows a cationic isotachophoretic system[16] in which the zone of a cation M^+ forming a complex MY^- with the counter anion Y^- migrates between zones of the leading and terminating cations L^+ and H^+ Certain concentrations of both free M^+ and the complex MY^- are present in zone M. determining the value of the effective mobility of M^+ The cation M^+ migrates forwards and recombines at the front boundary of its zone to form the complex The so-formed anionic complex migrates in the opposite direction, at the rear boundary the equilibrium is re-established by the decomposition of the complex

Depending on the rate of the complex decomposition reaction, we can distinguish three cases

(i) The decomposition of the complex is a very fast reaction The reaction may reach equilibrium within the interface between the zone of the acidic terminator H^+ and zone M In this case zone M is isotachophoretically stable and there is no loss of the analyzed substance owing to the chemical kinetics Such a migrating system is shown in Fig 5a

(ii) The decomposition of the complex is a slow reaction The decomposition of the complex is not fast enough for equilibrium to be attained within the zone boundary and the complex MY^- penetrates into the terminating zone Thus, zone M does not contain the whole amount of the metal sampled and is isotachophoretically unstable It is followed by a tail containing the metal in the form of cation M^+ and anion MY^- migrating in the terminating zone, see Fig 5b

(iii) The decomposition of the complex is a very slow reaction so that the complex can be considered as kinetically inert In this case the metal in the form of complex MY^- leaves the zone irreversibly, and can be modelled on the basis of the bleeding zone introduced[13] for anionic systems The decomposition (bleeding) rate is constant (see Fig 5c)

2 4   Isotachophoresis of kinetically inert species

A number of metal cations can form extremely strong complexes which can be subjected to electrophoresis or chromatography in a wide range of acids or bases without any risk of decomposition Such species behave in isotachophoresis as an ion-constituent comprising one sub-species only which does not decompose into other ones during the migration Its migration can thus be compared with the migration of simple ions of strong electrolytes Examples are the anionic migration of $Fe(CN)_6^{3-}$, Fe^{III}–EDTA or Al–EDTA, *cf*, Fig 2

2 5   Influence of acid–base and complex formation equilibria on the migration behaviour of zones

When metal salts (especially cations of the transition metals) are dissolved in
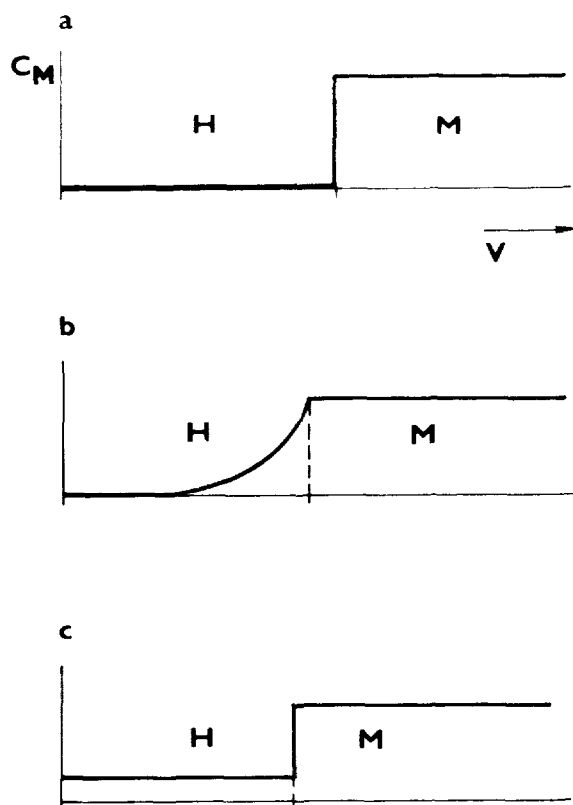
a

C_M

H

M

V

b

H

M

c

H

M

Fig 5 Stable (a) tailing (b) and bleeding zones (c) in cationic isotachophoretic systems v = Migration velocity

water, complicated mixtures of hydrolysis species may be formed For example, an aqueous solution of $Fe(NO_3)_3$ contains the species $Fe(H_2O)_5OH^{2+}$, $Fe(H_2O)_4(OH)_2^+$ and even $Fe(OH)_3^0$ which polymerizes upon aging In no case is the Fe^{3+} ion found to any extent in the solution (cf, ref 4)

In the possible reaction

$$Fe(OH)_3^0 + H_3O^+ \rightarrow Fe(H_2O)_4(OH)_2^+$$
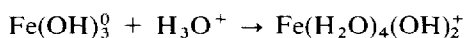
the species $Fe(OH)_3$ may be compared with a weak organic base B which can be protonated in the sense $B + H^+ \rightleftharpoons BH^+$ This fact is important especially when considering the correctness of migration of zones of inorganic cations and their mutual separability Here, the current theory[17][18] of analytical stability, correct migration of zones and of the separability of substances may be used, which has been developed for models of weak bases B ($B + H^+ \rightleftharpoons BH^+$) and weak acids HA ($HA \rightleftharpoons H^+ + A^-$)

A zone is analytically stable if it always contains the same amount of a given substance to be analyzed In isotachophoresis this means that the zone volume is constant and the zone migration is correct, i e , both the front and rear zone bound-

aries show self-sharpening properties (*cf*, ref 18) Neglecting instabilities due to slow chemical kinetics and the electrolyte arrangement (see section 2 3), the stability of isotachophoretic zones may be identified with the requirement of correct migration It may be expressed by the relationships (*cf*, Fig 6)

$$\bar{u}_{X,X} < \bar{u}_{L\ X} \tag{3}$$

$$\bar{u}_{T,T} < \bar{u}_{X,T} \tag{4}$$

where $\bar{u}$ is the effective mobility of a given substance (first subscript) in a certain zone (second subscript), *i e*, the effective mobility of substance X in the terminating zone is higher than the effective mobility of the terminator, and, simultaneously, the effective mobility of the leading substance in the zone of substance X is higher than the effective mobility of substance X in its own zone Any substance X fulfilling the relationships 3 and 4 migrates correctly —its zone is stable

In aqueous media, however, some complications may be expected due to the presence of the rapidly migrating H^+ and OH^- In any case H^+ and OH^- must be considered as the potential terminators for cationic and anionic isotachophoresis, respectively[18-21], and, by suitable selection of the leading electrolyte, the fulfilment of condition 4 must be ensured for $\bar{u}_{H\ H}$ and $\bar{u}_{OH,OH}$ in the terminating zones for cationic and anionic migration, respectively The definition and method of calculation of $\bar{u}_{H,H}$ and $\bar{u}_{OH,OH}$ can be found elsewhere[18,20]

The decision as to whether a substance X gives or does not give a correct isotachophoretic zone in a given electrolyte system has great significance in practice In cationic isotachophoresis, this is due to the fact that the ionic mobility of H^+ is of an order of magnitude higher than those of most other inorganic cations Condition 4 has here the form

$$\bar{u}_{H\ H} < \bar{u}_{M,H} \tag{5}$$

The value of $\bar{u}_{H.H}$ depends mainly on the pK of the counter anion used and on the concentrations of the leading ion and the counter ion in the leading electrolyte

Fig 7 shows the record of a cationic isotachophoretic analysis[20] of a model mixture containing sodium, lithium, Tris [tris(hydroxymethyl)aminomethane] and TBA (tetrabutylammonium) Correct isotachophoretic migration of the zones of all four components being separated occurs as condition 5 is fulfilled for all of them Fig 8 shows the analysis[20] of the same mixture in a system where the effective mo-
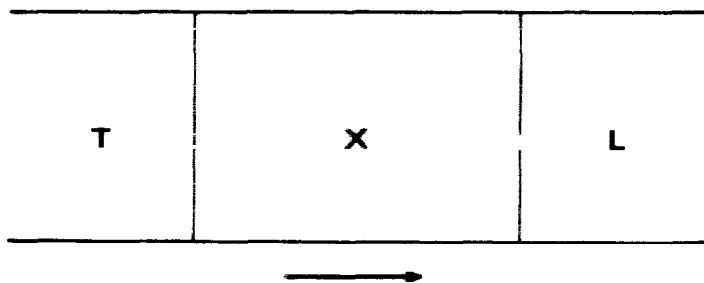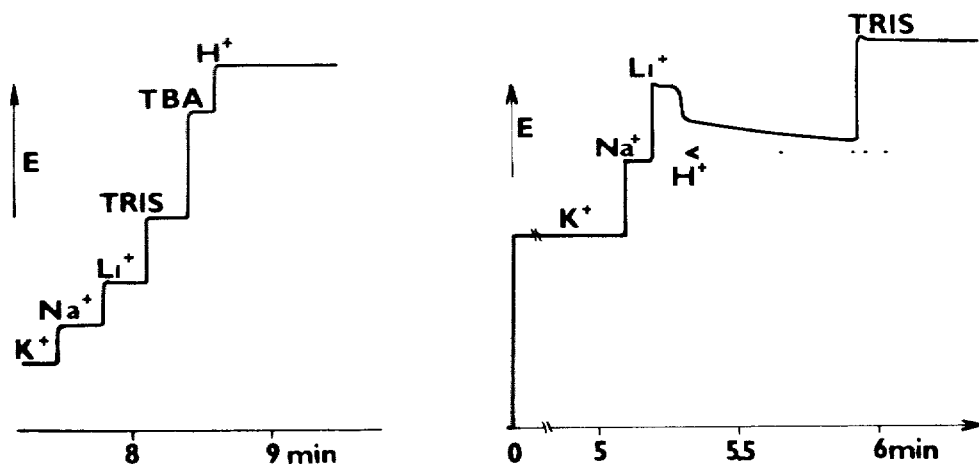
Fig 6 A correct isotachophoretic system

Fig 7 Isotachophoretic separation of a model mixture L = 0 01 M potassium acetate, T = 0 05 M formic acid E = Electric gradient After ref 20

Fig 8 Isotachophoretic separation of the same model mixture as in Fig 7 L = 0 01 M potassium formate + 0 01 M formic acid, T = 0 1 M Tris E = Electric gradient After ref 20

bility of H^+ has a higher value and condition 5 is fulfilled only for sodium Evidently, only sodium has a correct isotachophoretic zone Lithium exhibits an obscure zone distorted by the migration of the front of H^+, the latter is depicted by the arrow indicating the direction of movement at the step height level of H^+ when used as the terminator

Currently the problem of correct migration of zones is more general and studies are aimed at characterizing substances that can be analyzed correctly by employing the actual leading and terminating electrolytes, i e , to demarcate the region of stable zones For such purposes, the concept of the zone existence diagram was proposed and its use demonstrated[17][18] In a zone existence diagram the dependence of effective mobilities vs zone pH is shown, where for a given electrolyte system the region of points ($\bar{u}_{X,X}$, pH_X) corresponding to zones of substances analyzable in this system may be demarcated Correct zones are represented by a region within contours on which the above conditions 3 and 4 are just valid This concept has been suggested and worked out in detail for cases of organic bases and acids, but is of general validity

Fig 9 shows the zone existence diagram[18] for an anionic system formed by 0 01 M ammonium chloride (leading electrolyte) and OH^- (terminator) The region within the demarcating contours corresponds to correct zones of acids HA characterized by the values of $\bar{u}_{A,A}$ and pH_A The points on the curve descending from the point for Cl^- (leading zone) correspond to zones of strong acids, the points on the lower demarcating curve correspond to zones of weak acids for which the above condition with respect to OH^- as the terminator is invalid ($\bar{u}_{A,OH} = \bar{u}_{OH,OH}$) The network represents the pK_{HA} and u_A parameters of monohydric acids and allows to determine readily whether a given substance will or will not give a stable isotachophoretic zone in the given system Further, the diagram allows fast estimation of the properties of zones, for example, points corresponding to zones of various substances
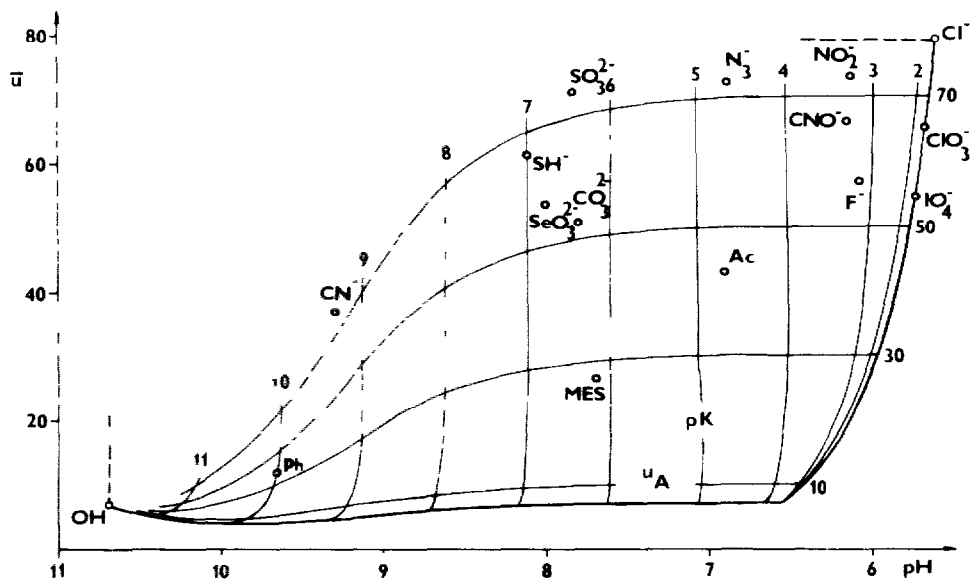
Fig 9 Zone existence diagram for the anionic isotachophoretic system with 0 01 M NH$_4$Cl as the leading electrolyte and OH$^-$ as the terminator After ref 18

may be inserted into the diagram, as is shown in Fig 9 for some inorganic ions and for two currently used terminators, acetate (Ac) and morpholineethanesulphonic acid (MES)

Zone existence diagrams may also be constructed for cationic systems, for a detailed study see ref 17

In isotachophoresis of inorganic cations, the required selectivity of the separation is currently reached by using complex formation with the counter ion An example of such a system[21] is shown in Fig 10, where a bivalent cation M^{2+} and
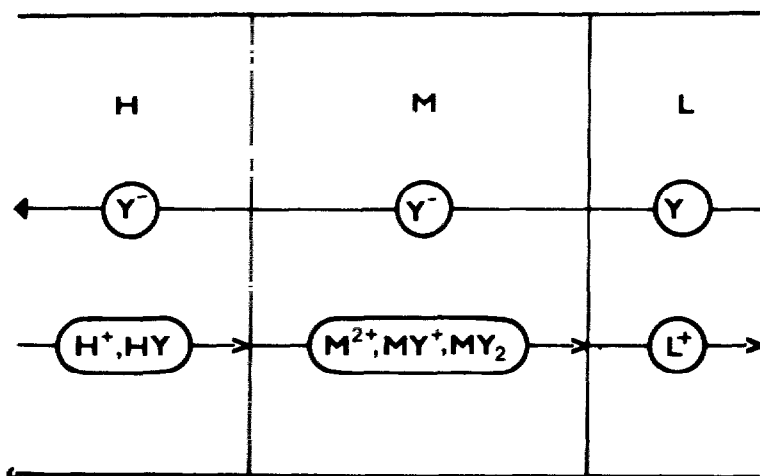

Fig 10 Isotachophoretic migration of cation M^{2+} with complex-forming counter ion Y$^-$ After ref 21
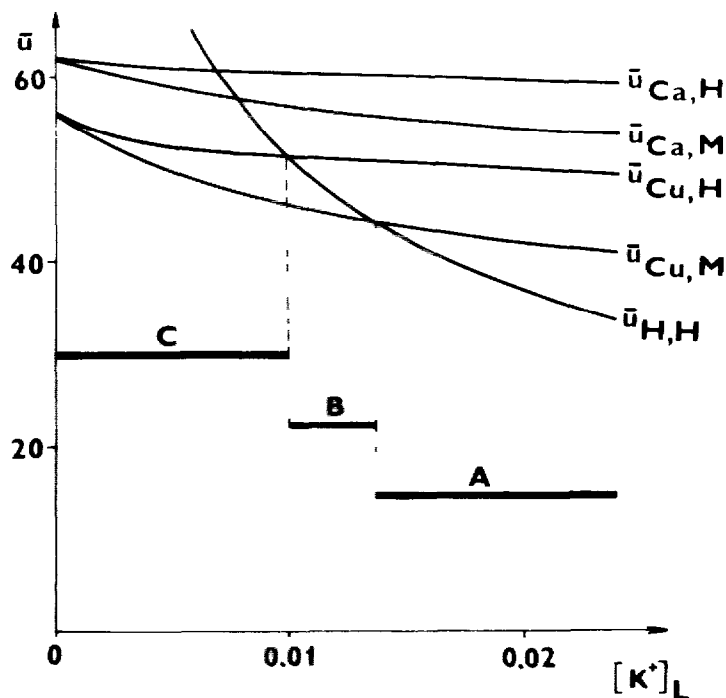
Fig 11 Calculated dependences of the effective mobilities of H^+, Ca^{2+} and Cu^{2+} on the concentration of the leading electrolyte After ref 22

the counter ion Y^- form the complexes MY^+ and MY_2 The criterion for correct migration of the zones of the analyzed substances has the form of condition 5 and in order to fulfil this (for a given complexing counter anion) the values of pH and concentrations of the components of the leading electrolyte are decisive

A more detailed discussion of the problem can be based on the graphical treatment[22] given in Fig 11 which shows the calculated dependences of the effective mobilities of H^+, Ca^{2+} and Cu^{2+} on the concentration of the leading cation It is seen that for both Cu^{2+} and Ca^{2+} the concentration axis may be divided into three sections as it is shown in Fig 11 for Cu^{2+} These sections correspond to three migration regimes of the copper zone

(A)   normal isotachophoresis $\bar{u}_{Cu H} > \bar{u}_{Cu,Cu} > \bar{u}_{H,H}$
(B)   mobility inversion $\bar{u}_{Cu,H} > \bar{u}_{H,H} > \bar{u}_{Cu,Cu}$
(C)   zone electrophoresis $\bar{u}_{H,H} > \bar{u}_{Cu,H}$

The experimental verification of this is shown in Fig 12 In the case a the zones show normal migration Case b corresponds to the point of intersection of the curves in Fig 11, case c to the inversion of mobilities for the copper zone In case d, copper migrates in a zone electrophoretic manner and the front of the zone of H^+ penetrates through its front boundary (see arrow) At the same time, the zone of Ca^{2+} showed normal isotachophoretic migration in cases a–c as expected from Fig 11

When using complexing counter ions, in practice it is often difficult to reach

a sufficiently low value of $\bar{u}_{H\,H}$ since most of the ligands are anions of low-pK acids This problem may be solved by the use of mixed counter ions[21], where both the needed ligand and a suitable weak anion ensuring the suppression of $\bar{u}_{H,H}$ are used as the components of the leading electrolyte

2 6 Separability of substances

The investigation of the separability of substances is not a simple problem There are well known cases where a pair of substances are not separated although they have the same effective mobilities in their own zones, e g , lactic and mandelic acids in an anionic system[23] Moreover, substances with different effective mobilities in their own zones may not be separate at all, e g , tetrabutylammonium and aniline in a cationic system[17]

Thus, the investigation of separability is based on the parameters $\bar{u}_{i,j}$[24] and is equivalent to the principle of determination of the migration order of zones of substances[17][18] If the migration order of a pair of substances i and j in a given electrolyte system can be determined unambiguously, then these substances are separable each from other Simple criteria hold for both possible migration orders

the zone of substance i migrates in front of the zone of substance j if

$$\bar{u}_{i,j} > \bar{u}_{j,j} \tag{6a}$$

$$\bar{u}_{j\,i} < \bar{u}_{i\,i} \tag{6b}$$

the zone of substance j migrates in front of the zone of substance i if

$$\bar{u}_{i,j} < \bar{u}_{j\,j} \tag{7a}$$

$$\bar{u}_{j\,i} > \bar{u}_{i\,i} \tag{7b}$$

If for a given pair of substances the combination of conditions (6b) and (7a) holds true, then the migration order cannot be determined, the substances form a steady state mixed zone and their separation is impossible (cf, ref 17)

For the determination of groups of substances which can be separated in a given electrolyte system from a given analyzed substance, it is possible to take advantage of the zone existence diagram and the so-called sequence contours The use of such a diagram may enable the prediction of not only separability and migration order but also inversions of mobilities and pH in the migrating system A detailed description of this approach can be found elsewhere[17,18]

3 SEPARATION OF METAL CATIONS

For this analytical problem isotachophoresis is one of a number of analytical methods which can be employed, e g . polarography, atomic absorption spectrometry, spectrographic and activation analysis The separation possibilities of isotachophoresis offer, however, a number of advantages and even very small differences in the chemical behaviour of elements may be used selectively to influence their effective mobilities and thus achieve the required separation
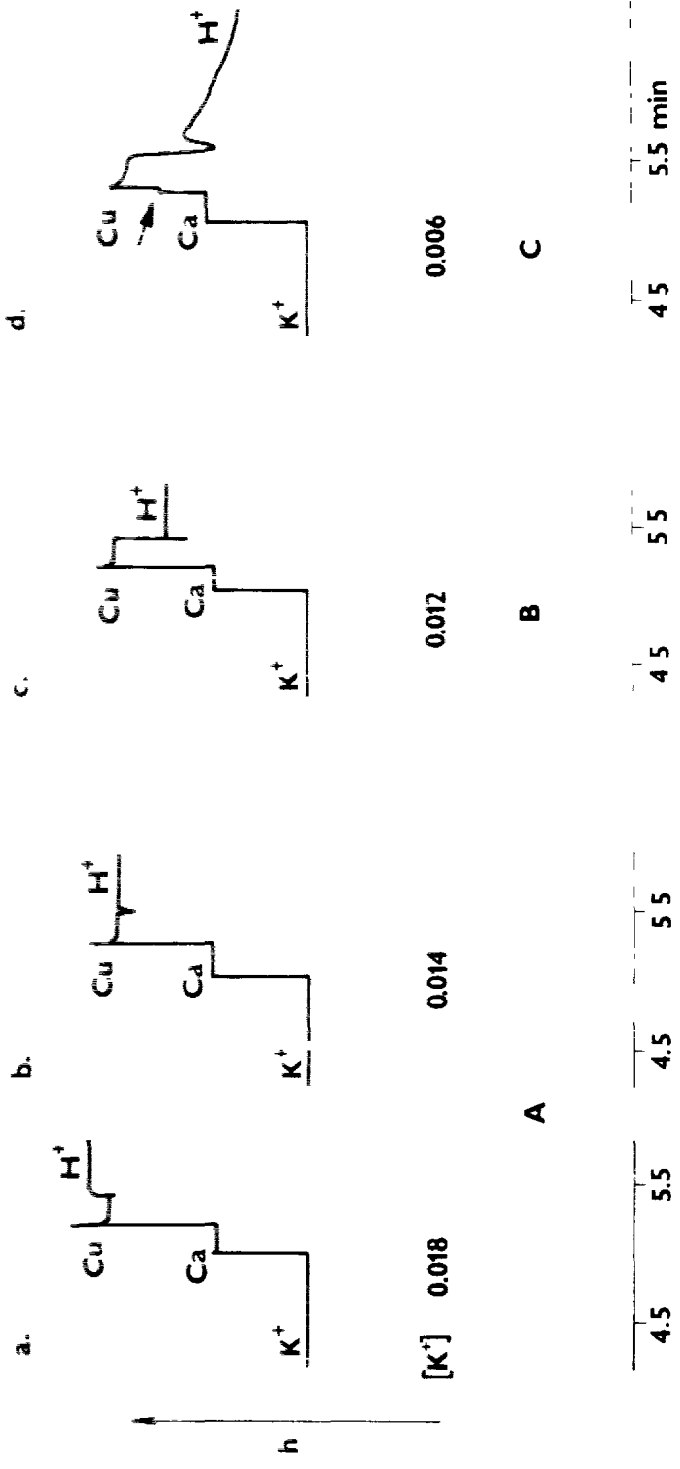
Fig 12 Experimental isotachophoregrams of Ca^{2+} and Cu^{2+} at various concentrations of the leading electrolyte L = potassium acetate, T = acetic acid h = Step height (see Fig 2) After ref 22

For influencing the effective mobilities of metal cations, changes in solvation of ions by using non-aqueous or mixed solvents (e g , methanol[25,26]), and especially electrolyte systems comprising fast reversible equilibria with a complexing counter anion[11,21,27 28] are used

The separation of alkali metals (also ammonium) is a difficult problem for isotachophoresis in aqueous solution This is caused by the very similar ionic mobilities of ammonium and potassium in water A model mixture of these cations has been separated by using methanol as a solvent in an apparatus with thermocouple detection[25,26] The separation of these substances in methanol with the use of commercial equipment and conductivity detection[29] is shown in Fig 13

The alkaline earth metal cations also have very similar ionic mobilities, and in order to separate them by isotachophoresis their effective mobilities must be influenced by complex formation for example by using CDTA (cyclohexane-1,2-diamine-N,N,N′,N′-tetraacetic acid)[27] or α-hydroxybutyrate[30] An example from the latter work is shown in Fig 14 where. moreover, Zn and Li are separated

The chemical similarity of lanthanides and the practical identity of their ionic mobilities has led to attempts to separate them using complexing counter ions Successful experiments were made with α-HIBA which yielded a complete separation of all lanthanides This separation was published by Nukatsuka et al [11] and belongs to the very important achievements of analytical isotachophoresis (see Fig 15)

In the analysis and preparative isolation of fission products an important step is the separation of lanthanides and actinides The use of counterflow isotachopho-
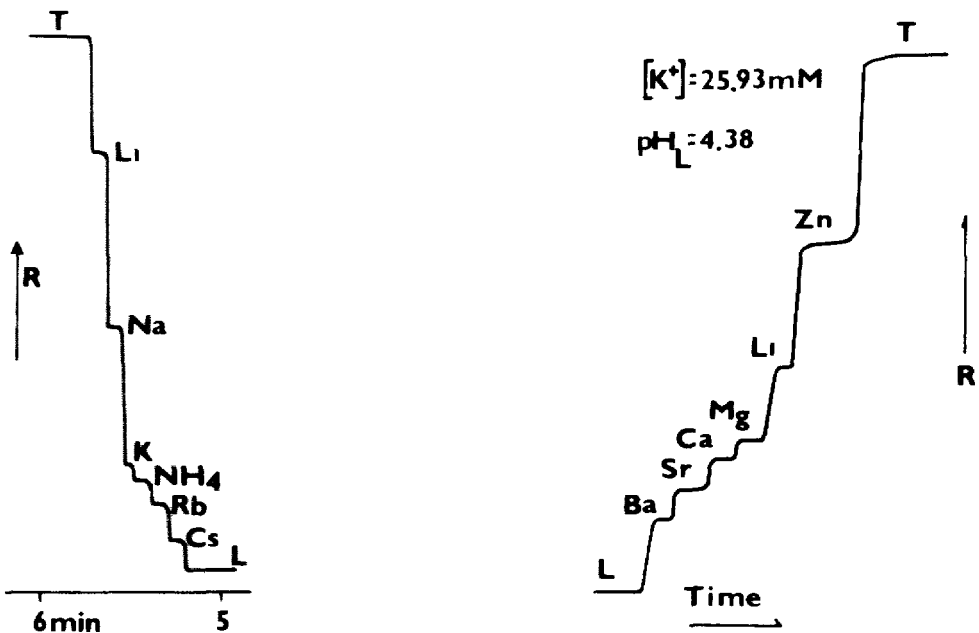

Fig 13 Separation of alkali metals and ammonium in methanol L = 0 0025 M HCl Solvent methanol–water (99 1, v/v) T = 0 001 M CuCl$_2$ R = Detector response After ref 29, with permission

Fig 14 Isotachopherogram of Ba^{2+}, Sr^{2+}, Ca^{2+}, Mg^{2+}, Li$^+$ and Zn^{2+} L = 0 02593 M K$^+$ + α-hydroxybutyric acid, pH = 4 38 T = ε-aminocaproate R = Detector response After ref 30, with permission
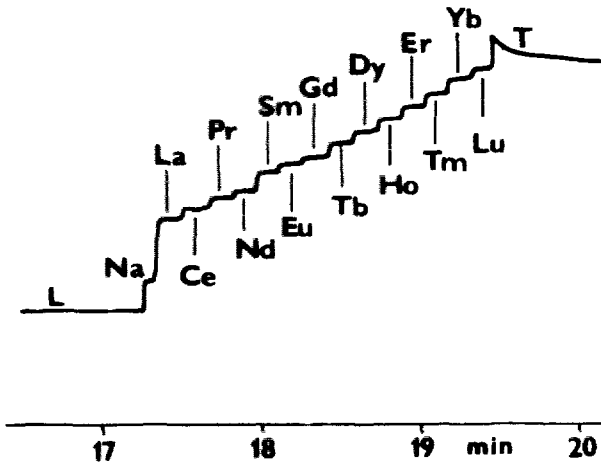
Fig 15 Isotachophoretic separation of lanthanides L = 0 027 M KOH, 0 015 M α-HIBA, acetic acid, pH = 4 92, 0 0025% polyvinyl alcohol, T = β-alanine After ref 11, with permission

resis ("Gegenstromelektrolyse") was studied Bilal and Reichmuth[31] on a model mixture containing one lanthanide (Nd) and one actinide (Am) The combination of Nd and Am was chosen because they possess the same charge and practically identical ionic radius, and thus represent the most difficult lanthanide and actinide pair to separate The successful preparative separation of 50 mg Nd and 80 mg Am was a very important demonstration of the potential of counter-flow isotachophoresis The same authors[32] separated milligram amounts of U, Np, Am and Cm in a 60-cm long cell with 2 M potassium acetate as the leading electrolyte, and performed a successful separation of lanthanides and actinides in highly active waste solution from a nuclear reactor Fig 16 shows the second step of the discontinuous separation[33]

For the separation and determination of metal ions, isotachophoresis offers a wide range of possibilities By using complex formation equilibria, the effective mobilities of the studied ions may be influenced almost without restrictions and thus the given separation may be optimized

Although the sensitivity of the method is not greater than some of the more established methods for the individual metals, a great advantage of isotachophoresis is the simultaneous determination of several metals in a relatively short time

The possibilities of isotachophoresis for the separation of multicomponent mixtures containing several analytical groups have so far been demonstrated only on
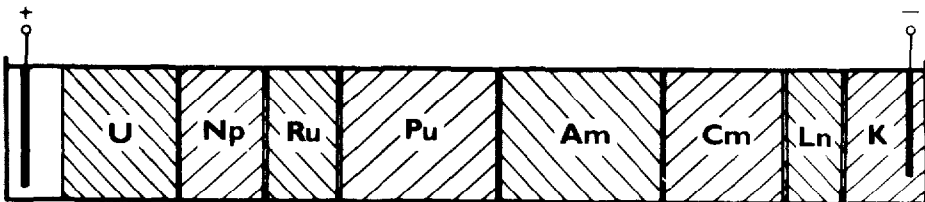

Fig 16 Separation of actinides from highly active waste (HAW) solution by means of counter-current ion migration Counter flow 0 5 M acetic acid + 0 05 M HNO₃ After ref 33, with permission
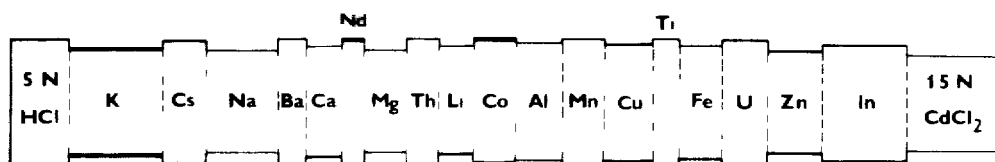
Fig 17 Separation of a multicomponent mixture of cations —shade projection of the glass capillary L = 5 M HCl T = 1 5 M CdCl$_2$ After ref 34

model mixtures The separation of a model mixture containing K, Cs, Na, Ba, Ca, Nd, Mg, Th, Li, Co, Al, Mn, Cu, Ti, Fe, U, Zn and In was described by Oshurkova et al [34] The shade diffraction picture of the mentioned separation is shown in Fig 17 Further examples of the separation of mixtures of metal ions with the use of complex formation equilibria are given in Figs 18 and 19 Fig 18 demonstrates[21] the use of mixed counter ions in order to control the migration of H$^+$ and complex-forming equilibria, and Fig 19 shows the use of α-HIBA for the separation and determination of some heavy metals with special relevance to environmental control[28]

4 SEPARATION OF SIMPLE ANIONS

In solutions containing simple anions the chemical equilibria are frequently more simple than in the case of metal cations The reason is that H$_3$O$^+$, protonated organic bases BH$^+$ or alkali metal cations may serve as the counter ion and the
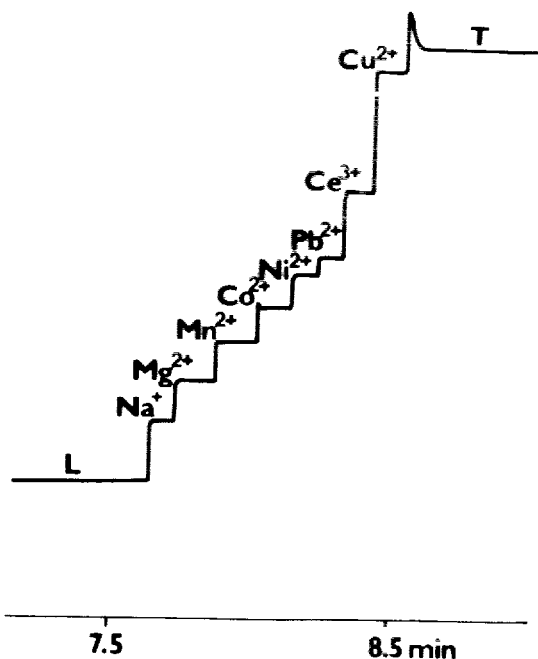

Fig 18 Isotachophoretic separation of a model mixture of cations by employing a mixed counter ion L = 0 05 M potassium lactate + 0 05 M acetic acid T = 0 1 M acetic acid After ref 21
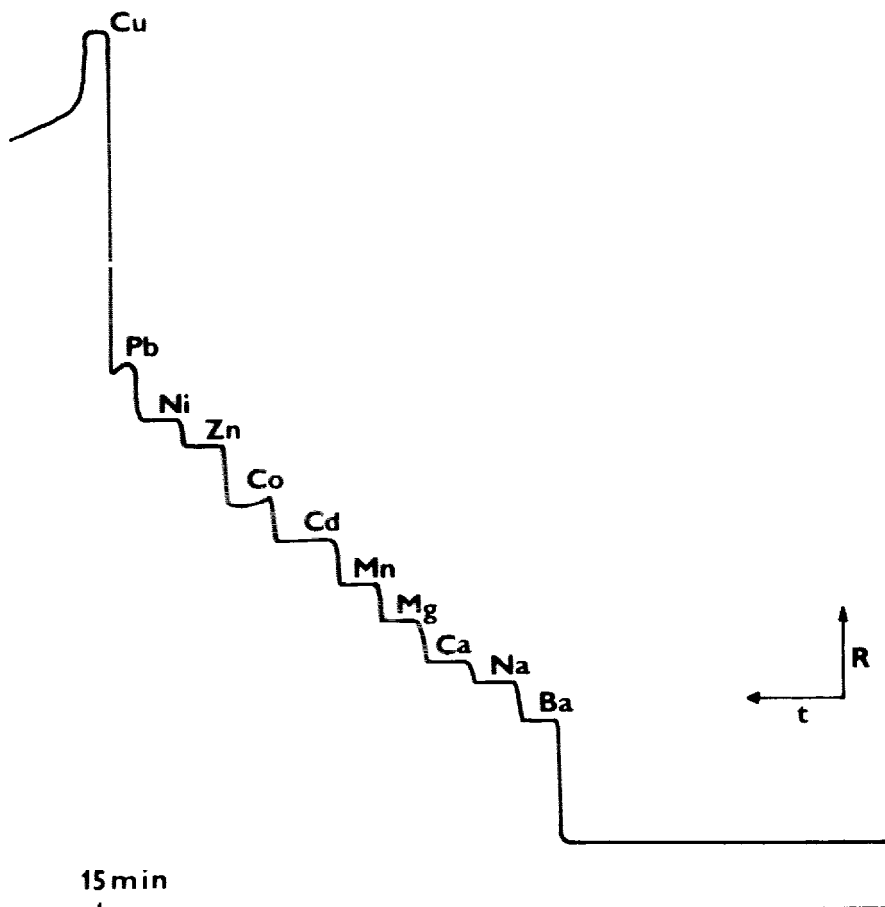
Fig 19 Separation of heavy metals by isotachophoresis L = 0 02 M potassium acetate + α-HIBA, pH = 4 3 R = Detector response After ref 28

formation of various complexes and hydrolysis products is thus eliminated On the other hand, in a number of cases, various anions derived from the same element, $e\,g$, NO_3^- and NO_2^-, $H_2PO_2^-$ and $H_2PO_3^-$, may occur For their separation, mild analytical procedures, $e\,g$, isotachophoresis, are needed which do not influence their mutual ratio in the original sample

For influencing the selectivity of the separations, acid–base equilibria for anions of weak acids and fast reversible equilibria with complex-forming metal cations for anions of strong acids are commonly used

The halides Cl, Br and I have very similar ionic mobilities in aqueous solution The presence of Cd^{2+}, however, may strongly influence their migration behaviour, $e\,g$, in $Cd(NO_3)_2$ as the background electrolyte for zone electrophoresis, halides migrate towards the cathode[35] The use of Cd^{2+} as the complexing counter ion in isotachophoresis[10] allows easy separation of Cl, Br and I in an aqueous medium (Fig 20) A very effective separation of all halides and other substances present in

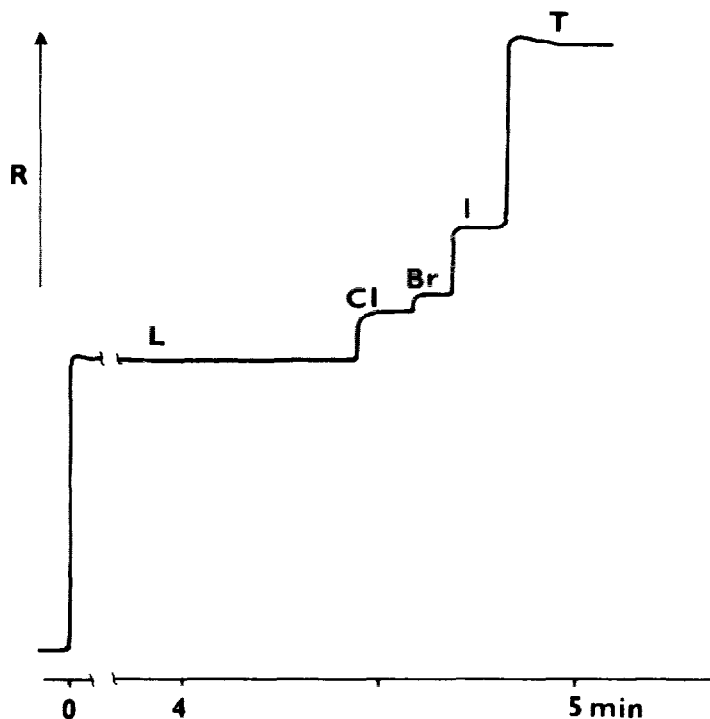Fig 20 Isotachophoretic separation of halides using complex formation with Cd^{2+} L = 0 006 M $Cd(NO_3)_2$, T = tartrate R = Detector response After ref 10

the solution after the absorption of the combustion products in elemental analysis after Schoniger[36] was achieved in methanol (Fig 21)[29]

The halogenates ClO_3^-, BrO_3^- and IO_3^- differ in their ionic mobilities due to their different molecular weights and may be separated very easily and quickly in an aqueous medium, see Fig 22

The separation of selenites and tellurites was published in 1970 by Konstantinov et al[38] They used a device where the electromigration proceeds in a glass capillary with the simultaneous application of a counter flow of the leading electrolyte The detection and evaluation of the analysis was performed by a diffraction method where the capillary was illuminated by a narrow beam of light parallel to its longitudinal axis and the shade diffraction picture of the capillary was observed

The separation of a mixture of chromates, molybdates, wolframates and perrhenates was performed by isotachophoresis on paper by Taglia and Lederer[39] who, at the same time, demonstrated the possibilities of analytical isotachophoresis on paper Besides the separation, Fig 23 shows clearly the concentrating effect of isotachophoresis, see the isotachophoretic zones of the first three substances analyzed which are much more narrow than the starting zone or than the ReO_4^- zone migrating in the elution mode A similar mixture containing dichromate, chromate, molybdate and wolframate in a glass capillary allowing the possibility of visual observation of the condensation of chromate to dichromate was separated by Bresler et al[40]

An important field of application of analytical isotachophoresis is the sepa-
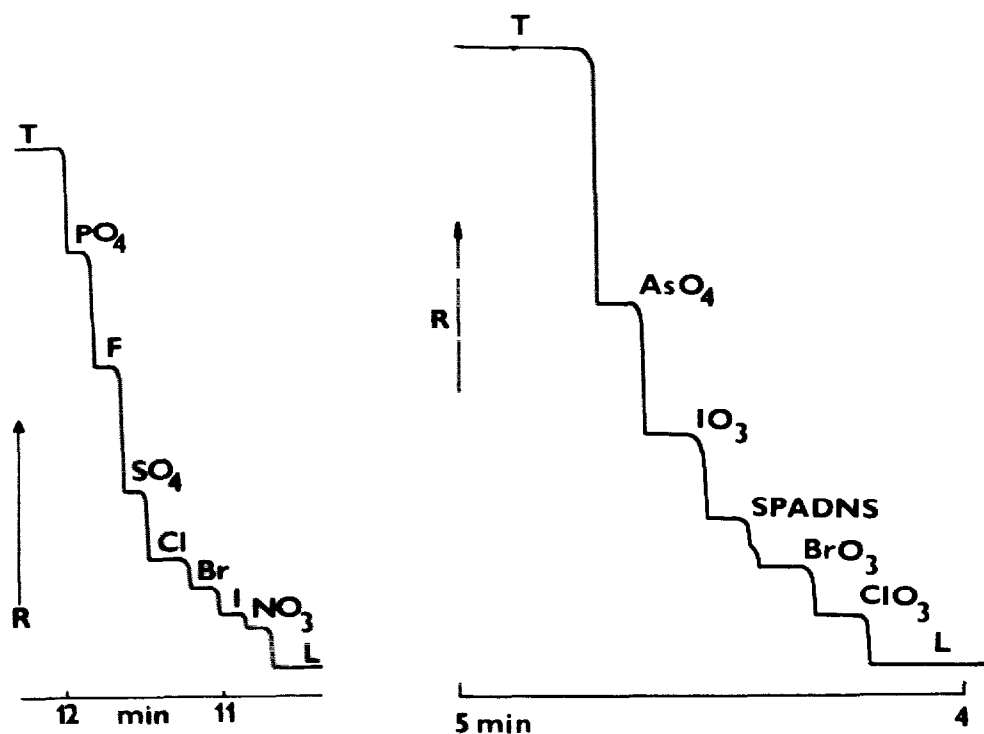
Fig 21 Separation of a model mixture of anions L = 0 001 M HClO$_4$ + N-ethylmorphine (EM), pH = 6 8, 0 1% methylhydroxyethylcellulose, solvent, methanol–water (9 1, v/v) T = 0 001 M caprylate, solvent, methanol–water (99 1, v/v) R = Detector response After ref 29, with permission

Fig 22 Separation of a model mixture with halogenates L = 0 0066 M HCl + aniline, pH = 4 2, T = acetate R = Detector response, SPADNS = sulphanilazochromotropic acid After ref 37

ration of oxygen-containing anions of the same element in various oxidation states The determination of an element without regard to the form present in solution is mostly based on sample pretreatment by a destructive method (oxidation melting, evaporation with HClO$_4$, etc) which transforms all forms into a single defined substance suitable for analytical determination The resolution and analysis of various valence forms of the same element is not usually possible with such methods and the
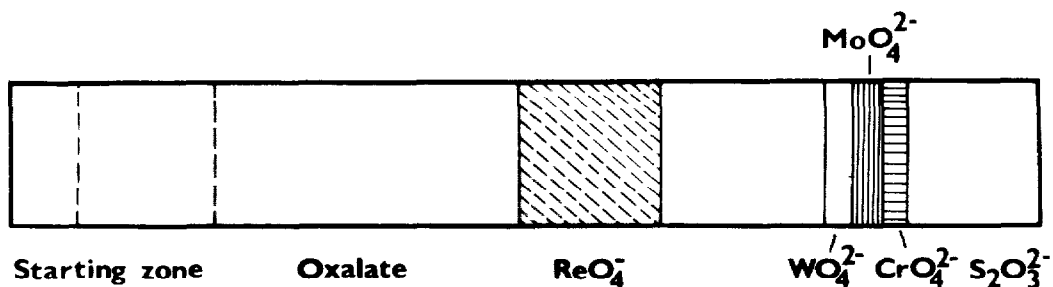

Fig 23 Isotachophoretic separation of chromate, molybdate, wolframate and perrhenate on paper L = 0 1 N Na$_2$S$_2$O$_3$, T = 0 1 N oxalate After ref 39, with permission
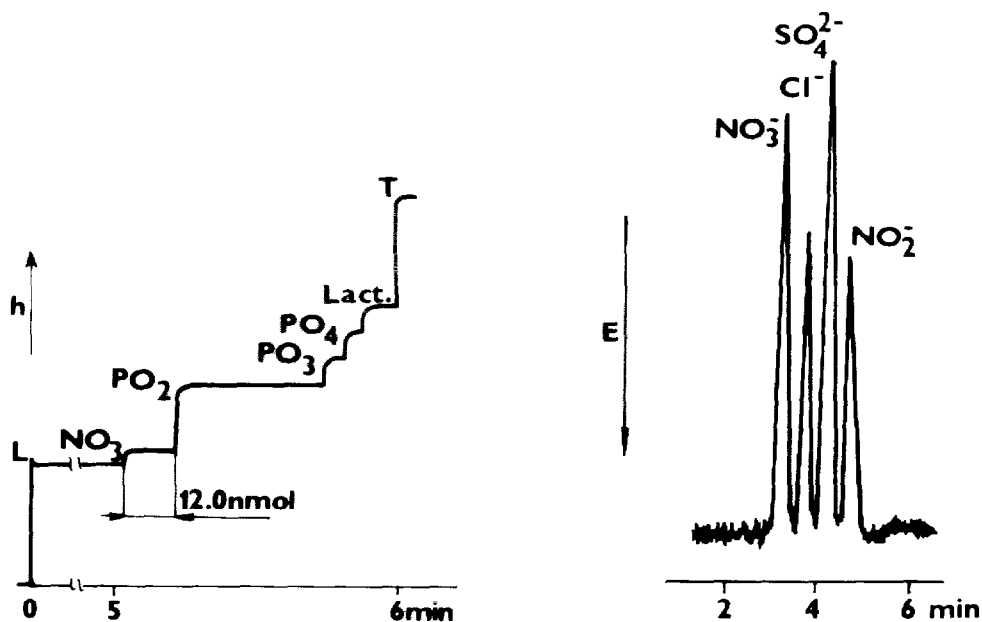
Fig 24 Analysis of a nickel plating bath containing nitrates, hypophosphites, phosphites, phosphates and lactates L = 0 01 M HCl + β-alanine, pH = 4 0 T = 0 01 M glutamic acid h = Step height (see Fig 2) After ref 41

Fig 25 Potential gradient record of the zone electrophoresis of anions using isotachophoretic equipment Background electrolyte 0 0025 M cadmium acetate E = Electric gradient After ref 44

selection of the conditions is more critical In isotachophoresis it is easy to select electrolyte systems which are practically inert with regard to oxidation or reduction of the analyzed compounds

Fig 24 shows an example of the separation[41] of hypophosphite, phosphite and phosphate in a nickel plating bath The nitrate content can be seen Sulphates and sulphites have already been successfully analyzed in practical samples For example, their determination in a mixture with fourteen other anions present in wine[42] and in pharmaceutical control[43] may be mentioned In the latter case the separation was carried out in water-30% acetone

Nitrite and nitrate are easily separated by isotachophoresis in model mixtures with Cl$^-$ as the leading ion Of interest from a practical point of view, is their determination in drinking water Here, isotachophoretic instrumentation with potential gradient detection and the electrolyte system developed[10] for isotachophoresis of halides and sulphates have been successfully used for capillary free-zone electrophoresis An example of the analysis of a model mixture is shown in Fig 25 where not only nitrate and nitrite but also chloride and sulphate are analyzed[44]

5 SEPARATION OF CONDENSED COMPOUNDS

The linear polyphosphates are represented by the general formula $M_{n+2}P_nO_{3n+1}$ where M corresponds to an univalent metal Short-chain polyphosphates ($n = 1-3$) have been separated and determined by several authors[45,46] These
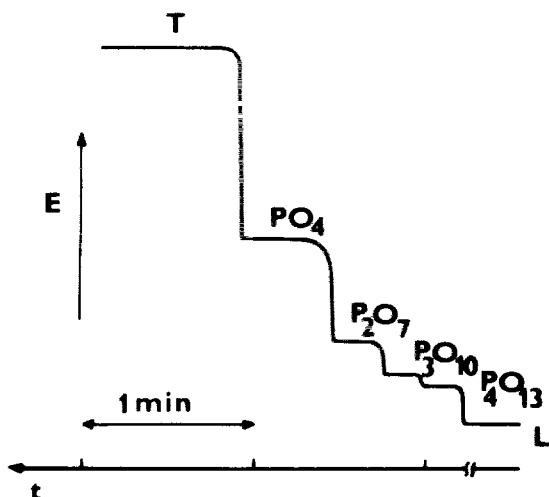
Fig 26 Isotachophoretic separation of linear condensed phosphates having $n = 1\text{-}4$ L $= 0\,01$ M HCl $+$ β-alanine, pH $= 4\,0$, T $=$ glutamic acid E $=$ Electric gradient After ref 15, with permission

polyphosphates are the main component of a number of fertilizers, detergents and water-softening agents Although polyphosphates readily undergo hydrolysis, $e\,g$, tetraphosphate in Fig 3, isotachophoresis allows quantitation of polyphosphates up to $n = 4$ owing to their slow hydrolysis rate An example of the separation of a model mixture[15] is shown in Fig 26

The long-chain polyphosphates $(n \geqslant 4)$ have so similar effective mobilities that their separation seems too difficult by isotachophoresis They are present in solution
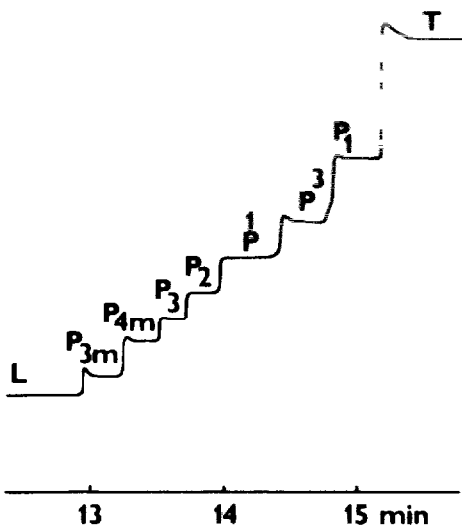

Fig 27 Isotachopherogram of various phosphorus oxoacids $P^1 =$ phosphinate, $P^3 =$ phosphonate, P_1 $=$ phosphate, $P_2 =$ diphosphate, $P_3 =$ triphosphate, $P_{3m} =$ cyclotriphosphate, $P_{4m} =$ cyclotetraphosphate L $= 0\,01$ M HCl $+$ histidine pH $= 5\,5$ $0\,1\%$ Triton X-100, T $=$ hexanoate After ref 48, with permission

as a mixture of oligophosphates of different chain lengths and the average chain length, $\bar{n}$, is usually employed to characterize them, especially for commercial purposes A simple empirical method was proposed[47] for the determination of $\bar{n}$, based on the linear correlation of the isotachophoretic step length per equivalent of P present vs log $\bar{n}$

The various phosphorus oxoacids, such as the linear, cyclic and lower phosphorus oxoacids, have been separated[48] with a leading electrolyte of 0 01 M HCl + Tris (pH = 5 5), in the migration order P_{3m}, P_{4m}, P_3, P_2, P^1, P^1 and finally P_1 (Fig 27) The separation and determination (in 20 min) of various phosphorus oxoacids containing two phosphorus atoms, such as $P(V)-O-P(V)$, $P(III)-O-P(III)$ and $P(III)-O-P(V)$, was reported by Nariai et al [49] Their procedure is suitable for the analysis of crude products and the study of the hydrolytic degradation of some condensed phosphates

The sulphur oxoacid ions such as sulphate, disulphate, peroxodisulphate, dithionate and tetrathionate can be determined simultaneously by using 10^{-2} M NaOH in 50% acetone as the leading electrolyte[50]

Isotachophoresis may give useful information also in the case of other condensed compounds During the hydrolysis of, e g, acidic solutions of vanadates(V), time-dependent amounts of individual polyvanadates are formed which may be separated by isotachophoresis[51]

The possibility of following the conversion of chromate into dichromate is described in Chapter 4

6 SEPARATION OF COORDINATION COMPLEXES

Many metal ions, especially those of the transition metals, Co, Rh, Pt, Cr, etc, may form extremely strong complexes with ammonia, amines, cyanides, thiocyanates and similar ligands During the solvolysis of such complexes, mixed ligand complexes are slowly formed which may be considered as considerably stable species and thus separable from each other The possibility of separation of very similar ionic complexes, e g, complexes of the same metal differing only in their ligands, has already been shown in the pioneering work of Preetz[52-54] who developed the method of counter-current electrophoresis ("Gegenstromionophorese") which is in principle counter-flow isotachophoresis This author clearly explained the basic principles of the separation according to mobilities, the same velocity of ions, the self-sharpening effect of the boundary and the adjustment of concentrations These explanations were verified experimentally, see, e g, Fig 28 which shows the separation of $IrBr_6^{2-}$ and $IrCl_6^{2-}$

For the separation of chemically similar and kinetically inert complex ions, the possibilities of isotachophoresis were demonstrated by Blasius and Wenzel[55] using a simple device ("Trennschlauchapparatur") They worked in a non-aqueous medium (formamide) with cellulose acetate gel as the supporting medium They also demonstrated the use of a colourless spacer (SCN^-) for the clearly visible separation of the coloured complexes $Cr(SCN)_6^{3-}$ and $Cr(CN)_6^{3-}$

The possibilities of two-dimensional continuous counter-flow isotachophoresis for the separation of very similar mixed ligand complexes were shown by Preetz et al [56] These workers reported the separation and isolation of complex ions of very
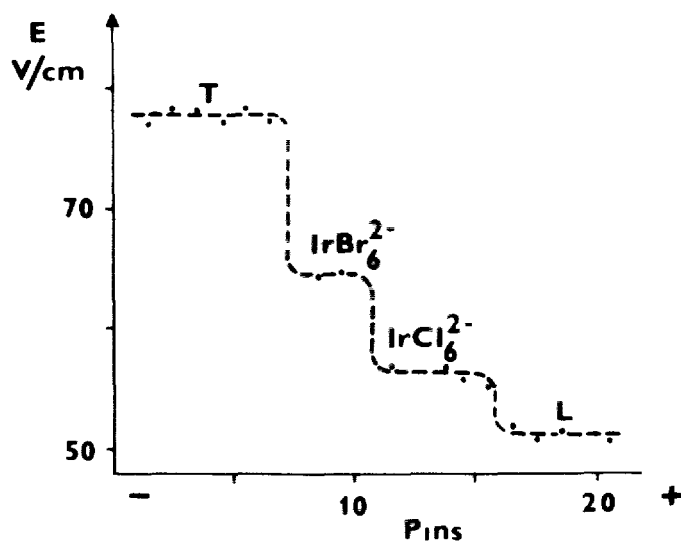
Fig 28 Isotachophoretic separation of $IrBr_6^{2-}$ and $IrCl_6^{2-}$ L = 0 01 M Li_2SO_4, T = acetic acid Counter flow 0 01 M Li_2SO_4 E = Electric gradient measured by the sensing electrodes (Pins) along the migration path After ref 53, with permission

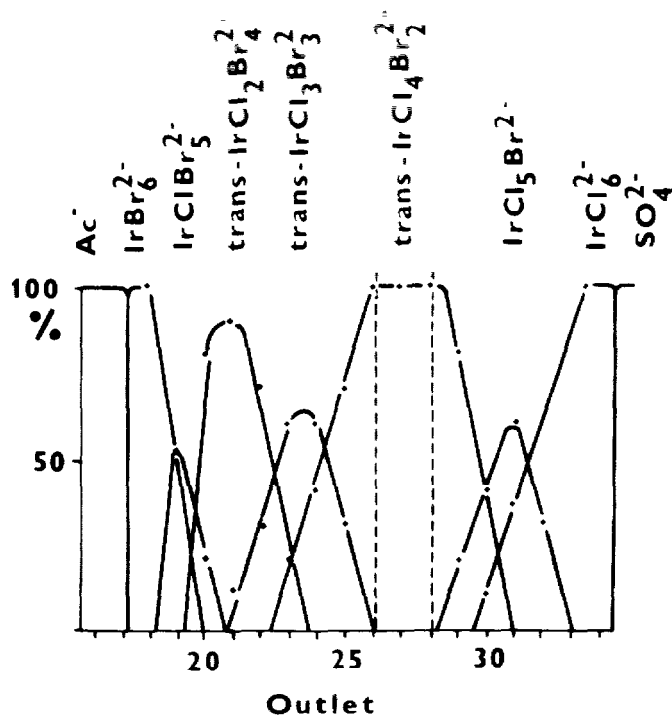Fig 29 Continuous isotachophoretic separation of complexes $[IrCl_xBr_{6-x}]^{2-}$, x = 0–6 L = 0 02 M K_2SO_4, T = 0 012 M potassium acetate Percentage of substances in outlet fractions vs outlet fraction number is shown After ref 56, with permission
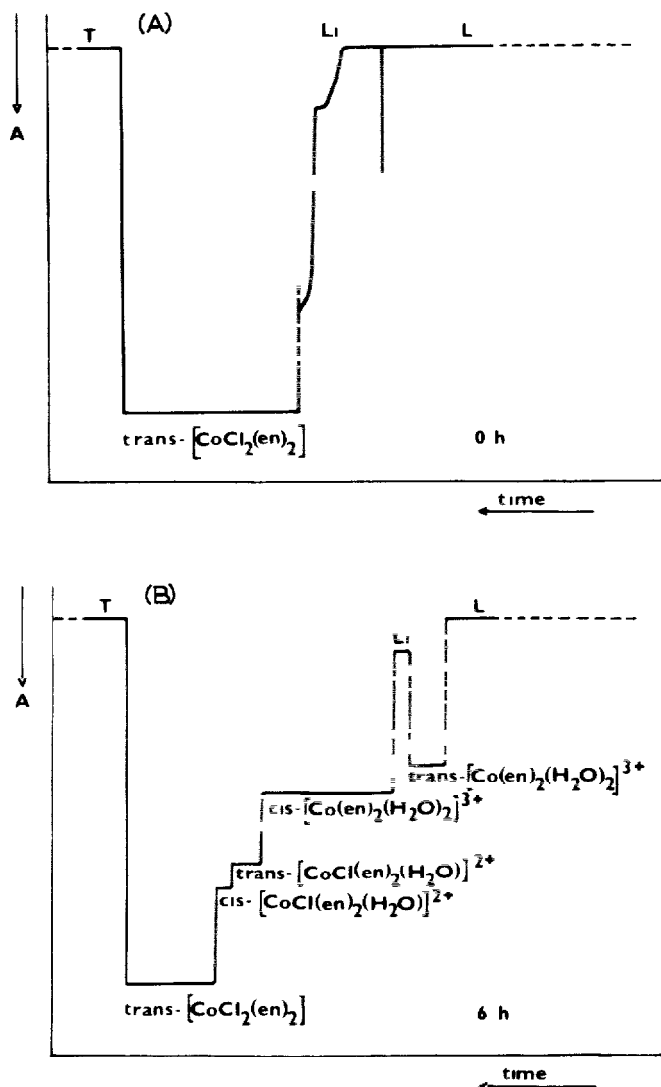
Fig 30 Isotachophoretic record of the aquation of the green complex *trans*-[CoCl₂(en)₂]Cl L = 0 01 *M* HNO₃, T = 0 01 *M* tetrabutylammonium bromide A, Start of the reaction, green sample solution, B, 6 h later, grey sample solution A = Absorbance After ref 57, with permission

similar mobilities ($\Delta u < 1\%$) and demonstrated the potential of the method by preparative isolation of mixed ligand complex ions of the type $[IrCl_xBr_{6-x}]^{2-}$, $x = 0–6$, see Fig 29

The possibilities of isotachophoresis in the separation of *cis* and *trans* isomers, were shown by Blasius et al [57] using as a model system the green complex *trans*-[CoCl₂(en)₂]Cl (en = ethylenediamine), where Cl⁻ is substituted by water Isotachophoresis allowed the monitoring of the kinetics of ligand exchange Analyses of samples of the mixture at reaction times of 0 and 6 h are shown in Fig 30
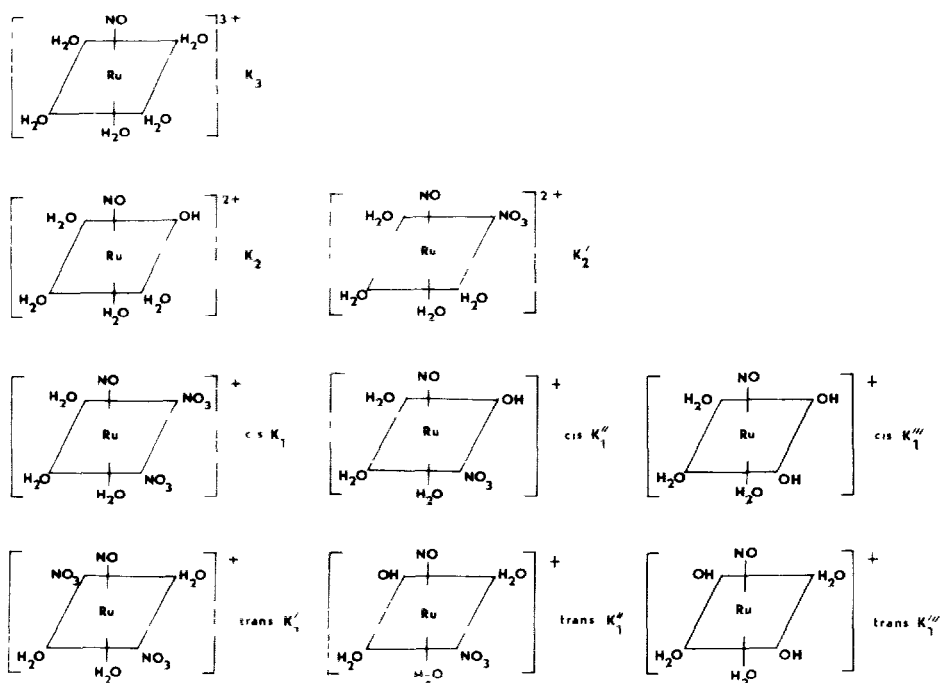
Fig 31 Cationic ruthenium nitrosyl nitrato complexes After ref 57

A successful separation and isolation of nine cationic nitrosyl nitrato complexes of ruthenium comprising three pairs of *cis–trans* isomers by cationic isotachophoresis was reported by Blasius *et al* [57], see Fig 31 The starting material, [Ru-NO(NO$_3$)$_3$(H$_2$O)$_2$]   2 H$_2$O. in nitric acid solution underwent ligand exchange of NO$_3^-$ for H$_2$O and formed cationic, neutral and anionic complexes The equilibrium is reached in *ca* 40 days at room temperature The UV record of a successful analysis is shown in Fig 32
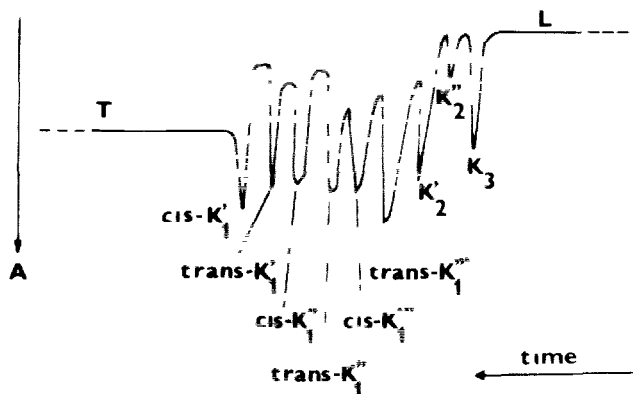

Fig 32 UV record of an isotachophoretic separation of ruthenium nitrosyl nitrato complexes L = 0 01 *M* HNO$_3$ T = 0 01 *M* tetraphenylarsonium chloride A = Absorbance After ref 57, with permission

7 SEPARATION OF MISCELLANEOUS COMPOUNDS

Isotachophoresis has also been successfully used in a number of other cases The separations of covalent compounds formed by substitution of H or OH in an inorganic compound by an organic radical must be mentioned The effectiveness of isotachophoresis in such cases may be demonstrated by the separation of homologous pairs of groups of various substances, e g , butyl phosphates[58] (Fig 33), phosphonoacetate and phosphonoformate[59] (Fig 34) and chlorinated cobaltocarboranes[60] (Fig 35)

8 PRACTICAL APPLICATIONS IN INORGANIC ANALYSIS

The application of analytical isotachophoresis covers a wide range from the separation of simple inorganic ions to the separation of complex and/or polymeric ions A number of surveys of such practical applications[1,3,61,62] cover the literature till 1979–1980 A number of important and stimulating separations published up to this time were included in the previous chapters This chapter is aimed at a critical evaluation of the current status and the prognosis of the future use of isotachophoresis in inorganic analysis

Isotachophoresis is and will be useful in inorganic analysis especially in cases where the problem involves the analysis of mixtures of ionic sub-species derived from one element, e g , mixed ligand complexes of metal cations, polyphosphates, nitrite and nitrate, mixtures of hypophosphite, phosphite and phosphate, etc In such cases, the classical methods of wet chemical analysis as well as a number of modern instrumentation methods, e g , atomic absorption spectrophotometry and flame photometry, fail since one or more steps in the analytical procedure are destructive and transform *de facto* all sub-species of an element into a single form suitable for analytical evaluation
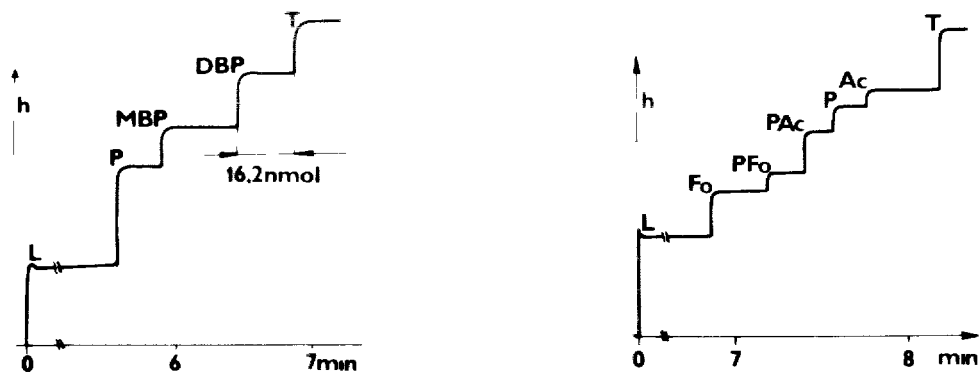

Fig 33 Isotachopherogram of a model mixture of phosphate (P), monobutyl phosphate (MBP) and dibutyl phosphate (DBP) L = 0 01 M HCl + histidine, pH = 6 0, T = morpholineethanesulphonic acid (MES) h = Step height (see Fig 2) After ref 58

Fig 34 Separation of a model mixture containing formate (Fo), phosphonoformate (PFo), phosphonoacetate (PAc), phosphate (P) and acetate (Ac) L = 0 01 M HCl + 0 019 M urotropine + 0 003 M CaCl$_2$, T = glutamic acid h = Step height (see Fig 2) After ref 59
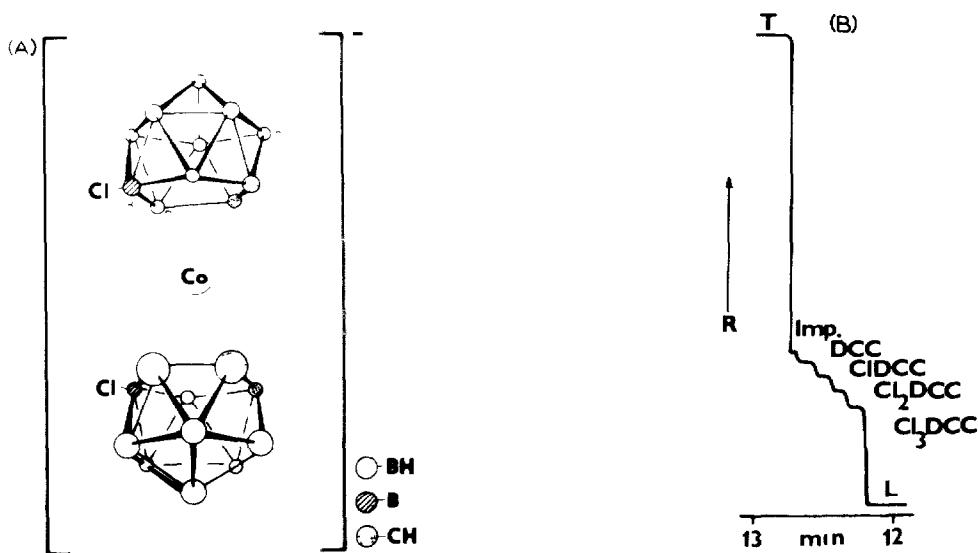
Fig 35 A, Structural formula of the dichloro derivative of the cobaltocarborane anion (Cl_2DCC) B, Isotachopherogram of the separation of chloro derivatives of DCC L = trichloroacetate + N-ethyl-morphine, pH = 6 4, 0 01% polyvinyl alcohol solvent ethanol–water (87 13, v/v), T = benzoate, pH = 7 0, solvent ethanol–water (95 5, v/v), Imp = impurity R = Detector response After ref 60, with permission

Especially in this field, isotachophoresis should be useful owing to its high separation efficiency without destructive sample treatment For example, the analysis of a new type of explosive[63], [2-(5-cyanotetrazolato)]-pentaammmecobalt(III) (called CP) and its main impurities from the synthesis, i e , (5-carbamoyltetrazolato)pen-
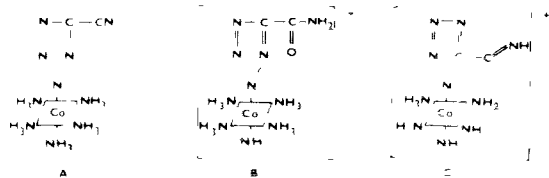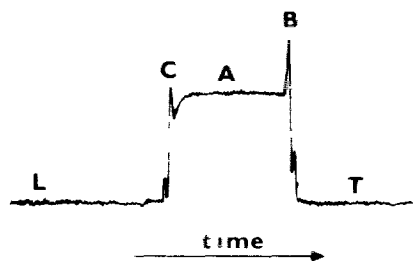

Fig 36 Example of the isotachophoretic separation of 7 5 nmol of 2-(5-cyanotetrazolato)pentaammme-cobalt(III) cation (CP) and its impurities L = 0 01 M potassium acetate, pH = 5 5, T = 0 01 M Tris After ref 63 with permission
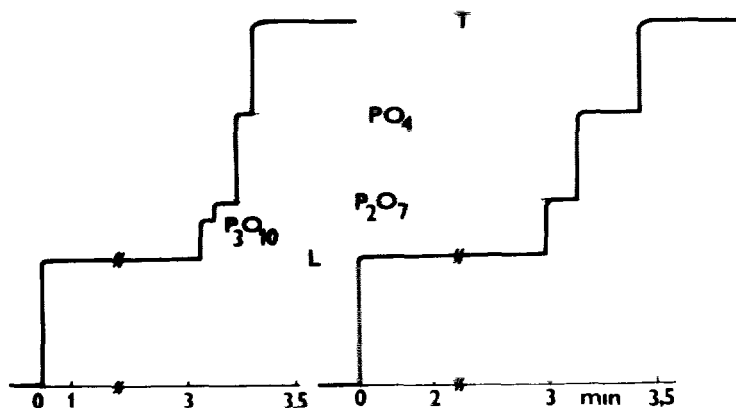
Fig 37 Isotachophoretic analysis of a liquid fertilizer L = 0 005 M HCl + histidine, pH = 6 0, T = glutamic acid After ref 45

taamminecobalt(III) perchlorate (called Amide) and (5-amidinotetrazolato)tetraam-minecobalt(III) perchlorate (called Amidine) by cationic isotachophoresis may be given This separation, see Fig 36, required about 20 min

An important factor is the speed of the isotachophoretic analysis which allows one to obtain information on the state in solution even from species involved in not instantly reversible equilibria For example, the analysis of $P_3O_{10}^{5-}$, $P_2O_7^{4-}$ and PO_4^{3-} in liquid fertilizers[45] (in Fig 37) required less than 4 min

In the separation of various elements, isotachophoresis is only one of a number of methods which can be employed Nevertheless, its potential lies especially in the analysis of similar ions derived from elements such as the lanthanides

From a practical point of view, the advantages of analytical isotachophoresis are that (i) it allows the analysis of inorganic substances even in presence of organic compounds (including colloids), which may cause considerable difficulties in other methods, such as the deterioration of liquid chromatography columns, (ii) microlitre sample volumes and a closed capillary system allow work even with toxic compounds, e g , the determination of cyanides in metal plating baths, etc

9 THE ISOTACHOPHORETIC SYSTEM AS A SOURCE OF INFORMATION ON CHEMICAL REACTIONS PROCEEDING WITHIN IT

Isotachophoresis in capillaries in free solution is already a well developed technique The theoretical basis has been elaborated to such an extent that relationships which permit the calculation of pH, effective mobilities, zone compositions, specific conductivity, level of the separation reached, etc , are available and have been incorporated in routine computer procedures[64-70], tables[71] and diagrams[17,18]

Thus not only theoretical predictions of the optimum electrolyte system enabling successful separations can be made but also isotachopherograms of the sample can be simulated[30 72] An example of the experimental and computer-simulated isotachopherogram[30] of a mixture of metal cations obtained with the use of a complexing counter anion is shown in Fig 38
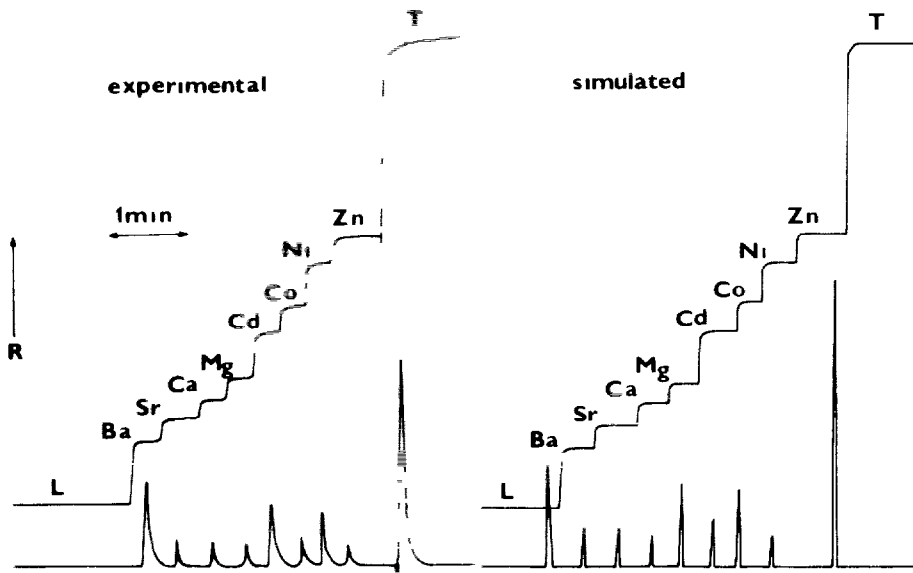
Fig 38 Observed and simulated isotachophoregrams of Ba^{2+}, Sr^{2+}, Ca^{2+}, Mg^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+} and Zn^{2+} L = 0 02074 M KOH + α-hydroxybutyrate, pH = 4 37, T = ε-aminocaproic acid (ε-AMC) R = Detector response After ref 30, with permission

9 1   Evaluation of constants of fast chemical equilibria

The starting point for the evaluation of acid dissociation constants from electromigration data is the equation describing the effective mobility of a given substances as a function of ionic mobilities, dissociation constants and the zone pH

In the basic arrangement of analytical isotachophoresis only the value of the relative effective mobility is a directly measurable quantity (from the signal of the potential gradient or conductivity detector), while the pH of the zone (different from that of the leading zone) is not directly obtainable by experiment This fact represents a serious complication, which, however may be overcome by using an iterative calculation method[-3] based on the dependence of the zone pH on the dissociation constant and on computer simulation of the isotachophoretic steady state

In practice, to achieve the maximum precision of the results, the following procedure is commonly adopted

(1) Measurement of the ionic mobilities under conditions of complete dissociation of the measured acid in its own zone

(2) Measurement of the effective mobilities under conditions of only partial dissociation of the acid in its zone, and subsequent computer simulation of the steady state in the zone using the above value of the ionic mobility The dissociation constant is taken as a variable and its final value corresponds to the best fit between the measured and simulated results

On the other hand, present theory and computer techniques allow a great deal of information to be obtained on the substances separated by processing of the experimental data This means the determination of dissociation constants and complex stability constants which characterize the appropriate chemical reactions, and of the

limiting values of ionic mobilities which characterize the individual ionic species
Besides these data, a more detailed study of the isotachopherogram under properly
selected working conditions provides not only information on the presence of chem-
ical reactions between the separated substances and the counter ion, but also allows
an estimate of the rates of these reactions

The advantage of isotachophoresis for all these measurements follows from
the nature of the method itself its separation possibilities extend even to mixtures of
substances or substances of low purity and, nevertheless, enable measurement of the
pure substance in its own zone, the separation of substances into individual zones
yields data on more substances in a single experiment, the capillary instrumentation
allows the use of microlitre volumes of sample solutions, the operation in free solution
eliminates the need for corrections for the chromatographic effect of a carrier

In a similar way[-4] the stability constants of complexes can be evaluated from
the isotachophoretic effective mobilities, with the only difference that the mobility
equation includes more terms and the computer simulation is thus more expensive,
cf refs 30 67 72 75

9 2   Information on the rates of chemical reactions

The pre-requisite for a correct isotachophoretic migration and the stability of
the zone of a given substance is that the reactions proceeding between this substance
and the counter ion are very fast If this is not so, then the zone will be unstable
which will be reflected in the quantitative analysis in such a way that the calibration
curve does not pass through the origin An example (cf, refs 9 and 16) is the cationic
isotachophoresis of La and Ho with CDTA as a component of the counter ion sys-
tem

La^{3+} reacts with CDTA to form the complex La–CDTA which dissociates
very slowly and migrates towards the anode This results in tailing of the lanthanum
zone, the calibration graph does not passs through the origin (see Fig 39) and,
moreover, the tail of the complex can be detected as a deformation of the plateau of
the zone following behind the lanthanum zone on the isotachopherogram, see Fig

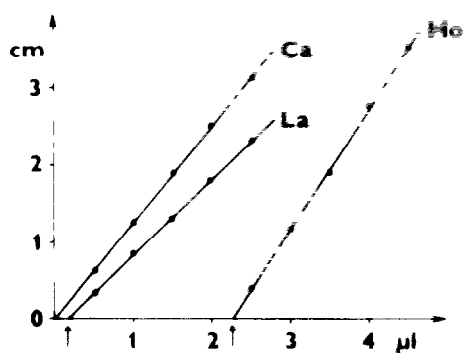Fig 39 Effects of not instantly reversible complexing equilibria on the stability of zones Experimental
calibration graphs of step length vs sample volume L = 0 05 M ammonium formate + CDTA, T =
formic acid (1) Ca^{2+} 0 002 M CDTA in the leading electrolyte, (2) La^{3+}, 0 002 M CDTA in the leading
electrolyte (3) Ho^{3+} 0 0005 M CDTA in the leading electrolyte After ref 16
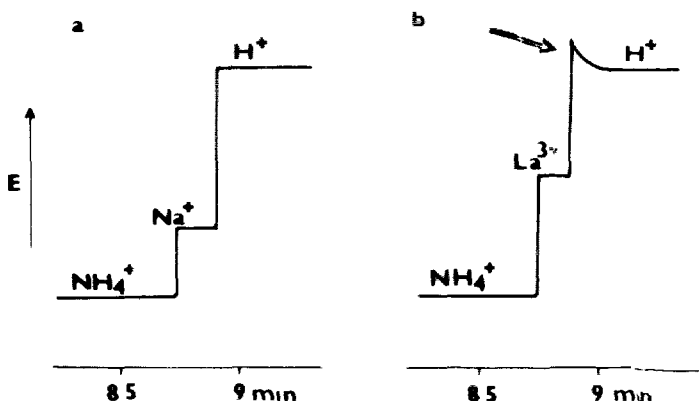
Fig 40 Experimental isotachophoregrams obtained with 0 002 M CDTA in the leading electrolyte, see
Fig 39 Samples Na$^+$ (a), La^{3+} (b) E = electric gradient After ref 16

40 Ho^{3+} reacts with CDTA to form a kinetically inert complex which migrates towards the anode This results in bleeding of the holmium zone whose calibration curve does not pass through the origin, see Fig 39 Owing to the bleeding, a constant trace of the complex is left behind the holmium zone and the plateau of the next zone is not distorted

It is seen that the isotachophoretic record may give qualitative information on the presence of slow reactions It may be expected (*cf*, ref 16) that isotachophoresis in combination with capillary zone electrophoresis performed with the same instrumentation will allow even quantitative evaluation of the rate constants of ionic chemical reactions

10 CONCLUSION

Isotachophoresis is an effective analytical separation technique with great potential in inorganic chemistry, especially due to the following properties

(i) in any given experiment a number of substances may be resolved (separated) At the same time, the separation efficiency is such that, *e g*, individual forms of mixed ligand complexes and or lanthanides as well as *cis* and *trans* isomers of coordination complexes may be separated

(ii) The separation is relatively fast so that even ionic species involved in not instantly reversible equilibria *e g* polyphosphates, may be separated

(iii) It is sensitive down to picomol amounts and owing to its concentrating effect is very advantageous for analyzing minor constituents in mixtures, it requires only little or no sample pretreatment

REFERENCES

1 P Bocek *Top Curr Chem* 95 (1981) 131
2 J Vacik and F M Everaerts in Z Deyl (Editor) *Electrophoresis Part A, Techniques* (*J Chromatogr Library Vol 18A*) Elsevier Amsterdam Oxford New York, 1979, Ch 10, p 193
3 F M Everaerts and Th P E M Verheggen in Z Deyl (Editor), *Electrophoresis Part B, Applications* (*J Chromatogr Library Vol 18B*) Elsevier Amsterdam, Oxford, New York, 1983, Ch 20, p 437

4 M Lederer in E Heftmann (Editor) *Chromatography Part B Applications* (*J Chromatogr Library, Vol 22B*) Elsevier Amsterdam, Oxford New York 1983 Ch 22, p 459

5 *Acta Isotachophoretica* Literature Reference List 1967 1980, LKB, Bromma, 1981

6 A Tiselius *Nova Acta Regiae Soc Sci Upps* 7 No 4 (1930) 1

7 D A MacInnes and L G Longsworth, *Chem Rev* 11 (1932) 171

8 L G Longsworth in M Bier (Editor) *Electrophoresis* Academic Press, New York, 1959, Ch 3, p 91

9 P Gebauer and P Boček, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, De Gruyter, Berlin, New York 1984, p 47

10 P Bocek I Miedziak M Deml and J Janak *J Chromatogr*, 137 (1977) 83

11 I Nukatsuka M Taga and H Yoshida *J Chromatogr*, 205 (1981) 95

12 P Boček M Deml and J Janak *Laborpraxis* 3 (1979) 18

13 P Gebauer P Boček M Deml and J Janak, *J Chromatogr*, 199 (1980) 81

14 P Gebauer and P Boček *Collect Czech Chem Commun*, 47 (1982) 1802

15 D Lučansky V Batora J Polonsky and J Garaj in F M Everaerts (Editor), *Analytical Isotachophoresis (Anal Chem Symp Ser Vol 6)* Elsevier Amsterdam, Oxford, New York, 1981, p 147

16 P Gebauer and P Boček *J Chromatogr* 299 (1984) 321

17 P Gebauer and P Boček *J Chromatogr*, 267 (1983) 49

18 P Boček and P Gebauer *Electrophoresis* in press

19 P Boček P Gebauer and M Deml, *J Chromatogr* 217 (1981) 209

20 P Boček P Gebauer and M Deml, *J Chromatogr* 219 (1981) 21

21 P Gebauer and P Boček *J Chromatogr* 242 (1982) 245

22 P Bocek P Gebauer and M Deml *Chem Listy* 78 (1984) 510

23 F M Everaerts and F E P Mikkers in A Adam and C Schots (Editors), *Biochemical and Biological Applications of Isotachophoresis (Anal Chem Symp Ser Vol 5)*, Elsevier Amsterdam, Oxford, New York 1980, p 1

24 P Gebauer and P Boček in B J Radola (Editor) *Elektrophorese Forum 83*, Technical University Munich 1983 p 276

25 J L Beckers and F M Everaerts *J Chromatogr* 51 (1970) 339

26 J L Beckers and F M Everaerts *J Chromatogr* 68 (1972) 207

27 I Nukatsuka M Taga and H Yoshida *Bull Chem Soc Jap*, 54 (1981) 2629

28 F M Everaerts, Th P E M Verheggen J C Reijenga G V A Aben, P Gebauer and P Boček, *J Chromatogr* 320 (1985) in press

29 M Koval *Thesis* Komensky University Bratislava, 1981

30 T Hirokawa T Matsuki, H Takemi and Y Kiso *J Chromatogr* 280 (1983) 233

31 B A Bilal and C Reichmuth, *J Inorg Nucl Chem* 34 (1972) 348

32 B A Bilal and C Reichmuth *Z Naturforsch Teil B* 36 (1971) 368

33 B A Bilal F Herrmann, K Metscher B Muhlig Ch Reichmuth and B Schwartz, in J D Navratil and W W Schulz (Editors) *Actinide Separations ACS Symp Ser, No 117*), American Chemical Society 1980 p 561

34 O V Oshurkova N V Chebotareva and I A Kozhurkina, *Zh Prikl Khim (Leningrad)*, 46 (1973) 776

35 M Lederer L Morpurgo and L Ossicini *J Chromatogr*, 50 (1970) 475

36 R Schonger *Mikrochim Acta* (1956) 869

37 M Deml P Bocek and J Janak, *J Chromatogr* 109 (1975) 49

38 B P Konstantinov O V Oshurkova and N V Chebotareva, *Zh Prikl Khim*, 43 (1970) 544.

39 V Taglia and M Lederer, *J Chromatogr* 77 (1973) 467

40 P I Bresler I A Ivanova, O V Oshurkova and G A Shitlerman, *Zh Anal Khim (Leningrad)*, 3 (1981) 593

41 P Boček S Pavelka M Deml and J Janak *J Chromatogr*, 151 (1978) 436

42 J C Reijenga Th P E M Verheggen and F M Everaerts, *J Chromatogr*, 245 (1982) 120

43 T Tatsuhara F Tabuchi, I Nishimura H Muro and F Ozoe, *Chem Pharm Bull*, 30 (1982) 1347

44 P Gebauer M Deml P Boček and J Janak *J Chromatogr*, 267 (1983) 455

45 P Boček B Kaplanova M Deml and J Janak *J Chromatogr*, 153 (1978) 287

46 F Mikkers W Van Gils and W Roos, in F M Everaerts (Editor), *Analytical Isotachophoresis (Anal Chem Symp Ser Vol 6)*, Elsevier Amsterdam Oxford New York, 1981, p 91

47 I Motooka H Nariai K Nakazaki and M Tsuhako *J Chromatogr*, 260 (1983) 377

48 T Yagı K Kojıma, H Nanaı and I Motooka, *Bull Chem Soc Jap*, 55 (1982) 1831
49 H Nanaı K Nakazakı M Tsuhako and I Motooka, *J Chromatogr*, 248 (1982) 135
50 K Fukushı G Kondoh K Hıro T Tanaka A Kawahara and S Wakıda, *Bunsekı Kagaku (Jap Anal)* 32 (1983) 362
51 M Drabık *Thesıs* Komensky Unıversıty Bratıslava 1983
52 W Preetz *Talanta* 13 (1966) 1649
53 W Preetz and H L Pfeıfer *Talanta* 14 (1967) 143
54 W Preetz *Naturwıssenschaften* 54 (1967) 85
55 E Blasıus and U Wenzel *J Chromatogr* 49 (1970) 527
56 W Preetz L Wannemacher and S Datta *Z Anal Chem*, 257 (1971) 97
57 E Blasıus K Muller and H Wagner *Z Anal Chem*, 311 (1982) 192
58 P Boček V Dolnık, M Deml and J Janak *J Chromatogr*, 195 (1980) 303
59 P Boček ın F M Everaerts (Edıtor) *Analytıcal Isotachophoresıs (Anal Chem Symp Ser, Vol 6)*, Elsevıer Amsterdam Oxford, New York 1981, p 203
60 M Koval, D Kanıansky L Matel and F Macašek, *J Chromatogr*, 243 (1982) 144
61 Y Shıogaı and T Yagı *Int Lab* 11 (1981) 69
62 S G Hjalmarsson and A Baldesteın *CRC Crıt Rev Anal Chem*, July (1981) 261
63 J M Lavoıe and P S Back *J Chromatogr* 264 (1983) 329
64 F M Everaerts and R J Routs *J Chromatogr*, 58 (1971) 181
65 J L Beckers, *Thesıs* Technologıcal Unıversıty, Eındhoven, 1973
66 F M Everaerts J L Beckers and Th P E M Verheggen, *Isotachophoresıs Theory, Instrumentatıon and Applıcatıons (J Chromatogr Lıbrary Vol 6)*, Elsevıer, Amsterdam, Oxford, New York, 1976
67 T Hırokawa and Y Kıso *J Chromatogr* 252 (1982) 33
68 T M Jovın *Ann N Y Acad Scı* 209 (1973) 477
69 J Vacık *Thesıs* Charles Unıversıty Prague 1980
70 L M Hjelmeland and A Chrambach *Electrophoresıs*, 4 (1983) 20
71 T Hırokawa M Nıshıno, N Aokı Y Kıso Y Sawamoto, T Yagı and J -I Akıyama, *J Chromatogr*, 271 (1983) D1
72 T Hırokawa and Y Kıso *J Chromatogr* 242 (1982) 227
73 Y Kıso and T Hırokawa *Chem Lett* (1980) 323
74 Y Kıso and T Hırokawa *Chem Lett* (1980) 745
75 T Hırokawa H Takemı and Y Kıso *J Chromatogr*, 280 (1983) 219