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Thin-layer gel filtration on pearl-condensed agar

Preliminary results

Gel chromatography has become a very important technique for separation and analysis of complex mixtures. Dextran gels (Sephadex) are used in column, and recently also in thin-layer techniques. Two convenient properties of thin-layer gel filtration are the reduced running time and small sample size.

The use of gel filtration on agar or agarose for the separation of high molecular substances has been introduced recently by POLSON[1]. Its primary advantages over dextran gel or polyacrylamide gel are the high exclusion limit and relative rigidity of agar gel even at very low concentrations. Pearl-condensed agar or agarose has been employed until now for column techniques[2–7]. We have tried to use this material in thin-layer techniques.

In this work the possibility of preparing pearl agar suitable for thin-layer gel filtration and its separation ability were studied. We used 4% pearl agar which had been prepared by a modified method according to HJERTÉN[8]. Fractionation experiments were performed with Blue Dextran 2000, which is often used in gel filtration for the determination of exclusion volumes of the chromatographic columns. Blue Dextran was separated into two peaks with a broad range of molecular weight (mol. wt. 10^5–10^6) on Sepharose 4 B (ref. 9). We had two reasons for the use of Blue Dextran for testing agar thin-layer gel filtration. It has a wide range of high molecular sizes and a marked blue colour.

Blue Dextran was a commercial preparation, batch No. "T_0-4474" (Pharmacia, Uppsala). A standard buffer 0.1 *M* Tris + 0.1 *M* NaCl, pH 7 (adjusted by HCl), was used in experiments both for gel filtration in column and for thin-layer techniques. Agar pearls were prepared as follows: A hot solution (about 90°) of 4% Difco agar in water (300 ml) was poured at once into a warm (50°) mixture of 470 ml toluene, 130 ml tetrachloromethane and 1 g Tween 60 (polyoxyethylene sorbitan monostearate). The mixture was subjected to vigorous mechanical stirring for about 30 min at 4000 r.p.m. in the homogeniser. After cooling to room temperature the stirring was stopped, the mixture was centrifuged 15 min at 3000 r.p.m. and the solution discarded. In the next step agar pearls were washed several times in water (approximately ten times) until complete removal of all organic solution was reached and then the pearls were separated according to their sizes by means of an appropriate sieve. The pearls of sizes 40–100 mesh were used for gel filtration on the column and those of 100–400 mesh for thin-layer technique.

Gel filtration was performed on a column of 4% agar pearls, equilibrated with the solution of standard Tris buffer. The column employed was 90 × 1.4 cm. A 2 ml portion of the 2.5% Blue Dextran solution was applied on the top of the column and the flow rate was adjusted to 5 ml/h. Fractions of 4–5 ml were collected and measured at 580 nm on the "Specol" spectrophotometer. Fractions selected for thin-layer gel filtration were concentrated by precipitation with methyl alcohol (1 Vol. of the fraction + 4 Vol. of CH_3OH). The whole solutions were discarded and the precipitates

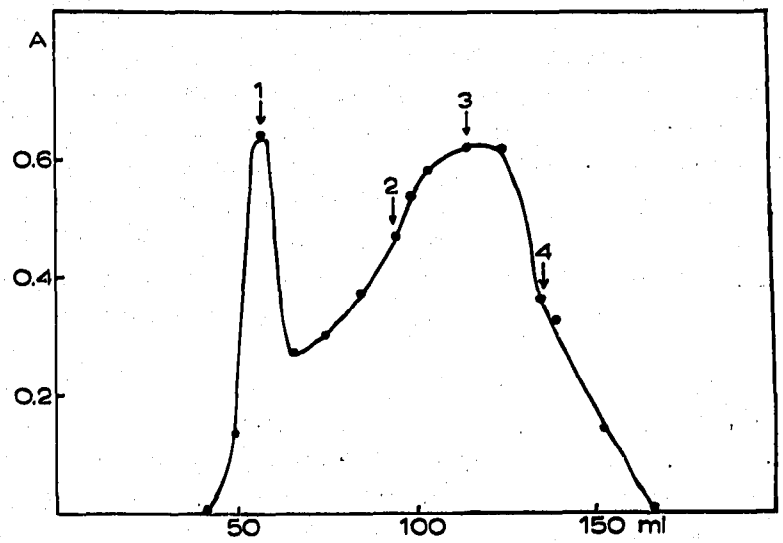
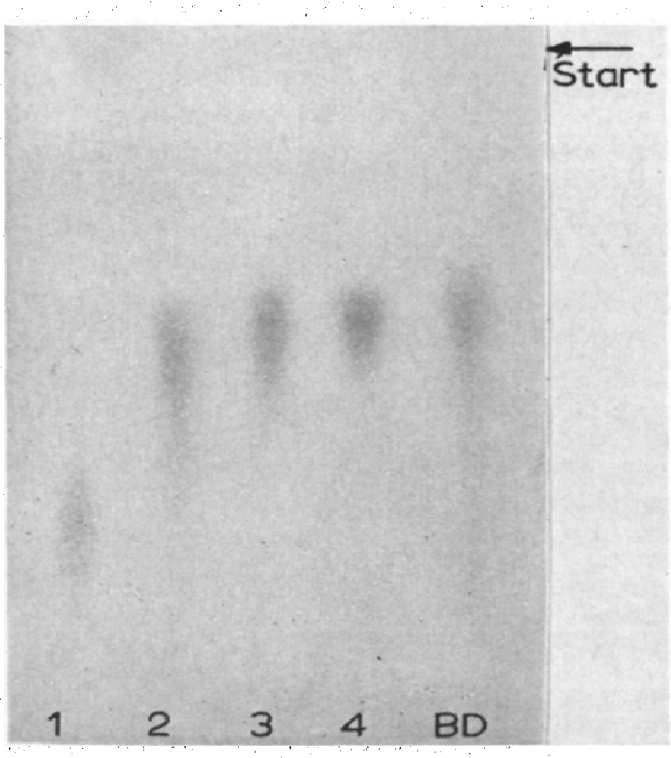


Fig. 1. Column gel filtration of Blue Dextran on pearl-condensed 4% agar, particle size 40–100 mesh. Column 90 × 1.4 cm, buffer 0.1 *M* NaCl + 0.1 *M* Tris + HCl, pH 7. The fractions selected for agar thin-layer gel filtration are indicated by arrows.



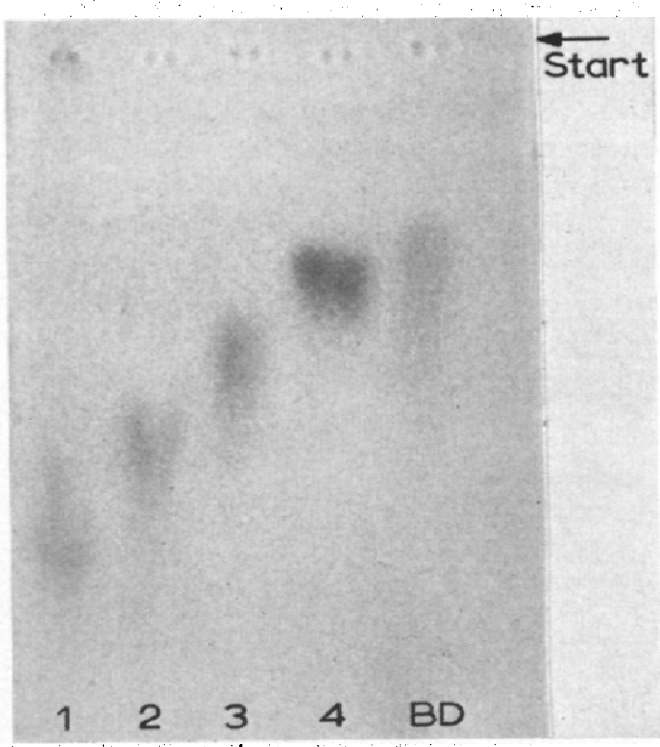


Fig. 2. Thin-layer gel filtration on agar of the Blue Dextran (BD) fractions from column gel filtration. Pearl-condensed 4% agar, particle size 100–400 mesh. Plates 8 × 14 cm, thickness of the layers 0.5 mm, buffer 0.1 *M* NaCl + 0.1 *M* Tris + HCl, pH 7, flow rate 1 cm/h, applied 20 μl of approx. 2–2.5% sample. For numbering of the fractions see Fig. 1.

Fig. 3. Thin-layer gel filtration on agar of the Blue Dextran (BD) fractions from column gel filtration. Applied: 20 μl of approx. 3.5–4% sample. Experimental conditions as in Fig. 2.

were dried at room temperature. The precipitates were dissolved approximately to 2–2.5% solutions by several drops of the standard Tris buffer.

The thin layers of agar pearls were prepared on 8 × 14 cm glass plates, thickness of the layers being 0.4–0.5 mm. The plates were run by descending chromatography in a closed chamber. The buffer reservoir and the upper end of the plate were connected by a filter paper wick of Whatman No. 3. Before a run was started the plates had to be equilibrated by allowing the Tris buffer to flow through the gel for 10 h. After equilibration, the samples (20 μl) were applied with a micropipette as round spots 5 mm in diameter. The flow rate through the gel of 1 cm/h was regulated by the angle of the plate (approx. 15°).

The elution pattern of Blue Dextran 2000 on a column with 4% agar pearls is given in Fig. 1. Blue Dextran separated into two peaks. The leading peak contains material of a very high molecular weight which is completely excluded from agar gel and indicates the void volume of the agar column. This elution profile is similar to that described in the Pharmacia Fine Chemicals booklet[9].

Fig. 2 shows thin-layer gel filtration on agar of selected fractions (indicated by arrows) of Blue Dextran from the agar column. The samples ran with decreasing rate in dependence on their decreasing molecular sizes. The visibility of the spots is limited by the concentration of Blue Dextran. We could not use more concentrated solutions because of the high viscosity of the samples which partially influenced their rate of flow. A high viscosity is marked especially in the first fraction. This fraction was running at a lesser rate than corresponded to its molecular size and a small part of the fraction stayed even on the start (see Fig. 3).

Our results with thin-layer gel filtration on agar indicate the possibility of using this method for simple and rapid orientation estimation of particle, aggregate and molecular sizes of proteins, modified and denatured proteins, nucleic acids, cell particles, viruses, etc. The main advantage of the agar thin-layer technique seems to be in the fact that small sample volumes are needed for the experiments. The results of further experiments with thin-layer gel filtration on agar will be reported later.

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