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## HIGH-SPEED ISOTACHOPHORESIS

### EFFECT OF DRIVING CURRENT ON THE QUALITATIVE INTERPRETATION OF ISOTACHOPHEROGRAMS

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#### SUMMARY

Joule heating has a significant effect on the temperatures of the zones in high-speed analytical isotachophoresis and thus the effective mobilities of species separated. The signal detected by a detector monitoring the electric field gradient or the electric conductivity of zones is dependent on the driving current used, and, moreover, the qualitative data (in terms of relative mobilities) obtained even in one run represent ratios of mobilities at mutually different temperatures. Relationships were derived for the effect of Joule heating on the qualitative data, and a procedure was proposed and experimentally verified for standardization of qualitative isotachophoretic data by extrapolating the data measured at various driving currents and thermostating temperatures to a given standard temperature and negligible Joule heating. The standardized values thus obtained represent a unified system of mutually comparable qualitative data and may serve as a valuable aid for identification.

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#### INTRODUCTION

In electrophoresis the substances separated are conveniently identified by their mobilities and migration rates. Among many factors influencing these electrophoretic parameters (for a review, see *e.g.* ref. 1) one of the most important is temperature. Hence, when absolute values of electrophoretic mobilities are determined the temperature of measurement must be known, and comparisons of mobilities as well as migration rates for identification purposes should be related to the same temperature (*cf.*, ref. 2). It must be borne in mind that the actual temperature in a zone is higher than the ambient value (thermostating temperature) owing to the wattage applied during the experiment.

The determination of mobilities in Tiselius moving boundary technique<sup>3</sup> and in free zone electrophoresis<sup>4</sup> is, in principle, based upon measuring the migration velocity of a zone in a buffered medium, where the electric field strength has the same value

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everywhere in the electrophoresis migration path (even when a temperature gradient is present) and the electrical conductivity in the migrating zone is that of the buffer solution. The Joule heat production is uniform along the migration path and different zones during one electrophoretic run migrate at the same temperature. Then all the values of the mobilities determined are related to the same temperature at which the conductivity of the buffer solution was measured (for details see ref. 4).

In zone electrophoresis in stabilizing media the determination of migration velocities provides the basic experimental data for identification. As in the previous case, all zones migrate at the same temperature. To assess the increase of the temperature due to the Joule heating during the electrophoretic run several procedures and relationships were proposed<sup>5,6</sup>; once the temperature increase has been determined, the actual temperature is known for all zones. Since the stabilizing media influence the migration in many ways (adsorption, tortuosity, etc.) the determination of absolute values of mobilities from migration velocities is difficult, and, for identification purposes, the relative migration rates and relative distances travelled are widely used<sup>7-9</sup>.

In isotachopheresis, the basic experimental data used for identification are not derived from migration velocities, however, they are derived from detector response to a suitable zone property. The potential gradient detector<sup>10,11</sup> and a.c. conductivity detector<sup>12-14</sup> are currently used for this purpose, where their signals are inversely proportional to the effective mobility of an ionic species in the detected zone. In the record of the signal, isotachopherogram, step-height corresponds to the signal magnitude, and, under constant conditions, identification of zones is carried out by comparing step-heights with those of standard substances. An inherent feature of isotachopheresis is that the electric field strength is different in different zones and, thus, Joule heat productions and mean temperatures are different in different zones even in one separation run. In such a situation, each one of the step-heights in the isotachopherogram corresponds not only to an individual species, but also to an individual temperature in the respective zone. Hence, the data measured in different types of columns at various driving currents and thermostating temperatures are difficult to compare and their use for identification is limited.

This paper aims to establish a procedure for standardization and unification of qualitative data provided by the potential gradient detector in analytical isotachopheresis. The procedure eliminates the effect of different Joule heatings in different zones and provides qualitative data representing relative effective mobilities at a given standard temperature.

## THEORETICAL

A compound, X, is characterized in an isotachopherogram, by a step-height,  $h_x$ ,

$$h_x = f_1 E_x = f_2 \frac{1}{\kappa_x} = f_3 \frac{1}{u_x} \quad (1)$$

where  $E_x$  and  $\kappa_x$  represent actual values of potential gradient and specific conductivity in the zone of species X,  $u_x$  denotes the effective mobility of species X and  $f_1$ ,  $f_2$  and  $f_3$  are instrumental constants of the detection system. To eliminate the

unknown factors  $f$ , two types of qualitative indices representing relative step-heights are conveniently used<sup>11,15-17</sup>

$$\Delta_X(R) = \frac{h_R}{h_X} = \frac{E_R}{E_X} = \frac{\kappa_X}{\kappa_R} = \frac{u_X}{u_R} \quad (2)$$

$$\delta_X(A, B) = \frac{h_X - h_A}{h_B - h_A} \quad (3)$$

where subscript  $X$  denotes the species in question and subscripts  $R, A, B$  represent suitable reference species separated in the same run. Since these indices can be mutually interconverted by using

$$\delta_X(A, B) = \frac{1/\Delta_X(A) - 1}{1/\Delta_B(A) - 1} \quad (4)$$

we only need one type of qualitative index. For simplicity, the index  $\Delta_X(R)$  has been chosen. The actual value of this index, which is determined directly from an isotachopherogram, corresponds to a fixed pair of different temperatures in the zones  $X$  and  $R$  and thus it is dependent on the driving current and thermostating temperature.

Let us call the standard state of zones the case where all zones have the same standard temperature,  $T_s$ . Such a standard state can be approached by carrying out the separation at negligible Joule heating and at thermostating temperature  $T = T_s$ . We can define a standard index,  $\Delta_X^s(R)$ ,

$$\Delta_X^s(R) = \frac{u_X^s}{u_R^s} = \frac{\kappa_X^s}{\kappa_R^s} \quad (5)$$

where  $\kappa^s$  and  $u^s$  represent specific conductivity and effective mobility under standard conditions. This standard index is dependent on the leading electrolyte composition only and is thus convenient for tabulation and for identification purposes. The more the actual temperature in zones deviates from the standard temperature the more the actual index,  $\Delta_X(R)$ , deviates from the standard index,  $\Delta_X^s(R)$ .

The actual temperature in a zone is the superposition of a thermostat temperature  $T_0$  and the increase due to the Joule heating. This increase can be expressed by the product  $Q \cdot P$ , where  $Q$  is the quotient representing the thermostating efficiency and  $P = E \cdot I$  is the power dissipated in a unit length of column used (for details see ref. 18). Assuming linear dependence of effective mobilities on the temperature, we can write for the actual specific conductivity

$$\kappa = \kappa^s (1 + \alpha Q \cdot P + \alpha \Delta T) \quad (6)$$

where  $\Delta T = T_0 - T_s$  is the difference between thermostating and standard temperatures, and the temperature coefficient,  $\alpha$ , involves the effect of temperature changes on the concentration of the ionic species in their zones.

Let us define a standard potential gradient,  $E^s$ , as the potential gradient in a zone under standard conditions when driving current  $I$  is applied

$$E^s = \frac{I}{Sx^s} \quad (7)$$

where  $S$  is the column cross-section. For a given species in its zone, the standard potential gradient is apparently dependent on the driving current and on the column cross-section. However, when determined for species X and R separated in one run, the ratio of  $E_X^s$  and  $E_R^s$  is given by:

$$\frac{E_R^s}{E_X^s} = \frac{x_X^s}{x_R^s} = \Delta_X^s(R) \quad (8)$$

$E^s$  may be determined by combining eqns. 7 and 6:

$$E^s = E(1 + \alpha\Delta T) + \alpha Q \cdot E^2 \cdot I \quad (9)$$

By measuring the actual values of  $E_X$  and  $E_R$  at driving current  $I$  and at thermostating temperature  $T_0$ , assuming the values of  $\alpha_X$ ,  $\alpha_R$  and  $Q$  are known, the application of eqn. 9 to species X and R in combination with eqn. 8 gives the required standard index  $\Delta_X^s(R)$ . The quotient  $Q$  can be eliminated by running two separations at different driving currents  $I_1$  and  $I_2$ , determining the values of  $E_{X1}$  and  $E_{X2}$  and by applying eqns. 9 and 7:

$$\frac{1}{Sx_X^s} = \frac{E_{X1}^s}{I_1} = \frac{E_{X2}^s}{I_2} = (1 + \alpha_X\Delta T) \frac{(E_{X1}/I_2) - (E_{X2}/I_1)}{E_{X1}^2 - E_{X2}^2} E_{X1}E_{X2} \quad (10)$$

The application of eqn. 10 to species X and R separated simultaneously, in combination with eqn. 8, gives the index  $\Delta_X^s(R)$ . The parameters  $\alpha_X$  and  $\alpha_R$  can also be eliminated by selecting the thermostating temperature to be equal to the required standard temperature and/or by choosing  $T_0$  as the standard temperature when data for identification are tabulated. Then, the standardization procedure which eliminates Joule heat effects is carried out by running two analyses at thermostating temperature  $T_0 = T_s$  and at driving currents  $I_1$  and  $I_2$ . In each isotachopherogram the step-heights of species X and R,  $h_{X1}$  and  $h_{R1}$ , and  $h_{X2}$  and  $h_{R2}$ , respectively, are determined, and the standard index is calculated by using a combination of eqns. 10, 8 and 1:

$$\Delta_X^s(R) = \frac{h_{R1}}{h_{X1}} \cdot \frac{h_{R2}}{h_{X2}} \cdot \frac{h_{X1}^2 - h_{X2}^2}{h_{R1}^2 - h_{R2}^2} \cdot \frac{(h_{R1}/I_2) - (h_{R2}/I_1)}{(h_{X1}/I_2) - (h_{X2}/I_1)} \quad (11)$$

## EXPERIMENTAL

The isotachopheretic column used consisted of a block of organic glass containing electrode chambers, injection port, control valves and sensing electrodes

of the potential gradient detector. The separations were performed in a capillary of rectangular cross-section, dimensions  $20 \times 0.1 \times 0.02$  cm, created by a groove in the organic block and covered with PTFE foil pressed on the block with a thermostatted metal plate. A high-voltage constant-current power supply controllable up to  $400 \mu\text{A}$  at a maximum of 16 kV was used. Potential gradients in zones were detected by a device consisting of a voltmeter with a high input resistance and recorded by means of a Servogor RE 571 line recorder (Goertz, Vienna, Austria). A detailed description of the isotachopheretic column, power supply and detection system can be found elsewhere<sup>11,19</sup>. The chemicals used were of analytical grade (Lachema, Brno, Czechoslovakia). The thermostatted metal plate was maintained at  $288^\circ\text{K}$  by means of circulating water. The leading electrode compartment was filled with  $0.005 \text{ M}$  NaBr adjusted to  $\text{pH} = 9$  with sodium hydroxide.  $0.0025 \text{ M}$  picric acid, similarly adjusted to  $\text{pH} = 6$ , was used as terminating electrolyte. A model mixture of nitrate, chlorate, bromate and iodate was used as sample. The separations were carried out at 350, 175 and  $87.5 \mu\text{A}$ .

## RESULTS AND DISCUSSION

The values of the index  $\Delta_x(\text{Br})$  were determined by using eqn. 2 and step-heights measured from isotachopherograms of the model mixture at driving currents of 350, 175 and  $87.5 \mu\text{A}$ . The value of  $350 \mu\text{A}$  was close to the maximum output of the constant-current power supply for a given electrolyte system. The power dissipated in the iodate zone at  $350 \mu\text{A}$  was *ca.*  $0.13 \text{ W/cm}$  and the corresponding temperature increase due to Joule heating was *ca.*  $21^\circ\text{K}$  (ref. 18). The temperature increase in the leading electrolyte at this current was *ca.*  $13^\circ\text{K}$ . The values of the standard index  $\Delta_x^s(\text{Br})$  were determined by using eqn. 11 and step-heights measured at 350 and  $175 \mu\text{A}$ . The effect of the driving current on the qualitative characteristics of the isotachopherogram is illustrated in Fig. 1, which shows the ratios  $h_x/h_{\text{Br}}$  obtained at 350, 175 and  $87.5 \mu\text{A}$ . It is obvious that driving current has a significant effect on the qualitative shape of the isotachopherogram, and the lower the effective mobility of

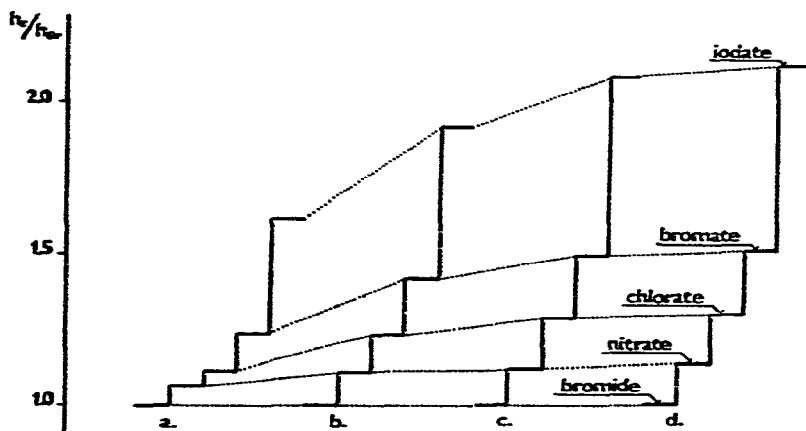


Fig. 1. The effect of the driving current on the qualitative characteristics of an isotachopherogram. Experimental values of  $h_x/h_{\text{Br}}$  were obtained at driving current,  $I$ , of  $350 \mu\text{A}$  (a),  $175 \mu\text{A}$  (b) and  $87.5 \mu\text{A}$  (c). Theoretical values of  $h_x/h_{\text{Br}} = 1/\Delta_x^s(\text{Br})$  corresponding to  $I = 0$  are also shown (d).

a species the larger is the effect upon relative step-height. Fig. 1 also shows the theoretical shape of the isotachopherogram obtained by plotting  $h_x/h_{Br} = 1/\Delta_x^*(Br)$ .

The experimental values of the indices measured at 350, 175 and 87.5  $\mu A$  are given in first three columns of Table I. The indices measured at 87.5  $\mu A$  can be considered as a good approximation of measurements at standard state, since the highest temperature increase due to Joule heating, *i.e.*, in the iodate zone, is *ca.* 1.3°K. The fourth column in Table I gives values of the standard index calculated by using eqn. 11 and data from analyses at 350 and 175  $\mu A$ . There is good agreement between the standard index and the index measured at negligible Joule heating. This means that the standard index can be considered as a good qualitative characteristic which is independent of the driving current used.

TABLE I

COMPARISON OF EXPERIMENTAL VALUES OF THE INDEX  $\Delta_x(Br)$  WITH CALCULATED STANDARD INDEX  $\Delta_x^*(Br)$

Species	$\Delta_x(Br)^*$			$\Delta_x^*(Br)$ (0 $\mu A$ )
	350 $\mu A$	175 $\mu A$	87.5 $\mu A$	
Bromide	1.00	1.00	1.00	1.00
Nitrate	0.94	0.90	0.89	0.88
Chlorate	0.90	0.81	0.78	0.77
Bromate	0.81	0.71	0.67	0.66
Iodate	0.62	0.52	0.48	0.47

\* Average from five determinations. Confidence interval  $\pm 0.01$ .

## CONCLUSIONS

The temperature in an isotachophoretic zone is the result of the superposition of Joule heating and the thermostating temperature. Owing to the different heat productions in different zones during a separation run, the actual values of the effective mobilities of ionic species in their zones shift non-uniformly from the values valid at thermostating temperature. Thus, small changes of the driving current may have a large effect on the shape of an isotachopherogram. Consequently, the values of the qualitative index  $\Delta_x(R)$  measured at different driving currents are significantly different.

The standardization procedure proposed eliminates the temperature differences among different zones by extrapolating actual values of their specific conductivities to a standard state where all zones have the same temperature. The values of the standard index calculated by eqn. 11 represent consistent mutually comparable data at the same standard temperature and in good agreement with data measured at negligible Joule heating. Thus the standardization procedure proposed can replace laborious time-consuming measurements at low driving currents when qualitative indices for identification are needed.

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