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INSTRUMENTATION FOR HIGH-SPEED ISOTACHOPHORESIS

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SUMMARY

The design and operating parameters of the individual elements of an isotachophoregraph were examined from the viewpoint of rapid quantitative analysis. An arrangement comprising an inlet port, separation column and detection cell was constructed, in which dead spaces were eliminated and the capillary and detection cell were efficiently cooled. This arrangement permits the time of isotachophoretic analysis from the instant of sample introduction to be shortened to 3–5 min, the development of the isotachophoregram proper taking 20–40 sec. The basis of the arrangement is a monolithic block of Perspex that comprises both electrode compartments, control stopcocks of the sampling device and the capillary. The latter is formed by a flat groove in the block, closed with a PTFE foil overlapping the groove and compressed on to the block with a thermostatted metallic plate. The detection cell is formed by part of the capillary and consists of two platinum contacts that extend into the groove in the monolithic block. The detector functions by sensing the electric gradient of the migrating zone. Recording was effected by means of a home-made voltmeter of high input resistance, with the detector input insulated electrically from the output of the recorder. The high-voltage source employed was also home made and yielded a stabilized current of up to 400 μA at 16 kV.

INTRODUCTION

The analytical utilization of isotachophoresis has received great interest lately. The selection of suitable conditions for separation[1–4] and the quantitative interpretation of isotachopherograms[5–7] have been considered. Also, considerable attention has been devoted to the instrumentation, the basic apparatus[8] being equipped with new detectors able to distinguish between even very narrow zones[9–14], an automatic device for measuring the length of the zone[15], an arrangement for the use and proportional control of the counter flow[11,16–18], resulting in a higher separation capability, and a commercial form being developed[12].

Although the above appurtenances represent substantial improvements over the original apparatus[8] and extend the operating possibilities appreciably, only some of the problems involved in analytical isotachophoresis can be solved by their incorporation. Thus, most of the papers published on isotachophoresis so far have had a

preparative or qualitative orientation. Further, when applying higher voltages in an effort to shorten the time of separation, which is now 30–90 min, spurious zones occurred owing to the inhomogeneity of the electrical and thermal regime.

The instrumentation of analytical isotachophoresis must meet the analytical requirements of separation methods. Particular requirements are speed of analysis, *i.e.*, the speed of separation and detection, quantitativeness of analysis, *i.e.*, the completeness of the separation of the individual components, unskewed detection of the individual zones, and quantitative interpretation of the record of separation. In routine analytical practice, the time of analysis often determines the utility of a method. In this paper, we describe the so-called high-speed analytical isotachophoresis, with which we have obtained analytical separations in several tens of seconds.

PROBLEMS IN ISOTACHOPHORESIS

The total time of analysis is given by the time elapsed from the introduction of the sample to the arrival of the zone of the terminating electrolyte in the detection cell. If the composition of the leading electrolyte is chosen according to the character of the sample to be analyzed, then the main possibility for speeding up the analysis consists in increasing the velocity of migration of the ions down the column. This can be achieved by employing higher electric gradients and, consequently, higher current densities. An increase in the electrical input obviously results in the production of a larger amount of the Joule heat in the column. This heat must be removed sufficiently rapidly by cooling the column, otherwise overheating occurs, which affects the stationary thermal regime in the column and convective mixing of the zones due to thermal differences.

Hence, increasing the speed of analysis by applying higher electric gradients is limited mainly by the maximum amount of heat that can be removed from the solution flowing under the influence of the driving current across the column wall into the thermostat. This aspect depends on the efficiency of cooling a column of a solution placed between two electrode compartments, and the limiting factor is therefore the section of the apparatus that is cooled the least. With the existing arrangements, this section is mainly the detector, in which a column of the solution several centimetres long is isolated from the thermostatting medium by the cylindrical walls of the detection cell, which are usually made of Perspex of a thickness exceeding 5 mm[10,11,13].

The speed of analysis is further limited by undesirable effects such as uncontrollable movement of the electrolyte along the column and diffusion into dead spaces in the column. With present instruments, potential sources of undesirable movement of the electrolyte are elastic deformations of the PTFE capillary, acetylcellulose membrane and connecting tubing. For the elimination of dead spaces, the column of the liquid in which the separation takes place should have as homogeneous a cross-section as possible. There must be no places with abrupt changes of diameter or with edges normal to the zone migration within the capillary, as they lead to the presence of spaces with a weak electric field in the direction of zone migration. The sources of dead spaces are mainly the disconnectable junctions between the injection port, capillary and the detection cell, where there are sharp edges and slots normal to the direction of the electric field at the interfaces between the parts. The diffusion of the

components into these spaces disturbs the course of separation. In order to restore sharp zone boundaries, it is necessary to increase the effective length of the capillary, thus increasing further the time of separation.

When applying higher electric gradients, the problem of the electrical isolation of the electrically conductive solution from the earthed parts of the apparatus, especially from the thermostat, is no longer negligible. The potential sources of electrical breakdown are mainly the disconnectable junctions.

The analytical value of the detection results is determined mainly by the sharpness of the zone boundary in the detection cell and the skew of it caused by the sensor. The least amount of skew is encountered with contact detectors and, of non-contact detectors, with the UV detector. With these detectors, it is particularly the dead volumes and temperature differences that cause the skew. The dead volumes can be reduced by removing the connections between the detection cell and the capillary and providing a homogeneous inner cross-section of the detection cell. Also, the configuration and shape of the sensing electrodes must not disturb the smoothness of the inner wall of the detection cell. Further, there should be no change, at the position of the detection cell, in the stationary temperatures regime in the column, *i.e.*, the solution in the cell must be cooled as efficiently as the solution in the capillary. The quantitative interpretation of the analytical record requires that the pH in the separated zones should be kept stationary. A disturbance of the steady state in the zone can be caused by an H^+ zone advancing in the capillary[16,19], formed due to the selectivity of the semi-permeable membrane or by artifacts from the electrode chamber passing through the membrane into the capillary. In order to eliminate these deleterious effects, it is necessary to provide between the semi-permeable membrane and the capillary or detection cell a buffer compartment of a sufficient volume, filled with fresh leading electrolyte before each analysis. A volume about ten times greater than that of the separation capillary is adequate[20].

A suitable approach appears to be the formation of a solid block comprising both electrode compartments, sample inlet port, non-elastic capillary and detection cell, thermostatted homogeneously throughout the entire volume.

EXPERIMENTAL

Apparatus

An apparatus designed for high-speed analytical isotachophoresis[21] is shown in Fig. 1. The basis of the arrangement is a monolithic block of, *e.g.*, Perspex, in which the electrode compartments, isolation semi-permeable membrane, buffer compartment, sample inlet port, control stopcocks, interconnection channels and the capillary with the detection cell and the connections to the detector are built-in directly by machining and sealing.

The capillary is formed by a flat groove milled directly into the lower wall of the monolithic block, closed with a PTFE foil overlapping the groove and compressed on to the block with a thermostatted metallic plate. The groove has a rectangular cross-section with a depth of 0.2 mm and a width of 1 mm. The length of the groove is about 25 cm and can be varied according to the length of the monolithic block. The detection cell is formed directly by part of the capillary at the position where platinum sensing contacts extend into the groove. These contacts are platinum wires

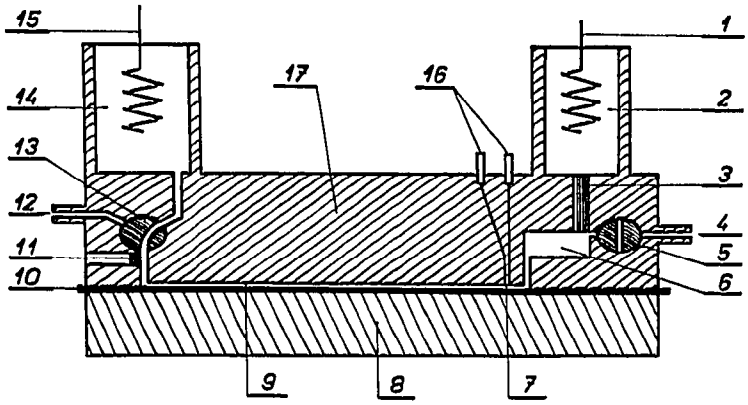

Fig. 1. Sectional view of the isotachophoretic column. 1 = Platinum electrode; 2 = electrode compartment; 3 = glass frit G4; 4 = tube for connecting the reservoir of the leading electrolyte; 5 = PTFE closing two-way stopcock; 6 = buffer compartment; 7 = scanning platinum electrodes; 8 = thermostatted metallic plate; 9 = flat capillary groove; 10 = PTFE foil; 11 = septum; 12 = drain tube; 13 = PTFE switching three-way stopcock; 14 = electrode compartment; 15 = platinum electrode; 16 = connectors to the detector; 17 = basic body of Perspex.

0.03 mm in diameter, separated from each other by about 0.05 mm in the longitudinal direction. The platinum wires extend into the groove, flush with it, at the level of the upper wall of the capillary. We can confirm the advantage of the recently described principle[10] of scanning potential gradients in the individual zones, measured as a voltage between the scanning electrodes. The semi-permeable membrane was formed by a glass frit (density G4) soldered into the monolithic block. The buffer compartment, with a volume of about 2 ml, was drilled out between the frit and the separation capillary in the block. The closing two-way and connecting three-way stopcocks with conical PTFE cores were created directly in the monolithic block. The connecting channels were drilled directly in the monolithic block and had a bore of about 0.8 mm. The sample inlet port was of ordinary construction, permitting samples to be introduced with a microsyringe through a septum.

A home-made high-voltage source[22] was used with the above isotachophoretic column and was capable of providing a stabilized d.c. current controllable up to 400 μA at about 16 kV. The detection device was a high input-resistance voltmeter, which also isolated electrically the high-voltage part of the measuring circuits connected to the detection cell from those for connecting the recorder. A Perkin-Elmer Model 1969 recorder was employed.

Operating conditions

Three model solutions containing a mixture of anions were employed for testing the performance characteristics of the instrument. The first solution contained some acids of the Krebs metabolic cycle, with the addition of oxalate as an internal standard and the trisodium salt of sulphanilazochromotropic acid (SPADNS) serving as an optical marker. The latter compound makes a deep-red zone clearly visible through the upper wall of the monolithic block, which makes it possible to measure directly the velocity of zone migration and to observe the establishment of equi-

librium. For this purpose, two lines 5 cm apart were marked beside the groove constituting the capillary, and the time required for the SPADNS zone to pass between them was measured with a stop-watch. The second sample was a mixture corresponding to the bath for chemical nickelizing, containing hypophosphite, phosphite, phosphate and lactate. Oxalate was added as an internal standard for quantitation. The third sample was a nine-component model mixture serving to test the resolving capability of the detection cell. The compositions of the mixtures and the operating conditions are specified in the figure legends.

Hydrochloric acid (0.0066 *M*) was used as the leading electrolyte, the pH of which was adjusted to 4.2 by using aniline as a source of buffering counter ions. The terminating electrolyte was 0.012 *M* acetic acid. All the chemicals were products of Lachema, Brno, Czechoslovakia. The electric driving current through the column was maintained at 200 μA. The course of separation was recorded at a chart speed of 16 cm/min. The temperature of the thermostat was 23°.

The quantitative interpretation of the records was carried out by the internal standard method, with the use of relative correction factors as described earlier[6]. The correction factors were obtained by analyzing standard mixtures of the components being determined.

RESULTS AND DISCUSSION

It can be seen from Fig. 1 that the capillary, in which the separation of a sample charge takes place, and the detection cell are cooled evenly. The arrangement does not involve connections that would produce dead spaces. The detection cell, which constitutes a homogeneous section of the capillary and has the same inner cross-section as the capillary, does not contain sites that could disturb the quantitativeness of detection. The entire arrangement is sufficiently mechanically rigid, which precludes elastic deformations and thus uncontrollable movement of the electrolyte in the capillary. There are no positions in the apparatus that would give an appreciable risk of electrical breakdown.

A record of the separation of the first model sample is shown in Fig. 2. It can be seen that the zone boundaries between the individual components are well developed and that the steps recorded can be evaluated analytically. The impurity apparent in the record is due to oxaloacetic acid. The entire separation is completed in less than 5 min from the introduction of the sample charge; the figure shows the situation within the 4-th and 5-th minute. The velocity of the migration of the zones down the capillary, as measured in the above-mentioned way, was 7.2 cm/min.

A record of the separation of the second model sample is shown in Fig. 3. The analysis of a mixture of acids of phosphorus, even in the presence of a lactate, is very difficult by classical chemical methods, and the quantitative results are not very good.

The third model mixture (Fig. 4) served for testing the resolving capability of the detection cell. It is apparent that ten zones of the separated components were detected reliably in about 30 sec. Also, a step due to an impurity present in the sample is apparent in the record. The length of this step in the record was about 2 mm, which corresponded to an actual length of about 0.3 mm as measured in the detection cell.

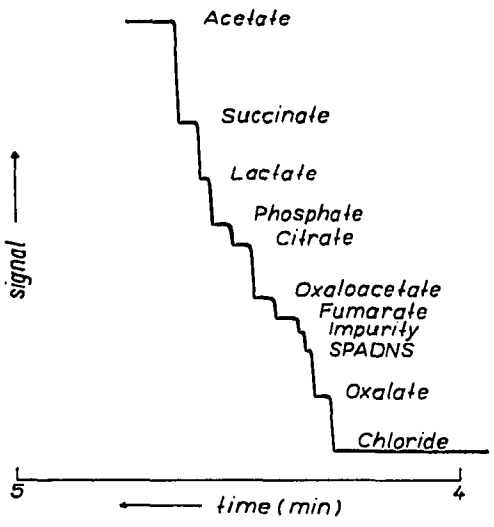

Fig. 2. Analysis of a model mixture of some acids of the Krebs cycle with the addition of SPADNS dye for determining the migration velocity. The volume injected was about 4 μl. The composition of the model mixture was $2.8 \cdot 10^{-4}$ *M* oxalic acid, $1.3 \cdot 10^{-4}$ *M* SPADNS, $3.3 \cdot 10^{-4}$ *M* fumaric acid, $7.4 \cdot 10^{-4}$ *M* oxaloacetic acid, $2.3 \cdot 10^{-4}$ *M* citric acid, $3.6 \cdot 10^{-4}$ *M* $NH_4H_2PO_4$ and $3.7 \cdot 10^{-4}$ *M* succinic acid. The figure shows only the record of the course of analysis between the fourth and fifth minutes.

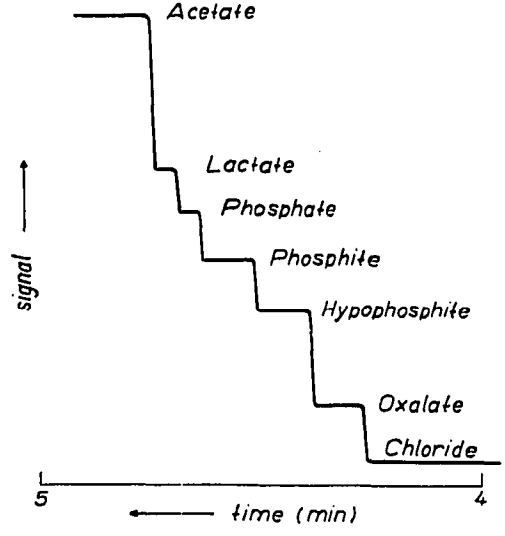

Fig. 3. Record of the analysis of a model sample of anions in a nickelizing bath with oxalic acid added as an internal standard. The composition of the model mixture was $1.22 \cdot 10^{-4}$ *M* NaH_2PO_2, $1.10 \cdot 10^{-4}$ *M* H_3PO_3, $0.54 \cdot 10^{-4}$ *M* $NH_4H_2PO_4$, $0.84 \cdot 10^{-4}$ *M* oxalic acid and $0.82 \cdot 10^{-4}$ *M* lactic acid. The volume injected was about 4 μl. The figure shows a record of the course of the analysis between the fourth and fifth minutes.

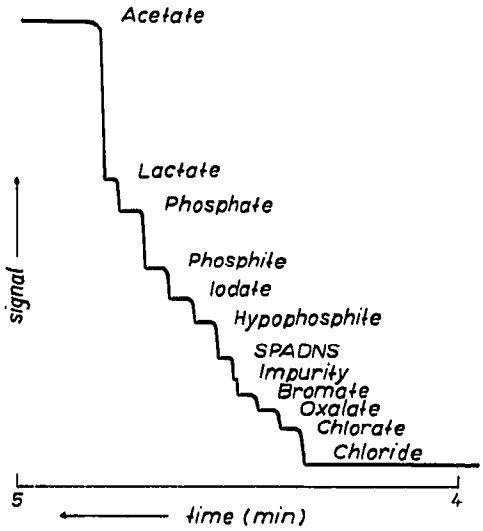

Fig. 4. Analysis of a ten-component mixture for testing the resolving capability of the detector. About 4 μl of the mixture were injected. The composition of the mixture was $6.0 \cdot 10^{-4}$ *M* $NaClO_3$, $4.2 \cdot 10^{-4}$ *M* oxalic acid, $6.0 \cdot 10^{-4}$ *M* $KBrO_3$, $3.0 \cdot 10^{-4}$ *M* SPADNS, $6.1 \cdot 10^{-4}$ *M* NaH_2PO_2, $6.0 \cdot 10^{-4}$ *M* KIO_3, $5.5 \cdot 10^{-4}$ *M* H_3PO_3, $4.2 \cdot 10^{-4}$ *M* $NH_4H_2PO_4$ and $4.1 \cdot 10^{-4}$ *M* lactic acid. The figure shows a record of the detection performed between the fourth and fifth minutes from sample injection.

The above separations are satisfactory, the velocity of the migration of the zones and the time of analysis being acceptable. Hence, from the point of view of the speed and efficiency of separation, the apparatus constructed satisfies the original aim. However, this alone would not be sufficient. The quantitativeness of the analyses was proved by means of the second model sample, as the quantitative analysis of mixtures of hypophosphites, phosphites and phosphates is also important from the viewpoint of the industrial practice. Table I shows the measured values of the relative correction factors with oxalic acid as a reference compound. The oxalic acid also served as an internal standard, added in a definite amount to the sample being analyzed. The results represent averages of three independent determinations. The deviation of the analytical results from the true values is apparently less than the

TABLE I

QUANTITATIVE EVALUATION OF AN ISOTACHOPHORETIC ANALYSIS

Ionic species	*Relative correction factor**	*Concentration in sample (mmole/l)*	*Concentration determined*** *(mmole/l)*	*Standard deviation*		*Deviation from true value*	
				mmole/l	*%*	*mmole/l*	*%*
Hypophosphite	0.715	1.220	1.225	0.015	1.2	+0.005	+0.4
Phosphite	0.784	1.100	1.092	0.015	1.2	−0.008	−0.6
Phosphate	0.809	0.450	0.449	0.005	1.1	−0.001	−0.2

* Oxalate was used as a reference compound.
** Average from three analyses.

standard deviation, which indicates the correctness of the analyses. The precision of the results, expressed as the percentage relative standard deviation, is about 1.2%. This precision is better than that attainable with present chemical methods.

CONCLUSIONS

It has been shown that the term high-speed isotachophoresis is now appropriate as a result of the decrease achieved in the time required for analysis.

It can be concluded that the criteria studied, *i.e.*, speed and quantitativeness, are obviously not the only ones to be considered. However, they can be taken as a good basis for a more general solution of the problems of the instrumentation of analytical isotachophoresis.

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