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ISOTACHOPHORESIS

SOME QUANTITATIVE ASPECTS OF THE SEPARATION OF ANIONIC MIXTURES

J. L. BECKERS AND F. M. EVERAERTS

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

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SUMMARY

The quantitative aspects of isotachophoretic analyses have been studied. The introduction of a calibration constant removes the necessity for constructing calibration curves for each ionic species. Calibration constants were determined for two electrolyte systems and the values obtained agreed with the theoretical values.

INTRODUCTION

In previous papers[1–3], the theory of electrophoretic separations has been described and some operational systems for the separations of anions and cations have been given. However, only the qualitative aspects have been studied. In this paper, studies on the quantitative aspects are described. Experiments were carried out to check the reproducibility and accuracy of the method.

In the previous work, the apparatus used was that described in ref. 4. The sample was introduced into the apparatus by means of a sample tap. The reproducibility, however, is much better when an injection block is used. To study the quantitative aspects, the instrument described in ref. 5 was therefore used. The sample is introduced into the instrument with a Hamilton micro-syringe in an injection block, as shown in Fig. 1.

All the chemicals used were of p.a. quality, obtained from Merck. Double-distilled water was used as the solvent.

THEORETICAL

For the quantitative determination of ionic species by isotachophoresis, calibration curves can be obtained experimentally. Theoretically, there should be a linear relationship between the zone length of an ionic species and the amount of the ionic species introduced. Calibration curves of each ionic species present in a sample and required to be separated must be measured, however. The introduction

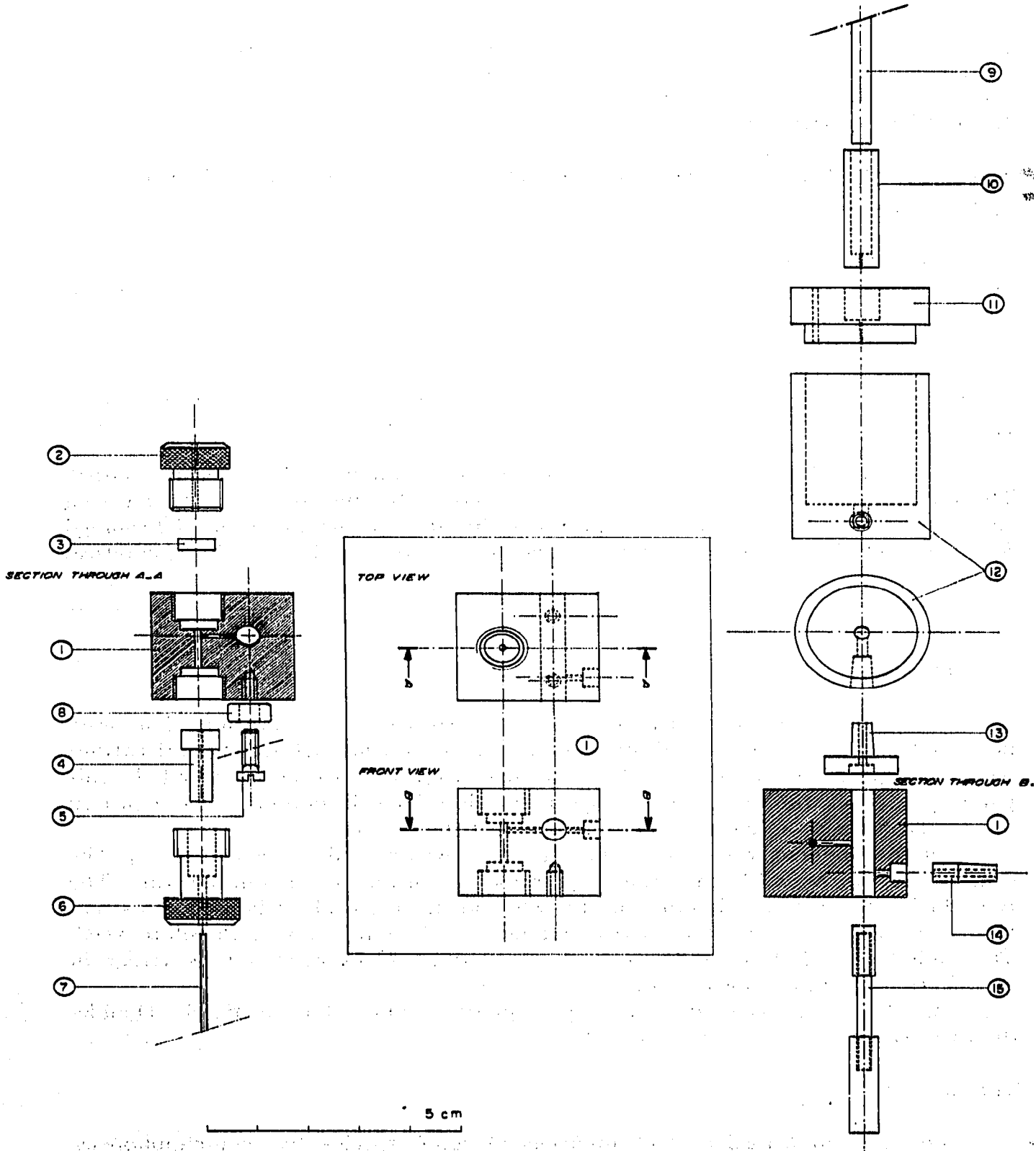

Fig. 1. Injection block and compartment for the terminal electrolyte. 1 = Injection block; 2 = bolt for fitting septum; 3 = septum; 4 = piece of Perspex for fitting capillary tube; 5 = screw for mounting injection block; 6 = bolt for fitting piece 4 and capillary tube; 7 = capillary tube; 8 = rubber O-ring; 9 = high-tension cable; 10 = piece of Perspex for mounting high-tension cable; 11 = cover for electrode compartment; 12 = electrode compartment; 13 = connection of electrode compartment with plunger compartment; 14 = connection towards drain; 15 = PTFE-covered plunger.

of a calibration constant, characteristic for all ionic species in a chosen system, simplifies the quantitative determinations. The calibration constant can be determined as follows.

The amount of an ionic species introduced in the apparatus is given by

$$Q = V_i \cdot C \tag{1}$$

where Q (mole) is the total amount of an ionic species, V_i (ml) is the volume of the sample injected and C (mole/cm^3) is the concentration of a particular ionic species present in the sample. The amount of a particular ionic species in the capillary tube will therefore be

$$Q = A \cdot C^* \cdot L \tag{2}$$

where A (cm^2) is the area of the capillary tube, C^* (mole/cm^3) is the actual concentration of the ionic species in its zone and L (cm) is the zone length of a particular ionic species.

Combining eqns. 1 and 2:

$$\frac{V_i \cdot C}{C^* \cdot L} = A \tag{3}$$

or

$$\frac{V_i \cdot C}{C^* \cdot L^*} = \text{constant} = K_{cal} \tag{4}$$

where K_{cal} is the calibration constant and L^* (sec) is the zone length as detected between two successive signals of the measuring thermocouple. In earlier papers[1,2], equations are given for the calculation of the actual concentration of an ionic species in its zone. This means that once the calibration constant, K_{cal}, is known, the concentration of an ionic species present in a sample can be calculated from the zone length. Not all calibration curves for each ionic species have to be measured separately.

In order to check the reproducibility of the analyses and to determine the calibration constants mentioned above, quantitative experiments were carried out in two different electrolyte systems with water as the solvent.

The calibration constant is not a constant for all systems. Some factors, such as variations in the concentration of the leading electrolyte, temperature and changes in the electrical current density, result in different potential gradients and hence affect the migration speed in the system. This effect produces different zone lengths for the same amounts of the ionic species in the different systems.

REPRODUCIBILITY

To estimate the reproducibility, ten times the zone length of formic acid (injected volume 3 μl of a 0.05 N solution) was measured in different experiments. The leading electrolyte was a solution of histidine and hydrochloric acid at pH 6.02. The concentration of the leading ion (chloride) was 0.01 N. The electric

current was stabilised at 70 μA. The terminator was a solution of glutamic acid.

The average zone length found was $\bar{L}^* = 311$ sec from ten experiments and the average deviation was 4 sec. Owing to the asymmetry of the step response, the zone length depends on the type of terminator used. Some experiments were therefore carried out with the same sample but with a different terminator (acetic acid). The average zone length then found was $\bar{L}^* = 307$ sec from five experiments and the average deviation was 3 sec. There was no significant difference when this value was compared with that obtained from experiments with glutamic acid as the terminator. Glutamic acid was therefore used as the terminator in all the other experiments.

TABLE I

CALIBRATION CONSTANTS AND ZONE LENGTHS WITH HISTIDINE–HYDROCHLORIC ACID AS THE LEADING ELECTROLYTE

Ionic species	*Concentration in the sample (mole/l)*	*Concentration in the zone (mole/l)*	*Injected volume (μl)*	*Detected zone length (sec)*	*Calibration constant ($K_{cal} \cdot 10^4$)*	*Deviation from average K_{cal}*	
						$\Delta K_{cal} \cdot 10^4$	%
Succinic acid	0.01	0.0051	4	163	0.4812	−1.73	−
Acetic acid	0.05	0.0085	3	358.5	0.4923	−0.62	−
Adipic acid	0.025	0.0046	3	335	0.4867	−1.18	−
Formic acid	0.05	0.0093	3	311	0.5186	2.01	
Iodic acid	0.05	0.0085	3	350	0.5042	0.57	
Lactic acid	0.031	0.0081	3	222	0.5172	1.87	
β-Chloro propionic acid	0.05	0.0081	3	370	0.5005	0.20	
Succinic acid	0.01	0.0051	3	119	0.4943	−0.42	−
Sulphamic acid	0.05	0.0090	3	335	0.4975	−0.10	−
Tartaric acid	0.025	0.0048	3	320	0.4883	−1.02	−
Acetic acid	0.05	0.0085	2	234	0.5028	0.43	
Adipic acid	0.025	0.0046	2	223	0.4874	−1.11	−
Iodic acid	0.05	0.0085	2	231	0.5093	1.08	
Maleic acid	0.05	0.0057	2	349	0.5027	0.42	
Tartaric acid	0.025	0.0048	2	213	0.4891	−0.94	−
Acetic acid	0.05	0.0085	1	120	0.4902	−0.83	−
Formic acid	0.05	0.0093	1	105	0.5120	1.35	
Average					0.4985	0.93	

DETERMINATION OF THE CALIBRATION CONSTANT

The calibration constant was determined from experiments carried out with histidine and hydrochloric acid at pH 6.02 as the leading electrolyte. The concentration of the leading ion (chloride) was 0.01 *N*. The electric current was stabilised at 70 μA. All zone lengths are listed in Table I. The third column in Table I shows the actual total concentrations of the ionic species, calculated by using the computer program mentioned earlier[2]. The last two columns show the deviations from the average K_{cal}. Reasonable values were obtained, which could be improved if more precise values were available for the mobilities.

TABLE II

CALIBRATION CONSTANTS AND ZONE LENGTHS WITH IMIDAZOLE–HYDROCHLORIC ACID AS THE LEADING ELECTROLYTE

Ionic species	*Concentration in the sample (mole/l)*	*Concentration in the zone (mole/l)*	*Injected volume (μl)*	*Detected zone length (sec)*	*Calibration constant ($K_{cal} \cdot 10^4$)*	*Deviation from average K_{cal}*	
						$\Delta K_{cal} \cdot 10^6$	%
Acetic acid	0.05	0.0075	3	467	0.4283	−0.82	−1.9
Adipic acid	0.025	0.0042	3	407	0.4388	0.23	0.5
Formic acid	0.05	0.0087	3	398	0.4327	−0.38	−0.8
Hydrofluoric acid	0.05	0.0087	3	409	0.4215	−1.50	−3.4
Iodic acid	0.05	0.0074	3	465	0.4364	−0.01	0.0
Lactic acid	0.0343	0.0069	3	340	0.4386	0.21	0.5
Maleic acid	0.05	0.0046	3	735	0.4437	0.72	1.6
Tartaric acid	0.025	0.0042	3	416	0.4290	−0.75	−1.7
Acetic acid	0.05	0.0075	2	308	0.4329	−0.36	−0.8
Formic acid	0.05	0.0087	2	255	0.4508	1.43	3.3
Maleic acid	0.05	0.0046	2	491	0.4428	0.63	1.4
Acetic acid	0.05	0.0075	1	154	0.4329	−0.36	−0.8
Formic acid	0.05	0.0087	1	129	0.4455	0.90	2.1
Average					0.4365	0.64	1.5

A similar determination of the calibration constant was carried out with imidazole and hydrochloric acid at pH 7.05 as the leading electrolyte. The concentration of the leading ion (chloride) was 0.01 *N*. The electric current was stabilised at 70 μA. All zone lengths measured in this system are listed in Table II. The last two columns show the deviations from the average K_{cal}. In this system also reasonable constancy of the calibration constant was obtained.

It should be remembered that the influence of the activity coefficients on the concentrations are neglected in the calculation of the actual concentration of the ionic species.

DETECTION LIMITS

From our experiments, we can state that the minimum detectable zone length is about 5 mm, using thermometric detection. This value can vary, depending on the heat-production of the adjacent zones, the electric current, the type of solvent used and some other minor factors.

The concentration of an ionic species in the capillary tube is about 0.01 g-equiv./l under the conditions used, and the cross-section of the capillary tube is about $1.6 \cdot 10^{-3}$ cm^2. This means the minimum amount of an ionic species that can be detected is about $8 \cdot 10^{-9}$ g-equiv. If the volume of the sample injected is 3 μl, the minimum concentration in the sample that can be detected is about $2.7 \cdot 10^{-3}$ g-equiv./l.

To illustrate the above, a separation of a mixture of 0.005 *N* oxalate, 0.01 *N* formate, 0.01 *N* acetate and 0.015 *N* β-chloropropionic acid in the system histidine–hydrochloric acid is shown in Fig. 2. Figs. 2a, b and c correspond to injected volumes

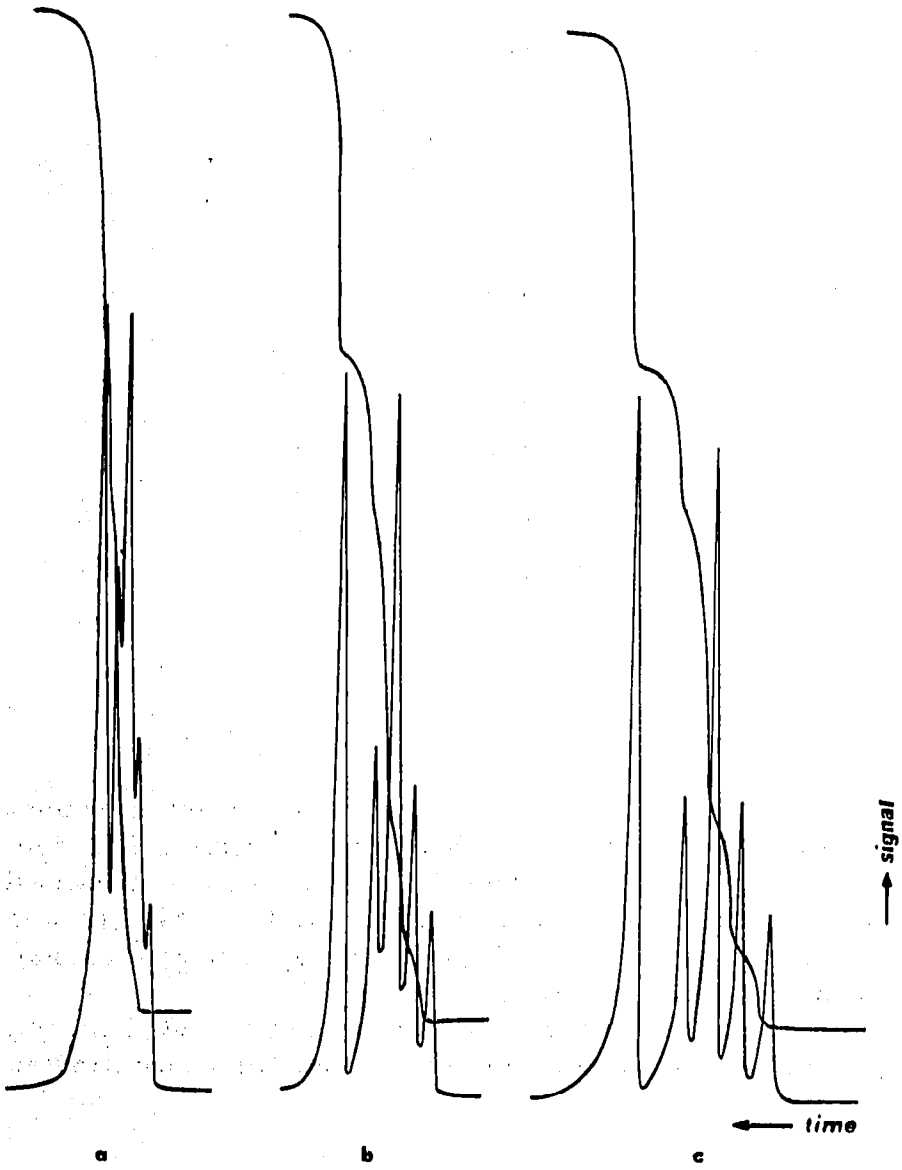

Fig. 2. Electropherograms for the separation of some anions in the system histidine–hydrochloric acid. The injected volumes were (a) 1, (b) 2 and (c) 3 μl.

of 1, 2 and 3 μl, respectively. This means that the amounts detected are $5 \cdot 10^{-9}$, 10^{-8}, 10^{-8} and $1.5 \cdot 10^{-8}$ g-equiv., respectively, for the different anions, if 1 μl is injected. It can be stated that Figs. 2b and c show a complete separation of the mixture to be separated, both quantitatively and qualitatively. All the quantitative information can still be deduced from Fig. 2a, for it should be remembered that for quantitative analyses the transition of zone boundaries is required. The differential signals give exactly the amounts of the ionic species introduced*. This in contrast with a gas chromatogram, with which Fig. 2a would be considered to represent an incomplete separation.

* The time interval between two succesive peaks, measured with a stop-watch, is given in seconds. The use of electronic equipment for measuring the time intervals more accurately can decrease these limits. The introduction of another type of detector can decrease these limits still further.

The detection limit for the minimum detectable amount can be decreased by using a leading electrolyte with a lower concentration. If the concentration of the leading electrolyte is decreased towards 10^{-3} N, the minimum detectable amount of an ionic species would be $8 \cdot 10^{-10}$ g-equiv., *i.e.*, a minimum concentration in the sample of $2.7 \cdot 10^{-4}$ g-equiv./l, if a volume of 3 μl is injected into the system.

The detection limit for the concentration in the sample can be decreased by injecting a larger volume, *e.g.*, with a sample tap. The sample taps that we commonly use have a volume of *ca.* 30 μl. Consequently, the minimum detectable concentration in the sample will decrease by a factor of 10. If the average concentration of the ionic species in the sample is low, a sample tap is therefore recommended*.

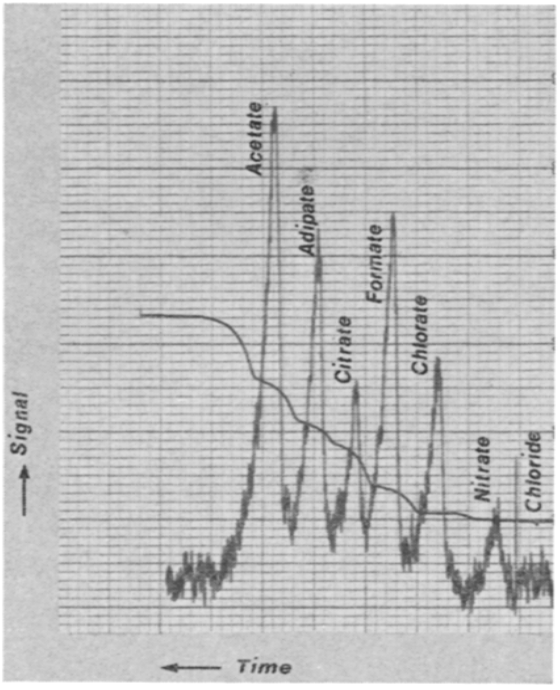

Fig. 3. Electropherogram of the separation of some anions with histidine–hydrochloric acid as the leading electrolyte at low concentration (10^{-3} N) and pH 6.02.

To study the possibility of carrying out analyses with a leading electrolyte of concentration 10^{-3} N, some experiments were performed with histidine and HCl at pH 6.02 as the leading electrolyte. The concentration of the leading ion (chloride) was 0.001 N. Because the driving potential available is limited, the electric current must be decreased to 7 μA. Fig. 3 shows the electropherogram for the separation of nitrate, chlorate, formate, citrate and adipate, with acetate as the terminator. A complete separation was easily obtained. A disadvantage may be the very small signals obtained from the thermocouples if the driving current is lowered too much. The signals must be amplified so that the signal-to-noise ratio decreases, as can be seen in Fig. 3.

* Large amounts of other ionic species present in the sample can disturb a complete separation because the steady state is difficult to attain.

DISCUSSION

It was shown that reproducible analyses can be carried out when the sample is introduced into the apparatus with a syringe. However, the construction of the injection block still causes some disadvantages in the introduction of the sample. The sample introduced is mixed with the leading electrolyte, and the sample will not form a plug separated from the leading electrolyte and the terminating electrolyte, as is the case when using a sample tap. This effect could be made visible by the injection of dyes.

The position of the leading electrolyte behind the sample, between the septum and the plunger compartment (Fig. 1), is another disadvantage. The ions with charges similar to those of the ions to be separated have to overtake the sample ions in order to give a complete separation, which means that the length available for the separation is not used only for the separation of the sample ions. In particular, difficulties can be expected if the mobility of the leading ions does not differ greatly from at least one of the ions present in the sample.

The time required for the analyses depends on the length of the capillary tube needed for the separation, the electric current used, the type of leading and counter ions present, the pH, the differences in the mobilities of the sample ions, the injected volume, the concentrations of the sample ions, etc. The time required for analyses was *ca.* 45–60 min under the conditions described.

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