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Detection electrodes for electrophoresis

The detection of boundary passages in electrophoresis has been the subject of much research. Utilizing the fact that each electrophoretic zone has a particular value for the electrical conductivity, and therefore a particular value for the Joule heat production, the thermometric detection method was the first to be thoroughly developed[1,2]. Other detection methods started from the direct measurement of the electrical conductivity[3–6], by using special electrodes mounted inside the narrow tube in which the electrophoretic separation was carried out. As it is necessary for stabilization of the zones to use tubes with internal diameters in the range 0.4–0.7 mm, the mounting of these electrodes is a skillful technique. Furthermore, the presence of such electrodes will influence the concentration profiles and will also interfere with the application of electrolyte counterflow techniques.

In the present paper, the use of an apparatus is described in which the electrode system is part of the wall of the narrow tube and which can overcome most of the above effects.

Experimental and results

Experiments were carried out in a narrow tube of PTFE (I.D. 0.45 mm; O.D. 0.7 mm) and in a narrow glass tube (I.D. 0.6 mm; O.D. 4 mm). The construction of the sensing cell for use with the narrow PTFE tube is shown in Fig. 1. In Fig. 1 the pieces (3) necessary for the fitting of the narrow tube[7] are made of brass in order to avoid an excessive temperature increase of the electrolyte. The pieces of Perspex (4) serve as liquid-tight mechanical connections between the brass fitting (3) and the electrode configuration (sandwich (6)). Part of the sandwich is shown scaled up to demonstrate the way in which the central insulating disc (Perspex) separates the two sensing electrodes (platinum). The electrical contact with the electrode is provided by a copper pin (5).

The construction of the sensing cell for use with the narrow glass tube is shown in Fig. 2. The platinum electrodes (3) are separated by a section of the capillary tube, and the sensing cell is stuck together with Shellac.

The driving current was supplied by a stabilized direct-current power source, supplying a current of between 40 and 150 μA, corresponding to potentials of the order of 10 kV.

For the measurement of the resistance of the liquid between the electrodes, a D.C. method and an A.C. method were used. The circuit for the D.C. measurements is shown in Fig. 3. In this method for the determination of the conductivity, the driving current was used, the resulting potential between the sensing electrodes (2) being a direct measure of the resistance. The input impedance of the voltmeter (3) was high enough to prevent the formation of disturbing gas bubbles at the sensing electrodes.

The A.C. circuit used is shown in Fig. 4. The generator (7), which was used at 810 Hz, was joined in series with a resistance (4) of 22 MΩ. With this arrangement, the A.C. current was not affected by changes in the resistance between the sensing electrodes. The capacitors (3), which were used for the separation of the circuit from the D.C. potential, were large enough (1 μF; 3500 V) to allow their A.C. impedances

to be neglected. The potential measured by the voltmeter (5) and registered by the recorder (6) therefore becomes a direct measure of the impedance between the sensing electrodes.

For comparison, the A.C. and D.C. methods were used with a distance of 2 mm between the sensing electrodes. In both cases the narrow tube was connected with

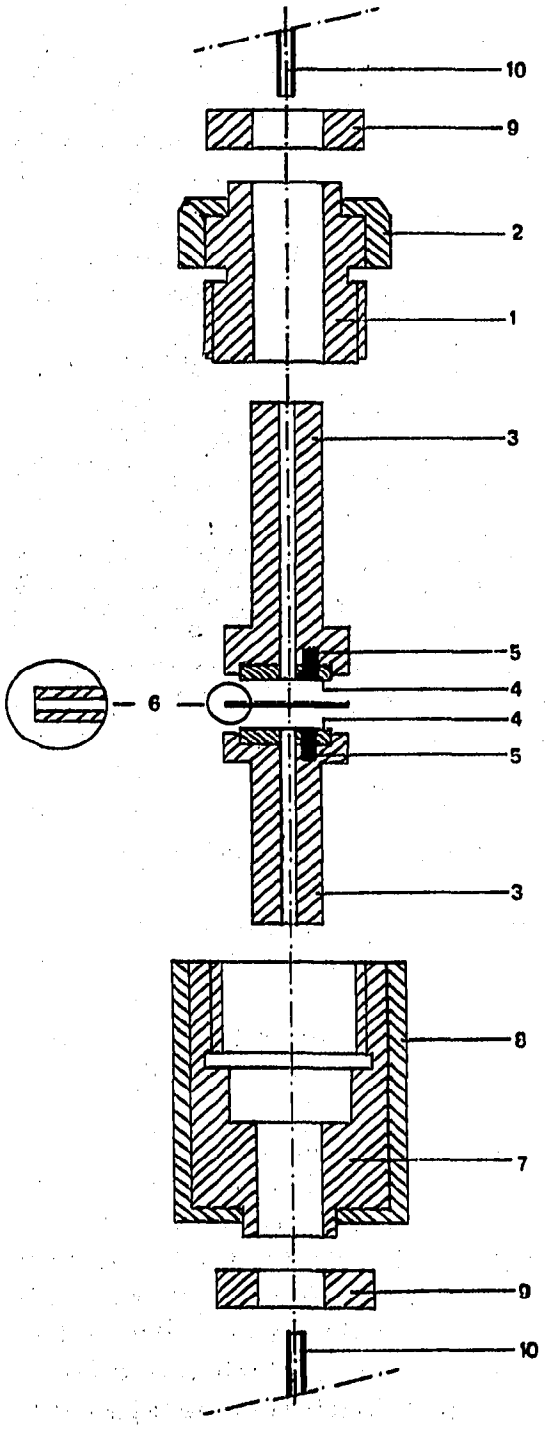

Fig. 1. The sensing cell used for the experiments with the narrow PTFE tube. 1 and 7 = Perspex used for electrical insulation; 2 and 8 = stainless-steel fitting; 3 = piece of brass for fitting the narrow tube; 4 = piece of Perspex for water-tight connection; 5 = copper pin for electrical contact; 6 = sandwich; 9 = stainless steel for external connections; and 10 = narrow tube.

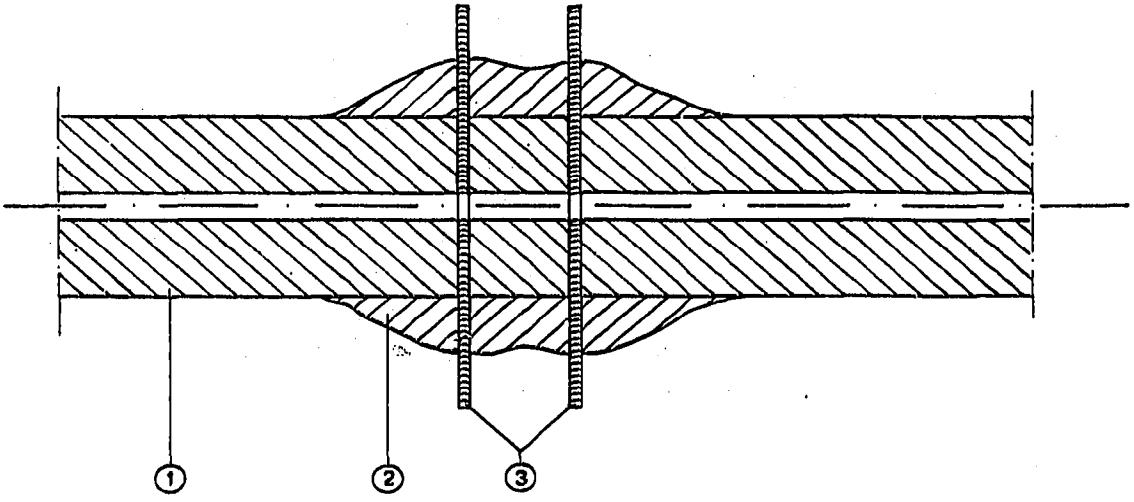

Fig. 2. The sensing cell used for the experiments with the narrow glass tube. 1 = Narrow tube; 2 = shellac; and 3 = platinum electrodes.

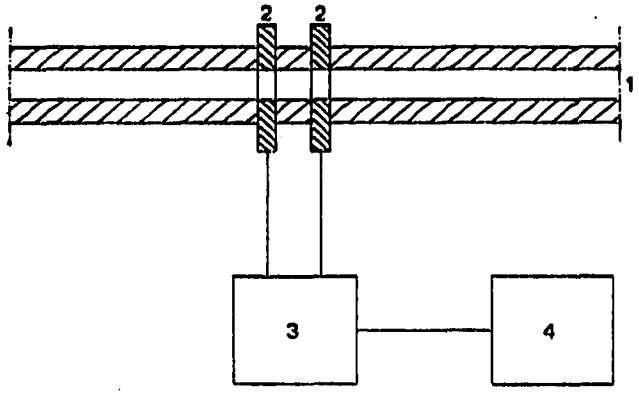

Fig. 3. Circuit for the D.C. measurements. 1 = Narrow tube; 2 = sensing cell; 3 = D.C. voltmeter; and 4 = recorder.

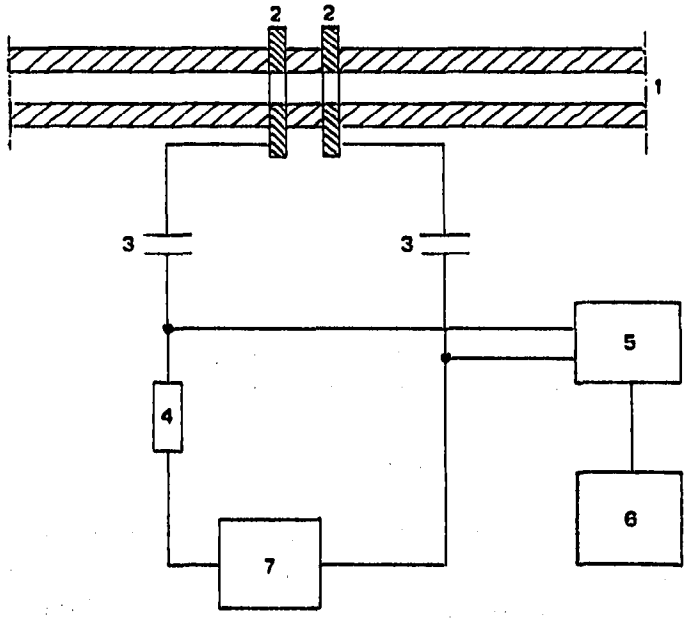

Fig. 4. Circuit for the A.C. measurements. 1 = Narrow tube; 2 = sensing cell; 3 = capacitors (1 μF, 3500 V) for separation from the D.C. voltage; 4 = resistor (22 MΩ); 5 = A.C. voltmeter; 6 = recorder; and 7 = A.C. generator.

an open and a closed electrode compartment. The electropherogram, shown in Fig. 5, was made with imidazole in hydrochloric acid (0.01 *M*) as the leading electrolyte. In order to increase the buffering capacity, an extra amount of imidazole was added so as to equalise the pH of the solution to the p*K* value of imidazole. The cathode compartment contained tartaric acid (0.01 *M*) as the terminator. The boundary velocity was 1.3 cm/min when a D.C. current of 60 μA was applied. Recording of the boundary passage was made separately in the D.C. and A.C. methods. Fig. 5 shows that the A.C. and D.C. electropherograms are comparable. The main part of the conductivity jump, related to the boundary passage, takes place within a time interval of 15 sec, which corresponds to a width of this boundary of 3 mm. This can be reconciled satisfactorily with the distance between the electrodes.

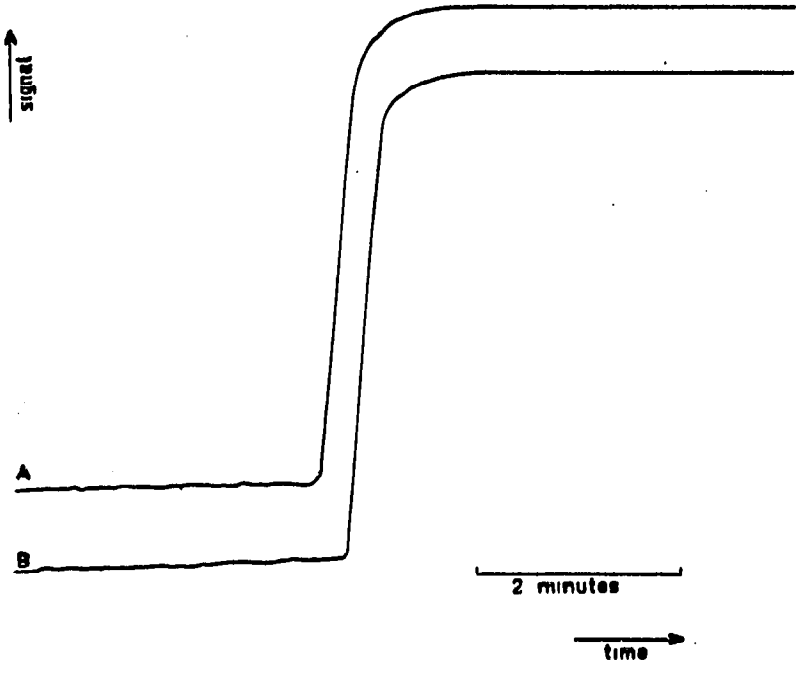

Fig. 5. Electropherograms (60 μA). Curve A: A.C. method; curve B: D.C. method.

Discussion

The A.C. method has the advantage that more information can be deduced from experiments at different frequencies. The results in Fig. 5 show a remarkable sharpness both for the A.C. and D.C. measurements. Comparison of this with the thermometric detection method[1,2] leads to the conclusion that an improvement of a factor of about 10 is obtained.

A pronounced advantage of the A.C. method is that the height of the boundary passage jump is independent of the electrophoretic driving current, so that during an analysis this driving current can be varied without interference from the relative height of the different steps.

The research is being continued by analysing the different factors that limit

the width of the boundary jump in order to increase the resolving power of the detection method.

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1 F. M. EVERAERTS AND A. J. P. MARTIN, *Anal. Chim. Acta*, 38 (1967) 233.
2 B. P. KONSTANTINOV AND O. V. OSHURKOVA, *Zh. Tekh. Fiz.*, 37 (1967) 1745.
3 W. PREETZ, *Talanta*, 13 (1966) 1649.
4 W. PREETZ AND H. L. PFEIFFER, *Talanta*, 14 (1967) 143.
5 O. HELLOW, *J. Electroanal. Chem.*, 19 (1968) 37.
6 S. FREDERIKSON, *Acta Chem. Scand.*, 23 (1969) 4.
7 F. M. EVERAERTS AND TH. P. E. M. VERHEGGEN, *J. Chromatogr.*, 53 (1970) 315.

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