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TEMPERATURE PROFILES IN CAPILLARY ISOTACHOPHORESIS*

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SUMMARY

The effects of experimental conditions (counterflow-rate, magnitude of the constant electric current, and difference between mobilities of leading and terminating electrolytes) on the quality of isotachophoretic separation has been investigated using thermometric detection. As with chromatographic techniques, the central moment of the second order computed by an analogue computer was chosen as the mathematical expression of the quality of separation. The effect of heat transfer through the capillary wall is discussed.

INTRODUCTION

Since Martin and Everaerts[2] described their apparatus, thermometric detection has become a universal detection method, and Everaerts[3] calculated equations for the temperature profiles between two zones. Experimental temperature profiles, however, do not yield information about actual concentration profiles in the capillary tube, owing to a time delay caused by heat transfer across the capillary wall. Profiles that correspond more closely to the true concentration are obtained by using conductivity detectors, which are also universal. The comparison between conductivity and thermometric detection shows that the central moment of the second order obtained by using a conductivity detector is smaller by nearly four orders of magnitude than that obtained with thermometric detection[4]. Recently, Coxon and Binder[5] have calculated the cross-sectional temperature distribution in the capillary tube. These workers calculated the profiles for various heating rates and for various thicknesses of capillary wall and established that the "bowing" of the cross-sectional temperature profiles increased as the wall thickness decreased.

This paper is an attempt to evaluate the effects of counterflow and electric current on the shape of the signal from the temperature detector.

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THEORY

In an isotachophoretic system, zones of separated ions move at the same instantaneous rate, and it is advantageous to keep the velocity of the zones constant during the experiments (or at least during the time when the boundary is passing the detector); this is ensured by using constant-current sources. A linear increase in the voltage applied to the system is achieved by the experimental arrangement (the most conductive leading electrolyte in the capillary tube is successively replaced by less conductive zones, the current density being kept constant). The applied voltage is increased proportionately with the migration rate. The constant of direct proportionality equals to a difference between the electric-field intensities in the terminating (E_2) and leading (E_1) electrolytes. This difference can be expressed in terms of the current density and specific conductivities of the electrolytes as follows:

$$\frac{\mathrm{d}U}{\mathrm{d}t} = (E_2 - E_1)v = \frac{\varkappa_1 - \varkappa_2}{\varkappa_1 \varkappa_2}\cdot i \cdot v \qquad (1)$$

where U is the driving voltage, t is the time, v is the migration rate, i is the current density and $\varkappa_1$ and $\varkappa_2$ are the specific conductivities of the first and last zones. This equation can be used for determining the migration rate or the position of the first boundary, for which it follows that:

$$x_t = \frac{U_t - U_{t_0}}{E_2 - E_1} \qquad (2)$$

where x_t is the co-ordinate along the tube axis on the x-scale, at time t and U_{t_0} is the initial driving potential. The central moment of the second order, used as a criterion for the quality of separation, is defined by the expression:

$$\mu(x - a)^n = \frac{\int (x - a)^n \mathrm{f}(x)\mathrm{d}x}{\int \mathrm{f}(x)\mathrm{d}x} \qquad (3)$$

If $a = 0$, the moment $\mu'(x-a)^n$ is the n-th moment about the origin. If $a = \mu_1$, the moment $\mu_n = \mu(x-\mu_1)$ is the central moment of the n-th order. Thus, we have:

$$\mu_2 = \mu_2' - (\mu_1')^2 \qquad (4)$$

and, for a transformation in which $u = (x - x_0)/h$, h being the transformation coefficient,

$$\mu'_{2,x} = h^2[\mu'_{2,u} - (\mu'_{1,u})^2] \qquad (5)$$

Integration by parts gives:

$$\mu'_{1,x} = \Big[x\mathrm{F}(x)\Big]_{-\infty}^{+\infty} - \int_{-\infty}^{+\infty} \mathrm{F}(x)\,\mathrm{d}x \qquad (6)$$

$$\mu'_{2,x} = \Big[x^2\mathrm{F}(x)\Big]_{-\infty}^{+\infty} - 2\int_{-\infty}^{+\infty} x\mathrm{F}(x)\,\mathrm{d}x \qquad (7)$$

F(x) is a primitive function of f(x). In an isotachophoretic system, the F(u) curves are being recorded, and it is necessary to calculate moments representing the actual temperature profiles in the capillary tube in order to obtain comparable results. We have to determine the transformation coefficient (h) where x corresponds to co-ordinates along the tube axis and u corresponds to co-ordinates in the F(u) graph. It follows from eqns. 1 and 2 that

$$h = \frac{vD}{q} \Big/ \frac{dU}{dt} \tag{8}$$

where D is the scale-reading on the u-axis and q is the sensitivity of the u-axis recorder.

By substituting $F(x) = 1 - \varphi(x)$, we obtain for the experimental curves:

$$\mu_1' = -\Big[x\varphi(x)\Big]_{-\infty}^{+\infty} + \int_{-\infty}^{+\infty} x\varphi(x)\mathrm{d}x \tag{9a}$$

$$\mu_2' = -\Big[x^2\varphi(x)\Big]_{-\infty}^{+\infty} + 2\int_{-\infty}^{+\infty} x\varphi(x)\mathrm{d}x \tag{9b}$$

EXPERIMENTAL

Measurements were performed in the apparatus described elsewhere[6-8]. Essentially, the apparatus consisted of a PTFE capillary tube 30 cm long (I.D. 0.4 mm; O.D. 0.6 mm); at one end of this capillary tube, a copper–constantan thermocouple was used for heat detection, and a second thermocouple in the middle of the tube served as a sensor for the counterflow controller (the counterflow was formed by different levels of electrolytes in the cathodic compartment and in a proportional level controller). A device for establishing constant counterflow and a constant-current source (0–100 μA) capable of operation at up to 3.5 kV were also included.

Concentration profiles in the capillary tube were transformed into temperature profiles by means of a thermocouple with low noise and heat capacity. It was convenient to record the resulting profiles in co-ordinates of temperature-applied voltage instead of applying the more frequently used chart with axes of temperature *versus* time. Thus, in order to calculate the moments, it was not necessary separately to determine new transformations for the abscissa if the counterflow magnitude was reset. In order to determine the transformation coefficient, the applied driving voltage was recorded; the first derivative, $\mathrm{d}U/\mathrm{d}t$, was also recorded to facilitate more accurate setting of the hydrodynamic flow.

The temperature profiles of the boundaries between the chloride ions, present in the leading electrolyte [histidine and its monochloride in a mole ratio of 1:1 (pH 6.1)] and the anions of some organic acids used as terminators were investigated, most experiments being carried out with a driving current of 50 μA. Only the boundaries between the leading electrolyte and glutamic acid were recorded at several driving currents (15, 30, 70 and 85 μA). Hydrodynamic flow either increased or decreased (counterflow) the migration rate.

The measured curves were treated by means of a MEDA 40 TB analogue computer (Aritma, Prague, Czechoslovakia). Two types of circuit were tried; the first one computed moments according eqn. 3, but the differentiator caused troubles and therefore eqns. 9a and 9b were computed by using the network shown in Fig. 1. The input signal was obtained from the temperature–voltage responses, *i.e.*, from F(u)

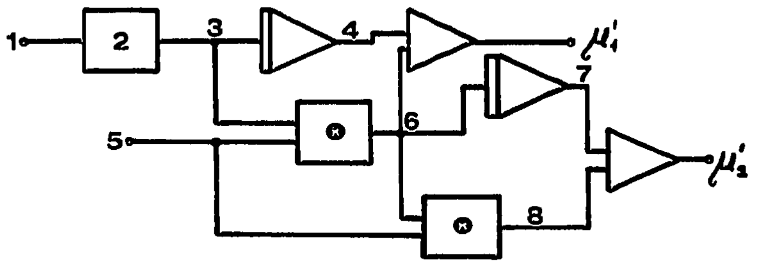

Fig. 1. Block diagram of circuit for analogue computation of moments. 1 = Signal from optical curve-follower; 2 = zero setting and normalization; 3 = $\varphi(x)$, 4 = $-\int \varphi(x)\mathrm{d}x$; 5 = x input; 6 = $x\varphi(x)$; 7 = $-2\int x\varphi(x)\mathrm{d}x$; 8 = $x^2\varphi(x)$.

functions, by means of an optical curve-follower. The accuracy of the second network was good, and the results were comparable with profiles calculated mathematically without the use of computer. Owing to the inadequate dynamic properties of the optical curve-follower, we could not use the computer for steep curves; these, therefore, were calculated mathematically. The boundary position was accepted as the μ_1' position in the capillary tube.

RESULTS AND DISCUSSION

The shapes of temperature profiles depend on the mechanism of heat transfer through the wall of the capillary tube and on the heat capacity of the thermocouple itself. Thus, temperature profiles are affected by the velocities of zones. If the migration rate is decreased, whether by the counterflow or by a decrease in the electric current, the detected signal becomes sharper (see Fig. 2). An attempt has been made[1] to calculate the distortion of the temperature profiles due to heat transfer by using pub-

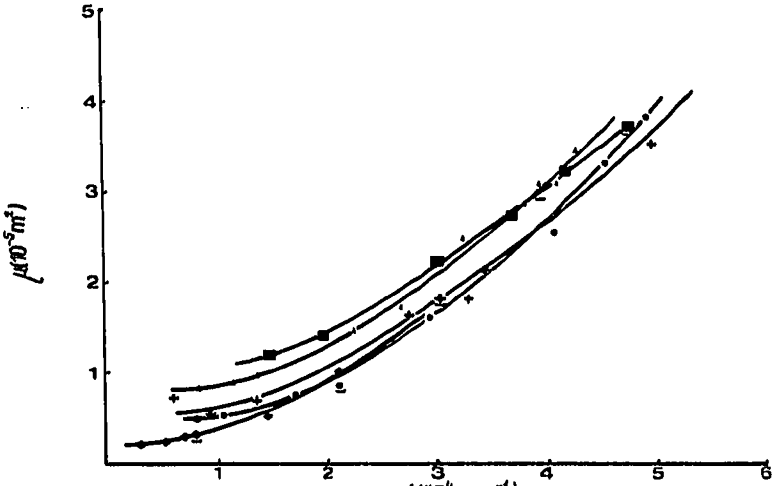

Fig. 2. Dependence of μ_2 on migration rate for various values of driving current. The underlined values were obtained when there was no hydrodynamic flow. ◆ = 15 μA; ● = 30 μA; + = 50 μA; ▲ = 70 μA; ■ = 85 μA.

lished equations of the profiles[3]. The coefficient of heat transfer was determined from data obtained in the isotachophoertic apparatus. Nevertheless, the calculated distorted profiles were sharper than the experimental ones. This could be explained as follows. The shapes of the temperature profiles depend not only on distortion due to heat transfer, but also on the cross-sectional distribution of temperature (the lower the mobility of a zone, the higher the "bowing" of the cross-sectional temperature profiles[4]). Because of these effects, the quality of separation (which is determined by the temperature detectors) deteriorates if the electric current is increased. Alternatively, deceleration by hydrodynamic flow leads to improvement of the values for μ_2 on the experimental curves, in spite of the defocusing effect of the counterflow. These results support the use of detectors based on different principles.

It would appear to be useful to retard the migration before the detector is passed by the boundary in order to obtain a sharper signal, provided that a thermocouple or a thermistor is used as detector.

The sharpness of the signal increases with the mobilities of the terminating ions (Fig. 3); this increase is due to strengthening of the self-sharpening effect. Results of zone separation with two different applied currents in times t' and t'' during one experiment showed that the separation quality was almost independent of t'/t''; only the magnitude of the counterflow was governing. This result is in agreement with previous considerations.

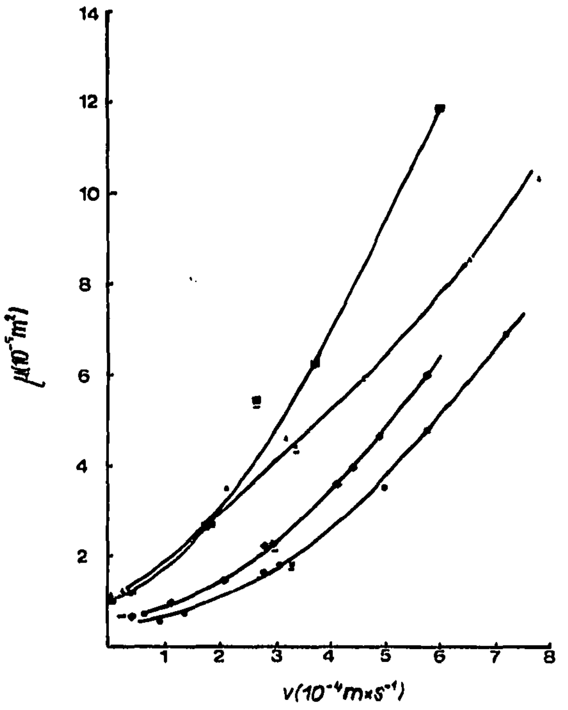

Fig. 3. Dependence of μ_2 on migration rate for various anions at 50 μA. The underlined values were obtained when there was no hydrodynamic flow. ■ = Formate; ▲ = maleate; ◆ = acetate; ● = glutamate.

The results show that, in an apparatus with a temperature detector, the counterflow enhances the quality of the signal detected, in spite of its defocusing effect. In addition to this, the counterflow increases the effective length of the capillary tube. Elimination of hydrodynamic flow in the direction of the ions undergoing separation is important, as parallel flow worsens the shapes of the zones.

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