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Note

Heterogeneities of blue dextran and differences among samples

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Blue dextran has been widely used in gel chromatography for checking the packing of gel columns and for the determination of void volumes, V_0. However, its physical properties have not been investigated in detail. The average molecular weight and molecular weight distribution do not represent the absolute molecular weight. However, the lack of such data should not be considered a problem as regards the practical use of gels with molecular weight exclusion limits of less than 10^6 and blue dextran may give the void volumes of columns satisfactorily.

In recent years, gels with the high exclusion limits have been utilized for various substances of high molecular weight. Some of the gels may give broad elution patterns with blue dextran and V_0 cannot be measured. The elution patterns from gels with intermediate exclusion limits may show a first peak with V_0 and other peaks or a plateau in a region with a large elution volume. For example, for a series of Sepharose gels V_0 values were reported by the manufacturers (Pharmacia) as being obtained from the first peak in the elution pattern of blue dextran[1]. Recently, it has been reported that the V_0 values of columns of Sephacryl S-400 and Sephacryl S-500, the exclusion limits of which may be higher than those of Sepharose, were determined from chromatograms of blue dextran[2].

However, the second peak or the plateau following the first peak in the elution pattern is inconvenient or interferes with the calibration of the columns. Therefore, we have fractionated blue dextran and used the fraction of large molecules for checking and calibration in order to obtain a more distinct band and V_0.

In the fractionation of blue dextran, we found that the molecular weight distribution in one sample was significantly different from those of other samples and inadequate for use in examining Sepharose CL-2B. Even the differences among other samples cannot be ignored in some instances. This paper reports on these differences in the context of the use of blue dextran for gel chromatography.

EXPERIMENTAL

Four samples of blue dextran 2000 (Pharmacia) were purchased in different years and were reprecipitated before use by pouring an aqueous solution into methanol. Gel filtrations for the evaluation of heterogeneity of molecular size were performed with Sepharose CL-2B for all samples and with Sepharose CL-6B for one sample. The column (100 × 1.5 cm I.D.) was fitted with a jacket of circulating water

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at 25°C. The solvent used was 0.1 *M* sodium chloride solution containing 0.02% of sodium azide. A 1-cm³ volume of 0.5% blue dextran solution was applied to the column and eluted at a constant flow-rate of 6 cm³ h⁻¹ (Sepharose CL-2B) or 12 cm³ h⁻¹ (Sepharose CL-6B). The polymer concentration in the eluent was measured with a Showa Denko Model Shodex RI flow-type differential refractometer equipped with a recorder. The viscosities of solutions in water containing sodium chloride at 25°C were measured using Ubbelohde-type capillary viscometers and the intrinsic viscosities, $[\eta]$, were evaluated. The absorptivity $A_{620}^{1\%}$, in the same solutions was also determined from absorbance measurements at 620 nm using a Shimadzu Model UV-200 spectrophotometer.

RESULTS AND DISCUSSION

The $[\eta]$ and $A_{620}^{1\%}$ data for the various samples of blue dextran are summarized in Table I. The decrease in $[\eta]$ with increasing NaCl concentration suggests a decrease in the charge effect (*e.g.*, ref. 3) of blue dextran as a polyelectrolyte. Other viscosity data for sample C at higher concentrations of sodium chloride indicated that the charge effect may be eliminated in sodium chloride solutions stronger than 0.5 *M*. Although the reason is not clear, $A_{620}^{1\%}$ depends on the sodium chloride concentration and is almost constant in sodium chloride solutions stronger than 0.5 *M*, corresponding to the dependence of $[\eta]$ on sodium chloride concentration.

The relationship between $[\eta]$ and molecular weight for blue dextran is unknown, but the analogy with the relationship for dextran[4–6] suggests that the differences in the average molecular weight, for example between samples C and D, may be considerable. The difference in $A_{620}^{1\%}$ is presumed to be due to the difference in the degree of substitution of the dye among the various samples.

Elution patterns from a gel column of Sepharose CL-2B are illustrated in Fig. 1. The first peak indicates the V_0 of the column and the proportion of large molecules in each sample excluded from the gel. The differences in the first peaks among the samples are very large: sample A had a small peak with V_0 being scarcely indicated; sample B gave a peak higher than that of sample A but smaller than that illustrated by the manufacturers[1]; sample C contained more large molecules than the others, producing a sharp, high peak, and might be preferable for examining this gel column;

TABLE I

INTRINSIC VISCOSITIES $[\eta]$ (cm³ g⁻¹) AT 25°C AND $A_{620}^{1\%}$ FOR SAMPLES OF BLUE DEXTRAN 2000 IN 0.1 AND 0.5 *M* NaCl SOLUTIONS

Each sample was purchased in the year indicated.

Sample			*[η]*		$A_{620}^{1\%}$	
Code	*Lot No.*	*Year*	*0.1 M NaCl*	*0.5 M NaCl*	*0.1 M NaCl*	*0.5 M NaCl*
A	8050	1973	–	46.8	–	8.46
B	5168	1980	50.5	46.1	–	9.63
C	GB19049	1982	59.0	53.0	9.75	9.37
D	ID30280	1985	47.8	43.6	9.16	8.70

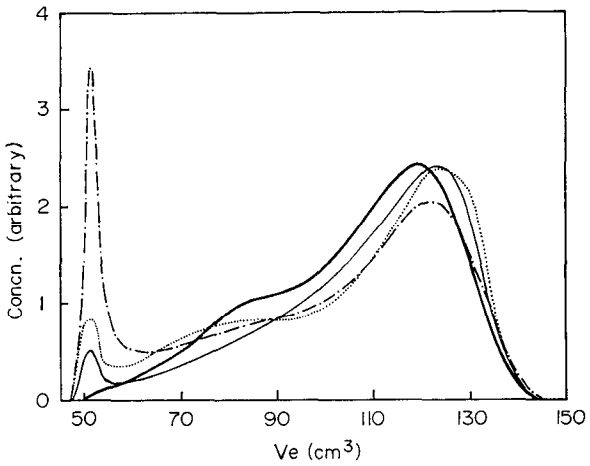

Fig. 1. Elution patterns from a column of Sepharose CL-2B for samples of blue dextran A (———), B (·····), C (—·—·—) and D (———). The column dimensions were 100 × 1.5 cm I.D. and 1 cm^3 of a 0.5% sample solution containing 0.1 *M* NaCl and 0.02% NaN_3 was applied. Elutions were carried out at a constant flow-rate of 6 cm^3 h^{-1} at 25°C. The void volume (V_0) and the inner volume (V_i) of the column were 51 and 145 cm^3, respectively.

on the other hand, sample D was not able to indicate V_0, and the front of the band was not visible during the elution through the column.

The right-hand ends of the last large peaks in Fig. 1 coincide with the inner volume, V_i, of the column, suggesting that small molecules eluting around the last peak could not be separated effectively with this column. Therefore, an apparent agreement of the last peaks does not indicate a similar distribution of small molecules in the samples.

For further characterization of sample D, elution from a column of Sepharose CL-6B was examined and the pattern is shown in Fig. 2, together with the elution

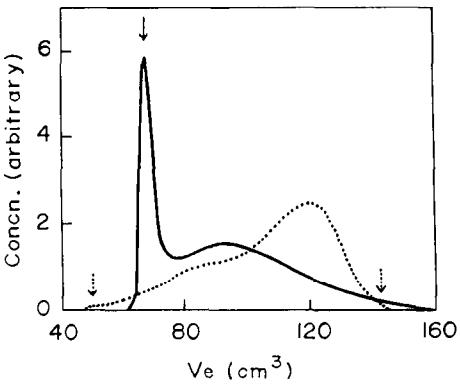

Fig. 2. Elution patterns of blue dextran D from gel columns of Sepharose CL-6B (———) and Cl-2B (·····). The columns and experimental conditions were as in Fig. 1, except that the flow-rate was 12 cm^3 h^{-1} for Sepharose CL-6B. The solid arrow indicates the V_0 of the Sepharose CL-6B column, of which the V_i was 177 cm^3 (not shown). The dotted arrows indicate the V_0 and V_i of the Sepharose CL-2B column.

pattern from Sepharose CL-2B as shown in Fig. 1 for the sake of comparison. About one third of the sample was eluted from Sepharose CL-6B in the first peak, and a corresponding amount was eluted from Sepharose CL-2B in a wide range between V_0 and the elution volume of *ca.* 100 cm^3. The second part of the elution pattern from Sepharose CL-6B contained about two thirds of the sample, but the end of the curve was far from V_i. The characteristics of sample D may be summarized as follows: (i) almost all of the large molecules contained in the samples are smaller than those that should be eluted around the V_0 of Sepharose CL-2B, but are large enough to be eluted in the V_0 of Sepharose CL-6B and other gels with lower exclusion limits such as the Sephadex series; (ii) small molecules in appreciable amounts contained in the sample may be smaller than those that could be separated with Sepharose CL-2B, but these are larger than the lower limit for separation with Sepharose CL-6B.

As is well known, the molecular weight distribution of blue dextran is broad[1]. The broad elution patterns of the gel filtrations observed in this work are to be expected. However, the differences in the elution patterns are unexpectedly significant. As mentioned before, sample D cannot be used as a reference material for examining columns of Sepharose CL-2B. Sample C is preferable for such a purpose, and the fraction that eluted around the V_0 of Sepharose CL-2B was separated and used satisfactorily for examining a series of Sepharoses. Sample A or B may be inadequate for checking the gel packing in columns of Sepharose CL-2B, because the first peak was so small and the following peak so large that the coloured band in the column was broad and the front of the band was scarcely visible. Only a small amount of the fraction could be separated for preparing samples of large molecules, as mentioned above. It could not be obtained from sample D.

Blue dextran 2000 is prepared from dextran 2000[1] having an average molecular weight of $2 \cdot 10^6$. The average molecular weight or molecular weight distribution of original dextran samples may be similar. If partial degradation of dextran molecules has taken place during the substitution reaction of the dye, the average molecular weight and molecular weight distribution of the blue dextran obtained should differ to some extent. It is not clear whether the differences observed are within or beyond the allowable differences among the blue dextran products, or whether samples C and D were extreme cases. It should be noted, however, that such differences in molecular weight and heterogeneities among the samples as reported here may be possible in other samples of blue dextran, and hence care or examination may be required in the use of blue dextran, *e.g.*, for the calibration of gel columns having high exclusion limits.

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