Thin-layer Gel Filtration

BENGT G. JOHANSSON and LARS RYMO

Department of Medical Biochemistry
University of Göteborg, Sweden

The introduction by Porath and Flodin [1] of the gel filtration method, generally applied as a column chromatographic technique, permits a very convenient way of separating substances of different molecular size. In order to achieve a simple method to test the ability of separating substances of biological interest by gel filtration using very small amounts of material we have investigated the possibility of using a thin-layer chromatographic technique which we will present in this paper.

Methods and materials. In this study Sephadex G-25, fine (water regain 2.4), Sephadex G-75, fine (water regain 7.6) and Sephadex G-75, particle size < 400 mesh, were used. The thin-layer plates were made with the equipment delivered by C. Desaga GmbH, Heidelberg. The spreader was used with the slit width adjusted to 0.5 mm.

Procedure. For Sephadex G-25 gel filtration 10 g of the gel was stirred with 50 ml of 0.1 M sodium chloride or with an appropriate buffer solution for one hour. The slurry was then transferred to the spreader and immediately spread over either four glass plates of the dimensions of 8 × 20 cm or two plates of the dimensions of 20 × 20 cm. After 10 min the plates could be removed from the plastic support without disturbing the uniformity of the gel layer. The plates could be used at once or stored in a moist chamber for one or two days. Preparation of plates with Sephadex G-75, fine, and Sephadex G-75 (particle size < 400 mesh) was performed in a similar manner, but using 5 g of the gel, which was intermittently stirred for 5 h with 70 ml of a buffer solution, usually 0.02 M sodium phosphate buffer, pH 7.0, containing 0.2 M sodium chloride.

The samples were applied at a distance of 4 cm from the end of the plate as spots with a diameter of 3—5 mm, which was possible by using volumes of 1—5 µl. The plates were placed in a paper electrophoresis unit (LKB 3276 BN, Stockholm) with one of the electrode chambers 7—10 cm higher than the other. A piece of filter paper (Whatman No. 3 MM) ensured contact between the gel and the buffer reservoir, giving a liquid flow through the gel. With a plate of the dimensions of 20 × 20 cm the electrophoresis unit could not be used; instead the plates were placed in a moist chamber leaning against a buffer reservoir with a filter paper contact as described above. The flow rates varied somewhat, but this did not interfere with the reproducibility of the runs. A suitable coloured substance, usually 2,4-dinitrophenyl aspartic acid (DNP-aspartic acid), was always applied to the plate as a marker of filtration velocity. Running times of 5 h for Sephadex G-25 and 8 h for Sephadex G-75 generally gave good separations. This corresponded to a travelling distance of about 10—15 cm for substances, which by column gel filtration were found to have R_F-values close to 1.0. After complete filtration the plates were dried in an oven at 80°C, giving a thin opaque film of the gel on the glass plates, and then sprayed with 0.3 % ninhydrin in ethanol or dyed with amido black (Amidoschwarz 10 B, E. Merck AG, Darmstadt) according to the following procedure. The plates were placed in a saturated solution of the dye in methanol-acetic acid-water, 80:10:10 (v/v), and left for one hour. Excess dye was removed by repeated washings in methanol-acetic acid-water, 70:15:15 (v/v), leaving a faint blue background in the gel. The plates were then dried at room tem-

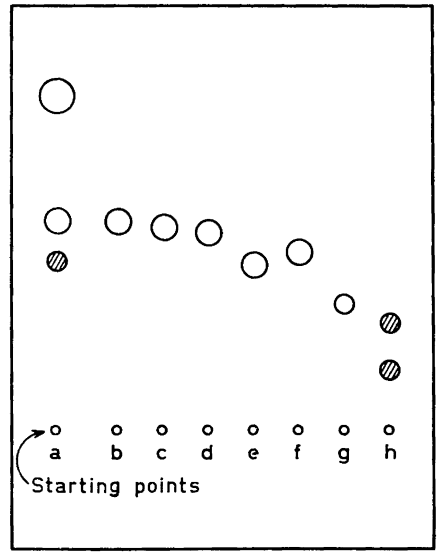

Fig. 1. Schematic representation of gel filtration on Sephadex G-25. a. Coeruleoplasmin and DNP-aspartic acid. b. Aspartic acid. c. Glycyl-glycine. d. Glycine. e. Phenylalanine. f. Tyrosine. g. Tryptophan. h. DNP-phenylalanine and DNP-tryptophan. Ninhydrin treatment of the plate.

perature. During the dyeing procedure a slight swelling of the gel was obtained, and it had a tendency to loosen from the plates. This, however, could be counteracted by careful handling.

Application of the method. Sephadex G-25 was used for the separation of a mixture of human coeruleoplasmin and DNP-aspartic acid as seen in Fig. 1. The separation was very sharp. Besides the yellow spot is seen a ninhydrin colouring substance coming from impurities in the DNP-amino acid preparation. Fig. 1 also shows the thin-layer filtration of some amino acids and amino acid derivatives. Glycyl-glycine moves with approximately the same rate as glycine. A slightly lower rate of movement is found for phenylalanine and tyrosine whereas tryptophan moves considerably slower than the other amino acids. The DNP-derivatives of phenylalanine and tryptophan are well separated and move slower than the corresponding amino acid. These results are in accordance with the fact that aromatic substances are retarded on the gel due to reversible adsorption [2].

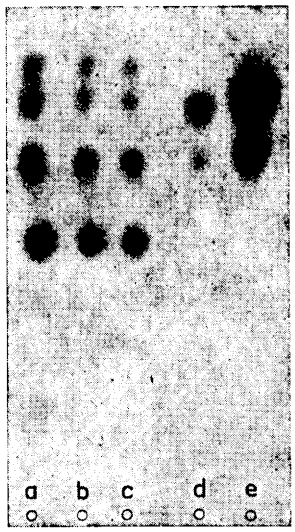

Fig. 2. Gel filtration on Sephadex G-75, fine. a—c. A mixture of bovine serum albumin β-lactoglobulin and α-lactalbumin. d, e. A sample of purified "Bence-Jones protein" from a pathological urine. Double amount of sample applied in e. The plate was dyed with amido black B.

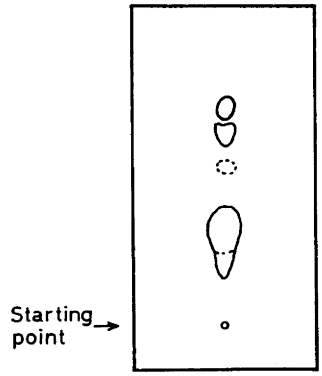

Fig. 3. Schematic representation of gel filtration of a tryptic hydrolysate of normal human γ-globulin on Sephadex G-75, fine. Ninhydrin colour.

An experiment using Sephadex G-75 is shown in Fig. 2. Artificial mixtures of bovine serum albumin, β-lactoglobulin and α-lactalbumin (molecular weights 69 000, 35 000 and 15 000) in equal parts are separated into four spots (Fig. 2a—c). In other experiments bovine serum albumin was found to give two spots. A sample of purified "Bence-Jones protein" gives two well separated spots (Fig. 2d, e). Filtration of a tryptic hydrolysate of human γ-globulin, finally, gives a separation in full accordance with the results obtained by column gel filtration [3] (Fig. 3).

The present method offers good possibilities of rapid comparative analyses by gel filtration and will be used in studies of human γ-globulin related proteins and their degradation products.

We are indebted to Miss Inger Karlsson for skilful technical assistance. The work was supported by a grant from the *Medical Faculty*, University of Göteborg.

A similar method for thin-layer gel filtration on Sephadex G-25 has quite recently been published by Determann[4].

1. Porath, J. and Flodin, P. *Nature* **183** (1959) 1657.
2. Porath, J. *Biochim. Biophys. Acta* **39** (1960) 193.
3. Hanson, L. Å. and Johansson, B. G. *Nature* **187** (1960) 599.
4. Determann, H. *Experientia* **18** (1962) 430.

Received October 8, 1962.