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Note

Sensitivity of a newly designed potential gradient detector for isotachophoresis

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The conductivity detector[1,2] and the potential gradient detector[3,4] have recently attracted attention as universal detectors for isotachophoresis. The former was found to have the disadvantages that it is necessary to change the operating conditions each time an electrolyte of different concentration is used, and that the results are influenced by the resistance of the sensing electrodes[5]. The latter, in contrast, has no such disadvantages and in fact has the advantage that identification of compounds can be easily accomplished from the ratio of potential gradients[5,6]. Neither of these two detectors presently available, however, ensures satisfactory sensitivity for practical application to analyses of trace components in biological samples.

With the intention of exploring the possibility of increasing the sensitivity, we have performed a series of experiments with the potential gradient detector in order to clarify the current-density dependence of bubble generation and the influence of the inner diameter of the sensing cell, the inner diameter of the capillary tube and the temperature and concentration of the electrolyte on the sensitivity.

EXPERIMENTAL

The equipment used was a Shimadzu IP-1 isotachophoretic analyzer[3].

Fig. 1 shows the sensing cell of the potential gradient detector. A PTFE disc insulator is sandwiched between two sensing electrodes (platinum discs). The sensing tube and the spacer tube have the same inner diameter, and the length of the spacer tube is about 100 times the gap (thickness of the PTFE insulator) between the electrodes, so that the diffusion of the samples into dead spaces existing at the joints is minimized.

RESULTS AND DISCUSSION

Generation of bubbles and current density

Fig. 2 shows the relationship between the current density and the time necessary for bubbles to be generated electrochemically on the sensing electrodes for different contact surface areas. The capillary tube and the sensing cell were filled with 0.01 *M* glutamic acid solution.

As the time necessary for bubbles to be generated is determined by the current

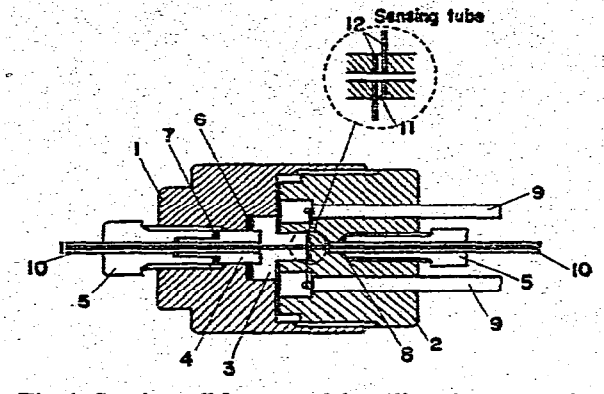

Fig. 1. Sensing cell for potential gradient detector. 1, 2 = Cell body (PTFE); 3 = spacer tube (PTFE); 4 = fitting guide (PTFE); 5 = fitting plug; 6, 7 = slip ring; 8 = PTFE sealing tape; 9 = PTFE-sleeved lead wire; 10 = capillary tube; 11 = PTFE disc insulator; 12 = sensing electrodes (platinum discs).

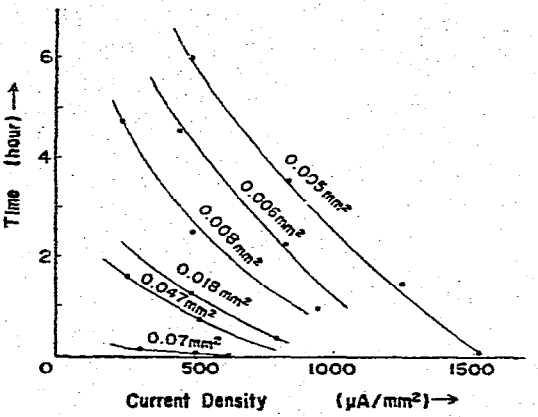

Fig. 2. Bubble generation time as a function of current density and the surface area of the sensing electrodes coming into contact with the electrolyte.

density and the contact surface area, the sensitivity can be enhanced or the analysis time can be reduced only to a certain extent by decreasing the inner diameter of the sensing cell. The smallest possible thickness of the platinum discs that could be used in our experiments was 0.01 mm and the smallest possible inner diameter of the sensing cell was 0.2 mm.

When these platinum discs and sensing cell were used, the greatest measurable migration current was 25–30 μA.

Sensitivity of sensing cell

Table I shows the influence on the sensitivity of the inner diameter of the capillary tube and the gap between the sensing electrodes. It shows that the minimum detectable amount is determined by the inner diameter of the sensing cell and the gap between the sensing electrodes, and that it is not influenced by the inner diameter of the capillary tube, within the ranges studied in our experiments.

TABLE I

MINIMUM DETECTABLE AMOUNT OF ADIPIC ACID

Leading electrolyte: 0.01 *M* histidine–0.01 *M* histidine·HCl. Terminal electrolyte: 0.01 *M* glutamic acid.

Inner diameter of sensing cell (mm)	*Inner diameter of capillary tube (mm)*	*Gap between sensing electrodes (mm)*	*Minimum detectable amount of adipic acid (g-equiv.)*
0.2	0.25	0.05	$1 \cdot 10^{-10}$
0.2	0.5	0.05	$1 \cdot 10^{-10}$
0.5	0.25	0.05	$5 \cdot 10^{-10}$
0.5	0.5	0.05	$5 \cdot 10^{-10}$
0.5	0.5	0.5	$1 \cdot 10^{-9}$
0.5	0.5	0.1	$8 \cdot 10^{-10}$

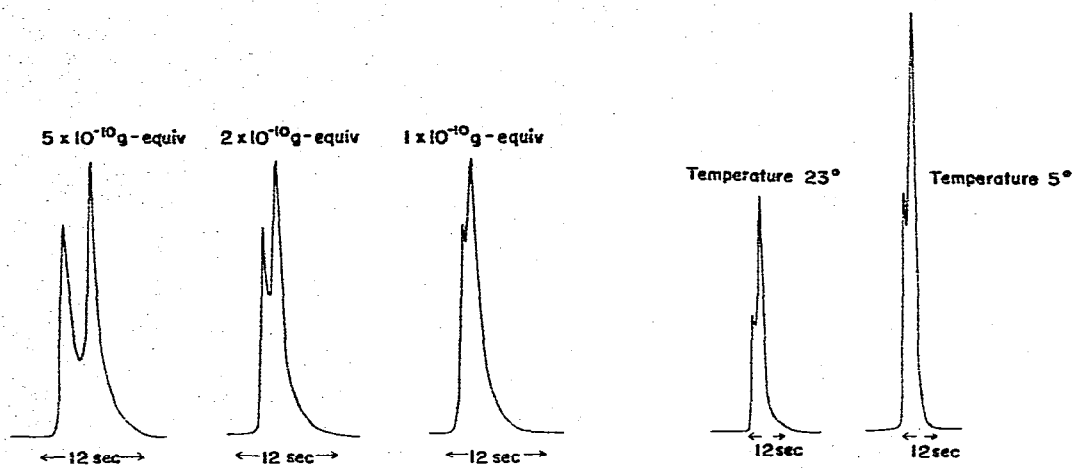

Fig. 3. Isotachopherograms of trace amounts of adipic acid. Leading electrolyte: 0.01 *M* histidine–0.01 *M* histidine·HCl. Terminal electrolyte: 0.01 *M* glutamic acid. Sensing cell: 0.2-mm I.D. Capillary tube: 0.25-mm I.D. Interval of sensing electrodes: 0.05 mm. Migration current: 25 μA. Thermostat temperature: 23°.

Fig. 4. Influence of thermostat temperature. Leading electrolyte: 0.01 *M* histidine–0.01 *M* histidine·HCl. Terminal electrolyte: 0.01 *M* glutamic acid. Sample: $1 \cdot 10^{-10}$ g-equiv. adipic acid. Migration current: 25 μA.

Fig. 3 shows an example of the detection of a trace amount of adipic acid. The detection limit under the conditions used was $1 \cdot 10^{-10}$ g-equiv.

When the thermostat temperature was set at 5°, using the same sensing cell, the potential gradient was increased 1.5-fold compared with that at 23°, and the peaks were higher, as shown in Fig. 4, but there was no distinguishable difference in the minimum detectable amount.

Fig. 5 shows the detection limit when 0.001 *M* leading and terminal electrolytes were used. It was found that addition of 0.05% of Triton X-100 (ref. 7) to the leading electrolyte makes it possible to detect $5 \cdot 10^{-11}$ g-equiv. of adipic acid as two peaks. From these experiments, it can be concluded that the enhancement of the

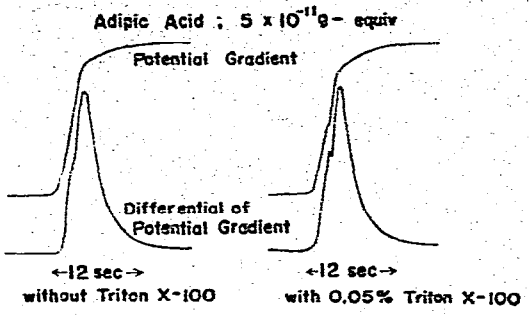

Fig. 5. Detection limit of sensing cell. Leading electrolyte: 0.001 *M* histidine–0.001 *M* histidine·HCl. Terminal electrolyte: 0.001 *M* glutamic acid. Migration current: 2.5 μA.

sensitivity by use of a low-concentration electrolyte cannot be recommended because it would decrease the sharpness of the zone boundaries.

It is expected that it will become possible to reduce the inner diameter of the sensing cell by minimizing the contact surface area of the sensing electrodes, and then to enhance the sensitivity further.

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REFERENCES

1 F. M. Everaerts and Th. P. E. M. Verheggen, *J. Chromatogr.*, 73 (1973) 193.
2 S. Stankoviansky, P. Čičmanec and D. Kaniansky, *J. Chromatogr.*, 106 (1975) 131.
3 T. Haruki and J. Akiyama, *Anal. Lett.*, 6 (1973) 985.
4 P. Boček, M. Deml and J. Janák, *J. Chromatogr.*, 106 (1975) 283.
5 M. Deml, P. Boček and J. Janák, *J. Chromatogr.*, 109 (1975) 49.
6 T. Haruki and J. Akiyama, *Pittsburgh Conference, Cleveland, Ohio, 1974*, No. 420.
7 L. Arlinger, *J. Chromatogr.*, 91 (1974) 785.