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RECENT DEVELOPMENTS IN ISOTACHOPHORESIS

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1 INTRODUCTION

Recently (2nd–6th September, 1984), the 4th International Symposium on Isotachophoresis, ITP-84[1], was successfully held in Hradec Králové, Czechoslovakia, and included basic and advanced courses[2]. The symposium illustrated that capillary isotachophoresis has successfully matured both as an analytical separation method and as a micropreparative method. Isotachophoresis is now a fairly advanced analytical method with a theory permitting the establishment of the possibilities of both its separation capabilities and its qualitative and quantitative aspects. In addition, advanced instrumentation allowing the realization of theoretical predictions is now available. From the viewpoint of information theory, isotachophoresis seems[3] to be comparable to other advanced separation methods, *e.g.*, liquid chromatography, as an identification method.

The number of published practical applications of analytical isotachophoresis continues to grow and we may presume that this method (together with ion chromatography) will soon dominate in the analysis of ionogenic substances in solutions. This conclusion is supported by a number of reviews that have appeared since 1981; the contents of these reviews can be characterized as follows:

(1) Brief descriptions of the basic principles of isotachophoresis[4–17] with appropriate examples of possible analytical applications[18–20], aimed, in a number of instances, at a certain field, *e.g.*, pharmacy[21–23], analysis of beverages[24], inorganic chemistry[25] or biochemistry[26].

(2) Reviews of applications of analytical isotachophoresis in a certain field, *e.g.*, the food industry[27] or the chemical industry[28]; analyses of compounds of a certain type[29], *e.g.*, inorganic compounds[30,31], amino acids, peptides and proteins[32–34] or nucleotides[35].

(3) Methodological reviews devoted, *e.g.*, to qualitative aspects[36], to the selection of electrolyte systems[37], to electrolyte systems for low-[38] and high-molecular-weight[39] compounds, to the isotachophoresis of complexes[40–43] or cations[44,45] or to classification of isotachophoresis from the viewpoint of moving boundary methods[46–48].

(4) Reviews of the physico-chemical principles of isotachophoresis[49–52].

(5) Reviews of instrumentation[53–56].

Unfortunately, the current literature on isotachophoresis is confused as the fundamental book on the subject[57] is 9 years old, there was a considerable delay in the publication of papers presented at the 3rd International Symposium on Isotachophoresis in Goslar[58], complete, critical reviews covering the recent isotachophoretic literature are lacking (the most recent reviews date back to 1981[59,60]; see also ref. 19) and, in 1980, the regular publication of a chronological literature reference list[61] was stopped.

The aim of this review is to summarize critically and as completely as possible original papers and reviews from the period 1981–84, particularly the last three international symposia on isotachophoresis (ITP-80, Eindhoven, The Netherlands; ITP-82, Goslar, F.R.G., and ITP-84, Hradec Králové, Czechoslovakia), and to characterize the present state of analytical isotachophoresis.

2 THEORY

2.1. Mathematical description of isotachophoresis

The basic description of isotachophoretic systems starts from general equations for the electrophoretic process, which include the following.

(i) transport processes in the presence of homogeneous chemical reactions in solutions of electrolytes (usually in a one-dimensional form, where time and migration path are independent variables);

(ii) the relationship between electric current and ion migration;

(iii) Ohm's law for electrolyte systems;

(iv) electroneutrality in solution;

(v) definition of the equilibrium constants;

(vi) definition of analytical concentrations of substances present in the form of various sub-species in the solution,

(vii) protolytic equilibria of the solvent (up to now always water).

The analytical solution of such a system of equations is very difficult even for a simple case and an explicit solution giving the dependence of the concentrations of various substances on time and migration path is apparently not obtainable. The present approach is to formulate and solve mathematical descriptions of simplified physico-chemical models, corresponding to calculations with one of the following aims:

(i) Calculation of the composition of equilibrium isotachophoretic zones and respective effective mobilities for qualitative and quantitative analysis. Here, diffusion is neglected and steady-state zones are assumed. Transport processes are described by algebraic equations and a mathematical description of the whole system is represented by a system of algebraic equations The required equations (for the case of weak polybasic acids and bases) and the iterative procedure (the so-called RFQ method) were described earlier[57]. The applicability of this method was extended to systems in which complex-forming equilibria[62] are present, including the case[63] where several ligands are coordinated to a metal. This method has recently been used for the calculation of the equilibrium zone characteristics in the construction of zone existence diagrams, which are important tools for the evaluation of zone stability and separability (see section 3.2).

(ii) Modelling of the separation dynamics. Transport processes are described here by partial differential equations and the system of equations is solved by computation. This enables the processes in an electrophoretic system to be modelled with respect to space and time. The physico-chemical model is considerably simplified and the corresponding simplified transport equations contain only a few terms.

The dynamics of the isotachophoretic process in a simple case of uni–univalent electrolytes, where transport equations included only the electromigration term, was studied in this manner[64,65]. The separation of a two-component sample, including various cases of non-ideal injection and the effects of an impurity present in the leading electrolyte or in the terminator, was described.

A more general procedure[66,67] also takes into consideration the influence of diffusion on the dynamics of the electrophoretic process. Relatively low current densities were used and the influence of diffusion on the character of the migrating

boundaries is well illustrated by the simulation results. Three fundamental boundary types appearing in the isotachophoresis of protolytes were analysed[68] by means of the above method: the classical sigmoid shape of the boundary between the two zones, the boundary with a decrease in the electric field intensity (appearing in the case of inversion of effective mobilities), and the boundary between a zone and the zone of a free weak acid (H^+ termination, see section 3.1). It can be concluded from the results that diffusion can, in some instances, disturb the isotachophoretic effect or to prevail over it, despite the fact that the basic model predicts stable zones.

Thormann and Mosher[69] dealt with the theoretical and numerical analysis of the shape and the width of the boundary between weak electrolytes. With the assumption of sufficiently weak electrolytes and equality of ionic mobilities, u, they obtained for the boundary width (characterized by the change in the molar fraction of one component from p to $1-p$) the relationship

$$W = (\rho' + 2) \ln\left(\frac{1-p}{p}\right)\left[\frac{FRTc_1 (u + u_X)}{I\rho'}\right] \tag{1}$$

[$\rho' = (K_1 - K_2)/K_2$, K_1 and K_2 are the dissociation constants, X = counter ion, T = absolute temperature, R = gas constant, F = Faraday's constant], from which it follows that the boundary sharpness improves with the difference in dissociation constants and with increasing current, I, whereas the increase in the concentration c_1 reduces the boundary sharpness. It is of interest that the theory here predicts an asymmetric shape of the boundary for weak electrolytes, which is also supported by computer-simulated model examples.

A very general approach has recently been presented[70] in which the system incorporating also the kinetics of chemical processes has been solved numerically. The simulation of model cases was limited, however, to the $Na^+ \rightarrow H^+$ (acetic acid) boundary, where, with regard to the high rate constants of acid–base reactions, no quantitative statement about the real width of the mentioned boundary could be made. Simulation with substantially lower rate constants indicated that the boundary width increases with the decreasing recombination rate constant.

(iii) Analytical solution for a certain simplified model. A general mathematical theory of electrophoresis, very widely conceived, and which has been presented only recently[71,72] should be mentioned here. This theory, however, when applied to isotachophoresis[72,73], is considerably simplified, including neglect of diffusion. The mathematical solution is performed only for the case of migration of strong or weak acids with H^+ as the counter ion. This leads to a series of Riemann invariants, the first one being constant in any position of the separation tube. This led Babskii and co-workers to the formulation of the concept of "electrolytic memory". The expression of the first invariant in the form

$$R_1 = \sum_i \frac{K_i c_i (u_H + u_i)}{K_i u_i} = \sum_{i,H} \frac{c_i}{r_i} \tag{2}$$

($r_i = u_i/u_H$) leads to its identity with the classical form[57] of the Kohlrausch regulating function, and "electrolytic memory" can therefore be understood only as the classical principle of the concentration adjustment formulated in another way.

2.2. Application of isotachophoresis to the evaluation of physico-chemical data

Primary information that can be obtained from isotachophoretic measurements and that serves for physico-chemical interpretations is the step height provided by a conductometric or potential gradient detector. This value reflects the specific conductivity and/or effective mobility of the compound in its zone. In general, it is a function of (i) the physico-chemical constants of separated compounds, and hence of ionic mobilities and equilibrium constants of the chemical equilibria involved, and (ii) the working conditions, especially the leading electrolyte composition, which determines the concentration and pH in the zone for given physico-chemical constants.

Obviously, for the evaluation of ionic mobilities and equilibrium constants it is necessary to know the effective mobility and the pH of the zone. The latter is, however, difficult to obtain by experiment. This problem was overcome by Kiso and co-workers[74–81], who developed a method based on iterative calculation starting from the dependence of the pH of the zone on the equilibrium constants and on computer simulation of the isotachophoretic steady state. To achieve the maximum accuracy of the results, ionic mobilities are measured first under conditions of total dissociation, then the complete simulation is performed with the values of the studied constants changing parametrically, until a good fit is obtained with the experimental dependence of the effective mobility on the parameters of the leading electrolyte. Kiso and co-workers applied the above method to the determination of the dissociation constants[74–76] of some weak acids and the stability constants of complexes of a number of cations with some organic hydroxy acids[77–80]. The ionic mobilities, dissociation constants, effective mobilities, effective charges, pH values of zones and slopes of calibration graphs were tabulated[81] for 287 anions in 31 electrolyte systems.

To calculate limiting ionic mobilities from experimental isotachophoretic data, a method was proposed[82] that is based on a computer solution of the system of equations describing the steady state and also including corrections for temperature and ionic mobility. A similar model was used by other workers[83], who evaluated the dissociation constants of weak acids from the dependence of experimental effective mobilities on the pH of the leading electrolyte.

A method[84] based on the measurement of the electric potential gradient was described for the determination of relative ionic mobilities. The arrangement used eliminates some interferences and leads to reproducible and precise results; the values are related to constant ionic strength in the zone.

For qualitative purposes, the measurement of the so-called "relative apparent mobility" by isotachophoretic experiments carried out at a constant voltage across the column with the use of the measured sample as the terminator was described[85–87].

The relative apparent mobility is obtained as the reciprocal of the time of passage of the boundary through the detector. The practical use of this parameter is, however, very restricted as it is not directly proportional to the electrophoretic mobility.

Determination of the Kohlrausch regulating function was described by Hjertén *et al.*[88], who used for its calculation the values of the mobilities and concentrations determined experimentally. For a fully dissociated bivalent anion (5-sulphosalicylate) they found good coincidence for the leading and the sample zones. For cationic analysis of the copper and cobalt zones with acetate as the counter ion they obtained different values, which was explained by the formation of complexes.

The possibility of determining complex formation constants of metals with neutral ligands was described by Stover[89], who reported the separation of alkali metals and ammonium with the use of 18-crown-6-ether neutral ligand in the leading electrolyte.

Fujishita *et al.*[90] described a method for the determination of relative ionic weight (M), which is based on an empirical correlation of experimental data. They showed that the currently used equation[91] containing $M^{-1/2}$ can be substituted, to a good approximation, by the expression $h_r = a + b\ M/|Z|$ where h_r is the relative step height and $|Z|$ the effective charge.

3 FUNDAMENTAL ANALYTICAL ASPECTS

In order to perform successful isotachophoretic analyses, it is necessary that a suitable electrolyte system, in which the sample substances separate, is available. Further, qualitative data permitting the identification of steps in the record and quantitative data permitting conversion of step lengths to analysed quantities should also be available.

3.1. Selection of electrolyte systems

The selection of an electrolyte system for every given analytical problem is aimed not only at a complete separation but also at the possibility of quantitative evaluation within the minimum possible time[92]. The following procedure was proposed for the selection of electrolyte systems[93]:

(i) a preliminary system is chosen;

(ii) whether all the substances in question, when analysed, form stable zones in this system is investigated; and

(iii) whether all of these substances can be separated from one another is investigated.

If the system according to (ii) or (iii) is not satisfactory, another system is taken and the procedure is repeated, starting from (i).

When all the zones are stable and all the substances are separable, it is necessary for the suitability of the electrolyte system to be tested by additional criteria:

(iv) by investigating whether the given electrolyte system is also convenient in the instrumentation used from the viewpoint of the amounts and ratios of the sample components; and

(v) when the procedure is developed for routine analysis, it is necessary to optimize it also from the viewpoint of the analysis time. It is possible, in principle, that the optimization procedure will again lead to the starting point (i).

3.2. Stability of zones

The stability of zones has two independent, but not unrelated, aspects, which are specified as follows.

(i) Correctness of isotachophoretic migration

From this viewpoint the zone is stable as long as it migrates between correctly

selected leading and terminating electrolytes where its front and rear boundaries are permanently sharp. The general condition of the sharp zone boundary is closely associated with the migration order and was formulated[94] for the boundary between the front zone X and the rear zone Y as follows:

$$\bar{u}_{X,X} > \bar{u}_{Y,X} \tag{3a}$$

$$\bar{u}_{X,Y} > \bar{u}_{Y,Y} \tag{3b}$$

where $\bar{u}_{X,Y}$ is the effective mobility of substance X in zone Y. Its validity suggests that substance X possesses a higher effective mobility than substance Y in both zones X and Y.

Provided that the zone fulfills the above conditions in the sense of migration between the leading and the terminating zones, then the system of electrolytes is considered to be correct[44].

From this viewpoint, the ions of the solvent play a significant role, as they can penetrate through the whole electrolyte system and disturb correct migration substantially. An aqueous medium, where both H^+ and OH^- have absolutely the highest ionic mobilities, is particularly concerned. The migration behaviour of H^+ in acidic cationic systems was described[95–97] and conditions were defined under which the system is correct with respect to H^+ and H^+ can serve as the terminator. Further, the concept of the effective mobility of H^+, $\bar{u}_{H,H}$, was introduced, with the aid of which correct migration can be described by condition 3b for Y = H. It holds in general that the effective mobility of H^+ increases with a decrease in the leading electrolyte concentration and in the pK_a value of the acid of the counter ion and with an increase in the content of the free acid in the leading electrolyte. The concept of the effective mobility of OH^- in anionic systems was formulated[93] in a similar manner.

The zone existence diagram was proposed[94] to define the range of substances that provide stable zones in a given system. Calculated effective mobilities of substances in their zones are plotted against the pH of these zones. With the aid of the parametric network in this diagram, the determination of equilibrium parameters of the zones of substances of known ionic mobilities and dissociation constants is simple. Hence the diagram permits a simple determination of zone stabilities of given substances, as the margin contours in the diagram define the range of existence of correct zones and thus the point corresponding to a sample substance must lie inside this area.

(ii) Analytical stability of zones

From this viewpoint the stable zone can be characterized as a zone that contains, independent of time, a constant amount of the analyte. This means that outside the sample zone proper no sample is present. Hence the zone of an analyte neither is enriched with this analyte from the leading or the terminating electrolyte nor decreases owing to migration of the analyte into the leading or terminating zone. At a constant composition of the leading electrolyte, the volume of the zone and its steady-state characteristics (concentration, pH) are also constant; in a tube of constant cross-section both the front and rear zone boundaries migrate at the same

The analytical stability of the zone is a natural property given by the chemical nature of substances, especially by the chemical stability of the separated substance in the subsequent zone. Analytically unstable zones can appear in practice particularly when the selectivity between the separated substances is affected by complex formation. According to the mechanism that ensures stability or causes the instability of a zone, zones can be classified into certain types[41,98], as follows.

(a) In simple stable zones, complex-forming reactions do not proceed and it is therefore migration of either uncomplexed metal cations or kinetically inert complexes.

(b) Equilibrium stable zones are characterized by the presence of fast complex-forming equilibria. The separated substance migrates as a mixture of various ionic sub-species that are chemically unstable outside the zone proper and are interconverted by fast reactions on the boundary.

(c) Tailing unstable zones are characterized by a slow reaction rate at the rea zone boundary that is comparable to the migration velocity. The existence of tailin zones was shown[98,99] for the cationic migration of lanthanum with the use of cy clohexanediaminetetraacetic acid as the counter ion. It follows from theoretica analysis that the amount of the substance escaping from the zone and subsequentl contained in the tail is proportional to the intensity of the electric field in the ne zone.

(d) Bleeding unstable zones are characterized by the fact that a certain component of the substance can leave the zone via the rear boundary and is chemically stable in the next zone. Bleeding zones were investigated[100,101] by studying the example of anionic migration of EDTA and NTA complexes. The theoretical model shows that the zone instability is proportional to the conditional complex stability constant (and hence to pH).

3 3. Separability of compounds

In the 1970s, the separability of substances was commonly investigated by a procedure based on the measurement of effective mobilities (or proportional detection signals) of separated substances in several systems and on finding a system where the differences in the effective mobilities obtained are maximal. This system was then considered as the separation optimum. In practice, this already classical procedure has often been combined with graphical processing, *e.g.*, of the $\bar{u} = f(pH_L)$ type. Recently, the possibility has been shown of obtaining the required dependences of effective mobilities by computer simulation[102,103] instead of by elaborate measurements. This procedure was used to find the separation conditions for some organic acids with the use of Ca^{2+} as a complexing counter ion and to find the conditions for the determination of trifluoroacetic acid in urine.

This procedure is obviously based on experimental determination and/or calculation of the mobilities of individual compounds in their zones (*i.e.*, parameters of the $\bar{u}_{i,l}$ type). As shown earlier[92,94], such a procedure is not generally correct and, in principle, it may fail. The reason is that the separability is affected by the effective mobilities of compounds in the mixed zone. Unfortunately, these values are not accessible by direct measurement. This difficulty can be overcome by a procedure[93,104] that converts the problem of separability into the problem of the determination of

migration order. This procedure, in principle, eliminates the necessity for knowing parameters of the mixed zone, as it starts from the evaluation of the equilibrium state. Data of two types are the basis here. (i) $\bar{u}_{i,s}$ and pH_i, corresponding to the evaluation of the steady state; and (ii) $\bar{u}_{i,j}$ determined by calculation from a known pH_j. If the compounds are separable, then their migration order can be determined unambiguously on the basis of conditions for their self-sharpening boundary (conditions 3a and 3b).

To evaluate the separability of a group of substances X from one given substance Y, the zone existence diagram[93,94,104] can be utilized, in which the sequence contours, corresponding to equalities in conditions 3a and 3b, are drawn through the point corresponding to the substance Y The whole diagram is then divided into regions containing points of compounds migrating in the X → Y and/or Y → X zone order and, moreover, a third region where both conditions 3a and 3b do not apply simultaneously and the zone order cannot be determined unambiguously. Here substances X and Y are not separable and create a stable mixed zone. As has been shown[94], the composition of such a mixed zone must be constant, which indicates that a sample will give, in principle, a zone of the pure substance that is in excess, migrating in front of the stable mixed zone. Although the risk of the confusion of such a pair of zones with a totally separated pair is high, calibration with a standard sample of variable composition usually permits a correct evaluation.

Another approach to the question of separability, including also quantitative aspects, was described by Gebauer and Boček[92]. They started from a model of the separation process of a binary mixture. The quantitative description of separability is based on the concept of selectivity expressed in terms of the relative difference in effective mobilities in the mixed zone. This quantity is accessible directly from a simple experiment and expresses the maximum separable volume of the mixed zone relative to the volume of the leading electrolyte. The concept of separation speed expresses the volume velocity of separation and makes it possible to take the analysis time into account. The two quantities mentioned above permit a simple and rapid optimization procedure, particularly for mixtures, where the problem can be simplified to the separation of a binary mixture.

3.4. Modifications of the isotachophoretic system

Recently, some papers have appeared that describe the theory and/or experiments for systems different from the basic "classical" structure of isotachophoretic systems.

The first modification represents a simplification in which, instead of a common counter ion, only a solvent ion (here H^+) migrates in the opposite direction. These systems were described earlier[105] ("buffer-free" isotachophoresis[106]). A more detailed description of such systems, starting from the application of the general moving boundary theory, has recently been presented[107,108]. The results obtained for equilibrium zone parameters extend existing simple theoretical models for basic systems and thus represent a more valuable contribution for practical applications than a complex mathematical elaboration[71] of the same system that provides comparable results.

The system in which a mixture of several compounds is used as the counter

ion is another possibility. A detailed description of such systems using counter ions consisting of up to six components has been published[109,110]. These systems provide a better correlation between the pH of the leading electrolyte and the effective mobilities of the separated compounds and hence permit a simple selection of the separation conditions by pH changes. Another case of the use of a mixed counter ion comes from the field of isotachophoresis of inorganic cations[97], where one counter ion provides complexing effects and the second counter ion provides sufficient buffering of the system, which the complex-forming counter ion of a strong acid is not able to ensure.

Further modifications of electrolyte systems were shown by the description[111,112] of "combined" systems (see also ref. 113), where the leading electrolyte contains a certain amount of the terminator or the terminator contains a certain amount of the leading compound, or both the possibilities are combined. In these systems, the number of compounds that migrate isotachophoretically decreases. This is considered to be the advantage of the system in the sense of an increase in the selectivity.

Although the systems mentioned above are sometimes designated as "non-isotachophoretic", they belong to the general concept of isotachophoresis. Even these systems permit isotachophoretic migration of stable isotachophoretic zones in the sense of the present understanding (see section 3.2).

3 5. Quantitative aspects

Some authors have recently treated quantitative analysis from the viewpoint of its simplification. The basic equation relating the zone length (in units of charge, Q, or time, t) with the amount of compound i, n_i:

$$Q_i = t_i I = n_i F/T_i \tag{4}$$

suggests that the calibration factor is given virtually only by the reciprocal transference number, T_i, of compound i in its zone.

With a suitable choice of the leading electrolyte, the influence of the variations in its composition on the accuracy of the determination of the transference number (and hence also of the calibration factor) was shown[114] to be negligible. The so-called tolerance diagrams, showing the influence of variations in pH and concentration of the leading electrolyte on the accuracy of anionic analyses, indicate that the tolerance range increases rapidly with increasing pH above 5.

Hirokawa and Kiso[115] used computer simulation to analyse the factors that affect the bias of the values of T_i in eqn. 4 caused by neglecting the influence of H^+ and OH^- ions. They also concluded that the simplification is justified (and the bias is in the range of a few percents) as long as the pH of the leading electrolyte is in the range 5–9. With a knowledge of respective ionic mobilities, the calculated calibration constants can then be used instead of the experimental calibration graph.

Eqn. 4 was also discussed by Svoboda *et al* [116] in connection with the possibility of using isotachophoresis as an absolute analytical method. In the view of Svoboda *et al.*, prerequisites are precise experimental values of the effective mobilities or the concentrations of substances in their zones.

An important factor, directly related to quantitative aspects, is the width of the zone boundary, which influences the detection limit of the analysis. Electroosmotic flow is one of the quantities that can influence the boundary width substantially. The possibility of affecting the electroosmotic flow (and hence the boundary sharpness) by the addition of surface-active agents to the leading electrolyte was studied[117] both theoretically and experimentally. The zeta potentials of the separation capillary walls were measured in commonly used solutions containing various non-ionogenic or cationic detergents. The boundary widths in a model mixture separated in these systems were also determined. The conclusion was that the disturbing effects can be eliminated best by means of a higher concentration and a lower pH of the leading electrolyte containing an additive admixture.

For evaluation of very short zones using UV detection, the zone area (in terms of integrated zone absorbance) was suggested[118] as a parameter suitable for quantitation.

4. INSTRUMENTATION

4.1. Introduction

The instrumentation aspects of isotachophoresis are well developed. Basic instrumentation for analytical isotachophoresis resulted from developments in the 1970s and a detailed description can be found in a comprehensive book[57] and a review[60]. The heart of the instrumentation is an isotachophoretic column, which is composed of a separation capillary, injection system, electrode chambers and detection cell. The column is connected to a stabilized power supply. The detector response to the isotachophoretic zones passing through the detection cell is processed in the detection device and the resulting signal is registered by a recorder as an isotachopherogram There are two types of design of the main separation unit, *viz.*, the isotachophoretic column. The first is of the module type[57], *i.e* , the components such as the injection system, electrolyte chambers and detection cell are independent modules and can be assembled as desired. The advantages of the modular equipment are its versatility and the possibilities of replacing various units and of adjusting the equipment to the demands imposed by a given analytical problem. In the second type[119,120], the isotachophoretic column is formed by a compact block and the separation capillary is created as a flat groove in this block. The compact equipment offers the advantages that both the separation capillary and the detection cell are thermostated with the same efficiency (the cell is made up from a part of the capillary without any connections or any changes in the inner diameter) and, moreover, a series of detection contacts can be placed along the separation path. The instrumentation used at present utilizes the advantages of both of the above possibilities. Various functional elements of the basic instrumentation have been developed fairly diversely since the end of the 1970s, and their present state can be discussed separately.

Micropreparative systems are also discussed separately; either their construction is a modification of the basic analytical equipment in continuous preparation or these systems differ from the basic analytical arrangement substantially in continuous preparation with hydrodynamic flow perpendicular to the direction of electromigration.

4.2. Injection system

An accurate and reproducible injection of a known and/or constant volume of the sample between of the leading and the terminating solutions is a fundamental requirement for the performance of quantitative analyses. By using the experience gained with chromatography, several satisfactory constructions are now available for sample injection, microsyringes and sampling valves made of electrically insulating material (*e.g.*, PTFE) being used to introduce the samples. Therefore, descriptions of new types of injection systems now appear only rarely; mostly sampling valves of simple construction[121] are involved or sampling systems are used the construction of which is motivated by special demands, such as sampling of extremely large sample volumes (up to 1 ml) (see section 4.3)[122].

In general, present-day sampling systems make it possible to inject volumes up to 30 μl and meet the demand of a sharply focused sample zone between the leading and the terminating solutions.

4.3. Separation capillary

The development of separation capillaries from the basic instrumentation to the present state has made considerable progress as a result of demands for an increase in the separation capacity for the purposes of analytical separations of mixtures of components with considerably different concentrations (*e g.*, body fluids). The separation capacity can be increased by extending the length of the separation capillary, but the analysis time and the maximum voltage required also increase. An elegant solution to this problem was the use of a concentration cascade[123] based on the principle of two different concentration levels of the leading electrolyte, where the separation capacity is increased in a chemical manner. The separation capillary is divided into two virtually independent units, which can be considered as two connected columns.

From the instrumental point of view, the column coupling system[124] frequently used today[54,55] has led to significant progress. Essentially, it consists of the pre-separation unit with a capillary of larger diameter (*e.g.*, 0.8 mm) equipped with the tell-tale detector and bifurcation block, behind which an analytical capillary of small inner diameter (*e.g.*, 0.2 mm) is connected. At the beginning of the analysis, the driving current passes through the pre-separation capillary only. At the end of this capillary, within a short distance before the bifurcation block, the tell-tale detector is located, which enables one to follow the sample migration and the level of the separation obtained. At a suitable moment, the driving current is switched so that it passes through the analytical capillary and thus introduces the required sample zones into it. This is analogous to the so-called "heart cutting" technique, a procedure used in chromatography to separate a group of microcomponents from macrocomponents. Microcomponents introduced into the analytical capillary are then further analysed separately. The use of the column coupling system also makes it possible to use different leading electrolytes in the pre-separation and analytical capillaries and thereby to affect the subsequent separation. The column coupling system is now being used with advanced home-made[57] and commercial[125], or commercial equipment can be adapted to this purpose[126] The column coupling system enables one to

separate mixtures containing components in ratios up to 1:1000 without increasing demands on the power supply voltage and without prolonging the analysis time in comparison with a simple system.

A mechanical manner of increasing the separation capacity is volume coupling[127], where the separation capillary is divided into two parts: a replaceable part of the capillary of an optionally larger diameter and thus also with a greater separation capacity and a permanent part with the detector. The parts are assembled with the aid of suitable centring rings without flexible sealings. This design provides simple adaptability of the equipment to the demand of a given analysis without extra demands on the voltage applied across the column.

Direct analysis of trace components in biological materials is, however, still beyond the possibilities of current isotachophoretic instrumentation. For this purpose, a column has recently been developed with which large sample volumes (up to 1 ml) can be analysed[121]. It permits the determination of microcomponents at concentrations up to 10^5 times higher than those of macrocomponents. The sample is introduced by a large-volume sampling valve and the pre-separation run is carried out in a channel with a larger cross-section (*e.g.*, 1 × 20 mm). Isotachophoretic zones are stabilized in this part of the column with the aid of a granulated hydrophilic gel. Major components of the sample are cut out from the separation channel in a similar manner to the procedure with the column coupling capillary system. Microcomponents are then introduced via a tapered channel into an analytical capillary (0.2 mm I.D.), where the final separation and detection are effected.

4.4. Detection

Detection is of key importance to practical applications of separation methods, and therefore also to isotachophoresis. For this reason, the detectors for isotachophoresis have been of central research interest since the beginning of the renaissance of isotachophoresis as an instrumental analytical method. The first universal on-line detector, which played a very significant role in the development of isotachophoresis, was the thermocouple detector[57], which senses the temperature of the migrating zones. Owing to its low resolving power (*i.e.*, its ability to detect zones shorter than about 5 mm) in the early 1970s. the thermocouple was replaced with universal contact detectors, which sense the electric resistance or potential gradient in zones.

The detection cell of these detectors is incorporated in the separation capillary and sensing platinum contacts are placed directly in its walls. The measured electric voltage or resistance is amplified by an electronic device and registered by the recorder as an isotachopherogram. The input part of the detection electronics is insulated galvanically from the output, *e.g.*, by means of a transformer[57] or an optical coupler. The design of the detection cells used allows zones shorter than 0.1 mm to be detected, which ensures the detection of zone volumes of the order of nanolitres in commonly used capillaries. A number of papers were devoted to the construction of suitable detection cells and to the processing electronics during the 1970s. Subsequently, descriptions of new designs appeared only rarely[128,129] and their contribution was the improvement or simplification of the fabrication technology of the detection cell.

Remarkable progress in the development of contact detections in recent years

was made by multi-channel detection[119,130,131]. Here, equidistant electrodes creating the sensing array are placed along the whole separation capillary. The electrodes are formed by vapour deposition of SnO_2 layers on a glass support plate, which serves as the bottom part of the separation capillary of the compact-type isotachopherograph. In the course of the analysis the voltage across the neighbouring contacts is scanned periodically with the aid of carbon contacts moved by a stepping motor. This zone-scanning system has been used mainly for automation of isotachophoretic analyses and for the investigation of separation dynamics (see section 4.6).

The disadvantage of contact detectors is the polarization of the sensing electrodes, which has an adverse effect on the utility of the signal magnitude for qualitative analysis (the step height is distorted). To solve this problem, a contactless high-frequency conductivity detector[132] was proposed in the 1970s. The measuring electrodes of this detector are placed on the outer walls of the separation capillary and the zone conductivity is measured through the capillary wall with the aid of a high-frequency voltage (1 MHz). Although this detection procedure is not yet used in practical applications, recent work[133] showed that an improved design of the electronic section and especially thermostatting of the whole system provide promising results. The equipment described here has considerable sensitivity (detection of zones 0.3 mm in length), which, however, depends of the electrolyte concentration. A concentration of 10^{-3} *M* Cl^- was found to be optimal for the leading electrolyte.

Detection based on differences in the refractive index of various zones was one of the first methods used in electrophoresis. In capillary isotachophoresis, the commercial instrumentation described most recently[134] is equipped with refractometric detection. This enables one to perform isotachophoretic analyses in glass capillaries of 0.1 mm diameter in which the zone migration is compensated with a counter flow of the leading electrolyte at a high concentration (of the order of moles per litre). Refractometric detection is performed in such a manner that the capillary is irradiated along its total length with a narrow light beam and the diffraction image is projected on a screen. The disadvantage of this system is the necessity to work with high electrolyte concentrations, which results in slow analyses (hours or tens of hours).

The development of a selective UV-absorption detector[57] for this technique was an outstanding contribution to the development of isotachophoresis in the 1970s. The UV detector is now a common component of commercial apparatus. In the ideal case, the zones of substances absorbing light at a selected wavelength (254 or 280 nm) are registered as rectangular pulses on passage through the detection cell. The UV detector was also proposed for universal detection[135,136] by using a counter ion the absorption of which is dependent on concentration and pH.

A commercial photometric detector for high-performance liquid chromatography (HPLC) was modified for the UV detection of isotachophoretic zones[137]. It permits the easy selection of wavelengths in the range 200–400 nm.

Simultaneous detection at two different wavelengths represented significant progress in the use of the UV detector for capillary isotachophoresis. Sometimes, when detecting zones of compounds that have similar molar absorptivities at a given constant wavelength, the resulting steps in the detection record can hardly be distinguished. In this instance, simultaneous measurement of UV absorption at two different wavelengths[138] enables one to obtain two mutually different qualitative data

on the same zone, which significantly aids in the identification of the compound in the given zone. The detector is designed in such a way that two independent and mutually perpendicular light beams of suitable wavelengths pass through a given point of the capillary — the detection cell. The changes in the intensities of the light beams are then detected and registered simultaneously.

A very simple UV detector has recently been developed[139] for use in commercial apparatus[125]. A suitable wavelength of the UV light of a contactless mercury lamp is selected with the aid of an interference filter and the light passes through a slit (0.2 × 0.3 mm) placed directly on the separation capillary. For detection a phototransistor is employed, in front of which is placed a wavelength shifter that converts UV light to the wavelength required by the phototransistor.

Great attention has recently been devoted to the development of new selective detectors for capillary isotachophoresis, which would facilitate the identification of compounds in the detected zones. Sensing of absorption spectra in isotachophoretic zones[140] is one of the possibilities. A common spectrophotometer is used, the measuring cuvette being replaced with a suitable window through which the separation capillary passes. On passage of the zone through the window, the analysis is interrupted and the absorption spectrum, which can then be compared with the spectrum of the pure compound, is recorded. To record the spectrum, it is necessary for the investigated zone to fill reliably the whole detection cell (the required zone length was 0.3 mm with the equipment described).

Fluorimetric detection is a highly selective method. In isotachophoresis, the equipment designed initially for the dual-wavelength UV detection was employed for fluorimetric zone detection[141]. Zones of fluorescing compounds or of compounds quenching counter-ion fluorescence were detected. Although the material of the separation capillary used forming the detection cell walls (PTFE) absorbs 96% of the incident light and, subsequently, also a portion of the passed or emitted light, good results were obtained with this arrangement for the reliable selective detection of a whole series of compounds, *e.g.*, B vitamins, quinine.

Radiometric detection of compounds labelled with radioactive isotopes[142] is a specific detection method. Its principle is the detection of the radiation emitted from the labelled compound zone passing the window of a Geiger–Müller (GM) tube. The material used for the separation capillary (PTFE) permits the detection of β-radiation, the energy of which is 0.7 MeV and higher (*e.g.*, ^{24}Na, ^{32}P, ^{36}Cl, ^{40}K, ^{42}K, ^{89}Sr). In experiments described[142], a separation capillary of I.D. 0.3 mm and a wall thickness of 0.13 mm was pressed into a slit of width 2 mm shielded with lead and situated directly on the inlet window of the GM tube. Although the optimum arrangement has probably not yet been achieved, the sensitivity obtained is considerable. For instance, in the case of $^{32}PO_4^{3-}$, the zone, which could not be distinguished in the conductivity record, was detected reliably with a radiometric detector.

Electrochemical detection, owing to its high sensitivity and specificity, is widely utilized in modern liquid chromatography. Its direct use in isotachophoresis is hindered by the presence of the driving electric field. Although this detection procedure has already been tested in isotachophoresis[143], where the electrochemical detector was attached to the separation capillary via an elution block in which the separated zones were flushed continuously into the detection cell, it seems likely that, after establishing a suitable geometrical arrangement and electric separation, iso-

tachophoresis will gain another sensitive specific detector. A two electrode arrangement (Pt, glassy carbon) was used and the detection limit found for easily oxidizable compounds (ascorbic acid) was about 1 pmole.

4.5. Electrode chambers, electrodes and power supply

Since the end of the 1970s, electrode chambers and the means of their connection to the separation capillary, electrodes and stabilized current supply have not changed significantly and detailed descriptions can be found in a book[57] and a review[60].

The chamber, filled with the leading electrolyte, is connected to the separation capillary via a semipermeable membrane (Cellophane). The terminator chamber is connected via a multi-way switching valve, which is open in the course of the analysis. Recent modifications have concerned only the material of the chambers, *e.g.*, simple equipment[144] made of fluorinated polymers [PTFE, poly(chlorotrifluoroethylene)] was developed for isotachophoresis in non-aqueous media.

A modification of the electrode chamber construction was described[145] in connection with anionic analyses at high pH. Here, interference by carbonates is a serious problem and can be solved[145] by using a closed system where the solutions in the electrode chambers are not accessible to atmospheric carbon dioxide.

The basic parameters of the sources of stabilized d.c. currents have not changed since the end of the 1970s[57]. A source with a maximum voltage of up to 30 kV, capable of supplying 10–500 μA of stabilized current, can be used for common applications.

4.6. Automation and data processing

Considerable attention has recently been devoted to simplification of the isotachophoretic analyser operation with the aid of control units that permit programmed control of the analysis and its record. Such control units are particularly suitable for work with column coupling systems[53,125,146]. They make it possible to programme the entire analysis in which the zone system obtained by a preliminary sample separation can be cut into four segments, two of which are further analysed and the other two are excluded from the subsequent analysis. A control unit[147,148], which permits flow programming and recorder control in the course of the analysis, was also described for the LKB commercial analyser[149].

Several procedures aimed at simplifying the quantitative evaluation of analyses have been proposed. The coulometric system[150] is based on an unambiguous relationship between the mass transfer by the electric current and the electric charge passed. Here, the analysis record resembles a common time record, but the time axis is replaced with the electric charge passed. The analysis record is independent of the separation equipment used and the separation current. The advantage of the principle is a high accuracy and uniformity of the output information, suitable for automation.

Another procedure for processing measured records is based on electronic evaluation of the step lengths by a modified chromatographic integrator (Shimadzu) or on an on-line microcomputer[148,151]. To permit the use of common chromatographic integrators for the quantitative evaluation of the isotachopherogram, a procedure

was developed for the conversion of the isotachopherogram into a record similar to a chromatogram[152,153]. In this instance, the time dependence of the signal, which is equal to dt/dh, where t is time and h the magnitude of the conductivity detector signal, is recorded. The conversion is performed by a microcomputer, which processes the signal of an A/D converter connected to the detector. Quantitative evaluation is then performed with a common chromatographic integrator.

An important contribution to qualitative assessment of the analysis record is based on computer processing of the signals from a dual-wavelength UV detector[138]. The processing is based on the calculation of the ratio of absorbances at different applied wavelengths in various zones. This ratio has a characteristic constant value for a certain group of related compounds, and then one can reliably interpret the analysis record. To investigate the course of the separation process and to evaluate automatically analyses immediately after the steady state has been reached, multichannel detection[130,154] is employed, where a detection array of sensing electrodes is in contact with the electrolyte along the separation path. The potential gradient distribution along the separation path is scanned by means of a moving contact at short time intervals, and is processed by a microcomputer. The processing is based on rapid periodical evaluation of the lengths of separate zones, (*e.g.*, every second). Constancy of zone lengths in several subsequent evaluations indicates that the steady state has been reached. The analysis is then evaluated and the results are printed out.

4.7. Micropreparative systems

Current capillary isotachophoretic analysers can be used for micropreparative purposes in a discontinuous arrangement only. Once the separation has been performed, the analysis is discontinued and the analysed compound zone is isolated by a suitable method. Either direct cutting of the capillary section containing the compound under analysis[155]or isolation with the aid of a microsyringe[156–158] can be used for this purpose. A fractionation valve[159], which is placed at the end of the separation capillary, was developed for the same purpose. When the zone in question passes through this valve, the driving current is switched off and the compound is flushed out for subsequent treatment. With a suitably selected amount of sample the yield is 90–100%. With the aid of those micropreparative techniques, isotachophoresis was coupled with mass spectrometry[160,161] and liquid chromatography[155].

The Tachofrac[162] is a device that permits the trapping of separated zones on a strip of cellulose acetate. This device is supplied with the LKB 2127 Tachophor commercial analyser.

Continuous free-flow isotachophoresis[163–165] is used for micropreparative purposes in an entirely different way. This technique, developed especially for micropreparative purposes, uses a laminary flow of thin (*ca.* 0.5 mm) layers of the liquid leading and terminating electrolytes for zone stabilization. The sample is pumped continuously between these two flows, electric current is applied and hence isotachophoresis proceeds perpendicularly to the electrolyte flows. At a suitably selected flow-rate and electric current, individual zones of the substances separated can be collected by means of a fraction collector at the outlet of the system. Up to several grams of pure chemicals can be prepared daily by this means. A commercially available apparatus[166] makes it possible to perform continuous isotachophoresis[167,168] in addition to *e.g.*, zone electrophoresis or isoelectric focusing.

The apparatus for isotachophoretic electrodesorption, developed mainly for the characterization and determination of biologically active compounds[169], was modified also for the micropreparative electrodesorption of monoclonal antibodies (for milligram amounts)[170]. Here, the adsorption element with an affinity sorbent is employed in which the protein in question is adsorbed. Then, this element is inserted into the isotachophoretic separation capillary and, in the subsequent isotachophoretic analysis, the determined protein is released by electrodesorption and, after the separation, is isolated with a microsyringe.

4.8. Commercial instrumentation

At present there are commercially available three analytical capillary isotachophoretic analysers and one instrument for continuous flow isotachophoresis.

The LKB Tachophor 2127[149], still available, was the first commercially available isotachophoretic analyser. It is equipped with a PTFE separation capillary, 0.5 mm in diameter, in lengths of 230–800 mm. The capillary is thermostated within the range 3–29°C. The sample is injected with the aid of a microsyringe via a septum. The power supply, with a maximum voltage of 30 kV, provides a stabilized d.c. current up to 500 μA. Either UV (254, 280, 340 and 365 nm) or conductivity detection is used. The Tachofrac fraction collector[162] is an optional device making it possible to trap separated zones on a cellulose acetate strip and subject them to additional, *e.g.*, immunological analysis. To simplify the operation, the instrument is equipped with a control unit. Replaceable elements of resistant TPX plastic are supplied for work with non-aqueous solvents.

Shimadzu (Japan) introduced the second commercially available apparatus for capillary isotachophoresis. The IP-1B apparatus[171] is supplied with a PTFE capillary, 0.5 mm in diameter, 200–1000 mm in length, with the possibility of thermostating at 5–20°C.

The later IP-2A apparatus[172] is equipped with capillaries of diameter 1 and 0.5 mm, with optional lengths. By this means (volume coupling) the separation capacity can be optimized for a given problem. The power supply, with a maximum voltage of 30 kV, provides up to 500 μA of stabilized d.c. current. The sample is injected with a microsyringe via a septum. The materials of construction used (PTFE, glass) permit the use of non-aqueous solvents. UV and potential gradient detection are used. In addition to the basic unit, equipment for establishing a counter flow and a device for electronic measurement of step lengths in the isotachopherogram are also available.

An analyser[125] equipped with a column coupling system is manufactured in Czechoslovakia. The pre-separation capillary, 0.8 mm in diameter, and the attached analytical capillary, 0.3 mm in diameter, are each 200 mm long. The sample is introduced either with an injection valve 25 μl in volume or with a microsyringe via a septum. The use of Perspex as the material of construction for electrode chambers restricts the working range to aqueous solutions. The power supply, with a maximum voltage of 16 kV, provides a stabilized d.c. current of up to 500 μA. Conductivity detection is used with the detection cells at the ends of both capillaries. The instrument is equipped with an automation unit controlling the column-coupling system and analysis timing.

Although the apparatus for continuous free-flow electrophoresis, the Elphor VaP 21[166], is not an analytical one, it can be classified as such in view of the separation efficiency obtained and its operation in a free solution. It makes it possible to perform isotachophoretic separations in a flat rectangular channel of optional dimensions, 50–200 × 250 × 0.3–1 mm, between two glass plates of good thermal conductivity. The sample is introduced continuously into the system at a flow-rate of 0.3–100 ml/h. A 90-piece fraction collector is placed at the outlet. The driving current power supply operates within the ranges 750 V/1330 mA to 3000 V/330 mA. The cooling system enables one to work in the temperature range 4–25°C and its cooling power allows the dissipation of up to 1200 W of electric power in the separation system. The apparatus is provided with a scanning UV detector (Elphor Scan) and for automatic control and evaluation of the separation an Apple II microcomputer with software on a floppy disc is available.

5 APPLICATIONS OF ISOTACHOPHORESIS

In the past, many reviews have been devoted to practical applications of isotachophoresis. The last review[60] covers the literature up to the end of 1980. This section is aimed at reviewing applications published since 1981 and earlier applications that were not included in the last review[60]. The section is divided up according to the origin of the samples, which enabled us to draw together the papers dealing with similar analytical problems, such as the presence of identical or similar major components or comparable analytical concentrations. In the field of clinical applications, the material is further classified according to the character of the analysed substances.

5.1. Analysis of body fluids

5.1.1. Inorganic anions

The heavy metals Ca, Fe, Zn, Pb, Cu and Al were analysed by isotachophoresis in deproteinated serum[173]. Prior to the analysis, the metals were concentrated by chromatography on Chelex. A selective complex-forming counter ion of α-hydroxyisobutyric acid was used to achieve the separation.

Sulphate was analysed in body fluids of urological patients[174]. Their urine was analysed after acidification with 0.1 *M* HCl and their plasma after ultrafiltration. The recovery of exogenic sulphate in plasma was 92–104% and in urine 87–103%.

5.1.2. Organic acids

Pyruvate, lactate, acetoacetate and β-hydroxybutyrate, *i.e.*, acids that play the most significant role in the acid–base balance, were determined in serum within 25 min by direct analysis[175,176]. A good correlation between isotachophoresis and enzymatic tests was found for the determination of lactate in plasma for both normal and elevated values[177]. Lactic acidosis, ketoacidosis and their mixed forms were thus easily distinguished. When the clinical state of the patient became critical, some additional acids appeared in the record. Both the patient's state and his chance of surviving could be evaluated reliably from the general profile of the isotachopherogram.

Citrate, which forms a complex with calcium ions and thus affects the formation of urinary calculi, was determined directly in urine[178]. To dissolve the citrate complex prior to the analyses, urine had to be kept at room temperature for at least 2.5 h. The minimum detectable amount was 0.2–0.4 nmole. The results correlated well with the enzymatic test with citrate lyase. The specificity of isotachophoretic analysis was confirmed by the disappearance of the citrate zone when analysing urine incubated with citrate lyase. Simultaneous determination of oxalate and citrate in 24-h urine was also described[179]. After adding $ZnCl_2$ to urine, a phosphate zone was also observed in the isotachopherogram and, in the urine of a patient suffering from oxalosis, glycolate was also found. Citrate was also determined in human semen plasma[180], physiological values ranged from 32 to 44 nmol/l.

Anions in urinary calculi were analysed by isotachophoresis in 14 min[181]. The calculus was ground and, after its dissolution in 1 *M* HCl, oxalate and phosphate were determined at pH 3.68 and, after dissolution in 1 *M* NaOH, urate, xanthine and cystine were determined at pH 7.7.

Methylmalonic acid levels were investigated in the urine of rats[182] and of patients with methylmalonic aciduria[183]. Regarding the detection limit of 0.5 μg and the physiological concentration range, methylmalonic acid was not found in the urine of healthy individuals.

Ascorbic acid was determined in leucocytes and in the urine of healthy persons[184]. The ascorbic acid zone was identified in the isotachopherogram by the prolongation of the zone after the addition of ascorbate and by the disappearance of the zone when the sample was incubated with ascorbate oxidase. Concentrations of 5–35 mg/l of ascorbic acid and 18–46 μg of ascorbic acid per 10^8 cells were found in undiluted urine by analysis in 30–35 min and, after ultrasonic treatment, in leucocytes.

The use of 95% methanol as the solvent permitted the determination of taurochendeoxycholic, taurocholic, glycocholic and glycochendeoxycholic acids in human bile[185].

A series of papers on the determination of oxalate in urine was supplemented with additional work[186–189]. The two most frequently used leading electrolytes (HCl–NaCl and HCl–β-alanine)[190] were compared. HCl–NaCl gave a higher reproducibility and a better recovery of oxalate.

Valproate, which is used for the treatment of primary epilepsy, was determined by direct isotachophoretic analysis of serum with a reproducibility of better than 2%[191].

Analytical isotachophoresis was also used[192,193] for the determination of acetate in the blood of patients during haemodialysis and the results were comparable to those obtained by an enzymatic method ($r = 0.95$), the reproducibility being 3.9%. The maximum acetate levels were found 180 min after the start of haemodialysis.

The content of trifluoroacetic acid, a metabolite of halothane, an inhalation anaesthetic, in the blood and urine of anaesthetized patients was monitored by isotachophoresis[103,194,195]. The presence of trifluoroacetate in urine could be detected by isotachophoresis eleven days after the operation.

Thiodiacetic acid, a major metabolite of carcinogenic vinyl chloride, was determined in the urine of persons exposed to vinyl chloride vapour[196,197]. The reproducibility of the analysis was about 3%, the minimum detectable concentration was $6 \cdot 10^{-6}$ mol/l and the analysis time was 45 min.

Formate was determined in serum and haemolysed blood after methanol intoxication with a reproducibility 3%[198]. The maximum concentration of formate in serum was 17 mmol/l.

The metabolites of ethylene glycol, *i.e.*, glycolate, glyoxalate and oxalate, were determined in the blood of intoxicated persons[199]. Whereas negligible amounts of glyoxalate and oxalate were found, the level of glycolic acid reached 17–29 mmol/l.

5.1.3. Amino acids and peptides

γ-Aminobutyric acid was determined in rat brain by cationic isotachophoresis[200]. Rat brain was homogenized, γ-aminobutyric acid was concentrated by ion-exchange chromatography and the eluate was then analysed for γ-aminobutyric acid with a detection limit of 1 nmol.

Cystathionine and its derivatives[201,202] were determined by anionic isotachophoresis in different rat tissues and in the urine of patients with cystathioninuria. The minimum detectable amount was about 1 nmol and the recovery was 92–95%.

Carnitine, which is important for activated fatty acid transfer and whose insufficiency in the organism manifests itself clinically, was determined in serum by cationic isotachophoresis[203]. The serum was incubated in alkaline medium, carnitine was trapped on an ion-exchange resin and 20–30 μl of the sample were analysed within 10–15 min with a detection limit of 0.2 nmol. The reproducibility was about 2.7% and the recovery was 108–116%. The method enables one to determine free carnitine and carnitine bearing short acyl groups. Levels of 42–62 μmol/l were found in the serum of blood donors.

Cysteine derivatives have been determined in human urine[204] and taurine in various rat tissues[205,206].

Isotachophoresis has been used several times for the determination of unidentified peptides in urine or the ultrafiltrate of serum from uraemic patients (or after fractionation of these samples by gel chromatography, gradient elution chromatography etc.)[207–212]. In the UV detector record characteristic zones were observed, the peptidic character of which was confirmed by amino acid analysis.

Tripeptide glutathione and its dimer glutathione disulphide were determined by isotachophoresis in homogenates from various rat tissues[213,214]. The purity of the isotachophoretic zones was checked by incubation of the sample with N-ethylmaleimide, dithiothreitol and glutathioreductase.

Iminopeptides were determined by isotachophoresis in the urine of patients with prolidase deficiency, which manifests itself clinically as iminopeptiduria[215]. Alkalinized urine was subjected to chromatography on Chelex, the fractions were evaporated to dryness and again chromatographed on Diaion. By applying this procedure to samples of urine from healthy persons, all peptides were removed. In the urine of patients with iminopeptiduria, five characteristic zones, which contained twelve dipeptides with terminal proline, were found by isotachophoresis.

Cytostatic methotrexate was determined in plasma by isotachophoresis[216,217]. Prior to the analysis, methotrexate in plasma was precipitated with silver ions and the precipitate was washed, dissolved in Tris buffer and analysed by anionic isotachophoresis. The minimum detectable concentration of methotrexate in plasma was 2.5 μg/l when the column coupling system was used.

5.1.4. Purines, pyrimidines and organic bases

Uric acid, an increased level of which in blood is a symptom of gout, was determined by isotachophoresis with a reproducibility of better than 2%[218]. The purity of the uric acid zone was illustrated by the disappearance of the zone after incubation of the sample with uricase. The results correlated well with those of an enzymatic determination ($r = 0.98$).

Everaerts and co-workers[219–223] determined purines and pyrimidines in urine, serum and in the ultrafiltrate of serum from healthy persons, hyperuraemic patients and patients with Lesch–Nyhan syndrome. Orotic, uric and hippuric acids, xanthine, hypoxanthine, allopurinol, guanosine and adenine were found in the isotachopherogram. In analysis of urine, steps due to orotidine and pseudoxanthine also appeared. When medical treatment with allopurinol was applied, steps due to oxopurinol and allopurinol also appeared[220]. In order to distinguish the zones in the UV record more clearly, non-UV-absorbing spacers were used. The purity of some zones (hypoxanthine, xanthine, allopurinol, uric acid) was checked by analysing samples incubated with enzymes that react with these compounds prior to the analysis[219,221].

Hypoxanthine, as a marker of tissue hypoxia, was determined in serum by isotachophoresis after deproteinization and ion-exchange chromatography[224]. An average hypoxanthine concentration of 0.91 μmol/l was found in the blood of 18 healthy blood donors; in the umbilical blood of newborns the hypoxanthine range was 3–75 μmol/l. The reproducibility was about 5% and the minimum determinable concentration was 10 nmol/l.

The composition of RNA bases from rat livers was determined by isotachophoresis after extraction and hydrolysis with KOH[225]. Isotachophoresis was used to study the influence of 5-fluoroorotate on nucleotide labelling with [^{3}H]orotate[226].

Theophylline, which is used to treat asthma, was determined in ultrafiltered serum[227]. For quantitation the absorbance of the spike between serine and bicine spacers was measured. The detection limit was 1 mg/l with an analysis time of 12 min. Theophylline bound to some proteins was also studied.

Cytostatic adriamycin and its main metabolite adriamycinol were determined in plasma after extraction with butanol[228]. Isotachophoresis did not separate these two cytostatics from one another.

The local anaesthetic trimecaine and both its des-ethylated metabolites were determined by isotachophoresis in plasma after deproteinization and extraction with chloroform[229]. The minimum determinable concentration was 50 μg/l and the reproducibility was 5%. The newer local anaesthetics heptacaine and its 2-alkoxy derivatives were determined in an analogous manner.

Quinine was determined in the extract of urine extracted with isopropanol–dichloromethane[230]. It was found that 2–15% of administered quinine was excreted by urine; the detection limit was 69 μg per 48 h.

Isotachophoresis was also used to determine spermine in semen[231].

5.1.5. Nucleotides and nucleosides

Nucleotide levels in muscles were determined in order to study their metabolism in Duchenne muscular dystrophy[232]. Suppressed levels of ATP, ADP and AMP were found in the isotachopherogram when muscle extract from patients with the above disease was analysed.

Isotachophoresis was also used to study the levels of ATP, diphosphoglycerate, phosphate and lactate in erythrocytes in stored blood[233,234]. The reproducibility of the analyses was better than 3% and enabled one to monitor concentration changes of these compounds during storage.

The contents of ATP, ADP, AMP, c-AMP, IMP, NADH, NAD^+, creatine phosphate, glucose-6-phosphate and pyruvate[235] were determined in heart and bone muscles of the dog, pig, frog and hamster. The values found were in agreement with literature data obtained by other methods.

Various rat tissues were analysed for ATP and ADP, using non-absorbing spacers[236,237]. The reproducibility of the determination of ATP was 2.2% and the recovery was 100–101%. Levels of UDP glucuronate in rat liver after narcosis with diethyl ether, halothane, enflurane, isoflurane and ketamine with diazepam were suppressed, as shown by an isotachophoretic study[238,239]. Isotachophoresis was used to study some nucleotides in human lymphocytes and in the culture of human fibroblasts[240]. Malignant hyperthermia was studied on a pig model. Levels of purine nucleotides, phosphocreatine, lactate and phosphate, as possible markers of malignant hyperthermia susceptibility[241], were investigated. Some further papers[242–245] also reported the determination of nucleotides, especially ATP.

Cytostatic 5-fluorouracil and its analogue 5-deoxy-5-fluorouridine were determined in plasma and serum by isotachophoresis, using a UV detector after deproteinization and ion-exchange chromatography[246]. Five minutes after their intravenous application (15 mg/kg body weight) the maximum concentration in plasma (120–260 μmol/l) was reached. The reproducibility of the analysis was 4–7%. A cytostatic arabinofuranosylcytosinetriphosphate was analysed in leukaemic cells after incubation and repeated washing[247]. The minimum detectable amount was 15 pmol with the use of the UV detection.

5.1.6. Proteins

In the past, protein analyses mainly involved the analysis of cerebrospinal fluid, particularly in cases of multiple sclerosis[248–254]. Isotachophoresis permitted the determination of the permeability of the blood–cerebrospinal fluid barrier for various IgG fractions and for albumin and also permitted a quantitative study of the synthesis of immunoglobulins G inside this barrier. Proteins were determined in cerebrospinal fluid in cases of neurinoma[255], meningitis[256], meningoencephalitis[248–250], acute leukaemia[257] and subarachnoid haemorrhage[250,258]. In the presence of high salt contents, dialysis of the sample is recommended prior to starting the analysis[259].

A number of studies have dealt with the isotachophoresis of serum proteins. To distinguish and characterize the zones better, mixtures of defined substances (amino acids and Good buffers) are frequently used as spacers[248–250,255,260–262]. Small, sharp peaks, the appearance of which is poorly reproducible, often appear in isotachopherograms of serum proteins. The existence of such peaks was explained as the presence of proteins that could have been denatured in the course of the isotachophoretic separation[263].

Isotachophoresis was used to determine immunoglobulins G in the serum of patients suffering from some infectious and non-infectious diseases. Significant differences were found in the composition of serum proteins of patients with liver cirrhosis and paraproteinaemia[255].

Serum lipoproteins pre-stained with Sudan Black B were determined by capillary isotachophoresis with photometric detection at 570 nm[264]. As the preparation proceeded in a free solution, chylomicrons did not remain at the start as in gel electrophoresis but migrated between low-density and very-low-density lipoproteins. High-density lipoproteins were successfully determined using UV detection[265,266].

Thrombin was determined in human blood by isotachophoresis within less than 20 min[267,268]. The purity of thrombin in the isotachophoretic zone was verified by incubation of the sample with heparin prior to the analysis; the thrombin zone disappeared from the isotachopherogram.

In order to determine proteins in body fluids, capillary isotachophoresis was combined with electrophoresis in agarose[269], with isotachophoresis in polyacrylamide gel[270] and with immunoelectrophoresis[271]. Further, isotachophoresis was applied to the determination of proteins in bovine serum, cerebrospinal fluid and bovine aqueous humour[272] and to the determination of crystallines in eye lens[273,274].

5.2. Drugs and their production

Arabonic acid, an intermediate in riboflavine production, was determined in samples of glucose oxidized in aqueous alkaline solutions with a reproducibility of 3.5%[275]. In addition to arabonic acid, oxaloacetic, formic, glycolic, lactic, gluconic and 3-hydroxypropionic acids were identified. The content of arabonic acid in mother liquors was about 0.1 mol/l and in crystalloids it was higher than 89%. Isotachophoresis was used for monitoring the enzymatic cleavage of deacetoxycephalosporin G, in which phenylacetic and 7-aminodeacetoxycephalosporanic acids[276] are produced; the latter is an intermediate in the preparation of new antibiotics of the cephalosporin type. The course of the enzymatic reaction could easily be studied by determining any of the three components of the reaction mixture.

Mucolytic S-carboxymethylcysteine was determined in syrups and capsules with a reproducibility of 0.7% and a detection limit of 10^{-10} mol[21]. In preparations kept for 6 weeks at 50°C, cysteinesulphinic acid was found as the main degradation product.

Sulphites, which are frequently added to drugs as antioxidants, and sulphates, produced by their oxidation, were determined by isotachophoresis, among other methods, in infusion solutions of amino acids[277]. The minimum detectable amounts were 0.2 mmol/l for sulphites and 0.1 mmol/l for sulphates.

The synthetic peptides alanylglutamine and tyrosinyllysyltyrosine, which are suitable for parenteral administration of glutamine and tyrosine because of their solubility in water and their thermal stability, were determined by isotachophoresis in less than 20 min[278–280].

Ascorbic and nicotinic acids, thiamine, pyridoxol, pyridoxal, pyrodoxamine and nicotinamide were determined in vitamin preparations by isotachophoresis[281]. The recovery was 93–101% and the reproducibility was better than 2%.

Isotachophoresis was also used to determine the following drugs: quinine[230], insulin[282], inorganic ions in crude drug preparations[283], valproate[284], codeine and phenyltoloxamine (reproducibility 1.0%, minimum detectable concentration 0.5 ppm)[285], the aminoglycoside antibiotics tobramycin, spectinomycin, clindamycin and lincomycin (minimum detectable amount 1.6 nmol, reproducibility 2%)[286], the

local anaesthetics procaine, lidocaine and tetracaine (reproducibility about 1.7%, detection limit 1.8 nmol)[287], ephedrine, norephedrine, norpseudoephedrine, phenylephedrine, carbinoxamine, methoxymethylmorphine and yohimbine (reproducibility 3–5%)[288], the peptide drugs saralasin, gonadorelin and protirelin (reproducibility about 2%)[289], ephedrine (reproducibility 1.0%), pyridine-4-aldoxime (minimum detectable amount 10 pmol)[290] and phosphonoacetate[291]. Paper isotachophoresis[292,293] with an on-line conductivity detector was used successfully for cationic and anionic determinations of various drugs (quinine, ephedrine, codeine and aminophenazone).

5.3. Food production

In this field, isotachophoresis has been used especially for determinations of organic acids, preservatives and flavours.

Isotachophoresis has proved to be an excellent analytical method for food preservatives, *e.g.*, sorbic, benzoic and propionic acids and *p*-benzoic acid esters (reproducibility better than 2%)[294–297], thiabendazole and diphenylol (reproducibility about 2%)[298], and food additives, *e.g.*, glutamate, GMP, IMP[299], mono-, di- and triphosphates, citrate, glutamic and ascorbic acids, glucono-δ-lactone in meat products (reproducibility about 1%)[300], glutamate in soup (reproducibility better than 2%)[301], cyclamate and saccharin sweeteners in dietetic drinks, tins and some other foodstuffs (reproducibility 0.4–2%)[302] and quinine in drinks (detection limit 5 mg/l)[230].

Great attention was devoted to determinations of acids in wines[303–309]. Acetic, succinic, gluconic, lactic, malic, malonic, phosphoric and sulphuric acids were determined in wines by using chlorate as the internal standard, and the reproducibility was about 2%[303].

The following acids were also determined in foods: citric acid in citrus fruit juices[310], succinic acid in fish extract[311], ascorbic and dehydroascorbic acids in citrus fruit concentrate (reproducibility 1–2%)[312], 2-pyrrolidone-5-carboxylic acid in tomatoes and tomato products[313], lactic, acetic, citric and phosphoric acids in sauerkraut brine (reproducibility about 1%, minimum determinable amount 3–5 ng)[314] and phosphoric, formic, citric, malic, glycolic, lactic and acetic acids in coffee[315].

Tetrodotoxin, which is the main toxin in poisoning by puffer fish, was determined by isotachophoresis in the fish extract with a limit of determination of 0.25 μg[316].

The histamine content in fish and fish products[317,318] was determined by isotachophoresis within 30 min. Isotachophoretic analysis of extracts from mackerel, tuna and herring (reproducibility about 1%, recovery 99%) revealed a rapid increase in histamine concentration after storage of the fish for 3–4 days at 4°C.

Isotachophoresis also proved to be very useful in the following food analyses; EDTA determination in mayonnaise and margarine (reproducibility 3–6%, minimum detectable concentration 10 ppm)[319], nucleotide determination in muscles of frozen carps[320], sinalbin and sinigrin determination in mustard seeds (reproducibility 2%, detection limit 2.4 nmol)[321], determination of inorganic acids in Miso[322], determination of cyanides in apricot kernel and plums and in products made from these fruits[323], determination of theanine, aspartic and glutamic acids in various species of green tea[324], and determination of amino acids in sweet pepper fruits[325].

5.4. Environmental analysis

The herbicide Asulam was determined in soil after extraction, centrifugation, filtration and repeated concentration[326] The detection limit was 0.5 ng. If preparative isotachophoresis was performed prior to the analysis, 0.02 ppm of Asulam could be detected and 0.1–0.2 ppm determined.

Cationic triazine and quaternary herbicides were determined by isotachophoresis in water and soil[327]. For the determination of quaternary herbicides (chlormequat, paraquat, diquat) the soil was boiled with 50% sulphuric acid, the herbicides were extracted into dichloromethane, the organic layer was evaporated to dryness and an aqueous solution of residue was analysed. The analysis gave a 70–80% recovery and the sensitivity of the method was *ca.* 10 ppb. Triazine herbicides (atrazine, simazine and prometryne) were derivatized to quaternary ammonium salts prior to analysis. The recovery was 95–98% and the limit of determination was 10 ppb

Phenol was determined in industrial waste waters by direct analysis with a detection limit of 0.1 ppm[328]. A 50-μl volume of the sample was required for the analysis and the analysis time was 20 min. Phenol concentrations of 76 ppm were found in waste waters and 33 ppm in purified waste waters.

Butylamine, which is used in numerous industrial applications, was determined in air[329]. The air was bubbled through 0.1 *M* HCl, the solution was evaporated in a stream of nitrogen and the residue was dissolved in water and analysed. The detection limit of the method was 1 nmol and the recovery was 99.5–118%. The time of the isotachophoretic analysis was 10 min.

Triethylamine and 2-dimethylaminoethanol were determined in air samples from polyuretane foam production[330]. The air was bubbled through 20 mmol/l HCl and the trapped amines were concentrated by adsorption on silica gel. The recoveries were 97–117 and 75–95% for triethylamine and dimethylaminoethanol, respectively.

Chloride, sulphate, nitrate, nitrite, fluoride and phosphate were determined in river water by isotachophoresis[331]. The reproducibility of the determinations of sulphate, nitrate and chloride was better than 2%, and that of nitrite, fluoride and phosphate was better than 5%. The detection limits were 60 ppm for nitrite and 30 ppm for phosphate and fluoride. The analysis time was 25 min.

Isotachophoresis was also used to determine sulphate, phosphate and organic acids in sewage[332], heavy metals (Fe, Cu, Ni, Cd, Co, Zn and Pb) in river water[205] and chloride, sulphate, nitrate, potassium, sodium, calcium and magnesium in surface water[333].

5.5. Chemical industry

In the analysis of industrial or, more often, potential industrial samples, isotachophoresis was used most frequently for the determination of phosphoric acid derivatives such as condensed phosphates[334–340], aminoalkylphosphonates[341] and acid alkyl phosphonates[342–344].

In connection with the growing use of biological technology for the preparation of new compounds, isotachophoresis was used to analyse lysine in fermentation broth[345], lower fatty acids[346] in cultures of microorganisms and organic acid products[347–349] from alkane fermentation. Some papers were devoted to the analysis of

acidic products from the degradation of saccharides[350–355] and methoxybenzene derivatives in degradation products from cellulose hydrothermolysis[356].

The analysis of waste water from metal coatings for Cu complexes[357], ballpoint pen ink for chloride and sulphate[358], cosmetic preparations for chondroitin sulphate[359], permanent waving lotion for thioglycolic and dithioglycolic acids and cysteine[360] and industrial samples for Fe^{2+} and Fe^{3+} (ref. 361), the determination of chlorocarboxylic acid produced by phenol chlorination[362], sulphur oxo acids in sodium sulphide[363], inorganic ions in crude phosphoric acid[364], the veterinary drug amprolium in feedstuffs[365], sulphur oxo acids[366], the explosive 2-(5-cyanotetraazolato)pentaminecobalt(III)[367] and halogenated cobaltocarboranes, used as extraction agents in nuclear chemistry[368], are additional industrial applications of isotachophoresis.

5.6. Miscellaneous

Isotachophoresis was used in enzymology to measure prolidase activity in the serum of iminoaciduric patients[369] and to study the enzymatic transfer of sulphate[370], and to study glucuronidation[371], the metabolism of ascorbic acid[372] and methionine[373], nucleotide-dependent enzymatic processes[374] and reactions of glutathione-S-reductase[375] and adenylate kinase[376].

In protein chemistry, isotachophoresis served to study substitution of dog serum albumin[377], to investigate the bound of 8-aniline-naphthalenesulphonic acid to serum albumin[378], to determine enzyme–immunoglobulin conjugates used in enzyme immunoassay[379], to determine proteins[380], to control the synthesis of the C-terminal pentapeptide bombinine[381,382], to determine virus membrane glycoproteins[383], to study the influence of lyophilization on the protein composition in reference sera[384], to investigate formate dehydrogenase inactivation[385,386], and to determine fetuin in nasal secretion[387] and nuclear proteins in regenerating rat livers[388].

In peptide chemistry, isotachophoresis was applied to the determination of low-molecular-weight compounds in peptide preparations[389–391], the reduced and oxidized forms of glutathione[392] and lysylalanine[393] and to the investigation of the deiodination of iodotyrosine during peptide hydrolysis[394].

In the chemistry of nucleotides, isotachophoresis was applied to the determination of nucleotides produced by microorganisms after high-voltage and ultrasonic treatment[395], to the determination of adenine nucleotides[396,397] and to the control of nucleotide synthesis[398].

Isotachophoresis further served to analyse venom from spiders and snakes[399,400] and stinging insects[401,402], plant alkaloids[403] and allergens[404,405], royal jelly for 10-hydroxy-Δ^2-decenic acid[406], incubated human saliva for putrescine, cadaverine and agmatine[407,408], the composition of the polar lobe of the first cleavage stage of *Nassarius reticulatus* embryos[409], products from the reaction of ammona and acetone[410], humic acid[411], dithiocarbamates[412], alginates[413], hyaluronates[414], cyclohexylamine[415], hydroxybiphenyl conjugates[416], 5-aminolevulic acid and its derivatives porfobilinogen and 4,5-dioxovaleric acid[417], formaldehyde and formic acid[418], acids produced by *Candida albicans*[419] and sialic acid[420]

Successful separations of model mixtures suggest possibilities of using isotachophoresis in practice. Cationic analyses in the past 4 years include separations

of alkali metals and ammonium in a methanolic medium[421], using associates with 18-crown-6-ether[89,422] of varying stability, separations of alkaline earth metals with cyclohexanediaminetetraacetic acid[423] and α-hydroxybutyrate[79] counter ions, separations of K, Na, Ca and Mg with sulphate[120] and the N-oxide of nitrilotris(methylphosphonic) acid[333] counter ions, the complete separation of lanthanides with α-hydroxyisobutyrate as a complex-forming counter ion[424] and the separation of a mixture of Ba, Na, Ca, Mg, Mn, Cd, Co, Zn, Ni, Pb and Cu[173].

Anionic isotachophoretic separations of inorganic compounds include separations of iodide, bromide and chloride and $[Fe(CN)_6]^{3-}$, $[Fe(CN)_6]^{4-}$ and perchlorate with the addition of α-cyclodextrin to the leading electrolyte[422], the separation of chloride, bromide and iodide in a methanolic medium[421] and the investigation of condensation of chromate into bichromate[134] and the formation of various polyvanadates[425].

Separations of coordination compounds include those of *cis* and *trans* isomers produced by the pyrolysis of the green complex of *trans*-$[CoCl(en)_2]Cl$ in which Cl was replaced with water[426], nine cationic nitrosylnitrato complexes of ruthenium with three pairs of *cis-trans* isomers produced by acidic hydrolysis of $[RuNO(NO_3)_3(H_2O)_2]$[426–428] and chelates of Fe(II), Co(II), Cu(II) and Ni(II) with 1,10-phenanthroline in acetonitrile medium[429].

Fatty acids[430], nucleotides with Mg^{2+} counter ion[431], polyamines with citrate counter ion[432] and organic acids[433] were separated among organic model mixtures.

Finally, we can also mention some papers[434–438] that involve isotachophoresis to a small extent and do not fit the above classifications.

6. SUMMARY

This paper is summarizing the contributions to the analytical capillary isotachophoresis published during the period 1981–1984. It characterizes the present state of the method and covers theory, fundamental analytical aspects, instrumentation and applications.

Special attention was payed to the fundamental analytical aspects, and a detailed discussion is given of the selection of electrolyte systems, stability of zones and separability of substances.

The present commercial instrumentation is also briefly described.

7. ADDENDUM

To cover more recent progress in isotachophoresis as represented by the last International Symposium on Isotachophoresis, ITP-84, as completely as possible, the preparation of the manuscript was based on the book of the symposium abstracts[1] and on the actual presentations in the lectures and poster sessions. Hence this text includes a number of papers that did not appear in the symposium volume (*J. Chromatogr.*, Vol. 320, No. 1). On the other hand, owing to some differences between the actual symposium presentations and the final proceedings, which appeared just when this manuscript was submitted for publication, a few papers from this volume must be referred to here. They concern the use of the concentration cascade technique on the commercial LKB instrument[439], desorption isotachophoresis[440], UV detection

at 206 nm[441], the determination of mobilities and pK values[442], dynamics of isotachophoretic separations[443] and the determination of sodium in serum[444].

REFERENCES

1 P Boček (Editor), *Symposium Abstracts, 4th International Symposium on Isotachophoresis, September 2–6, 1984, Hradec Králové, Czechoslovakia*, Institute of Analytical Chemistry, Czechoslovak Academy of Sciences, Brno, 1984.
2 P Boček (Editor), *Isotachophoresis — Basic Course, Advanced Course, ITP-84, September 2–6, 1984, Hradec Králové, Czechoslovakia*, Institute of Radioecology and Applied Nuclear Techniques, Plant for Development and Production of Nuclear Instruments, Spišská Nová Ves, 1984
3 E Kenndler, presented at the *Fourth International Symposium on Isotachophoresis, Hradec Králové, September 2–6, 1984*
4 Z. Prusík, in O Mikeš (Editor), *Laboratory Chromatographic Methods*, SNTL, Prague, 1980, p 572 (in Czech).
5 J Vacík and F M Everaerts, in Z Deyl (Editor), *Electrophoresis, A Survey of Techniques and Applications, Part A, Techniques*, Elsevier, Amsterdam, Oxford, New York, 1979, p 193.
6 E Kenndler, *Oesterr Chem Z*, 82 (1981) 61
7 C Ferri, *ICP*, 10 (1982) 107
8 C J Holloway and I Trautschold, *Z. Anal Chem*, 311 (1982) 82.
9 K. Katoh and H. Miyazaki, *Bunseki*, No 9 (1982) 659
10 T A Koumitze and I. N Papadogianne, *Chem Chron., Genike Ekdose*, 47 (1982) 273
11 F M Everaerts, F E. P Mikkers, Th P. E M Verheggen and J Vacík, in E Heftmann (Editor), *Chromatography, Fundamentals and Applications of Chromatographic and Electrophoretic Methods, Part A, Fundamentals and Techniques*, Elsevier, Amsterdam, Oxford, New York, 1983, p. 331
12 Y Kiso and T Hirokawa, *Kagaku (Kyoto)*, 37 (1983) 394
13 Y Kong, *Yaowu Fenxi Zazhi*, 3 (1983) 311
14 T Stiefel, *Anal Taschenb*, 3 (1983) 139
15 J Vacík, in J Zýka (Editor), *New Trends in Analytical Chemistry*, Volume 1, SNTL, Prague, 1983, p. 9 (in Czech)
16 J Vialle, M Chevalier and M. Marichy, *Analusis*, 11 (1983) 99
17 Z Deyl and J Hořejší, *New Compr Biochem.*, 8 (Separ Methods) (1984) 415
18 Y Shiogai and T Yagi, *Int Lab*, 11 (1981) 69
19 E Kenndler, *Trends Anal. Chem.*, 2 (1983) 202.
20 J Vialle, *Trends Anal Chem*, 3 (1984) 61
21 J Lang and B Büchele, *Acta Pharm. Technol.*, 26 (1980) 237
22 R Jannasch, *Pharmazie*, 36 (1981) 231
23 F. M. Everaerts and Th P. E M Verheggen, in C. F Simpson and M. Whittaker (Editors), *Electrophoretic Techniques*, Academic Press, London, 1983, p 149
24 P Offizorz, E Krüger and K Rubach, *Monatsschr Brauwiss*, 37 (1984) 168
25 J Maslowska and J Kolys, *Zesz. Nauk Politech Lodz, Chem. Spozyw.*, 411 (1983) 145
26 A. Zhu, *Shengwu Huaxue Yu Shengwu Wuli Jinzhan*, 58 (1984) 53
27 T Yagi, *Shokuhin To Kagaku*, 25 (1983) 73
28 T Kameo, *Kagaku To Kogyo (Osaka)*, 57 (1983) 465
29 Z Deyl (Editor), *Electrophoresis, A Survey of Techniques and Applications, Part B, Applications*, Elsevier, Amsterdam, Oxford, New York, 1983.
30 M. Lederer, in E Heftman (Editor), *Chromatography, Fundamentals and Applications of Chromatographic and Electrophoretic Methods, Part B, Applications*, Elsevier, Amsterdam, Oxford, New York, 1983, p 459.
31 P Boček and F. Foret, *J. Chromatogr*, 313 (1984) 189.
32 C J Holloway and V. Pingoud, *Electrophoresis*, 2 (1981) 127
33 P Delmotte, *Sep Purif Methods*, 10 (1981) 29
34 P. M. S. Clark and L. J Kricka, *Advan. Clin Chem.*, 22 (1981) 247
35 C J. Holloway and J Luestorff, *Electrophoresis*, 1 (1980) 129
36 F Everaerts, J Reijenga and T. Verheggen, in D Stathakos (Editor), *Electrophoresis '82*, Walter De Gruyter, Berlin, New York, 1983, p 293

37 P. Boček and P Gebauer, in P Boček (Editor), *Isotachophoresis — Basic Course, Advanced Course, ITP-84, September 2–6, 1984, Hradec Králové, Czechoslovakia*, Institute of Radioecology and Applied Nuclear Techniques, Plant for Development and Production of Nuclear Instruments, Spišská Nová Ves, 1984, p. 16.
38 V Dolník and P Boček, in P Boček (Editor), *Isotachophoresis — Basic Course, Advanced Course, ITP-84, September 2–6, 1984, Hradec Králové, Czechoslovakia*, Institute of Radioecology and Applied Nuclear Techniques, Plant for Development and Production of Nuclear Instruments, Spišská Nová Ves, 1984, p 30.
39 Z Prusík and V. Kašička, in P Boček (Editor), *Isotachophoresis — Basic Course, Advanced Course, ITP-84, September 2–6, 1984, Hradec Králové, Czechoslovakia*, Institute of Radioecology and Applied Nuclear Techniques, Plant for Development and Production of Nuclear Instruments, Spišská Nová Ves, 1984, p 49.
40 M. Tazaki, S Nagahama and K Ueno, *Kagaku (Kyoto)*, 38 (1983) 674.
41 P Gebauer and P Boček, in P Boček (Editor), *Isotachophoresis — Basic Course, Advanced Course, ITP-84, September 2–6, 1984, Hradec Králové, Czechoslovakia*, Institute of Radioecology and Applied Nuclear Techniques, Plant for Development and Production of Nuclear Instruments, Spišská Nová Ves, 1984, p 78
42 P Gebauer and P Boček, *Chem Listy*, 77 (1983) 483.
43 P Boček, in F M Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p. 143
44 P Boček, P Gebauer and M. Deml, *Chem Listy*, 78 (1984) 510
45 P Boček, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 31.
46 L M. Hjelmeland and A Chrambach, *Electrophoresis*, 3 (1982) 9.
47 A Chrambach and L. M Hjelmeland, in H Hirai (Editor), *Electrophoresis '83*, Walter De Gruyter, Berlin, New York, 1984, p 81
48 A Chrambach, *J. Chromatogr*, 320 (1985) 1
49 W Thormann, *Separ Sci Technol.*, 19 (1984) 455
50 F. M. Everaerts and F E P. Mikkers, in F M. Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p 1
51 J. Vacík, in P Boček (Editor), *Isotachophoresis — Basic Course, Advanced Course, ITP-84, September 2–6, 1984, Hradec Králové, Czechoslovakia*, Institute of Radioecology and Applied Nuclear Techniques, Plant for Development and Production of Nuclear Instruments, Spišská Nová Ves, 1984, p 1
52 J Vacík, *New Compr Biochem.*, 8 (Sep Methods) (1984) 29
53 D. Kaniansky and P Havaši, *Acta Fac. Rerum Nat Univ Comenianae, Chim.*, 31 (1981) 137
54 F M Everaerts, Th. P E M Verheggen and J. C Reijenga, *Trends Anal Chem*, 2 (1983) 188.
55 D. Kaniansky and P Havaši, *Trends Anal Chem*, 2 (1983) 197
56 D Kaniansky and P. Havaši, on P Boček (Editor), *Isotachophoresis — Basic Course, Advanced Course, ITP-84, September 2–6, 1984, Hradec Králové, Czechoslovakia*, Institute of Radioecology and Applied Nuclear Techniques, Plant for Development and Production of Nuclear Instruments, Spišská Nová Ves, 1984, p 122
57 F M Everaerts, J L Beckers and Th P E. M. Verheggen, *Isotachophoresis, Theory, Instrumentation and Applications*, Elsevier, Amsterdam, Oxford, New York, 1976
58 C. J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984
59 P Boček, *Top Curr. Chem.*, 95 (1981) 131
60 S G Hjalmarsson and A Baldesten, *CRC Crit. Rev Anal. Chem.*, July (1981) 261
61 *Acta Isotachophoretica, Literature Reference List 1967–1980*, LKB, Bromma, 1981
62 T. Hirokawa and Y Kiso, *J Chromatogr.*, 242 (1982) 227.
63 T. Hirokawa, H Takemi and Y. Kiso, *J Chromatogr*, 280 (1983) 219.
64 J Vacík and V Fidler, in F. M. Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p 19
65 V. Fidler, J Vacík and Z Fidler, *J Chromatogr*, 320 (1985) 167
66 M. Bier, O A Palusinski, R A Mosher and D A Saville, *Science*, 219 (1983) 1281.
67 M. Bier, O A Palusinski, R A. Mosher, A Graham and D A. Saville, in D Stathakos (Editor), *Electrophoresis '82*, Walter De Gruyter, Berlin, New York, 1983, p 51
68 R A Mosher, W Thormann and M Bier, *J Chromatogr*, 320 (1985) 23.

69 W. Thormann and R A Mosher, *Trans Soc Computer Simul*, 1 (1984) 83
70 P Radi, *Electrophoresis*, 5 (1985) 195.
71 V. G Babskii, M Yu Zhukov and V. I Yudovich, *Mathematical Theory of Electrophoresis*, Naukovaya Dumka, Kiev, 1983 (in Russian)
72 M. Yu. Zhukov and V I Yudovich, *Dokl. Akad Nauk SSSR*, 267 (1982) 334.
73 M. Yu. Zhukov, *Mol Biol (Kiev)*, 36 (1984) 28
74 Y Kiso and T Hirokawa, *Chem Lett*, (1980) 323
75 T Hirokawa and Y. Kiso, *J Chromatogr*, 252 (1982) 33.
76 T Hirokawa, M. Nishino and Y Kiso, *J Chromatogr*, 252 (1982) 49
77 Y Kiso and T Hirokawa, *Chem Lett*, (1980) 745.
78 T Hirokawa and Y. Kiso, *J Chromatogr*, 248 (1982) 341
79 T Hirokawa, T Matsuki, H Takemi and Y Kiso, *J Chromatogr*, 280 (1983) 233
80 T Hirokawa, N. Aoki and Y Kiso, *J Chromatogr*, 312 (1984) 11
81 T Hirokawa, M. Nishino, N Aoki, Y. Kiso, Y Sawamoto, T Yagi and J. Akiyama, *J Chromatogr*, 271 (1983) D1.
82 V Zadražil, J Vacík and V. Fidler, presented at the *Fourth International Symposium on Isotachophoresis, Hradec Králové, September 2–6, 1984*
83 V Kašička, J. Vacík and Z. Prusík, *J Chromatogr*, 320 (1985) 33.
84 J Pospíchal, M Deml, Z Žemlová and P. Boček, *J Chromatogr.*, 320 (1985) 139
85 H Carchon and E. Eggermont, *Electrophoresis*, 3 (1982) 263
86 H Carchon and E. Eggermont, *Arch Int Physiol Biochem*, 90 (1982) B97
87 H A Carchon and E Eggermont, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 23
88 S Hjertén, L -G. Oefverstedt and G. Johansson, *J Chromatogr.*, 194 (1980) 1.
89 F S Stover, *J Chromatogr.*, 298 (1984) 203
90 O Fujishita, S Higuchi, M Yoshikawa, T Aoyama and M Horioka, *Chem Pharm Bull*, 31 (1983) 2134
91 Y Kiso and T Hirokawa, *Chem Lett*, (1979) 891
92 P Gebauer and P. Boček, *J. Chromatogr*, 320 (1985) 49.
93 P. Boček and P. Gebauer, *Electrophoresis*, 5 (1984) 338
94 P. Gebauer and P Boček, *J Chromatogr*, 267 (1983) 49
95 P Boček, P. Gebauer and M. Deml, *J Chromatogr*, 217 (1981) 209
96 P Boček, P Gebauer and M. Deml, *J Chromatogr.*, 219 (1981) 21
97 P Gebauer and P Boček, *J Chromatogr*, 242 (1982) 245.
98 P Gebauer and P Boček, in C. J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 47
99 P. Gebauer and P Boček, *J Chromatogr*, 299 (1984) 321
100 P. Gebauer, P Boček, M Deml and J Janák, *J. Chromatogr*, 199 (1980) 81
101 P Gebauer and P Boček, *Collect Czech Chem. Commun.*, 47 (1982) 1802
102 T Hirokawa and Y Kiso, *J. Chromatogr*, 257 (1983) 197
103 T. Hirokawa, H Takemi, Y. Kiso, R Takiyama, M. Morio, K Fujii and H Kikuchi, *J Chromatogr.*, 305 (1984) 429
104 P. Gebauer and P Boček, in B. J Radola (Editor), *Elektrophorese Forum '83, Kurzfassungen der Vorträge und Poster*, Technische Universität München, Freising-Weihenstephan, 1983, p 276
105 E Schumacher and T Studer, *Helv Chim Acta*, 47 (1964) 959.
106 P Ryser, *Mitt Geb Lebensmittelunters. Hyg*, 67 (1976) 56
107 L. M. Hjelmeland and A. Chrambach, *Electrophoresis*, 4 (1983) 20
108 Z. Buzás, L M Hjelmeland and A Chrambach, *Electrophoresis*, 4 (1983) 27
109 P. J Svendsen and C Schafer-Nielsen, in B. J Radola (Editor), *Electrophoresis '79*, Walter De Gruyter, Berlin, New York, 1980, p 265
110 C. Schafer-Nielsen, P. J. Svendsen and C. Rose, *J Biochem Biophys. Methods*, 3 (1980) 97
111 C Schafer-Nielsen and P. J Svendsen, in B J Radola (Editor), *Electrophoresis '79*, Walter De Gruyter, Berlin, New York, 1980, p 275.
112 C Schafer-Nielsen and P J. Svendsen, *Anal. Biochem.*, 114 (1981) 241
113 F Mikkers and F Everaerts, in F. M Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p 1
114 F Everaerts, N Groot and F Mikkers, in R C Allen and P Arnaud (Editors), *Electrophoresis '81*, Walter De Gruyter, Berlin, New York, 1981, p 743

115 T Hirokawa and Y Kiso, *J. Chromatogr.*, 260 (1983) 225
116 M Svoboda, X Svobodová and J. Vacík, presented at the *Fourth International Symposium on Isotachophoresis, Hradec Králové, September 2–6, 1984.*
117 J C Reijenga, G V. A Aben, Th P E M Verheggen and F M. Everaerts, *J Chromatogr.*, 260 (1983) 241
118 M. Svoboda, X Svobodová and J Vacík, *J Chromatogr*, 273 (1983) 228.
119 P Boček, M Deml and J. Janák, *J. Chromatogr.*, 106 (1975) 283
120 E Schumacher, W Thormann and D Arn, in F M. Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p 33
121 D Kaniansky, *Thesis*, Komenský University, Bratislava, 1981
122 V. Dolník, M Deml and P Boček, *J Chromatogr.*, 320 (1985) 89
123 P. Boček, M Deml and J. Janák, *J. Chromatogr*, 156 (1978) 323
124 F M Everaerts, Th. P E. M. Verheggen and F. E. P Mikkers, *J Chromatogr*, 169 (1979) 21
125 *CS Isotachophoretic Analyser*, ÚRVJT, Spišská Nová Ves, Czechoslovakia, 1982
126 G Eriksson, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 145.
127 Th P. E. M Verheggen and F M Everaerts, *J Chromatogr*, 249 (1982) 221
128 F Nishiyama, T. Hirokawa and Y Kiso, *Bull Chem. Soc Jap*, 54 (1981) 933.
129 D Kaniansky, M Koval and S Stankoviansky, *J. Chromatogr*, 267 (1983) 67.
130 E Schumacher, D Arn and W Thormann, *Electrophoresis*, 4 (1983) 390
131 W. Thormann, *Thesis*, University of Berne, 1981.
132 B Gaš, M Demjaněnko and J Vacík, *J. Chromatogr*, 192 (1980) 253
133 J Vacík, J Zuska and I Muselasová, *J Chromatogr*, 320 (1985) 233
134 P I Bresler, I A Ivanova, O. V Oshurkova and G A Shtilerman, *Zh Anal. Khim*, 36 (1981) 593.
135 L Arlinger and H. Lundin, *Protides Biol Fluids Proc Colloq.*, 21 (1973) 667
136 J C Reijenga, A. A G. Lemmens, Th P E M Verheggen and F M Everaerts, *J Chromatogr.*, 320 (1985) 67
137 J Tamchyna, J Zuska and J. Vacík, *J Chromatogr*, 320 (1985) 241
138 J. C. Reijenga, Th P. E M Verheggen and F. M. Everaerts, *J Chromatogr*, 267 (1983) 75
139 P. Havaši and D Kaniansky, presented at the *Fourth International Symposium on Isotachophoresis, Hradec Králové, September 2–6, 1984.*
140 D Hanibalová, J Vacík and V Fidler, *J. Chromatogr*, 320 (1985) 185
141 J C Reijenga, Th P E. M. Verheggen and F M Everaerts, *J Chromatogr.*, 283 (1984) 99.
142 D Kaniansky, P Rajec, A Švec, P Havaši and F Macášek, *J. Chromatogr*, 258 (1983) 238.
143 D. Kaniansky, P Havaši, J. Marák and R. Sokolík, presented at the *Fourth International Symposium on Isotachophoresis, Hradec Králové, September 2–6, 1984*
144 M Deml, J Pospíchal and P Boček, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p. 11.
145 Th P E. M. Verheggen, J C Reijenga and F M Everaerts, *J. Chromatogr*, 260 (1983) 471
146 F. E. P Mikkers, *Thesis*, University of Technology, Eindhoven, 1980
147 H Schwendtner, W. Engelhardt and P O Schwille, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter. Berlin, New York, 1984, p 17
148 F S Stover, K L. Deppermann and W. A Grote, *J. Chromatogr*, 269 (1983) 198
149 *LKB Tachophor 2127*, LKB, Bromma, Sweden, 1983.
150 P Boček, M Deml, J. Štikarovský and J Janák, *Panel Discussion, 2nd Danube Symposium on Progress in Chromatography, Carlsbad, Czechoslovakia, 1979*
151 D. Arn, E Schumacher and W. Thormann, presented at the *Fourth International Symposium on Isotachophoresis, Hradec Králové, September 2–6, 1984*
152 J C Reijenga and D M. J Kroonenberg, in F M Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p. 217
153 J C Reijenga, W van Iersel, G. V. A Aben, Th P E M Verheggen and F M. Everaerts, *J Chromatogr*, 292 (1984) 217.
154 W Thormann, *J Chromatogr*, 334 (1985) 83.
155 A. C Schoots and F M Everaerts, *J Chromatogr*, 277 (1983) 328
156 S. Kobayashi, Y Shiogai and J. Akiyama, in F M Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p 47
157 T Yamada, A Talbot, Y Iijima, Y. Itano and F. Kosaka, *Acta Med. Okayama*, 36 (1982) 399

158 W. Thorn, F Blaeker and E. Weiland, *J Chromatogr.*, 210 (1981) 319
159 D. Kaniansky, V. Zelenská and I Zelenský, *J Chromatogr* , 256 (1983) 126
160 E Kenndler and D Kaniansky, *J Chromatogr.*, 209 (1981) 306.
161 E. Kenndler and E. Haidl, presented at the *Fourth International Symposium on Isotachophoresis, Hradec Králové, September 2–6, 1984*
162 *LKB Tachofrac*, LKB, Bromma, Sweden, 1983
163 H Wagner and V Mang, in F M Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p 41.
164 H Wagner and V Mang, in C. J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 357
165 H Wagner, V Mang, R Kessler and W Speer, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 347
166 *Elphor VaP 21*, Bender und Hobein, Munich, 1984
167 H. Wagner and R Kessler, in D Stathakos (Editor), *Electrophoresis '82*, Walter De Gruyter, Berlin, New York, 1984, p 303
168 H Wagner, V Mang and D Schönenberger, presented at the *Fourth International Symposium on Isotachophoresis, Hradec Králové, September 2–6, 1984.*
169 V Kašička and Z. Prusík, *J Chromatogr.*, 273 (1983) 117
170 Z Prusík and V. Kašička, *J Chromatogr* , 320 (1985) 81
171 *IP 1B, Capillary Type Isotachophoretic Analyzer*, Shimadzu, Kyoto, 1974
172 *IP 2A, Capillary Type Isotachophoretic Analyzer*, Shimadzu, Kyoto, 1979
173 F M Everaerts, Th. P E M. Verheggen, J C Reijenga, G. V A Aben, P Gebauer and P Boček, *J Chromatogr* , 320 (1985) 263
174 W Tschöpe, R Brenner and E. Ritz, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 207
175 V Dolník and P Boček, *J Chromatogr* , 225 (1981) 455.
176 V. Dolník and P Boček, *J Clin. Chem. Clin Biochem.*, 19 (1981) 654
177 D. Walterová, Z Stránský, J Bartek and V Seidlová, *Biochem Clin. Bohemoslov* , 12 (1983) 147
178 W Tschope and E. Ritz, *J Chromatogr* , 221 (1980) 59
179 W Tschope and E Ritz, in F. M Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p. 63
180 K Ogata, K Murakami, S. Tanabe and T Imauari, *Rinsho Kagaku*, 10 (1981) 136.
181 G Bruchelt, H Oberritter and K H. Schmidt, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 229
182 T Kawata, T Inui, A Maekawa and T. Suzuki, *Vitamins*, 54 (1980) 11
183 H. Mikasa, K Sasaki and H Kodama, *J. Chromatogr* , 190 (1980) 501
184 H. Oberritter, G Bruchelt and K. H Schmidt, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p. 237
185 J C. Reijenga, H. J L. A Slaats and F M Everaerts, *J Chromatogr* , 267 (1983) 85
186 W Tschope and E. Ritz, in L H Smith, W G Robertson and B Finlayson (Editors), *Urolithiasis*, Plenum, New York, 1981, p 951.
187 K. H Schmidt, V Hagmaier, D Hornig, J P Vuilluemier and G Rutishauser, in J G Brockis and B Finlayson (Editors), *Urinary Calculus*, PSG, Littleton, 1981, p 395
188 K Schmidt, V. Hagmaier, G Bruchelt and G Rutishauser, in L H. Smith, W. G Robertson and B. Finlayson (Editors), *Urolithiasis*, Plenum, New York,, 1981, p 959
189 K Schmidt, V Hagmaier, G Bruchelt and G Rutishauser, *Urol Res* . 8 (1980) 177
190 N Schwendtner, W Achilles, W Engelhardt, P O Schwille and A Sigel, *J Clin Chem Clin. Biochem.*, 20 (1982) 833
191 F Mikkers, Th. Verheggen, F Everaerts, J Hulsman and C. Meijers, *J Chromatogr* , 182 (1980) 496
192 S Moch, D Schohn and H Jahn, in C. J. Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p. 217
193 P Faguer, N. K Man, D. Pierrat, G Jehenne and J L Funck-Brentano, presented at the *Fourth International Symposium on Isotachophoresis, Hradec Králové, September 2–6, 1984*
194 M. Morio, K. Fujii, R Takiyama, F Chikasue, H Kikuchi and L Ribarić, *Anesthesiology*, 53 (1980) 56.
195 R Takiyama, *Masui To Sosei*, 19 (1983) 141
196 L. Křivánková, E Samcová and P Boček, *Electrophoresis*, 5 (1984) 226

197 L. Křivánková, P Boček and E. Samcová, *Prac Lek* , 36 (1984) 163
198 S Øvrebø, D Jacobsen and O M Sejersted, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 261
199 S Øvrebø, D Jacobsen and O M. Sejersted, presented at the *Fourth International Symposium on Isotachophoresis, Hradec Králové, September 2–6, 1984.*
200 T. Ageta, H Mikasa, K Kojima and H Kodama, *J. Chromatogr* , 233 (1982) 361
201 H. Kodama, N. Mizoguchi, K Sasaki and H. Mikasa, *Anal. Biochem* , 133 (1983) 100
202 H Kodama, H Mikasa and T Ageta, in H Hirai (Editor), *Electrophoresis '83*, Walter De Gruyter, Berlin, New York, 1984, p 585.
203 C Dragsholt and K B Yderstraede, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p. 253
204 H. Kodama, M Yamamoto and K Sasaki, *J Chromatogr.*, 183 (1980) 226.
205 H. Mikasa and H Kodama, *Ganryu Aminosan*, 2 (1979) 329.
206 H. Mikasa, T. Ageta, N Mizoguchi and H Kodama, *J Chromatogr* , 202 (1980) 504
207 J Gróf and J Menyhárt, in F. M Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p 99
208 J Gróf, J Menyhárt, Z. Ribai and M. Idei, *3rd International Symposium on Isotachophoresis, Goslar, 1982, Symposium Abstracts*
209 A C. Schoots, F E. P Mikkers, H A Classens, R de Smet, N van Landschoot and S. M. G. Ringoir, *Clin Chem* , 28 (1982) 45
210 R de Smet, N van Landschoot, G van der Stiggel and S. Ringoir, *Int J. Artif Org* , 6 (1983) 67
211 J Menyhárt and J Gróf, *Acta Chir. Acad. Sci Hung* , 22 (1981) 47
212 J Gróf, J Menyhárt, I. Szikla, A Pajor and K Vallent, presented at the *Fourth International Symposium on Isotachophoresis, Hradec Králové, September 2–6, 1984.*
213 H Mikasa, T Ageta and H. Kodama, *Ganryu Aminosan*, 4 (1981) 267
214 H Mikasa, T Ageta, N Mizoguchi and H Kodama, *Anal Biochem.*, 126 (1982) 52
215 H Mikasa, K Sasaki, H Kodama, J Irata and M Ikeda, *J. Chromatogr* , 305 (1984) 204
216 O. Driesen and H Beukers, in F M Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p. 69
217 O. Driesen, H Beukers, L Belfroid and A Emonds, *J Chromatogr.*, 181 (1980) 441
218 Th. Verheggen, F Mikkers, F Everaerts, F Oerlemans and C de Bruyn, *J Chromatogr* , 182 (1980) 317
219 F Oerlemans, C de Bruyn, F Mikkers, Th. Verheggen and F. Everaerts, *J Chromatogr.*, 225 (1981) 369.
220 C. de Bruyn, F. Oerlemans, F Mikkers, Th Verheggen and F Everaerts, in F M Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p 55
221 F Oerlemans, C de Bruyn, F Mikkers, Th Verheggen and F Everaerts, in F M Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p 189
222 F Oerlemans, Th Verheggen, F Mikkers, F Everaerts and C de Bruyn, in A Adam and C Schots (Editors), *Biochemical and Biological Applications of Isotachophoresis*, Elsevier, Amsterdam, 1981, p. 63
223 F Oerlemans, R de Abren and C de Bruyn, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 245
224 B Gustavsson, R -M Olsson and J Waldenström, *Anal Biochem* , 122 (1982) 1
225 G. Eriksson, in C. J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 145
226 G Eriksson and U Stenram, in C. J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 135.
227 J C Reijenga, A Gaykema and F E P Mikkers, *J Chromatogr.*, 287 (1984) 365
228 H Akedo and K Shinkai, *J Chromatogr* , 227 (1982) 262.
229 Z. Stránský, Z Chmela, P. Peč and L Šafařík, *J Chromatogr.*, 342 (1985) 167
230 J C. Reijenga, G V. A. Aben, A. A. G. Lemmens, Th P E M Verheggen, C H M. M de Bruyn and F M Everaerts, *J Chromatogr* , 320 (1985) 245
231 A. Tsutsumi, H Kodama and H Ishiru, *Igaku No Ayumi*, 129 (1984) 15
232 F Oerlemans, C van Bennekom, C de Bruyn and S Kulakowski, *J Inher Metab. Dis* , 4 (1981) 109.
233 A Talbot, *Acta Med Okayama*, 36 (1982) 407
234 A Talbot, *Acta Med Okayama*, 36 (1982) 431

235 M Aomine, M Arita, S Imanishi and T Kiyosue, *Jap J. Physiol.*, 32 (1982) 741
236 J. A Pérez, F Mateo and E. Meléndez-Hevia, *Electrophoresis*, 3 (1982) 102
237 G Eriksson, *Anal. Biochem.*, 109 (1980) 239.
238 G Eriksson and D. Stråth, *FEBS Lett.*, 124 (1981) 39
239 P I Christensson and G. Eriksson, presented at the *Fourth International Symposium on Isotachophoresis, Hradec Králové, September 2–6, 1984*
240 G Eriksson, A Malmstrom, B. Sárnstrand, G Jonsson and R Pero, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 153
241 C A. van Bennekom, F. T Oerlemans, M Verburg and C. de Bruyn, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 165.
242 M. Aomine and M. Arita, *Gen Pharmacol*., 15 (1984) 145
243 J Punkt and R. Hacker, *J Chromatogr*, 320 (1985) 105.
244 J Gróf, I. Sziklai and J Menyhárt, *Kiserl Orvostud*, 34 (1982) 22
245 Z. Li, X. Ding and K Xu, *Shengwu Huaxue Yu Shengwu Wuli Jinzhan*, 58 (1984) 72
246 B. Gustavsson, O. Almersjo, M. Berne and J. Waldenstrom, *J Chromatogr.*, 276 (1983) 395
247 J. Lilliemark, A Baldesten and C Peterson, *Curr. Chemother Immunother*, 2 (1982) 1312
248 K G. Kjellin and L. Hallander, in A. Adam and C. Schots (Editors), *Biochemical and Biological Applications of Isotachophoresis*, Elsevier, Amsterdam, 1980, p 239.
249 L. Hallander and K G Kjellin, in A. Adam and C Schots (Editors), *Biochemical and Biological Applications of Isotachophoresis*, Elsevier, Amsterdam, 1980, p. 245
250 L Hallander and K G. Kjellin, in F M Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p. 129
251 W W. Tourtellotte, A. R Potvin, B I Ma, R W. Baumhefner, M. J Walsh, P Dickstein, T Ingram, T. Cowan, P Shapshak and P. Delmotte, *Neurology*, 32 (1982) 261
252 W. W Tourtellotte, R W Baumhefner, P Dickstein. G Cowhig, P Delmotte and A. R. Potvin, *Neurology*, 30 (1980) 398
253 D. Caputo, *J Neurol*, 229 (1983) 55.
254 W W Tourtellotte, T. J Ingram, P Shapshak, S M Staugaitis and H O Reiber, in *Experimental Allergic Encephalomyelitis A Useful Model for Multiple Sclerosis*, Alan R Liss, New York, 1984, p 379.
255 C. J Holloway, W Heil and E. Henkel, in R C Allen and P. Arnaud (Editors), *Electrophoresis '81*, Walter De Gruyter, Berlin, New York, 1981, p 753
256 H. E M Smuts, B. W. Russell and J W Moodie, *J Neurol. Sci*, 56 (1982) 283.
257 D Del Principe, G. Biancini, C Colistra, A Menichelli, C D'Arcangelo and G Mancuto, presented at the *Fourth International Symposium on Isotachophoresis, Hradec Králové, September 2–6, 1984*
258 K G. Kjellin and L Hallander, in F M Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p. 121
259 K G Kjellin and L B. Hallander, *J Biochem Biophys. Methods*, 7 (1982) 47
260 T Yagi, K Kojima, M. Yagi and Y. Kajita, in H Hirai (Editor), *Electrophoresis '83*, Walter De Gruyter, Berlin, New York, 1984, p 503
261 C J. Holloway, R V Battersby, B. Büssenschutt, G Bulge and J Lüstorff, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 273
262 T Hine, in H Hirai (Editor), *Electrophoresis '83*, Walter De Gruyter, Berlin, New York, 1984, p 541.
263 E. Kojima, T Manabe and T Okuyama, in H Hirai (Editor), *Electrophoresis '83*, Walter De Gruyter, Berlin, New York, 1984, p 511.
264 G Schmitz, U Borgmann and G Assmann, *J Chromatogr.*, 320 (1985) 253
265 M Bojanovski and C J Holloway, in R C Allen and P Arnaud (Editors), *Electrophoresis '81*, Walter De Gruyter, Berlin, New York, 1981, p 809
266 M. Bojanovski and C J Holloway, *J Clin Chem Clin Biochem.*, 19 (1981) 620
267 D Del Principe, G Mancuso, C D'Arcangelo, A Menichelli, P M Strappini and S. Del Vecchio, *Thromb. Res.*, 20 (1980) 137
268 D Del Principe, G Mancuso, G Biancini, C D'Arcangelo, A Menichelli, M Persiani, P M Strappini and E. V. Cosmi, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 297
269 R Jarolke and M Siegert, in C J. Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984. p 325

270 K. G. Kjellin and L. B Hallander, in C J. Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 337.
271 E Borriss and S Husmann-Holloway, in C. J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 305
272 J. Bours, P Delmotte and G J. Binkhorst, in A. Adam and C. Schots (Editors), *Biochemical and Biological Applications of Isotachophoresis*, Elsevier, Amsterdam, 1980, p. 89.
273 J Bours, H -D Zauzig and H Rink, in A Adam and C Schots (Editors), *Biochemical and Biological Applications of Isotachophoresis*, Elsevier, Amsterdam, 1980, p 207
274 J Bours and P Delmotte, in A Adam and C. Schots (Editors), *Biochemical and Biological Applications of Isotachophoresis*, Elsevier, Amsterdam, 1980, p 221
275 V Dolník, P Boček, L Šístková and J Korbl, *J. Chromatogr*, 246 (1982) 343.
276 V. Dolník, P Boček, L Šístková and J Korbl, *J. Chromatogr*, 246 (1982) 340
277 T Tatsuhara, F Tabuchi, I Nishimura, H. Muro and F Ozoe, *Chem. Pharm Bull*, 30 (1982) 1347
278 P. Stehle, P Kühne, P Pfaender and P Furst, *J. Chromatogr*, 249 (1982) 408
279 P. Stehle, P Pfaender and P Fürst, *J Chromatogr*, 294 (1984) 507.
280 P. Stehle, B Kühne, P Pfaender and P Fürst, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 73
281 R. Röben and K Rubach, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p. 109
282 A Baldesten, in D Brandenburg and A Wollmer (Editors), *Insulin, Chemistry, Structure and Function of Insulin and Related Hormones*, Walter De Gruyter, Berlin, New York, 1980, p 207
283 K. Kojima and T Yagi, *Shoyakugaku Zasshi*, 36 (1982) 280
284 T Tatsuhara, *Yakugaku Zasshi*, 102 (1982) 988
285 J Lang and B Buchele, in F M Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p 75
286 H Klein and R Teichmann, *J. Chromatogr*, 250 (1982) 152
287 H. Klein, *Drug Res*, 32 (1982) 795.
288 H Klein and R Teichmann, *Pharm Z*, 127 (1982) 447
289 R Jannasch, *Pharmazie*, 38 (1983) 379
290 R H Jannasch, *3rd International Symposium on Isotachophoresis, Goslar, 1982, Symposium Abstracts*
291 P Boček, in F M Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p 203
292 A Barcuchová and V Jokl, *Cesk. Farm*, 29 (1980) 226
293 A Barcuchová and V Jokl, *Cesk. Farm*, 32 (1983) 12.
294 K Rubach, Ch Breyer and E Kirchhoff, *Z Lebensm.-Unters.-Forsch*, 67 (1982) 31
295 D Eichler and K Rubach, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 117
296 K Rubach and Ch Breyer, *Getreide Mehl Brot*, 35 (1981) 91
297 S Donhauser, K Glas and B Gruber, *Monatschr Brauwiss.*, 37 (1984) 252
298 C Weiss and K Rubach, in C J. Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 125
299 K Matsushima, Y. Oshima, M Yamamoto and K Sugisawa, *Nippon Shokuhin Kogyo Gakkai-Shi*, 29 (1982) 631
300 H Klein, *Fleischwirtschaft*, 61 (1981) 1
301 S Chauvet and M N Desormeaux, *Ann Falsif Expert Chim Toxicol*, 76 (1983) 23
302 K Rubach and P Offizorz, *Deut Lebensm -Rundsch*, 79 (1983) 88
303 J C. Reijenga, Th P. E M Verheggen and F. M. Everaerts, *J Chromatogr*, 245 (1982) 120
304 J. Farkaš and M Koval', *Kvasny Prum.*, 28 (1982) 256
305 K Pruša and O. Smejkal, *Kvasny Prum*, 29 (1983) 7
306 J Farkaš and M Koval', *Vinohrad*, 20 (1982) 160.
307 J Farkaš and M Koval', *Vinohrad*, 20 (1982) 186
308 S Chauvet and P Sudrana, *Analusis*, 11 (1983) 243
309 H Klein and H. G. Stettler, *Wein-Wiss*, 39 (1984) 51
310 P E Shaw, B S Busling and C. W. Wilson, *J Agr Food Chem*, 31 (1983) 182
311 H Fukuba and T Tsuda, *Eiyo To Shokuryo*, 33 (1980) 147.
312 K. Rubach and Ch Breyer, *Deut Lebensm -Rundsch*, 76 (1980) 228.
313 E. Goto, A Maokawa and T Suzuki, *Eiyo To Shokuryo*, 33 (1980) 225
314 H Klein, *Ind Obst -Gemuseverwert*, 67 (1982) 31

315 A Scholze and H G Maier, *Lebensmittelchem Gerichtl Chem*, 36 (1982) 111.
316 K Shimada, M Ohtusuru, T. Yamaguchi and K Nigota, *J Food Sci*, 48 (1983) 665
317 K Rubach, P Offizorz and Ch. Breyer, *Z Lebensm.-Unters -Forsch*, 172 (1981) 351
318 P. Offizorz and K Rubach, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 103
319 Y Ito, M Toyoda, H Suzuki and M Iwaida, *J Ass. Offic Anal. Chem.*, 63 (1980) 1299
320 H. Fukuda and T Tsuda, *Kaswigaku Zasshi*. 30 (1979) 410
321 H Klein, *Z Acker-Pflanzenbau*, 150 (1981) 349
322 H Kayanara, H Yasuhira and T Mochizuki, *Miso Kenkyusho Kenkyu Hokoku*, 21 (1980) 34
323 K Kojima, T Yagi and T. Okuda, *Shoyakugaku Zasshi*, 36 (1982) 280.
324 Y. Shiogai, T Yagi and J. Akiyama, *Bunseki Kagaku (Jap. Anal)*, 26 (1977) 701
325 T Yamaguchi and M. Fukuda, *Mukogawa Joshi Daigaku Kiyo Shokumotsu-Hen*, 27 (1980) 15.
326 D. Kaniansky, V Madajová, M Hutta and I. Žilková, *J Chromatogr*, 286 (1984) 395.
327 Z. Stránský, *J Chromatogr.*, 320 (1985) 219.
328 P. A Pfeifer, G K Bonn and O. Bobleter, *Z Anal Chem.*, 315 (1983) 205
329 L Hansén, J. Sollenberg and K. Wiberg, *J Chromatogr.*, 312 (1984) 489.
330 L Hansen, B. Kristiansson and J Sollenberg, in C. J. Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p. 81.
331 I. Zelenský, V. Zelenská, D Kaniansky, P Havaši and V Lednárová, *J Chromatogr*, 294 (1984) 317.
332 S. Matsui, R Yamamoto and S Ide, *Mizu Shori Gijutsu*, 21 (1980) 993
333 J Vacík and I Muselasová, *J Chromatogr*, 320 (1985) 199
334 I Motooka, H Nariai, K. Nakazaki and M. Tsuhako, *J Chromatogr.*, 295 (1984) 533
335 T Yagi, K Kojima, H Nariai and I Motooka, *Bull Chem. Soc. Jap*, 55 (1982) 1831.
336 H. Naraiai, K Nakazaki, M. Tsuhako and I. Motooka, *J Chromatogr.*, 248 (1982) 135
337 F. Mikkers, W. van Gils and W. Roos, in F M Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p 91.
338 D Lúčanský, V Bátora, J Polonský and J Garaj, in F M. Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p 147.
339 J Polonský, D Lúčanský and L' Repášová, in F. M Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p 195
340 I Motooka, H Nariai, K Nakazaki and M Tsuhako, *J Chromatogr*, 260 (1983) 377.
341 E A Fitzgerald, *J. Chromatogr Sci.*, 21 (1983) 188
342 P Boček, V Dolník, M Deml and J Janák, *J. Chromatogr*, 195 (1980) 303
343 P. Boček, V Dolník, M. Deml and J. Janák, *Chem Prum*, 30 (1980) 642.
344 Y Shiogai and T Yagi, in C. J. Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 95.
345 V Madajová, D Kaniansky, E Čižmárová and M. Hudec, *J. Chromatogr*, 320 (1985) 131
346 J. Polonský, L' Repášová, D. Šmogrovičová and V Táborský, *Ropa Uhlie*, 25 (1983) 599.
347 V Madajová, D Kaniansky, Z Radej and A Eszenyiova, *Ropa Uhlie*, 23 (1981) 481.
348 V Madajová, D Kaniansky, Z. Raděj and A Eszéniova, *J Chromatogr*, 216 (1981) 313.
349 Š Bálint and M. Jurčová, presented at the *Fourth International Symposium on Isotachophoresis, Hradec Králové, September 2–6, 1984*
350 L'. Repášová, J. Polonský, M Košík and Š Vodný, *J Chromatogr.*, 286 (1984) 347.
351 L' Repášová and J Polonský, *Ropa Uhlie*, 26 (1984) 341
352 J. Polonský, L' Repášová, A Pekarovičová and M Košík, *J. Chromatogr*, 320 (1985) 111.
353 Y Okatani, Y Sagara and Y. Takeda. *Sanfujinka No Sekai*, 33 (1981) 934
354 D S. Ryder, D R. Woods, M Castian and C A Masschelein, *J Amer Soc. Brew Chem.*, 41 (1983) 125.
355 G. K. Bonn, P A Pfeifer, H Hormeyer and O Bobleter, *Z Anal. Chem*, 318 (1984) 30.
356 P Pfeifer, G. Bonn and O Bobleter, in C J Hollowy (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 89
357 T Ohira, T Kikuchi, T Kuwabara and S. Kaneko, *Niigata-Ken Kogyo Gijutsu Senta Kenkyu Hokokusho*, (1982) 75
358 S Murai, T. Ozawa and Y Maki, *Bunseki Kagaku (Jap Anal.)*, 33 (1984) 229.
359 S. Yamamoto, D Ohta and Y Morikawa, *Bunseki Kagaku (Jap. Anal)*, 31 (1982) 251
360 H Mizuguchi, M. Hagi, K Ohara and Y Kawase, *J SCCJ*, 16 (1983) 144

361 V A. Luginin and L A. Rzhevina, *Probl Sovrem Anal Khim.*, (1981) 207
362 S Onodera, T. Udagawa, M Tabata, S. Ishikura and S. Suzuki, *J. Chromatogr* , 287 (1984) 176
363 T. Yagi, K Kojima and T. Haruki, *J. Chromatogr* , 292 (1984) 273
364 I Zelenský, E Šimuničová, V. Zelenská and D Kaniansky, presented at the *Fourth International Symposium on Isotachophoresis, Hradec Králové, September 2–6, 1984.*
365 L. Křivánková and P Boček, *Electrophoresis*, 6 (1985) 143
366 K Fukushi, G. Kondoh, K Hiiro, T. Tanaka, A Kawahara and S. Wakida, *Bunseki Kagaku (Jap. Anal.)*, 32 (1983) 362.
367 J M Lavoie and P S Back, *J. Chromatogr.*, 264 (1983) 329
368 M Koval', D Kaniansky, Ľ Mátel and J Macášek, *J Chromatogr.*, 243 (1982) 144
369 H Mikasa, J Arata and H. Kodama, *J Chromatogr* , 310 (1984) 401.
370 E. Anhalt and C J Holloway, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 197.
371 C J Holloway, S. Husmann-Holloway and G Brunner, in F M Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p 25
372 G Bruchelt and K Schmidt, in F M Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p 137.
373 F. Teggmeier and C J. Holloway, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p. 175
374 S Husmann-Holloway, E. Anhalt, J Lüstorff and C. J Holloway, in R C. Allen and P. Arnaud (Editors), *Electrophoresis '81*, Walter De Gruyter, Berlin, New York, 1981, p. 781
375 R. V. Battersby and C J. Holloway, *Z Anal Chem* , 317 (1984) 748
376 J Lüstorff and C. J Holloway, in F M Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p 179
377 R Einarsson, R. Karlsson and E Åkerblom, *J Chromatogr.*, 284 (1984) 143
378 C J. Holloway and G Bulge, *J. Chromatogr* , 234 (1982) 454
379 S Linpisarn, P M S Clark, L. J Kricka and T P Whitehead, in R C Allen and P. Arnaud (Editors), *Electrophoresis '81*, Walter De Gruyter, Berlin, New York, 1981, p 767
380 K Yasakawa, K. Kojima, T Manabe and T. Okuyama, in H. Hirai (Editor), *Electrophoresis '83*, Walter De Gruyter, Berlin, New York, 1984, p 119
381 K Friede and C J Holloway, *Electrophoresis*, 2 (1981) 116
382 C. J Holloway and K. Friedel, in R C Allen and P Arnaud (Editors), *Electrophoresis '81*, Walter De Gruyter, Berlin, New York, 1981, p 821
383 K. Lovgren, L Jun-Fang, A Baldesten and B Morein, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 291
384 P M S. Clark, T P Whitehead and L J Kricka, in F M. Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p. 109
385 A M Yegorov, A P Osipov, M. M Dikov and A Y Karulin, in F M Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p 167
386 M M Dikov, A. Y Karulin, A P Osipov and A M Yegorov, *Bioorg Khim.*, 5 (1979) 1217
387 A. Baldesten and B Morein, in F M. Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p. 115
388 J. L Pipkin, W G Hinson, J F Anson and J L. Hudson, *Cell Biol Int. Rep* , 6 (1982) 205
389 J. W van Nispen, P. S L. Janssen, B C. Goverde and H M Greven, in K Brunfeldt (ERditor), *Peptides 1980*, Scriptor, Copenhagen, 1981, p 731
390 J W van Nispen, P S. L Janssen, B C Goverde, J C. M. Scherders, F van Dinther and J A J Hannink, *Int J Peptide Protein Res* , 17 (1981) 256
391 P S L Janssen and J W van Nispen, *J Chromatogr* , 287 (1984) 166
392 C J. Holloway and R V Battersby, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 193
393 H Fukuba and Y. Tsuda, *Nippon Eiyo Shokuryo Gakkaishi*, 36 (1983) 373.
394 C J Holloway, B Bussenschutt and V Pingoud, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p. 187
395 H Hulsheger, S Husmann-Holloway, E Borriss and J Potel, in C J. Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p. 157
396 E Anhalt and C J Holloway, in F M Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p. 159

397 J Lustorff and C J Holloway, in R. C. Allen and P Arnaud (Editors), *Electrophoresis '81*, Walter De Gruyter, Berlin, New York, 1981, p 797
398 E M. Gavrilova, M. M Dikov, A. P. Osipov, N I. Kiseleva and T G Mitrochina, in F. M Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p 173
399 C G Kent, A T Tu and C R. Geren, *Comp Biochem Physiol*, 77B (1984) 303
400 A T Tu, J Stermitz, H Ishizaki and S Nonaka, *Comp Biochem Physiol.*, 66B (1980) 249
401 R. Einarsson and U Moberg, *J Chromatogr*, 209 (1981) 121
402 R. Karlsson and R Einarsson, *Anal Lett.*, 15 (1982) 909
403 D Walterová, V Preininger and V Šimánek, *Planta Med*, (1984) 117
404 R Einarsson and R Karlsson, *Int Arch Allergy Appl Immunol*, 68 (1982) 222.
405 C H M. M de Bruijn, J C Reijenga, G V A Aben, Th P E M Verheggen and F. M Everaerts, *J Chromatogr*, 320 (1985) 205.
406 H Goto and Y Muto, *Yakugaku Zasshi*, 101 (1981) 185
407 K. Kirimura, Y Wadagaki and N Sakai, *Shigaku*, 70 (1982) 1
408 K. Kirimura, Y Wadagaki and Y Misturo, *Jap J Oral Biol.*, 23 (1981) 896
409 C A M. van Dongen, F E P. Mikkers, Ch de Bruyn and Th P E M. Verheggen, in F. M Everaerts (Editor), *Analytical Isotachophoresis*, Elsevier, Amsterdam, 1981, p 207
410 D Lúčanský, E Kománová, A. Pukáčová and E Kardoš, presented at the *Fourth International Symposium on Isotachophoresis, Hradec Králové, September 2–6, 1984*
411 N R Curvetto and G Orioli, *Plant Soil*, 66 (1982) 205.
412 H Tetsumi, Ch. Matsumoto and M. Sumi, *Nippon Kagaku Kaishi*, (1984) 780
413 S. Yamamoto, T Ohta, Y Morikawa and M. Yokouchi, *Bunseki Kagaku (Jap Anal)*, 33 (1984) 58.
414 S Yamamoto, T Ohta and Y. Morikawa, *Bunseki Kagaku (Jap. Anal)*, 31 (1982) 557
415 Z. Stránský, V Šimánek and D Walterová, *Acta Univ Palacki Olomuc., Fac Med*, 104 (1983) 83
416 S Nambaru and T. Yamaha, *Eisei Shikensho Hokoku*, 100 (1982) 77
417 R Fukae, Y Shioi and T Sasa, *Anal. Biochem*, 133 (1983) 190
418 H Mitsumata and H Oguro, *Bunseki Kagaku (Jap Anal.)*, 31 (1982) 646
419 L P. Samaranayake, D A Weetman, D. A M. Geddes and T. W MacFarlane, *Microbios*, 35 (1982) 91
420 E Weiland, W Thorn and F. Bläker, *J. Chromatogr.*, 214 (1981) 156
421 M. Koval', *Thesis*, Komenský University, Bratislava, 1981
422 M Tazaki, M Takagi and K. Ueno, *Chem Lett*. (1982) 639.
423 I Nukatsuka, M Taga and H Yoshida, *Bull. Chem Soc Jap*, 54 (1981) 2629
424 I Nukatsuka, M Taga and H Yoshida, *J Chromatogr*, 205 (1981) 95
425 M Drábik, *Thesis*, Komenský University, Bratislava, 1983.
426 E Blasius, K Muller and H. Wagner, *Z. Anal Chem.*, 311 (1982) 192
427 E Blasius, J. P Glatz and W Neumann. *Radiochim Acta*, 29 (1981) 159
428 E. Blasius, K. Muller, W Neumann and H Wagner, *Z Anal Chem*, 315 (1983) 448.
429 H. Yoshida and Y Hirama, *J. Chromatogr*, 298 (1984) 243.
430 M. Koval', D Kaniansky, M. Hutta and R. Lacko, presented at the *Fourth International Symposium on Isotachophoresis, Hradec Králové, September 2–6, 1984*
431 I Nukatsuka and H Yoshida, *J Chromatogr*, 237 (1982) 506
432 V Dolnik, M. Deml and P Boček, in C J Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter De Gruyter, Berlin, New York, 1984, p 55
433 T Yamamoto and T. Yamakawa, *Bunseki Kagaku (Jap. Anal.)*, 30 (1981) T 93
434 A Biber and K. Hempel, *J Biochem Biophys. Methods*, 5 (1981) 237
435 O. V Oshurkova and Yu S. Parilov, *Ispol'z Metod Termobarogeokhim Poiskakh Izuch. Rud Mestorozhd*, (1982) 115
436 G. Azzar, A. Degiuli, J P. Benedetto and R Got, *J Chromatogr.*, 213 (1981) 177
437 H Huebers, E. Csiba, B Josephson, E Huebers and C Finch, *Proc. Nat Acad. Sci U S*, 78 (1981) 621
438 A M. C. Davies, *Anal Proc.*, 21 (1984) 64.
439 F S Stover, *J. Chromatogr*, 320 (1985) 45.
440 V Kašička and Z Prusík, *J. Chromatogr*, 320 (1985) 75.
441 Th P E. M Verheggen, F. M. Everaerts and J C Reijenga, *J. Chromatogr*, 320 (1985) 99
442 J. L Beckers, *J Chromatogr.*, 320 (1985) 147
443 Z Fidler, V. Fidler and J Vacík. *J Chromatogr*, 320 (1985) 175.
444 A. A G Lemmens, J. C. Reijenga, F M. Everaerts, R T P. Janssen, J A. R J. Hulsman and C A M. Meijers, *J Chromatogr.*, 320 (1985) 193.