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ISOTACHOPHORESIS

QUANTITATIVE ASPECTS OF THE SEPARATION OF MIXTURES OF ANIONS

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SUMMARY

Quantitative aspects of isotachophoretic analysis have been considered and particular attention was paid to the regions of low concentration, necessitating the use of automatic measuring equipment. Zones of pure samples were studied and the mutual influences of the zones on each other were examined. The appearance of mixed zones is briefly discussed.

INTRODUCTION

In a previous paper¹, some quantitative aspects of the separation of mixtures of anions have been described. The use of an injection block, instead of supplying a fixed volume of sample via a tap, facilitates work with sample solutions of different concentrations. Because thermometric detection was applied in the earlier work¹, a minimum zone length of 0.5 cm was required in order to permit a satisfactory detection by this method. The highest linearity and reproducibility were found, however, when small amounts of sample were introduced in the capillary, resulting in small zone lengths. The use of a high resolution detector² permitted much more work to be carried out in the region of low concentrations in which we were particularly interested, and the present work is therefore a continuation of that described earlier¹.

EXPERIMENTAL

We used an instrument for the automatic recording of the zone lengths, as described by Mulder and Zuska³, which enabled small time intervals to be used between successive peaks (with a minimum of 0.3 sec).

For the study of the mutual influence of the zones, a test mixture of three anions (nitrate, chlorate and acetate) was prepared.

Doubly distilled water was used as a solvent and all the chemicals used were of p.a. grade (E. Merck, Darmstadt, G.F.R.).

While in the previous work¹ two different leading electrolytes (operational

systems) were tested, in this study only the system histidine and histidine·HCl at pH 6 was used (the concentration of hydrochloric acid was 0.01 *N*), because the system itself does not influence the reproducibility of the analysis.

In order to make the time of analysis as short as possible (10 min), a capillary of about 15 cm was used for the separation of the mixture.

Because the injection is made in the leading electrolyte and not on the boundary between the leading and terminating electrolytes, a considerable time is needed in order to separate the chloride from the injected sample, especially if nitrate is present or the sample is concentrated. Owing to the short length of the capillary used, mixed zones are formed if nitrate at a concentration of 0.1 *N* is injected. However, as mentioned above, our main interest was in the region of low concentrations.

Calibration

As described previously¹, a calibration constant can be applied in order to remove the necessity for constructing calibration curves for each ionic species. This calibration constant can be defined as:

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

where

K_{cal} = the calibration constant

V_i = the volume of the sample injected (ml)

C = the concentration of a particular ionic species (mole/cm³)

C^* = the actual concentration of the ionic species in its zone (mole/cm³)

L^* = the zone length of a particular ionic species, measured in seconds

RESULTS AND DISCUSSION

Tables I, II and III show the results of the analyses with nitrate, chlorate and acetate. In addition to the pure components, mixtures of them were also injected. The concentrations were chosen such that a 0.5- μ l volume could be injected each time.

TABLE I

ZONE LENGTHS AS FOUND EXPERIMENTALLY AT CONCENTRATIONS OF 0.0125 *N*

<i>Ion</i>	L^*						$K_{\text{cal}} \cdot 10^4$	
NO ₃ ⁻	13.5	—	—	14.1	—	13.8	14.1	0.448
ClO ₃ ⁻	—	13.5	—	13.8	14.1	—	13.8	0.467
CH ₃ COO ⁻	—	—	15.6	—	15.9	16.2	16.2	0.462

TABLE II

ZONE LENGTHS AS FOUND EXPERIMENTALLY AT CONCENTRATIONS OF 0.025 *N*

<i>Ion</i>	L^*						$K_{\text{cal}} \cdot 10^4$	
NO ₃ ⁻	27.9	—	—	28.2	—	27.9	28.2	0.448
ClO ₃ ⁻	—	28.5	—	28.2	27.9	—	28.2	0.457
CH ₃ COO ⁻	—	—	32.1	—	31.8	31.8	31.8	0.459

TABLE III
ZONE LENGTHS AS FOUND EXPERIMENTALLY AT CONCENTRATIONS OF 0.05 *N*

<i>Ion</i>	<i>L</i> *						<i>K_{cat} · 10⁴</i>	
NO ₃ ⁻	57.3	—	—	56.7	—	56.7	57.3	0.443
ClO ₃ ⁻	—	56.7	—	57.3	57.6	—	57.9	0.452
CH ₃ COO ⁻	—	—	63.6	—	62.7	62.7	64.8	0.460

The current in all analyses was stabilized at 70 μ A. Glutamic acid was used as the terminating electrolyte at a concentration of approximately 0.01 *M*. Average results from two experiments are listed in the tables.

The actual concentrations of the ionic species in the zones, in the steady state, moving behind the leading electrolyte were computed with a computer program described by Beckers⁴. As expected, a completely linear relationship, between zone-length and amount of component injected, was found in the region of low concentrations.

For the computation of the calibration constants, an average of the values listed in Tables I, II and III was taken. The deviations from the average calibration constant can be ascribed to the use of a microsyringe for sample introduction. These values are listed in Table IV. The results obtained agree with those presented earlier¹.

TABLE IV
CALIBRATION CONSTANTS DETERMINED FROM THE VARIOUS ELECTROPHEROGRAMS

<i>Ion</i>	<i>Concentration (N)</i>	<i>K_{cat} · 10⁴</i>	<i>Deviation in K_{cat} · 10⁷</i>	<i>% Deviation</i>
NO ₃ ⁻	0.0125	0.448	-7	1.4
ClO ₃ ⁻		0.467	+12	2.6
CH ₃ COO ⁻		0.462	+7	1.3
NO ₃ ⁻	0.025	0.448	-7	1.4
ClO ₃ ⁻		0.457	+2	0.4
CH ₃ COO ⁻		0.459	+4	0.9
NO ₃ ⁻	0.05	0.443	-12	2.7
ClO ₃ ⁻		0.452	-3	0.7
CH ₃ COO ⁻		0.461	+6	1.3
Average		0.455		1.43

The use of an automatic device for printing the time intervals between successive peaks proved the expected linearity of the results, which was only partially proved in the previous work¹.

The zones do not have a mutual influence on each other, as shown in Table IV. Mixed zones were found between the pure sample zones if too high concentrations were applied.

Fig. 1 shows the step heights, as found in the electropherograms, as a function of the ratio of the concentrations of the sample constituents. A linear relationship between the nitrate and chlorate zones was found, whereas a non-linear relationship was obtained between the chlorate-acetate and nitrate-acetate zones. This effect

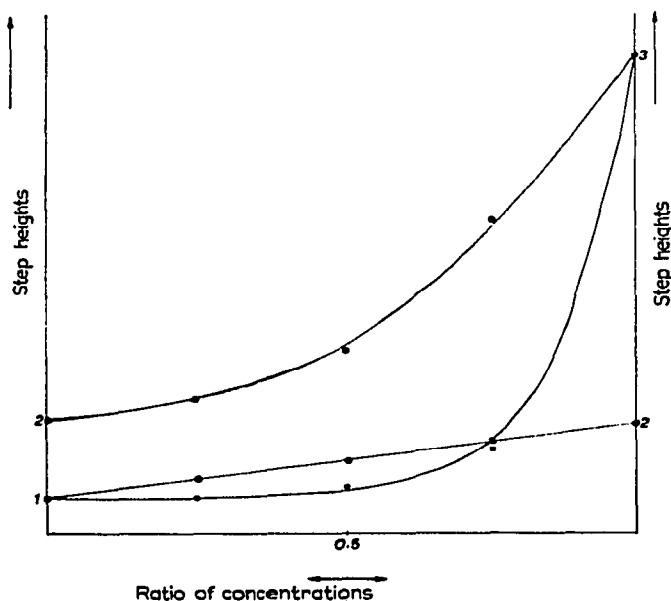


Fig. 1. Step heights of some mixed zones as measured in isotachophoretic experiments: 1 = nitrate; 2 = chlorate; 3 = acetate.

cannot be ascribed to the difference in pH values of the successive zones: for chloride, nitrate, chlorate and acetate pH values of 6.02, 6.03, 6.04 and 6.12, respectively, can be expected.

The difference of only 0.1 pH unit with respect to the p*K* value of acetic acid cannot explain the large difference in conductivity, as recorded by the measuring electrodes. A more reasonable explanation must be found in the temperature dependence of the conductivity. Between the nitrate–acetate and chlorate–acetate zones, a much more pronounced temperature difference is registered than between the nitrate–chlorate zones. Table V shows that the nitrate and chlorate ions in particular are more influenced than the acetate ion by a change in the temperature. The influence of temperature variations on the p*K* value of especially the cation (counter-ion) must also be taken into consideration.

TABLE V

DEPENDENCE OF CONDUCTIVITY ON TEMPERATURE

C = concentration (mole/l); *T* = temperature (°C); *Λ* = conductivity ($\Omega^{-1} \text{ cm}^2/\text{g-equiv.}$).

<i>Ion</i>	<i>C</i>	<i>T</i>	<i>Λ</i>	<i>Λ/°C</i>
NO ₃ ⁻	0.01	0	34.4	~1.7
	0.01	35	92.8	
ClO ₃ ⁻	0.01	0	30.2	~1.1
	0.01	35	67.5	
CH ₃ COO ⁻	0.01	0	17.5	~0.74
	0.01	35	43.2	

Because mixed zones may obscure the isotachopherogram and hinder both qualitative and quantitative interpretations, Fig. 1 shows that the step height of a mixed zone may differ from one particular case to another.

Although in the application of the universal conductivity detector the recording, especially in this particular case, of the mixed zone is influenced by the pH, this small variation in pH can be amplified if other types of detectors are used, *e.g.*, a UV detector. Changes in pH can have a greater influence on the absorption of chemical components than on the conductivity.

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