# Notes

## Preparative thin layer gel filtration

Gel filtration is a technique which has become increasingly popular for sample purification and the analysis of complex mixtures[1]. The cross-linked dextran Sephadex (AB Pharmacia, Uppsala, Sweden) has been employed extensively in column techniques[2,3] and more recently in thin layer techniques[4–6]. Two advantages of the thin layer technique are those of reduced running time and small sample size. However, one of the major disadvantages of this method has been that of the location of the resolved components. The methods which have been previously described usually result in the destruction of the sample[6]. The method described in this communication presents a means of circumventing this problem through the use of the dye Lissamine Rhodamine B-200 as the location reagent.

*Materials and methods*

The gel plates were prepared in the following manner: The dextran Sephadex G-200 was constituted as a slurry in a *M*/15 Sorenson phosphate buffer, pH 7.8. The initial buffer volume to dry weight of dextran ratio was 25:1 (ml/g). After permitting the dextran to swell for 72 h, an additional volume of the phosphate buffer was added, this aliquot containing the Lissamine Rhodamine B-200 (Imperial Chemical Industries Ltd., Manchester, England) at a concentration of 1.2 mg/ml. The final buffer volume to dextran weight ratio was 30:1, while the final concentration of the dye was 0.2 mg of the dye per milliliter of slurry. Application of the slurry to the 15 × 30 cm glass plates was accomplished by a modification of the technique described by BARON AND ECONOMIDIS[7]. This technique consisted of fixing the plate into position by a double thickness of masking tape along the periphery of the length of the plate. The slurry was then poured along one of the free edges of the plate, and spread by means of a 0.5 × 20 cm glass rod, held firmly against the tape and slid in a smooth, even motion. After removal of the tape, the plate was placed in the chamber and connected to the buffer reservoir by a double thickness of Whatman No. 1 filter paper in an arrangement similar to that described by DETERMANN[4]. The eluting buffer consisted of the Sorenson buffer described above containing the Lissamine Rhodamine B-200 dye at a concentration of 0.2 mg/ml. Separations were performed at an angle of 12° for a period of 9 h. After completion of the run, the plates were permitted to air-dry at 5°, then exposed to an ultraviolet light source. The light source employed by the author was that of a "Black Raymaster" (George Gates & Co., Franklin Sq., Long Island, N.Y.). Following the location of the resolved components by ultraviolet light, the removal of these samples was accomplished through the addition of 0.1 ml of the phosphate buffer. The re-hydrated gel was then transferred by means of spatula to a small test tube and an additional 0.25 to 0.50 ml of the buffer was added to the sample. Elution of the samples from the gel was permitted to proceed for approximately 6 h at 5°.

Standard protein solutions consisting of Papain (Worthington Biochem. Corp., Freehold, N.J.), β-lactoglobulin (Pentex Corp., Kankakee, Ill.), Bovine Serum Albumin (Nutritional Biochem. Corp., Cleveland, Ohio), Egg Albumin (Nutritional Biochem. Corp.) and Porcine Thyroglobulin (Sigma Chem. Co., St. Louis, Mo.) at a 1 mg/ml concentration were applied to the plates in 10 μl volumes. Normal guinea pig serum samples were applied in 5, 10, and 15 μl samples. Isolation of the $\gamma_2$-globulin fraction of these sera was accomplished through the addition of 10 and 15 μl volumes of a two-thirds ammonium sulfate-saturated serum sample to the equilibrated plates.

Analyses of the eluted samples were performed by means of the immunoelectrophoretic technique of GRABAR AND WILLIAMS[8] and by the gel double diffusion technique of OUCHTERLONY[9]. The antiserum employed for these analyses was that of a rabbit anti-guinea pig globulin antiserum (Nutritional Biochem. Corp., Cleveland, Ohio).

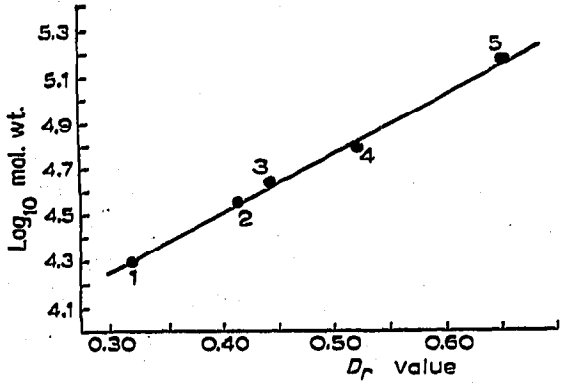


Fig. 1. $D_r$ Values obtained for standard protein solutions:

| *No.* | *Sample* | *$Log_{10}$ mol. wt.* | *$D_r$ value* |
|---|---|---|---|
| 1 | Papain | 4.301 | 0.316 |
| 2 | β-Lactoglobulin | 4.556 | 0.422 |
| 3 | Egg albumin | 4.653 | 0.448 |
| 4 | Bovine serum albumin | 4.770 | 0.530 |
| 5 | Bovine γ-globulin | 5.204 | 0.655 |

*Results and discussion*

Upon exposure to ultraviolet light, the zones containing the sample fractions are easily seen as darkened areas against a bright orange background. This technique has proven capable of detecting total protein concentration levels as low as 0.2 μg of a standard bovine serum albumin solution. The method of application of the gel slurry to the plates affords results which are quite reproducible, as evidenced by the graph in Fig. 1. A linear relationship is seen to exist when the $D_r$ value (ratio of the migration distance of the retarded species to that of the excluded species) is plotted against the log of the molecular weight of the retarded protein, a relationship similar to that described by ANDREWS[10]. The fractionation of the normal sera yielded zone

patterns similar to those described by JOHANSSON AND RYMO[5]. Elution of the second zone, as seen in Fig. 2, results in the isolation of components which, when analyzed immunoelectrophoretically, indicate the presence of the 7S-type globulins. However, elution of the first zone (normally the albumin zone) of the ammonium sulfate-precipitated sample yields $\gamma_2$-type globulins when immunoelectrophoretically analyzed (see Fig. 2). The results of the double diffusion analyses indicate that the isolation procedures employed are sufficiently mild as to minimize the possibility of sample denaturation.

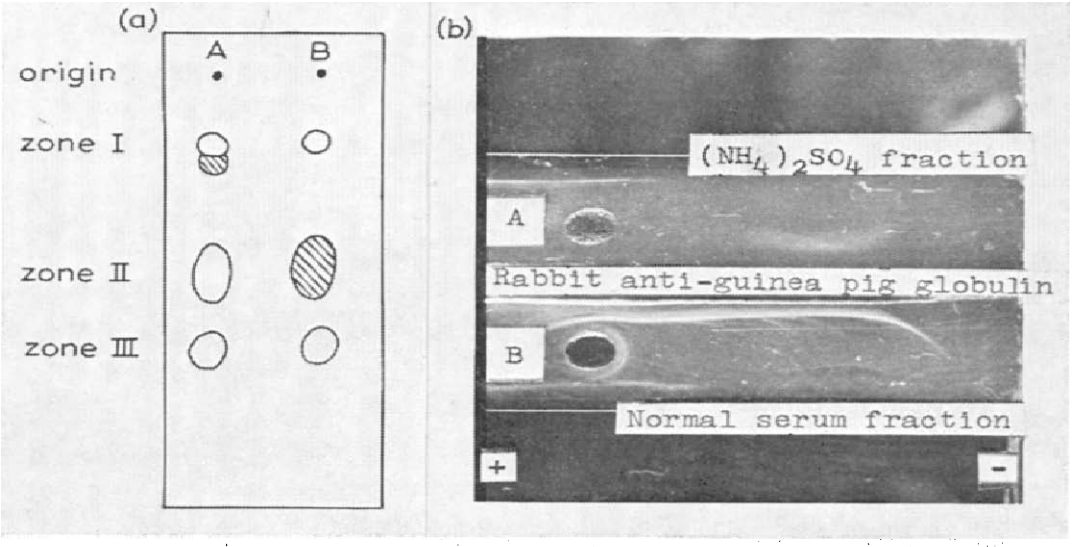


Fig. 2. Immunoelectrophoretic analyses of eluted serum globulin fractions. Shaded areas in (a) represent eluted plate segments.

The employment of the dye Lissamine Rhodamine B-200 as the location reagent in a fluorescence-quenching technique permits the investigator to employ thin-layer gel filtration as a preparative technique. Because of its sensitivity and ease of performance in reproducible fashion, application of this method could well be extended to "thick-layer" as well as two-dimensional (electrophoresis, elution) techniques, and should prove useful to those working with sample quantities too small to be conveniently applied to columns.

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1 P. FLODIN, *Dextran Gels and Their Application in Gel Filtration*, Pharmacia, Uppsala, 1962.
2 J. PORATH, *Biochim. Biophys. Acta*, 39 (1960) 193.
3 T. P. KING AND P. S. NORMAN, *Biochemistry*, 1 (1962) 709.
4 H. DETERMANN, *Experientia*, 18 (1962) 430.
5 B. G. JOHANSSON AND L. RYMO, *Acta Chem. Scand.*, 16 (1962) 2067.
6 T. WIELAND AND H. DETERMANN, *J. Chromatog.*, 28 (1967) 2.
7 D. N. BARON AND J. ECONOMIDIS, *J. Clin. Pathol.*, 16 (1963) 484.
8 P. GRABAR AND C. WILLIAMS, *Biochim. Biophys. Acta*, 10 (1953) 193.
9 O. OUCHTERLONY, *Arkiv Kemi Mineral. Geol.*, 268 (1948) 1.
10 P. ANDREWS, *Biochem. J.*, 91 (1964) 222.

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