Journal of Chromatography, 165 (1979) 87–101
Chromatographic Reviews
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHREV. 117BB4

CAPILLARY ISOTACHOPHORESIS

P. DELMOTTE
National Centre for Multiple Sclerosis, 16 Van Heylenstraat, Melsbroek (Belgium)

CONTENTS

1. Introduction 87
2. Apparatus 89
3. Choice of operating conditions 91
4. Some practical aspects 92
5. Practical applications 93
 5.1. Organic acids 94
 5.2. Nucleic acids 95
 5.3. Antibiotics 96
 5.4. Amino acids 96
 5.5. Peptides 98
 5.6. Proteins 98
6. Conclusions 100
7. Summary 100
References 101

1. INTRODUCTION

It is well known that the basic principles underlying many modern analytical techniques were enunciated several decades before their practical applications. In most instances, this was mainly due to the unavailability of materials and equipment necessary for the practical realization of the basic ideas.

This is especially true of electrophoretic techniques. The advent of different kinds of plastic materials, the development of new components and ideas in electronics and the synthesis of new chemicals contributed to bridging the gap between basic principles and daily use. Isotachophoresis is no exception. Although theoretical studies were first published over 50 years ago[1,2] and some use of the principle was made in disc electrophoresis[3], the practical application of isotachophoresis is still in its infancy.

Isotachophoresis is no replacement for the other electrophoretic techniques and its possibilities are not restricted to the biochemical field. In many instances, where rapid and precise analyses of samples with fairly uniform composition must be made, isotachophoresis is becoming the method of choice. Provided that the compounds of interest can be made to move in an electric field, isotachophoresis, with its wide range of operating conditions and detection possibilities, should at least be considered.

Zone electrophoresis, in one or more of its variations depending on the support materials, size and combinations with other techniques, is familiar to most workers.

Isoelectric focusing, although introduced in practice more than a decade ago, is

much less well known. Even if the possibilities of isoelectric focusing are more restricted than those of zone electrophoresis, its superiority in some fields has been proved. The most characteristic feature of this technique is that a true steady-state equilibrium can and must be reached. The method has two outstanding advantages: a very high reproducibility and a useful concentrating effect. Its practical application is restricted almost exclusively to the field of protein chemistry.

Isotachophoresis is the latest of the electrophoretic techniques. The discussion of its theoretical aspects is beyond the scope of this paper and interested readers are referred to a complete treatise on the subject which appeared in 1976 [4].

In zone electrophoresis, the charged molecules constituting the sample are separated under the influence of an electric field into more or less well separated zones in a uniform supporting electrolyte. Each zone moves with a characteristic speed, depending mainly on the charge of the molecules, the ease with which they move through the supporting material and the electric field strength. Each zone is overtaken and diluted continuously by the electrolyte ions, which leads to a continuous broadening of the zones and hence a decrease in the sensitivity.

Isotachophoresis, on the other hand, makes use of a discontinuous system: two different electrolyte solutions are used. The first electrolyte contains an ion (the leading ion) with the same charge sign as that of the sample ions to be separated, but with an effective electrophoretic mobility higher than that of the fastest moving of the sample ions. The second solution contains the terminating ion. It too has the same charge sign, but an effective electrophoretic mobility lower than that of the slowest moving of the sample ions to be separated; a common counter ion, chosen for its buffering capacity at the desired separation pH, is used for both electrolyte solutions.

The mixture of sample ions is brought between the two electrolyte solutions so that the conditions are such that all of the different sample ions always remain sandwiched between the leading and terminating ions. At the same time as the leading and terminating ions move under the influence of the electric field, the sample ions arrange themselves in order of their electrophoretic mobilities. Fig. 1 gives a schematic representation of the different stages of an isotachophoretic separation experiment.

In isoelectric focusing, once the equilibrium has been reached, the different sample components stop and are focused at well defined positions of the stationary pH gradient.

In isotachophoresis, on the other hand, the equilibrium is a dynamic one: the leading and terminating ions move with a constant velocity and the sample ions, also at the same speed, move in an equilibrated manner between them; hence the derivation of the name of this technique (= moving with the same speed).

Several remarks concerning this dynamic equilibrium must be made:

(a) If the different sample ions have sufficiently spaced effective electrophoretic mobilities, then each component will form a separate zone. The different zones follow each other without interruption.

(b) The classification of the different zones is based only on the electrophoretic mobility.

(c) From the Kohlrausch equation[2], it follows that the concentration of the leading ion and the effective mobility of a sample ion are the only factors that determine the concentration of an ion in its zone. It can thus be concluded that for a given set of experimental conditions, the zone length is a direct measure of the amount of a

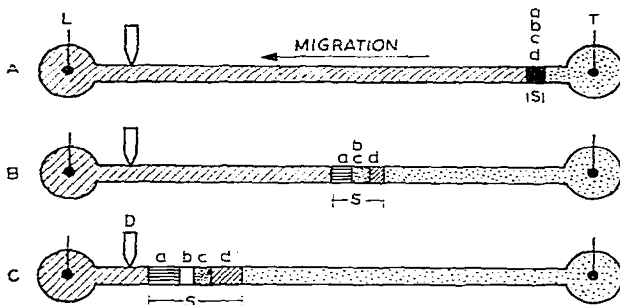

Fig. 1. Different stages of an isotachophoretic separation experiment. L = Leading electrolyte; T = terminating electrolyte; D = detector; S = sample with four components. A, Start condition; B, mid-way, with partial separation of sample ions; C, just prior to detection, all sample ions are separated.

component present in the system. Another important consequence of these properties is the concentrating effect of isotachophoresis. Indeed, if a component is present at very low concentration then, at equilibrium, this component will be concentrated in a very narrow zone. Detection of trace amounts of some components in complex mixtures can often be achieved.

(d) The potential gradient in a given zone is dependent on the mobility of the ion in that zone. The voltage drop per unit length of a zone remains constant over the whole length of a zone. As the current is kept constant, this implies that the heat generated in the different zones can give useful qualitative information about the component in a given zone.

The most important requirements for analytical isotachophoresis can be formulated as follows:

(1) Isotachophoretic separation experiments must be carried out under strictly controlled conditions.

(2) The reliability and accuracy that can be obtained in isotachophoresis are a function of the general operating conditions.

(3) The chemicals used to prepare the electrolyte solutions must be of analytical-reagent grade and be further purified by recrystallization, distillation or other procedures.

(4) The detection systems should be completely without drift and noise.

(5) All of the operating parameters (voltage, current, temperature, electrolyte system and pH) should be recorded precisely.

(6) The duration of an isotachophoretic run depends on the operating conditions, the electrolyte solutions and the sample composition. An average analysis time of between 10 and 20 min is typical.

2. APPARATUS

As isotachophoresis in a capillary tube is by far the most interesting analytical application of this technique, only this system will be considered in detail here.

In Fig. 2, the basic equipment needed for an isotachophoretic experiment is presented. The following components can be considered:

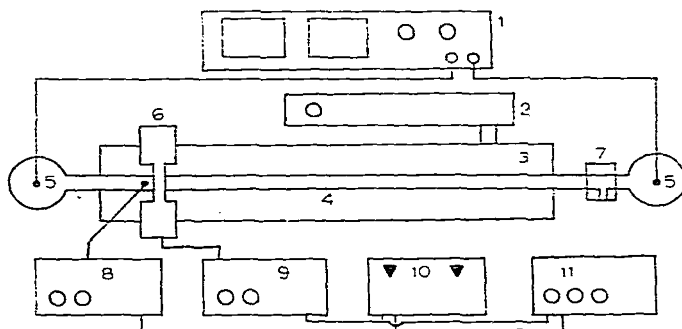

Fig. 2. Equipment for capillary isotachophoresis. 1 = Power supply; 2 = thermostat; 3 = thermostated environment for capillary; 4 = capillary; 5 = electrolyte vessels; 6 = UV detector assembly; 7 = injection port; 8 = thermometric signal amplifier; 9 = UV signal amplifier; 10 = two-pen recorder; 11 = electronic peak surface integrator.

(i) The actual separation takes place in a glass or PTFE capillary tube of less than 0.5 mm I.D.

(ii) The temperature of the capillary is kept constant either by winding it around a metallic cylinder or by immersing it in a liquid. The temperature of the cylinder or liquid must be carefully controlled.

(iii) On each side, the capillary is connected to an electrolyte vessel provided with a platinum electrode.

(iv) On one side of the capillary, provision must be made to allow for sample introduction. This can be done through a septum, as in gas chromatography, or by a multi-port valve system.

(v) A high-voltage power supply capable of delivering 500 μA at up to 20–30 kV is needed. The constant current regulation of the power supply must be extremely well designed. The electrical current through the capillary is very important as it determines not only the time of analysis, but also the temperature and the zone boundary sharpness. Further, as the zone length gives quantitative information about the amount of the components present in the sample, it is extremely important that the actual speed with which all ions are forced through the capillary remains absolutely constant.

(vi) One or more detectors must be provided. The most popular detection systems are thermometric, potentiometric and UV absorption.

We shall not consider the practical details of all of the components here, but the detectors should be considered more closely.

By far the most important detail that distinguishes capillary isotachophoresis from most other electrophoretic techniques is that detection of the separated components forms an integral part of the analytical unit. In zone electrophoretic and isofocusing experiments, detection of the separated components is carried out after the actual separation and often takes more time and effort than the separation stage itself. In isotachophoresis, the results of the separation experiment are immediately available and in most instances not only qualitative but also quantitative information can be obtained.

Temperature measurement on the outside of the capillary tube is the most

universal detection system. As already explained, under a given set of experimental conditions, the heat produced in a zone is dependent on the effective mobility of the ion in that zone. By expressing the heat produced in a certain zone as a percentage of the difference in heat produced in the leading and terminating electrolytes, qualitative information about the ion in a zone is obtained. This value is referred to as the step height (for an example, see Fig. 8). On the other hand, as the different ions form zones with sharp boundaries, it follows that the zone boundaries are characterized by sharp temperature changes. These temperature changes can be used to detect the passage from one zone to the other and hence to obtain information about the zone length. This in its turn is a measure of the amount of a component present in the sample. The practical usefulness of the thermometric detector is hampered by its slow response: heat must pass through the capillary wall. Differentiation of the thermal signal can sometimes be an aid in increasing sensitivity.

Potential gradient detectors consist of electrodes inserted through the capillary wall. The voltage drop between the two electrodes is measured with an independent instrument. As already stated, the potential gradient in a given zone depends on the effective mobility of the ion in that zone. The voltage drop between the electrodes can therefore be used to obtain qualitative information. Also, as with the thermometric detector, the sudden shift in potential gradient from one zone to the next can be used to detect zone boundaries.

Instead of the potential drop between two closely spaced electrodes, the conductivity of the solution can be monitored by resistance measurement, using an alternating current between the electrodes.

In any case, extreme care should be taken to control all leak currents between the electrodes.

A third type of detector makes use of UV absorption. In the dynamic equilibrium stage of an isotachophoretic experiment, all ions move with the same speed in individual zones and only the counter ion is mixed with them. Hence the measurement of the UV absorption can give valuable information about some of the ions and at the same time serve to detect zone boundaries.

In some instances, it is even possible to detect the boundaries between two consecutive non-UV-absorbing zones. Indeed, it is extremely difficult to prepare electrolyte solutions without trace amounts of UV-absorbing material in them. As these impurities are present in trace amounts, they generally form extremely narrow UV-absorbing zones sandwiched between non-UV-absorbing zones of interest. Zone boundaries then show up as spikes on the otherwise flat UV baseline (for an example, see Fig. 3B).

In practice, the UV absorption must be made directly on the capillary tube itself. The total internal volume of the complete capillary is so small (of the order of 40 μl) that no enlargement for a UV-absorbing cell, as in liquid chromatography, can be used. Therefore, stringent conditions are put on the light source, the UV detector and the signal amplifier.

3. CHOICE OF OPERATING CONDITIONS

Several factors must be considered in choosing a suitable electrolyte system for the optimal separation of a given sample. The separation of the different com-

ponents of a sample is primarily influenced by the difference in effective electrophoretic mobility of the ions. All operating conditions influencing the mobilities must be optimized in order to achieve rapid and complete separation of the components of interest.

Mathematical equations governing all the factors that influence an isotachophoretic experiment have been worked out[4]. However, in many instances, not all of the basic data necessary for application of the equations are available. The choice of suitable electrolyte systems will more often than not be a matter of experience and intuition. Positive and negative influences must be weighed against each other.

We cannot go into great detail here, but the most important factors are the following:

(a) The choice of the solvent. The replacement of water by deuterium oxide or a non-aqueous solvent can sometimes improve a separation.

(b) The choice of the buffering counter ion. This must be chosen with consideration of the desired separation pH and offers maximal buffering capacity at that pH.

(c) The choice of the leading ion. A prerequisite for isotachophoresis is that the leading ion must have a mobility higher than that of any of the sample ions of interest. However, its mobility must not be too high otherwise, and this is especially true for large molecules with low mobilities, it may be that insufficient time is allowed for all of the sample ions to reach a true dynamic equilibrium.

(d) The choice of the terminating ion. In most instances, it will have a lower effective mobility than that of the slowest of the sample ions of interest. In some special instances, however, a terminating ion with a mobility such that some of the sample ions are overtaken by it may be of help in simplifying the separation pattern.

(e) Additives, such as mobility spacer ions, stabilizers, surface-active agents and internal standards, can be chosen for addition to the system to suit individual needs.

4. SOME PRACTICAL ASPECTS

As yet there is little choice in the commercial apparatus for analytical isotachophoresis, probably because of the exacting demands put on most of the components needed to form a complete and versatile analytical ensemble.

High-voltage power supplies, d.c. signal amplifiers, thermostating equipment and recorders that satisfy most of the demands of capillary isotachophoresis can be bought separately. However, the construction of the actual analytical sub-unit, consisting of the mounted capillary, the sample injection system and the electrolyte vessels, can only be constructed in advanced workshops with the highest standards.

The different electrolyte solutions must be prepared with the utmost care. Not only can impurities in the chemicals show up as unwanted peaks or zones, but many of the separation parameters can be influenced by them. One of the most important factors, and perhaps the most often overlooked, is the actual surface condition of the inside wall of the capillary. Some of the sample components and/or impurities in the electrolyte solutions can influence this condition. Complete or patchwise coating of the capillary wall with these components can drastically alter the duration of an experiment (electroendosmosis), the voltage reached during detection (loss of bound-

ary sharpness), change in temperature profiles (current leakage) and drift in the UV baseline by slow release of previously absorbed components from the coating. Even analytical-reagent grade chemicals in most instances need one or more additional purification steps. Blank runs without sample should be made regularly, especially after a new batch of electrolyte solutions has been made up.

The composition of the sample, other than the components of interest, must be kept under control. The ionic composition of the sample should be such that the chosen separation pH of the leading electrolyte is not altered appreciably by sample injection.

One of the main advantages of capillary isotachophoresis is the absence of a support material, as in zone electrophoresis. (We compare here capillary isotachophoresis with zone electrophoresis in a support. There is of course also capillary zone electrophoresis which does not have all advantages, and isotachophoresis in supports which has some disadvantages.) This has several advantages: electroendosmosis is reduced to a very low value; irreproducibility of results, due to variations in the physical and chemical properties of a support material, is eliminated; the effective mobility of the charged molecules is not affected by the passage through the support material.

The concentrations of the electrolyte solutions are normally in the range 0.01–0.005 mole/l. The lower limit of detection for a given compound becomes lower as the concentration of the leading electrolyte is lowered. Unfortunately, below 0.001 mole/l, the contribution of the proton and the hydroxyl groups to the migration becomes appreciable. For the same reason, separation pH values outside the range 3–10 are less suitable.

5. PRACTICAL APPLICATIONS

Capillary isotachophoresis has already found a wide range of applications in many different fields of research, production and control. (For literature surveys see Everaerts *et al.*[4] and the LKB reference lists.) It is impossible to give a comprehensive list, but among the most important are the following:

(a) Pollution control: detection and measurement of inorganic and organic compounds.

(b) Process control: metal ions, organic radicals.

(c) Quality control: amino acids, organic acids, antibiotics, organophosphates, etc.

(d) Research: proteins, peptides, amino acids, organic acids, nucleic acids.

The practical applications presented below were selected with the sole aim of stimulating interest in this new analytical technique.

The separation by isotachophoresis of cationic species seems to have attracted the least attention. Everaerts *et al.*[4] described several electrolyte systems that can be used to separate different metal ions and organic compounds with amino groups. Either hydrogen or potassium ions are used as the leading ion with Tris as the terminating ion. Depending on the chosen separation pH, acetic or cacodylic acid can be used as the counter ion. Thermometric detection is most often used. Separations of anionic species have attracted much wider interest.

5.1. Organic acids

Speed, high sensitivity, minimal sample preparation and the combination of direct qualitative and quantitative information are all contributory factors that render capillary isotachophoresis interesting for the study of organic acids, especially in biological solutions.

In Fig. 3A, the application of isotachophoresis in the fermentation industry is presented. Only 2 μl of a 1:500 dilution of crude fermentation broth, obtained during the industrial production of citric acid, was injected. Typical experimental conditions were as follows: leading electrolyte, 5 mM HCl, 0.3% methylcellulose, titrated to pH 3.85 with β-alanine; terminating electrolyte, 5 mM caproic acid; analysis time, 10–20 min, and detection, UV at 254 nm and thermometry.

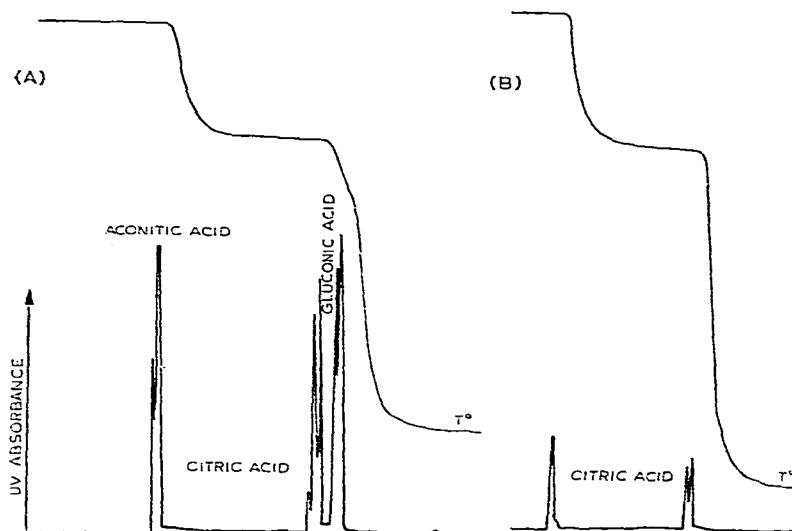

Fig. 3. (A) Isotachophoretic separation of crude fermentation broth in citric acid production. (B) Identical separation of a sample from a batch before the last purification step. T° = thermometric signal (for conditions, see text).

Fig. 3B presents the results obtained under the same conditions by injection of 2 μl of a 1:100 dilution of the concentrate before the last purification step. It is interesting to note that, with a similar electrolyte system at pH 2.45, it is possible to achieve a quantitative separation of citric acid and isocitric acid.

Somewhat similar problems arise in the food industry and Fig. 4A illustrates this[5]. From the injection of a few microlitres of a de-gassed soft drink, the citric acid and ascorbic acid contents can be directly evaluated. The effect of contact with atmospheric air on the ascorbic acid level of the same soft drink is shown in Fig. 4B.

Fig. 5 illustrates the determination of the organic acid content of a fruit yoghourt preparation. The presence or absence of some of the organic acids enables one to judge the fruit content of these commercial preparations.

Recently, problems in human dental hygiene have been succesfully approached by capillary isotachophoretic analysis of the organic acids produced by the growth of microorganisms.

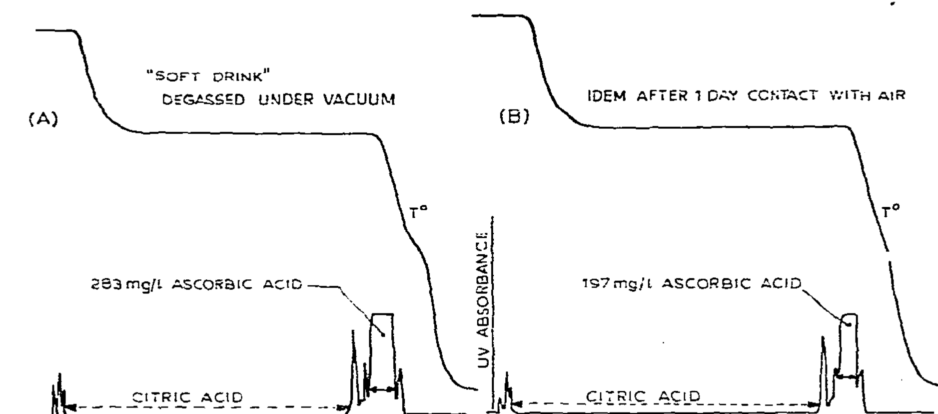

Fig. 4. (A) Isotachophoretic separation experiment in which an untreated sample of a de-gassed commercial soft drink was injected. (B) Separation under the same conditions as in (A), with the same de-gassed soft drink after contact with atmospheric air for 24 h.

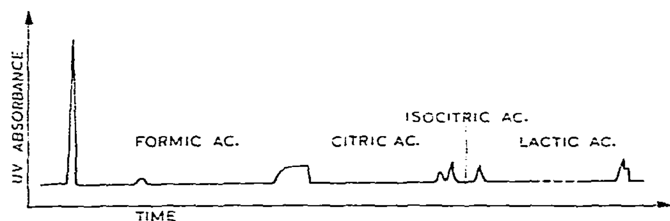

Fig. 5. Result obtained on isotachophoresis of a commercial preparation of fruit yoghourt.

A completely different kind of application can be found in the isotachophoretic determination of phenylglyoxalic, mandelic, hippuric and methylhippuric acids in the urine of human subjects after occupational exposure to styrene, toluene and xylene[6]. The *o*-, *m*- and *p*-isomers of methylhippuric acid can be partially separated. After ether extraction of the urine, all four acids can be measured accurately by isotachophoresis. Even amounts as small as 0.5 nmole can be determined in about 20 min.

5.2. Nucleic acids

Analytical isotachophoresis is an excellent method for the qualitative and quantitative determination of nucleotides[7,8]. The different phosphates of adenosine, cytidine, guanosine and uridine can be easily separated from each other. Measurement of zone lengths gives direct quantitative information about the nucleic acid composition of the sample. Fig. 6 shows the results obtained with a synthetic mixture of 13 nucleic acids when analysed in a 63-cm capillary in about 40 min. Detection was effected by UV absorption at 254 nm. Virtually the same electrolyte system as described above for organic acids was used. The separation pH was 3.89 and about 1.5 nmole of each nucleotide was used (4.5 nmole for c-AMP).

As reported by Gustafsson[13], 5-fluorouracil can be measured in human serum at levels that are of clinical interest. This problem is of interest in human cancer therapy.

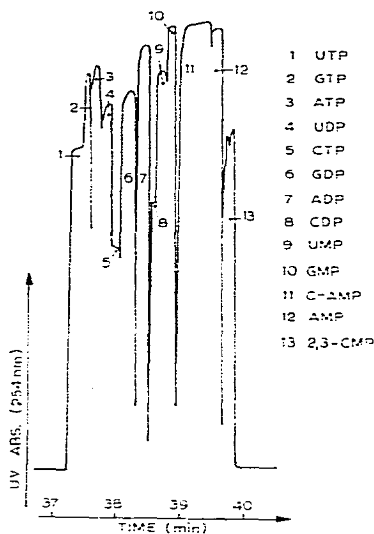

Fig. 6. Isotachophoretic separation of a synthetic mixture of 13 nucleic acids in a 63-cm capillary. Separation pH, 3.89.

5.3. *Antibiotics*

Analytical isotachophoresis is an excellent technique for the quality control of antibiotics such as penicillins and tetracyclines. Despite the small differences in mobility, due to their similarity in structure, they can be separated and quantified. A typical example is the separation of the penicillins carbenicillin, flucloxacillin, ampicillin and amoxicillin. Chloride ion was used for the leading electrolyte, adjusted to pH 7.2 with Tris and with 0.2% methylcellulose as anti-convection additive. The terminating ion was 10 m*M* β-alanine, adjusted to pH 10.3 with barium hydroxide. Other conditions were capillary length, 43 cm; temperature, 11°; total separation time, 32 min; sample, about 2 μg of each antibiotic; detection, UV absorption at 254 nm. The results of such a separation experiment are shown in Fig. 7.

Another example of a similar separation is the analysis of the ingredients of an antibiotic preparation for infusion. As revealed by isotachophoresis, the pharmaceutical mixture contained, in addition to a tetracycline and doxycycline, ascorbic acid, sorbitol and several other UV-absorbing substances in smaller amounts.

5.4. *Amino acids*

The establishment of the amino acid composition is of interest in many fields of research and industry. Nearly all separation techniques have been tried: liquid chromatography, gas chromatography and electrophoresis are among the most important. Despite having received little attention until now, isotachophoresis seems to be an interesting addition to this range of techniques.

To change from one operational system to another in isotachophoresis takes little time (normally not longer than required for rinsing and refilling the capillary and electrode vessels) and the total analysis time is very short (*ca.* 10 min). With this in mind, it can be stated that the application of two different systems to the same sample will lead to more useful information than when one tries to separate all of the

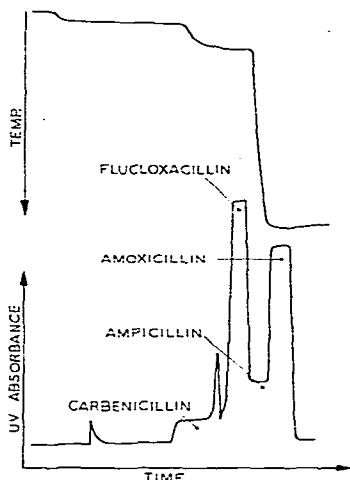

Fig. 7. Isotachophoretic separation of an antibiotic preparation. Lower trace = UV absorption; upper trace = thermometric signal.

amino acids in one run. With a separation pH above 8, most of the amino acids will have an effective mobility for a separation according to the isotachophoretic principle. Most of them will migrate anodically.

Fig. 8 presents the results of an isotachophoretic separation experiment[4] on eight amino acids. The experimental conditions were as follows: leading electrolyte, 0.004 *M* 5-bromo-2,4-dihydroxybenzoic acid adjusted to pH 9 with β-alanine; terminating electrolyte, 0.01 *M* β-alanine adjusted to pH 10.5 with barium hydroxide; electric current, 50–100 μA. It can be seen that both the thermal step height and the UV-absorption trace contribute to the interpretation of the results.

Robinson and Rimpler[9] recently reported the determination by capillary isotachophoresis of aspartic acid, asparagine, glutamic acid and glutamine. An ultrafiltrate of human serum was injected without further treatment.

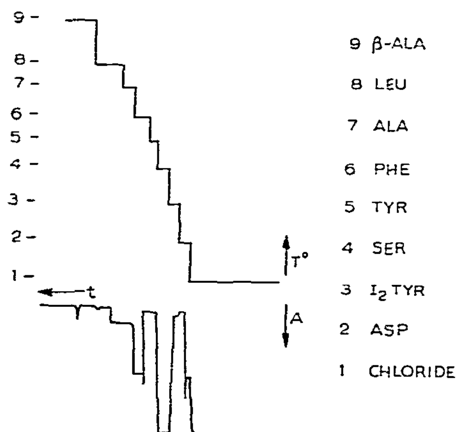

Fig. 8. Isotachophoresis of a mixture of amino acids. Upper trace = thermometric signal; lower trace = UV absorption.

5.5. Peptides

Most of the analytical systems elaborated for amino acid analysis can also be used for the separation of small peptides. The field of biologically active peptides has developed enormously during the last decade. As many of these compounds have potential use in human medicine, their synthesis and purification is important. The same advantages for amino acid analysis by isotachophoresis make this technique ideally suited to the detection and measurement of the small peptides. Capillary isotachophoresis has already been applied to most of the naturally occuring peptides such as oxytocin, vasopressin and somatostatin. Typical results obtained by capillary isotachophoresis during the purification of the vasoactive intestinal peptide (VIP) are shown in Fig. 9. In each instance, about 30–40 μg of material were injected and the total analysis time was about 9 min.

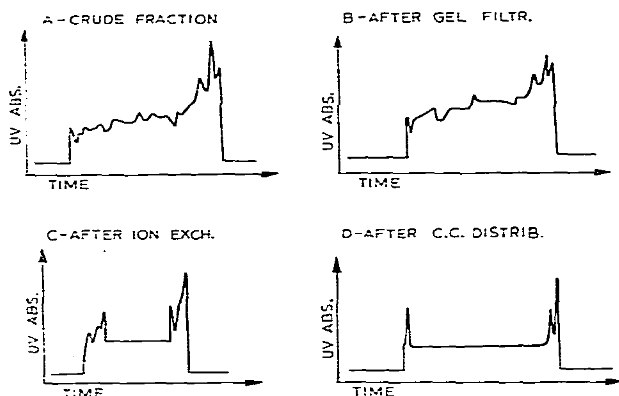

Fig. 9. Isotachophoretic separation of vasoactive intestinal peptide during different stages of the purification procedure. Leading ion, 0.005 M KAc titrated to pH 5.1 with HAc, 0.25% methylcellulose; terminating ion, 0.005 M α-alanine; capillary length, 23 cm; separation time, 9 min.

5.6. Proteins

The application of capillary isotachophoresis to proteins has been investigated intensively only during the last 2 years. Under suitable operating conditions, the different protein species of a sample arrange themselves between the leading and terminating electrolytes according to their net electrophoretic mobilities. Not much useful information can be gained from such an experiment because the UV beam, however narrow, cannot resolve the different protein zones, which follow each other without interruption and all of which absorb the UV light. In order to arrive at useful separation patterns, a spacer mobility gradient must be created between the leading and terminating electrolytes. The different protein species constituting the sample are then interspaced with other ions that have mobilities in the same region as those of the proteins under separation. A prerequisite is that these spacer ions must show no UV absorbance and that there must be a sufficient number of them with different mobilities[10].

The commercial preparations of synthetic peptides used for isoelectric focusing are suitable as spacer preparations. Some of the amino acids can also be used as

discrete spacers to split up a complex protein separation pattern into moie easily interpretable sub-groups.

If the operating parameters are sufficiently optimized and a true dynamic equilibrium has been reached, then the integration of the peak surfaces produced by pure protein species is directly proportional to the absolute amount of protein in the peaks[11].

Fig. 10A shows a typical separation pattern obtained with only 0.6 μl of human serum. The large bıoad peak is albumin and all of the peaks with mobilities slower than that of the amino acid valine are mobility sub-fractions of immunoglobulin G. The pattern of immunoglobulin sub-fractions depends on the composition of the mobility spacer gradient used, but it is extremely reproducible.

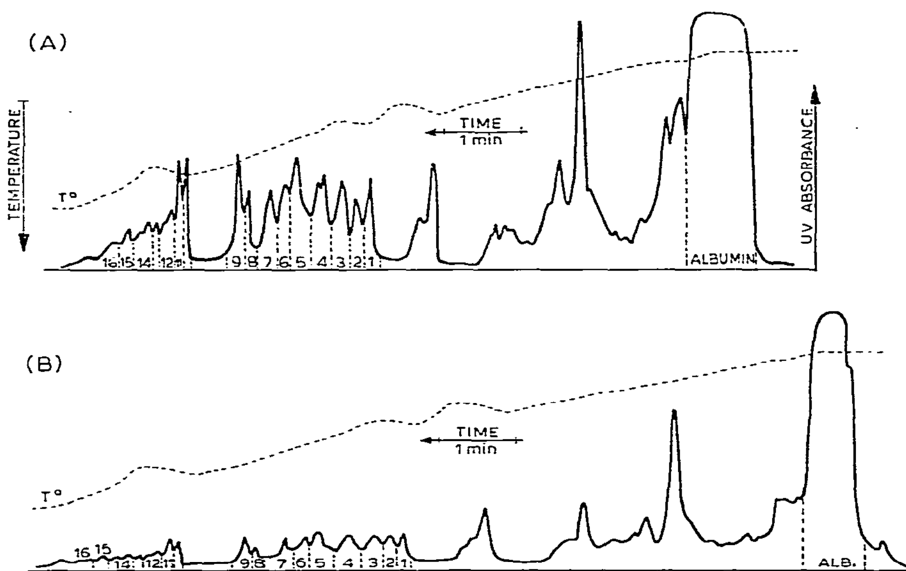

Fig. 10. Isotachophoresis of proteins. Upper pattern, 0.6 μl of human serum; lower pattern, 2 μl of 15-fold concentrated cerebrospinal fluid from the same subject. G = glycine spacer; V = valine; A = β-alanine. Leading electrolyte, 0.005 *M* morpholinoethanolsulphuric acid adjusted to pH 9 with aminopropanediol, 0.4% methylcellulose; terminating electrolyte, 0.005 *M* animocaproic acid adjusted to pH 10.8 with barium hydroxide; temperature, 12°; capillary length, 23 cm; separation time, 25 min.

Fig. 10B presents the pattern obtained by injection of 2 μl of about 15-fold concentrated cerebrospinal fluid from the same subject. As the peak surfaces are a direct measure of the amount of protein, and by taking into account the sample volumes and concentration factor, the permeability coefficients of the blood/cerebrospinal fluid can be calculated for several protein species.

Fig. 11A is the separation pattern obtained from the soluble eye lens proteins from a young mouse. Fig. 11B shows the same results obtained under the same conditions but with the proteins from the lens of a very old mouse. Influence of age on the protein composition can thus be demonstrated in a very short time, using very small

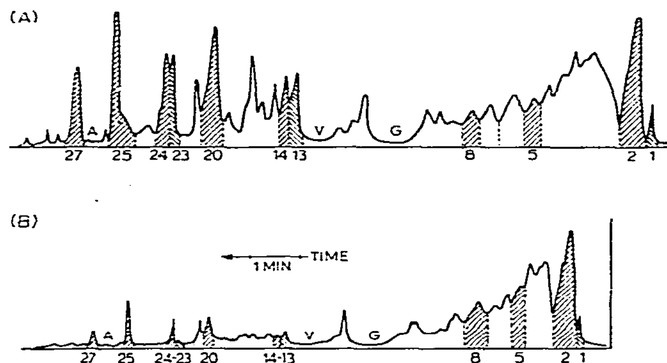

Fig. 11. Isotachophoresis of soluble eye lens proteins from mouse. Injected: 2 μl of 1% solution. Conditions as in Fig. 10. (A), very young mouse; (B), very old mouse.

amounts of protein and without making use of denaturation or coloration of the separated protein fractions.

6. CONCLUSIONS

A fraction collector device applicable in capillary isotachophoresis has recently become available[12]. The sample zones are transferred to a moving cellulose acetate strip without appreciable loss of resolution. This renders possible the application of special detection and identification techniques. Owing to the extremely small amounts of material concerned, the most promising application of this technique lies with radioactively labelled compounds.

Preparative isotachophoresis in columns of polyacrylamide gels is a new, standardized, high-resolution technique, more specifically suited to protein chemistry. Preparative isotachophporesis can compete with many other protein separation techniques. Much valuable time, effort and materials can certainly be saved if the separation conditions for such preparative work are first studied by capillary isotachophoresis.

7. SUMMARY

Isotachophoresis is an electrophoretic technique in which the different sample ions are separated, under the influence of a strong electric field, according to their effective electrophoretic mobilities; all ions are, at equilibrium, moving with the same constant speed between two different electrode ions.

The separated components are detected directly in the capillary, either by thermometry, potentiometry, resistance measurement or by UV light absorption. Practically any charged molecule (organic acids, nucleic acids, antibiotics, amino acids, peptides and proteins) can be separated and in most instances be quantified, in a very short time, by this new analytical technique.

REFERENCES

1 W. C. D. Whetham, *Phil. Trans. Roy. Soc. London, Ser. A*, 184 (1893) 337.
2 F. Kohlrausch, *Ann. Phys. (Leipzig)*, 62 (1897) 209.
3 L. Ornstein and B. J. Davis, *Ann. N.Y. Acad. Sci.*, 121 (1964) 321.
4 F. M. Everaerts, J. L. Beckers and T. P. E. M. Verheggen, *Isotachophoresis*, Elsevier, Amsterdam, 1976.
5 G. Neumann, *Meeting "Fruitsaps and Fruitdrinks", Heidelberg-Eppelheim, September 19–21, 1977.*
6 J. Sollenberg and A. Baldensten, *J. Chromatogr.*, 132 (1977) 469.
7 B. Sjödin, A. Kopwillem and J. Karlsson, *Scand. J. Clin. Lab. Invest.*, 35 (1975) 699.
8 D. C. Gower and R. C. Woledge, *Sci. Tools*, 24 (1977) 2.
9 D. V. Robinson and M. Rimpler, *J. Clin. Chem. Clin. Biochem.*, 16 (1978) 1.
10 K. G. Kjellin, U. Moberg and L. Hallander, *Sci. Tools*, 22 (1975) 3.
11 P. Delmotte, *Sci. Tools*, 24 (1977) 33.
12 L. Arlinger, *J. Chromatogr.*, 119 (1976) 9.
13 B. Gustafsson, in press.