Journal of Chromatography, 261 (1983) 127–129
Elsevier Science Publishers B.V., Amsterdam — Printed in the Netherlands

CHROM. 15,699

Note

Electrophoresis of thiols in cellulose gels

II. Application of cellulose gel membranes

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(First received November 29th, 1982; revised manuscript received January 17th, 1983)

The resolution of thiols in cellulose gel rods followed by their estimation has been described in a previous paper[1]. There is however a need for a rapid preliminary estimation of the number of thiols and their relative mobilities in the sample. For that purpose, electrophoresis in cellulose gel membranes immersed in a toluene bath has been successfully applied. In order to facilitate the preparation and use of cellulose gel membranes, a glass fibre strip is employed as a framework.

EXPERIMENTAL

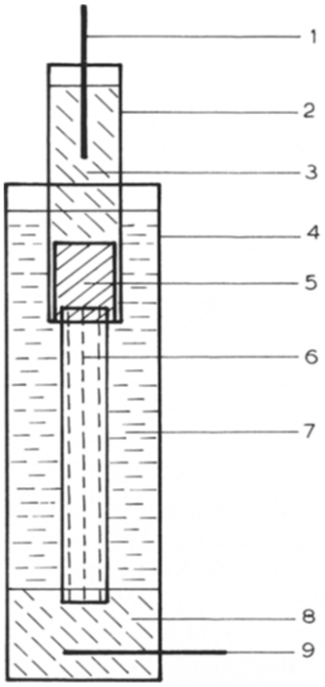
Sheets (10 × 25 cm) of glass fibre paper (GF81, Koch-Light) were immersed in viscose containing additives[1], pressed between two glass plates to remove the excess of viscose and air-bubbles and set aside for solidification. The membranes obtained after separation of the glass plates were washed with water, hot sodium sulphite, again with water and finally covered with ethylene glycol to prevent drying.

For the present purpose, the membranes were cut into strips (2 × 12 cm), equilibrated with the buffer and blotted between sheets of clean filter-paper just prior to use. At one end of the strip a perpendicular stroke was made and filled with the sample containing some phenol red. The strip was then fixed in an incision of the cellulose gel stopper (previously equilibrated with the buffer) which after smearing with glycerol was packed into a glass tube and placed in the electrophoretic cell as indicated in Fig. 1. The free end of the strip must be in contact with the buffer at the bottom. The glass tube (2) was then filled with the same buffer and the current switched on. The toluene bath must be cooled to keep the temperature within 18–19°C. Electrophoresis was performed at a current of 5–8 mA and a gradient of 50–90 V/cm.

To locate the resolved thiols the cellulose gel strip was pressed against a reactive paper strip prepared by saturation of Whatman No. 1 paper with one of the following reagents:

(1) 2 ml of tetraacetoxymercurifluorescein (0.2 mg/ml) with 1 ml of uric acid solution (0.2 mg/ml) and 2 ml of 25% (w/v) triethanolamine, prepared just before use.

0021-9673/83/$03.00



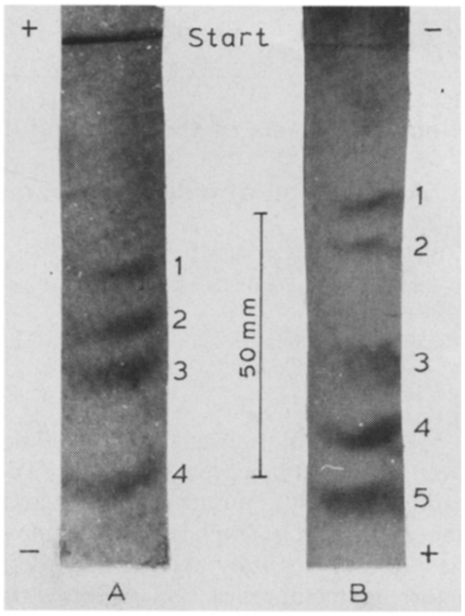


Fig. 1. Electrophoresis cell for cellulose gel strips: 1 and 9 = electrodes; 2 = glass tube (100 × 22 m); 3 and 8 = buffer; 4 = glass vessel; 5 = a stopper prepared from cellulose gel (30 × 22 mm); 6 = cellulose gel strip; 7 = toluene bath cooled with a water cooler.

Fig. 2. Distribution patterns obtained by electrophoresis at 55 V/cm in a cellulose gel membrane and located by contacting with HMB–dithiofluorescein paper. Buffer A, time 55 min: 1 = glutathione; 2 = penicillamine; 3 = cysteine; 4 = homocysteine. Buffer B, time 15 min: 1 = glutathione; 2 = captopril; 3 = 2-mercaptopropionic acid; 4 = mercaptoacetic acid; 5 = thiomalic acid.

(2) 30 mg of dithiofluorescein dissolved in 5 ml of 0.1 *M* potassium hydroxide, filtered and added to 10 ml of 0.01 *M* *o*-hydroxymercuribenzoic acid (HMB) to give a persistent blue colour, followed by addition of 5 g of triethanolamine and dilution to 25 ml with water. This reagent and reactive papers can be stored for 1 month but should be protected from light. The dry reactive papers should be sprayed before use with a solution of 25% (w/v) triethanolamine.

Reagent 1 locates thiols as fluorescent spots under UV light, reagent 2 as blue spots.

The distribution patterns for some thiols are shown in Fig. 2, and the corrected relative mobilities are summarized in Table I. The minus indicates migration towards the cathode. Phenol red always migrates towards the anode at a rate of 0.0051 $cm^2/V \cdot min$. In acidic solution, thioglucose does not migrate at all, but at pH 7 it has a negative charge resulting from complexing of the borate ion.

Let us define the relative osmotic mobility by

$$k = S_o/S_r$$

TABLE I

CORRECTED RELATIVE MOBILITIES OF THIOLS WITH PHENOL RED AS REFERENCE AND THIOUREA AS IMMOBILE SUBSTANCE

Buffers: A, acetic acid (7.5 ml/l) + formic acid (2.5 ml/l) + ethylene glycol (50 ml/l), pH 2.5, $k = -0.05$; B, triethanolamine (10 g/l) + boric acid (20 g/l) + Na_2EDTA (2 g/l) + ethylene glycol (100 ml/l), pH = 7.0, $k = -0.24$.

Thiol	*Buffer A*	*Buffer B*
Cysteamine	−3.2	−1.8
Cysteine methyl ester	−2.0	−0.17
Homocysteine	−0.48	+0.10
Cysteine	−0.33	+0.19
Penicillamine	−0.28	+0.24
Glutathione	−0.22	+0.94
Thioglucose	0.00	+0.35
Captopril	+0.11	+1.2
Acetylcysteine	+0.26	+1.4
Thiosalicylic acid	–	+1.6
Mercaptoacetic acid	–	+2.1
2-Mercaptopropionic acid	–	+1.8
2-Mercaptoethanesulphonic acid	+2.2	+2.2
Thiomalic acid	+0.31	+2.5

where S_o and S_r are the distances from the start line of the immobile (thiourea) and reference (phenol red) substances. The value of k determined in buffer A is −0.05 and in buffer B, −0.24, indicating the negative charge of the cellulose gel.

The relationship between the corrected, u, and uncorrected, $u' = S/S_r$, relative mobilities is given by

$$u = \frac{S - S_o}{S_r - S_o} = \frac{u' - k}{1 - k}$$

where S is the distance of the investigated thiol from the start line. Once the relative osmotic mobility has been determined, the corrected relative mobilities can be calculated from uncorrected ones.

Assuming 0.5 cm between the bands as the full resolution, the time of resolution can be calculated from

$$t_{res} = \frac{100}{(u_1 - u_2)G} \text{ min}$$

where G is the voltage gradient in V/cm. The time of resolution for cysteine and penicillamine in buffer A at $G = 80$ V/cm is 25 min.

REFERENCE

1 M. Wroński, *J. Chromatogr.*, 248 (1982) 363.