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Note

Theoretical and practical limitations of capillary isotachophoresis in trace analysis

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The separation principle of isotachophoresis, carried out in different media (acrylamide gel, paper) can be used for qualitative and semi-quantitative analysis. Continuous free-flow isotachopohoresis is used for preparative purposes. It has been possible to carry out trace analyses since the introduction of capillary-tube isotachophoresis in the 1960s. In this paper, we shall use the term "isotachophoresis" to mean capillary-tube isotachophoresis.

The performance of any analytical technique is characterized in terms of accuracy, precision and dynamic range. In isotachophoresis the determination of the dynamic range is complicated by its dependence on the sample composition. Often associated with the dynamic range is the minimum detectable amount, which is especially important in trace analysis. Whereas in chromatography there is a common trend to use, whenever possible, more specific detectors in trace analysis, in isotachophoresis the detector sensitivity is determined by factors other than, for example, noise. Here, sensitivity is determined by the leading electrolyte concentration and the detector cell volume. Moreover, completely different interpretation is necessary to use specific detection in a more sensitive manner. The gain is a factor of 10 and not more, even in principal-component analysis.

The detection limit with universal detectors is consequently determined by both operational and equipment parameters, because zone-volumes are measured. There are a number of theoretical and practical constraints as to the operational conditions (field strength, concentration) that will be discussed. The equipment parameter (cell volume) clearly has constructional limitations, but theoretical insight has revealed that there is no point in further decreasing the cell volume because of the inherent uncertainty of the zone-volume, owing to diffusion and a number of convective disturbances from temperature gradients and electroosmosis. Evaluation of both operational and constructional limitations has shown that the absolute detection limit, that can theoretically be attained, lies 2–3 decades below that of present-day isotachophoretic equipment.

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OPERATIONAL LIMITATIONS

The minimum detectable amount, q_{min}, with universal detection in isotachophoresis is given by[1,2]:

$$q_{min} = \pi r_0^2 lc \tag{1}$$

in which r_0 is the capillary internal radius, l the detector cell length and c the separand concentration in its zone: it is clear that all three can be minimized. The separand concentration c is determined by using the Kohlrausch law, the separand mobility and the leading electrolyte composition. With a transport number of the leading electrolyte of 0.5 or greater (necessary for separation efficiency), the value of c can decrease to *ca.* 0.5 times that of the leading electrolyte. For reasons of a buffering capacity the latter should be in the order of 10^{-3} M or more.

When minimizing the detector cell length, it should be remembered that there is an inherent uncertainty in the exact location (or moment of detection) of a zone-boundary. This uncertainty arises from three phenomena: electroosmosis, diffusion and convection due to the axial temperature gradient. In comparison with the latter the radial temperature gradient can be neglected.

Electroosmosis leads to convection of the liquid, which in turn causes an axial pressure gradient accross the zone-boundary. This gradient is zero only at distance $r_0/\sqrt{2}$ from the capillary axis[3] and gives rise to a non-planar zone-boundary. Therefore, certain precautions may be necessary: decreasing the zeta-potential of the wall, increasing the viscosity or decreasing the dielectric constant of the liquid[3]. The curved profile can otherwise range over a length of 10^{-4} m or more.

Although diffusion in isotachophoresis is counteracted by the mechanism of self-correction, the steady-state zone-boundary composition is determined by diffusion, as described by several authors[4,5].

The length of the diffusion-controlled layer is given by:

$$\Delta l_D = 4mRT/\Delta mFE \tag{2}$$

where $\Delta m/m$ is the relative mobility difference of the two separands. R the gas constant, T the absolute temperature, F the Faraday constant and E the average field strength. In this relation, which gives the uncertainty in the location of the zone-boundary, the diffusion coefficient, D, is included in the mobility by means of Einstein's approximation, $D = mRT/F$, for monovalent ions. At normal working temperatures, a mobility difference of 10% and a field strength of 10^4 V m^{-1}, the diffusion length Δl_D is equal to 10^{-4} m.

Another disturbance arises from the stepwise increase in temperature from one zone to another. The length of this disturbance can be approximated by (see ref. 3, p. 38):

$$\Delta l_C = \rho C_p m \Delta T/j \tag{3}$$

where ρ is the density, C_p the specific heat of the liquid, ΔT the temperature difference and j the current density. For a separand with mobility of $50 \cdot 10^{-9}$ m^2 V^{-1} sec^{-1} in water at $j = 10^3$ A m^{-2}, Δl_C is $2 \cdot 10^{-4}$ m per degree temperature difference.

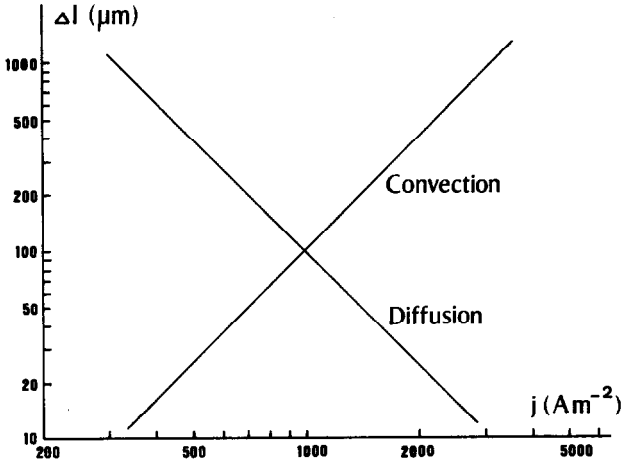

Fig. 1. The length of the convective (Δl_C) and diffusive disturbance (Δl_D) in isotachophoresis as a function of the current density. Conditions for this example are mentioned in the text. A capillary of 0.2 mm I.D. was used.

Zone-to-zone temperature differences were determined experimentally in capillaries with different inner diameters[6]. In a capillary with $r_0 = 10^{-4}$ m and at the current density mentioned, the temperature difference amounts to 10°K between leading and terminating electrolytes. With an approximate zone-to-zone difference of 0.5°K, the length of the convective disturbance, Δl_C, is 10^{-4} m. When optimizing the current density in this respect, an increase would seem attractive from eqn. 3. However, experiments have shown that ΔT is proportional to j^2. Consequently, a higher current density even increases Δl_C. Alternatively, a lower current density, through E, increases Δl_D. Fig. 1 illustrates these effects. It was shown that, under the conditions described Δl_D and Δl_C are of the same order of magnitude *i.e. ca.* 10^{-4} m.

CONSTRUCTIONAL LIMITATIONS

It was shown that operational limitations give rise to an inherent uncertainty in the zone length of 10^{-4} m, so that it is unnecessary to decrease the detector cell length below this value. According to eqn. 1, the detector cell volume can also be minimized if a smaller radius is used. Several problems may arise: construction of the cell, coupling to the separation system and the danger of clogging. There is an additional fundamental limitation. The electric double-layer thickness, δ, for dilute solutions of monovalent electrolytes at room temperature can be approximated by:

$$\delta = 0.3 \cdot 10^{-9}/\sqrt{c} \tag{4}$$

where c is the concentration (mol l^{-1}) and δ in metres. When $c = 10^{-3}$ M, δ is 10^{-8} m. With a capillary radius, r_0, of 10^{-5} m, wall effects can be neglected provided that electro-osmosis is suitably accounted for[7]. The effect of surface conductance can short-circuit the isotachophoretic process. The relative contribution of surface conductance is approximated by[7]:

$$2\kappa^{\sigma}/r_0\kappa = 2\varepsilon^2\zeta^2/\eta\delta r_0\kappa \tag{5}$$

where κ^σ is the surface conductance per unit of wall circumference, κ the bulk conductance, ε the dielectric constant, ζ the zeta-potential of the wall and η the viscosity of the liquid. With a zeta-potential reduced to 10^{-2} V, with $\varepsilon = 80 \cdot 10^{-12}$ F m^{-1}, $\eta = 10^{-3}$ N m^{-2} sec, $\delta = 10^{-8}$ m, $r_0 = 10^{-5}$ m and $\kappa = 10^{-1}$ S m^{-1}:

$$2\kappa^\sigma / r_0 \kappa = 1.3 \cdot 10^{-7} \quad (6)$$

Under these conditions, the effect of surface conductance can indeed be neglected. A decrease of the radius of presently used capillaries from 10^{-4} to 10^{-5} m can consequently be considered from a theoretical point of view.

CONCLUSIONS

The theoretical limit of detection with universal detection in isotachophoresis, given by eqn. 1, is limited by an inherent uncertainty in the zone length measured, owing to both diffusion (Δl_D) and convection (Δl_C). The order of magnitude is 10^{-4} m, with a minimum detectable zone length of $3 \cdot 10^{-4}$ m. The double-layer thickness (10^{-8} m at 10^{-3} *M*) should be negligible compared with the capillary radius, which in turn should be greater than 10^{-5} m. The concentration in the steady state, *c*, should be 10^{-3} *M* or higher.

It can therefore be concluded that the theoretical detection limit with universal detection consequently obtained amounts to 10^{-13} mole for monovalent ions, or 100 femtoequivalents. This value is still 2–3 decades below that of present-day isotachophoretic equipment.

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