Potential Gradient Detector for Capillary Type Isotachophoresis

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Synopsis. The potential gradient detector previously reported has been improved as regards mechanical stability and the contaminated electrodes can be easily exchanged with new ones. The linearity of step-height holds below the electrode voltage of 1.2 V (0.005 mol dm^{-3} aq HCl). The detection limit was 2.5×10^{-10} mol for adipic acid.

In isotachophoresis, the ratio of potential gradients of sample zone (E_V) to leading zone (E_L) can be measured by means of a potential gradient detector (PGD). The ratio, R_E, correlates with the step-height (h) in the isotachopherogram and the effective mobility (m) of sample and leading ion as follows:

$$R_E = E_V/E_L = h_V/h_L = \bar{m}_L/\bar{m}_V, \quad (1)$$

where V and L denote the sample and leading zones, respectively.

The R_E values obtained are useful for the evaluation of aboslute mobility,[1,2] dissociation constants[2] and complex stability constants.[3] For the measurements of R_E, the drift of baseline in the isotachopherogram should be suppressed as little as possible. The drift might be due to the following: (A) Inadequate choice of electrolyte system and/or pH of leading electrolyte. (B) An undesirable electric pass among sensing electrodes and ground due to leak of electrolytes and/or high humidity. (C) The inactive electrodes of PGD contaminated by electrode reaction; An exchange of the damaged sensing electrodes is preferable, since the PGD once contaminated will not give a stable base line even when it is rinsed well. The defects due to acryl resin[2] were removed: The previous model was not strong mechanically; due to thermal melting on boring, it was difficult to pass electrolytes with a uniformed bore and no organic solvent could be used.

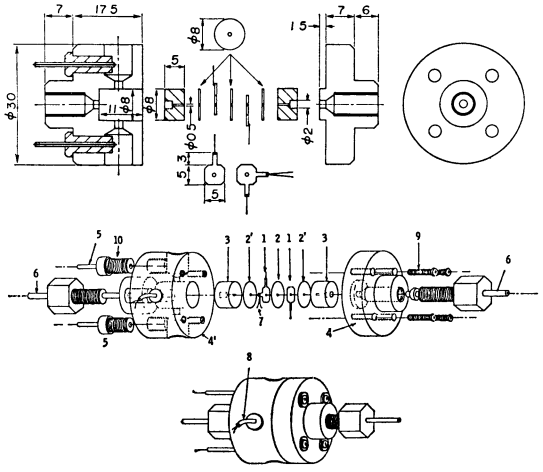

Fig. 1. Potential gradient detector.
1: Pt sensing electrodes, 2: PTFE insulator, 2′: PTFE gaskets, 3: PTFCE holders, 4 and 4′: Cell bodies (polycarbonate), 5: Lead wires, 6: PTFE capillary tube, 7 and 8: Cu-constantan thermocouple, 9: Fixing screws, 10: PTFE bushings.

Construction of PGD Cell. Figure 1 shows the mechanical construction and an exploded view of the PGD cell. The cell bodies (4,4′) are made of polycarbonate resin and turn on a rod, the holes in the bodies being for screws (9), and plugs to fit the capillary tube to the cell, and lead wires to the sensing electrodes (5). The sensing electrodes (1) are made of platinum disk of 20 μm thick. A copper-constantan thermocouple soldered to one of the electrodes (7) is to monitor the temperature of electrolyte. The holders (3) for sandwiching the electrodes are made of poly(trifluorochloroethylene) (PTFCE), which is harder than poly(tetrafluoroethylene) (PTFE). PTFE disks of 50 μm thick were used as an insulator of the electrodes (2) and gaskets (2′). The diameter of the pass of electrolyte is 0.5 mm. The bored electrodes were carefully trimmed with sandpaper (# 2000) and rinsed with water, 0.1 mol dm^{-3} HNO_3, distilled water and ethanol. The holders, insulators, and gaskets were also rinsed with ethanol and dried.

One of the holders (3) was inserted into the cell body (4′), a centering rod of 0.5 mmϕ being inserted into the holes. The gaskets, electrodes, insulator and the other holder were inserted and settled. The other body was then fixed tightly with the screws (9) and the rod was pulled out carefully. The lead wires, supported by PTFE bushings (10), were soldered to the sensing electrodes. When construction was complete, the pass was rinsed again.

Check on PGD. First, the linearity between the input voltage applied and the observed step-heights was checked using the recorder of an isotachophoretic analyzer (Shimadzu Seisakusho Ltd., IP-1B) and continuous voltage generator (0 to 2 V).

A 0.005 mol dm^{-3} aq HCl was used as an electrolyte in the pass of the capillary tube and the PGD. The pH was adjusted to 6 by addition of histidine. When there was no electric leakage and the sensing electrodes were clean, the base line of the electropherogram did not drift for 1 h with migration current of 50 μA (drift, less than a few percent of the step-height of base line). Sometimes, an upward drift due to electric leakage among the electrodes and ground, and a downward drift due to electric leakage between the electrodes or the contaminated electrodes were observed.

Since the electric resistance of the buffered electrolyte is constant in the thermostatted capillary tube, the migration current and the voltage detected should be linearly correlated until the sensing electrodes are polarised. The linearity of step-heights on migration was checked as follows. The migration current was varied from 25 to 200 μA at regular intervals of 25

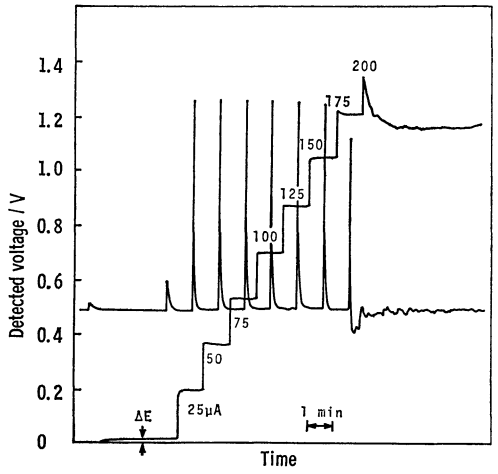

Fig. 2. Electropherogram for the check on linearity of step heights.

μA and the step-heights *vs.* time were recorded (Fig. 2). The irregular step-heights on migration due to polarisation of the electrodes were recorded at 200 μA. However, the linearity held below 175 μA, at which the electrode voltage corresponded to *ca.* 1.2 V. The value agrees with the decomposition voltage of aq HCl. If the electrodes were contaminated or the electric leakage took place in the pass of electrolyte, no normal linear relation could be obtained. Thus, the present PGD gives the correct R_E values of given samples, when the voltage between the sensing electrodes does not exceed the decomposition voltage of sample or solvent. ΔE in Fig. 2 shows an example of the asymmetrical potential which appears at the opening of a short-circuiting switch for PGD. Ideally, the value should be null, but sometimes a slight shift was observed. For estimation of the exact R_E value, the step-height of ΔE should be subtracted from the step-heights of leading, samples and terminating zones. Use of an internal standard will give more reliable R_E values.

Detection Limit. Adipic acid was used in order to check the quantitative limitation according to the procedure reported by Akiyama and Mizuno.[4] The leading electrolyte was 0.01 mol dm^{-3} aq HCl containing 0.2% Triton X-100, the pH of which was adjusted to 6.0 by adding histidine. The terminating electrolyte was 0.01 mol dm^{-3} glutamic acid. The pH was adjusted to *ca.* 6 by adding histidine. Figure 3 shows the differential curves of the isotachopherograms obtained for the injected sample. The quantitative limit was *ca.* 5×10^{-10} mol and the detection limit 2.5×10^{-10} mol.

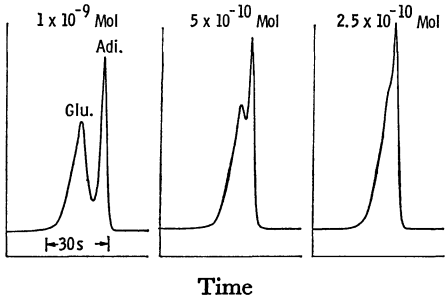

Fig. 3. Differential curves of isotachopherograms of adipic acid for the check on detection limit. Adi: adipic acid, Glu: glutamic acid.

According to a computer analysis of isotachophoresis for the injected sample of 1×10^{-9} mol, the total concentration in the sample zone and the zone length were estimated to be 0.00433 mol dm^{-3} and 0.1176 cm, respectively. The isotachophoretic velocity can be expressed as follows:

$$v = E_a\bar{m}_a = J\bar{m}_a/\kappa_a \text{ cm/s}, \quad (2)$$

where E_a is the potential gradient and $\bar{m}_a$ the effective mobility, J the current density and κ_a specific conductivity. When the current applied is 25 μA, J is 1.273×10^{-2} A/cm^2. The simulated $\bar{m}_a$ and κ_a were 42.96×10^{-5} cm^2/V s and $5.593\times10^{-4}/\Omega$ cm, respectively. Substitution of these values into Eq. 2 gives the isotachophoretic velocity 9.8×10^{-3} cm/s. The passing time of the zone through the detector expected was 12 seconds ($0.1176/9.8\times10^{-3}$). This agrees with the observed one (*ca.* 12 s.) as shown in Fig. 3. The maximum resolving power of 5×10^{-10} mol corresponds to the zone length of 588 μm, which is 8 times the effective gap of the electrodes of 70 μm.

The resolving power of 2.5×10^{-10} mol reported by Akiyama and Mizuno[4] for the same inner diameter and thickness of insulator is twice as large as that of our result. The difference may be due to the thickness of our electrodes (20 μm), since it would affect the degree of sharpness of differential curves. A better resolution might be obtained using thinner eletrodes, a smaller inner diameter of PGD, and lower concentration of leading electrolyte than those in the present experiment.

References

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