

STATIONARY MEAN TEMPERATURES AND RADIAL TEMPERATURE PROFILES IN CAPILLARY ISOTACHOPHORESIS

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The increase of the mean temperature of the zones effected by the Joule heat was compared for three types of columns for isotachophoresis, with equal migration velocities of the separated components. A method employing the experimental mean temperature rise per unit electric power was suggested for the calculation of the radial temperature profiles in symmetrically cooled columns with circular cross sections. The radial temperature profile was calculated and plotted for a particular case corresponding to common working conditions. The difference between the temperature in the column centre (temperature maximum) and that at the column wall was found to be essentially lower than the overall temperature increase in the zone represented by the difference of the mean temperature of the zone and the thermostat temperature, which in the case treated amounted to 4.5 K.

The problems of electrophoretic techniques involve inherently also the effects associated with the generation of the Joule heat in the electrophoretic separation system. This Joule heat brings about not only a temperature increase during the electrophoresis (relative to the temperature of the thermostat or to the starting currentless state), but also the occurrence of temperature gradients. The temperature increase causes also changes of electrophoretic mobilities of the separated substances. More substantial heatings may result in a decomposition of the substances present in the column or in objectionable irreversible changes such as denaturation of proteins in the sample, *etc.* In closed column systems the heating can give rise to bubbles breaking the current circuit and so interrupting the course of the electrophoresis. The temperature gradients imply variations in the density of the medium, which results in convection disturbing the sharpness of the zone boundaries. All the temperature effects appear also in isotachophoresis, the latest electrophoretic technique, the capillary modification of which appears now as a promising analytical¹ and micropreparative² method.

During isotachopheresis, substances of different effective mobilities are separated from one another; a system results with zones following each other closely and migrating with the same velocity³. The effective mobility of a substance migrating in an isotachopheretic zone conforms to the mean temperature of the zone; from the temperature we are often able to infer in advance whether or not the desired separation will take place⁴. An increase of the mean temperature can preclude the separation of substances displaying a small difference of mobilities, since in the zone of the substance possessing lower mobility the temperature increase is higher and the mobility difference decreases. The deterioration of the boundary sharpness of the isotachopheretic zones aggravates the detection of very short zones, so that the potentialities of the high-resolution detectors cannot be fully utilized⁵⁻⁹. Striving for rapid isotachopheretic analyses¹⁰ results in application of high voltage gradients and so in the use of high electric current densities within the column. In this manner, however, a considerable Joule heat is generated, with all the negative consequences. If the current circuit is broken by a gas bubble, the voltage of the constant current supply, commonly used in isotachopheretic instrumentation, rises up to the maximum; the insulation of the column may then break down, whereby the column would be destroyed. An efficient and uniform cooling of the column is therefore a prerequisite for high-speed isotachopheresis. A measure of the cooling efficiency is the column temperature rise over the temperature value of the thermostatic medium. A recalculation of such a temperature rise in different column types to equal migration velocity and thus to equal duration of analysis allows a comparison of the cooling efficiency in these columns. It is important to know the radial temperature profiles, too, as they affect the character of the detector signal and thus its qualitative as well as quantitative interpretation; this has been pointed out in the study¹¹ comparing detection with a UV and a conductivity detector. The role of the radial temperature gradients in the range of the zone interface has been described only qualitatively¹².

In order to assess the effect of the temperature phenomena, we compare in this work the heating in the steady-state for three types of isotachopheretic columns at equal migration velocities; in addition, we suggest a practical method of determination of the radial profiles in symmetrically cooled columns of circular cross sections.

THEORETICAL

For a description of the increase of the steady-state mean temperature of isotachopheretic zones we have introduced⁴ the quantity \bar{Q} defined as

$$\bar{Q} = (\bar{T} - T_0)/P, \quad (1)$$

where \bar{T} is the steady-state mean temperature in the zone, T_0 is the temperature of the thermostatic medium, and P is the electric power dissipated over a unit length of the column. For a comparison of various column types from the point of view of the thermostatic efficiency we have applied the quantity $\bar{Q}S$, where S is the cross section of the column filled with the electrolyte. This quantity represents the increase of the mean temperature caused by unit mean power in a unit volume. The quantity \bar{Q} is virtually constant for a column in a wide range of thermostat temperatures and of dissipated power. The value \bar{Q} being once determined for the column treated,

the increase of the mean temperature of the zones can be determined in advance for different working conditions, and, in addition, the determination of the radial temperature profile in symmetrically cooled columns of circular cross section can be appreciably simplified.

Consider a linear dependence of the electrolyte conductivity on the temperature:

$$G_T = G_0[1 + \alpha(T - T_0)], \quad (2)$$

where G_T and G_0 are the electric conductivities at the temperatures T and T_0 , respectively, and α is the temperature coefficient of the electrolyte electric conductivity (usually about 0.02 K^{-1}). For a model of the isotachophoretic zone where the equipotential surfaces are planes perpendicular to the direction of the zone migration, the heat transfer in the radial direction inside the column of circular cross section can be described by the equation^{13,14}

$$(d^2T/dr^2) + (1/r)(dT/dr) + (P_v/k_1)[1 + \alpha(T - T_0)] = 0, \quad (3)$$

where r is the radial distance, T the temperature, P_v the electric power dissipated at the given potential gradient in a unit electrolyte volume whose conductivity corresponds to the temperature T_0 , and k_1 is the thermal conductivity coefficient of the electrolyte. Denoting R_1 and R_2 the inner and outer diameters of the capillary, k_2 the thermal conductivity coefficient of the capillary walls, and H the coefficient of heat transfer to the cooling medium, we can rewrite Eq. (3), regarding the heat passage within the column walls, in the form^{4,14}

$$T(r) = T_0 - \alpha^{-1} + AJ_0(\beta r) \quad (4)$$

with

$$\beta = (P_v \alpha / k_1)^{1/2} \quad (5)$$

and

$$A = \alpha^{-1} \{ J_0(\beta R_1) - (k_1/k_2) \beta R_1 J_1(\beta R_1) [\ln(R_2/R_1) + (k_2/R_2 H)] \}^{-1}, \quad (6)$$

where $J_0(\beta r)$ and $J_1(\beta r)$ are Bessel functions.

It is often difficult to determine the H and P_v values, as these quantities are not immediately experimentally accessible. The problem can be, however, solved by employing the experimentally accessible P and \bar{Q} values. The power P is given by the relation¹³

$$P = 2\pi P_v \int_0^{R_1} [1 + \alpha(T - T_0)] r dr = 2\pi \alpha k_1 \beta R_1 J_1(\beta R_1). \quad (7)$$

Combining Eqs (1) and (2) we obtain an expression for the dependence of the conductivity on the electric power:

$$G_T = G_0[1 + \alpha \bar{Q}P]. \quad (8)$$

Regarding the electric potential in the radial section as constant we can write

$$P = P_v[1 + \alpha \bar{Q}P] \pi R_1^2. \quad (9)$$

By this equation the P_v value is determined. A combination of Eqs (5) and (9) leads to the value β and in combination with Eq. (7) also to the value A in the forms

$$\beta = \{\alpha P / [(1 + \alpha \bar{Q}P) \pi R_1^2 k_1]\}^{1/2}, \quad (10)$$

$$A = [1/2J_1(\beta R_1)] [(1 + \alpha \bar{Q}P) P / \pi k_1 \alpha]^{1/2}. \quad (11)$$

Having measured the \bar{Q} value we are able to determine the steady-state radial temperature profiles for a column rather simply by employing Eqs (4), (10), and (11). The k_1 value can be approximated for aqueous solutions by thermal conductivity of water, the α value is usually available from tables. The P value represents the working conditions chosen, *i.e.* the potential gradient and the driving current.

EXPERIMENTAL

The rise of the steady-state mean temperature with the power introduced was measured as described previously⁴; the electrolyte resistance in the column and the dissipated power are measured simultaneously. The measurements were performed for different thermostat temperatures in three types of hitherto used columns whose cross sections are drawn schematically in Fig. 1. The separation part of the first column (Column A, Fig. 1) was represented by a rectangular groove (1 × 0.2 mm), milled out in a plexiglas block, which was pressed tightly on a thermostatted metal block covered with PTFE foil about 0.2 mm thick. A detailed description can be found elsewhere¹⁰. The second column (Column B, Fig. 1) was manufactured from a capillary of circular cross section, wound up in a groove on the surface of an aluminium cylinder cooled with water. The cross section of the capillary amounted to $1.8 \cdot 10^{-7} \text{ m}^2$, the wall thickness was approximately 0.12 mm. The capillary with the aluminium cylinder was placed in an air bath. A detailed description of this type equipment is given elsewhere¹⁵. As the third column (Column C, Fig. 1) served a PTFE capillary of an instrument LKB 2127 Tachophor (LKB, Bromma, Sweden). The capillary, circular cross section $2.4 \cdot 10^{-7} \text{ m}^2$, was immersed in the coolant.

RESULTS AND DISCUSSION

The experimental \bar{Q} and $\bar{Q}S$ values for Columns A, B, C (Fig. 1) are given in Table I. This table comprises also the values of the electric current, power, and the corresponding heating calculated for a zone of glutamic acid, with 0.01M-HCl + 0.02M

β -alanine as the leading electrolyte, the migration velocity being considered always the same, *viz.* $4.17 \cdot 10^{-4} \text{ m s}^{-1}$ (2.5 cm/min). The data tabulated evidence that the elevation of temperature in the isotachophoretic capillary columns has to be taken into account, as an increase by 5 K can bring about a change of the mobilities of strong electrolytes by as much as 10%. In the case of weak electrolytes the situation may be further complicated by the temperature dependences of the dissociation constants¹⁶. The magnitude of the heating is determined by the contact with the thermostatic medium and the geometry of the cross section of the electrolyte column.

TABLE I

Comparison of the Values \bar{Q} , $\bar{Q}S$, Electric Current I , Electric Power Dissipated Over a Unit Column Length P , and the Mean Temperature Increase Relative to the Thermostat Temperature $T - T_0$, Calculated for the Same Migration Velocities

Column	\bar{Q} K m W^{-1}	$\bar{Q}S$ $10^{-7} \text{ K m}^3 \text{ W}^{-1}$	I μA	P W m^{-1}	$\bar{T} - T_0$ K
A	1.85 ± 0.04	3.90 ± 0.20	110	2.7	5.1
B	5.10 ± 0.10	9.17 ± 0.50	100	2.5	12.7
C	1.36 ± 0.03	3.24 ± 0.20	130	3.3	4.5

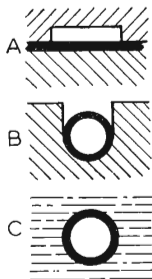


FIG. 1

Schematic Picture of the Cross Sections of the Compared Types of Isotachophoretic Columns

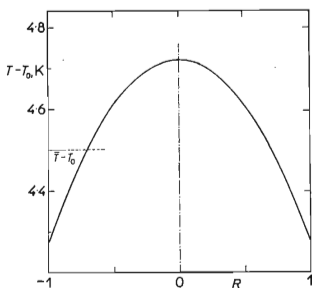


FIG. 2

Radial Distribution of the Temperature Increase $T - T_0$ (K) in the Stationary State Inside the Type C Isotachophoretic Column (LKB 2127 Tachophor)

Fig. 2 shows the steady-state radial profile of temperature calculated by means of Eqs (4), (10), and (11) and employing data of Table I for Column C, the k_1 and α values used being $0.603 \text{ W m}^{-1} \text{ K}^{-1}$ and 0.0232 K^{-1} , respectively⁴. From the figure the difference between the temperatures in the capillary centre and at the capillary wall is seen to amount to 0.44 K , while the increase of the mean temperature under these conditions is about ten times higher (4.5 K – see Table I). Thus in the common working conditions the variation of mobilities in the radial direction due to the temperature gradient is negligible; on the other hand, the increase of the mean temperature relative to the thermostat temperature cannot be neglected. All the values used for calculations of the effective mobilities of the separated components (e.g. the limiting mobilities, equilibrium constants, constants of the Debye–Hueckel–Onsager relation) should be properly corrected for these temperature changes¹⁷.

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