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J. M. Barrales-rienda[a]; A. Horta[b]; E. Saiz[b]
[a] Sección de Química Física Macromolecular Instituto de Plásticos y Caucho C. S. I. C., Madrid, Spain [b] Departamento de Química Física Facultad de Ciencias, Universidad Complutense, Madrid, Spain

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Molecular Weight Distribution of a Clinic Dextran

J. M. BARRALES-RIENDA

Sección de Química Física Macromolecular
Instituto de Plásticos y Caucho
C.S.I.C.
Madrid 6, Spain

and

A. HORTA and E. SAIZ

Departamento de Química Física
Facultad de Ciencias
Universidad Complutense
Madrid 3, Spain

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ABSTRACT

A sample of clinic dextran ($\overline{M}_n$ = 54,000) has been fractionated by precipitation at 25°C in the system water/ethanol. Seven fractions were isolated in the usual way and freeze-dried. Fitting of the molecular weight distribution to the exponential functions of Tung and Schulz gives a good approximation, but it is improved by using a linear combination of the exponential, logarithmic, and normal functions, according to a method recently developed.

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INTRODUCTION

Dextran is a macromolecule that is produced when cultures of bacteria of the Acetobacter, Betabacterium, Leuconostoc, or Streptococcus families are grown on a medium containing sucrose. One of the greatest applications of dextran, apart from its increasingly growing use as the stationary phase for GPC in cross-linked form, is as a partial substitute for blood plasma, specifically as a volume expander. Its pharmacological applications are direct consequences of its physicochemical properties. Undoubtedly, all depend not only on molecular weight but also on the details of its distribution.

Clinic dextran is usually obtained by hydrolytic partial depolymerization and subsequent fractionation of the dextran that is produced by industrial fermentation in a culture of Leuconostoc mesenteroides. The product from partial hydrolysis gives a dextran with molecular weight in the range needed for clinical applications. Specifications for clinic dextran are strict. In part, this is due to the fact that the higher molecular weight species are retained by the human body for longer periods than required, while the lower molecular weight species are excreted in the urine and are not useful as blood plasma volume expanders.

Because it is a product obtained biosynthetically and subsequently hydrolyzed, it should have a molecular weight distribution not easily represented by simple distribution functions. Therefore, a detailed analysis of it using more elaborate methods is in order. Recently, a new method of analysis of molecular weight distributions, which is based on a linear combination of the logarithmic, exponential, and normal functions, has been proposed [1]. It has been successfully applied to fractionation data of poly(methyl acrylate) [1, 2], poly(m-chlorostyrene) [3], and poly(p-chlorostyrene) [3]. There is no doubt that it can also present advantages in the description of molecular weight distributions of the type of clinic dextran.

The purpose of the present work is twofold. On the one hand, to determine experimentally the molecular weight distribution of clinic dextran by means of a fractionation technique. On the other hand, to carry out an analysis of its distribution by making use of the usual simple functions and of the linear combination method.

Dextran has been fractionated by a variety of methods, i.e., precipitation [4-9], extraction [8, 10], chromatography [11-13], ultrafiltration [14, 15], sedimentation [12, 16], turbidimetric titration [17, 18], and, more recently, GPC [19-27]. The method most often employed as a preparative tool, apart from GPC, has been fractional precipitation using water as solvent and a highly polar organic liquid, such as methanol, ethanol, or acetone (in order to achieve complete miscibility), as precipitant. The efficiency of this preparative

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procedure is well established for dextran. This is the method we have chosen for our analytic purposes.

EXPERIMENTAL

Material

The sample of clinic dextran used in this work was supplied by Dr. A. D. Filipe, Laboratorio de Física Médica e Radioisótopos del Hospital do Ultramar of Lisbon (Portugal). It was fractionated without any preliminary treatment.

Fractionation

As solvent/precipitant system we used bidistilled water/ethanol. The fractionation was carried out by a conventional precipitation technique, starting with a 1% aqueous solution (10 g/1000 cm^3). Addition of precipitant up to cloudiness was followed by dissolution and thermal requilibration at 25°C. The fractions precipitated in a gel-like state. They were redissolved in water, freeze-dried, and subsequently dried at 25°C and 10^{-4} Torr for 24 hr. The fractionation data are given in Table 1 for comparison purposes.

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Characterization

The fractions were characterized viscometrically, using the Mark-Houwink equation obtained by Senti et al. [5] for fractionated dextran in water at 25°C:

$$(\eta) = 1.09 \times 10^{-1} \overline{M}_n^{0.50} \quad (cm^3/g) \tag{1}$$

Molecular weights calculated according to this equation are included as the fifth column of Table 1. We have not observed separation of crystals while fractionating, (not even during the isolation of fractions in the low molecular weight region) such as the one reported by Jeanes et al. [28] for the fractionation by precipitation of low molecular weight dextrans.

ANALYSIS OF THE DISTRIBUTION

The molecular weight of a fractionated polymer is represented by means of theoretical functions containing parameters which can be

TABLE 1. Fractionation Data of a Clinic Dextran[a,b]

i	m_i	w_i	$(\eta)_i$	M_i
1	0.9000	0.0901	42.5	183,000
2	2.2650	0.2269	37.0	137,000
3	1.1820	0.1184	32.3	104,000
4	1.7529	0.1756	28.8	83,000
5	2.0585	0.2062	24.2	58,300
6	0.5287	0.0530	17.9	32,000
7	1.2959	0.1298	12.8	16,400

[a] i = fraction number; m_i = grams precipitated; $w_i = m_i / \Sigma m_i$; $(\eta)_i$ = intrinsic viscosity (cm^3/g); M_i = molecular weight. Fractionation yield = 99.83%.

[b] $\overline{M}_n = 1/\Sigma(w_i/M_i) = 54{,}000$

$\overline{M}_w = \Sigma w_i M_i = 90{,}300$

$\overline{M}_z = \Sigma w_i M_i^2 / \Sigma w_i M_i = 117{,}000$

$\overline{M}_{z+1} = \Sigma w_i M_i^3 / \Sigma w_i M_i^2 = 134{,}000$

adjusted to the fractionation data. In order to obtain such parameters, two alternative procedures are usually followed. For the first one, the determination is made in such a way that the mean deviation between the distribution and the fractions is a minimum. For the second one, the theoretical function is forced to reproduce the values of certain averages of molecular weight (moments of the distribution) calculated from the fractions. In the case of two-parameter functions, the method of Tung [29], applied to the generalized exponential function, and the method of Wesslau [30], applied to the logarithmic function, are two examples of the first procedure. Both methods arrive at the proper parameters by looking for the best fit of the distribution (expressed in such a way as to be linear in log M) to the fractions. A typical example of the second procedure is the exponential function of Schulz [31], whose parameters are easily fixed from the experimental values of the averages $\overline{M}_n$ and $\overline{M}_w$.

The method proposed by two of us [1] advantageously combines both alternative procedures. It uses a linear combination of two-parameter functions which allows the simultaneous and independent adjustment of

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four parameters in a simple manner. Two of these parameters are fixed with the experimental values of $\overline{M}_n$ and $\overline{M}_w$. The other two parameters are chosen in one of two ways: either making the theoretical values of $\overline{M}_z$ and $\overline{M}_{z+1}$ of the distribution coincide with the experimental ones (Method A), or imposing the condition that the mean square deviation of the integral distribution with regard to the accumulated fractions be a minimum (Method B). To describe the molecular weight distribution of our dextran as precisely as possible, we have applied all these methods to the fractionation data and have obtained the following results.

The method of Wesslau is not applicable to our macromolecule since the plot of the accumulated fractions vs log M on probabilistic paper is far from linear. The plot according to the method of Tung has been represented in Fig. 1. As can be seen, it also shows some curvature but still gives a satisfactory linear fit with slope = 1.55, intercept = -7.78, and standard deviation = 0.06. The function of Schulz is directly adjusted with the values of $\overline{M}_n$ and $\overline{M}_w$ of Table 1. Methods A and B of the linear combination give the following molecular weight distributions for our dextran

$$W_M = 1.02\, W_M^E - 0.19\, W_M^L + 0.17\, W_M^N \qquad \text{(Method A)}$$

$$W_M = 2.03\, W_M^E - 0.91\, W_M^L - 0.12\, W_M^N \qquad \text{(Method B)} \tag{2}$$

where W_M represents weight distribution and the superscripts E, L, and N, denote the exponential (Schulz), logarithmic, and normal two-parameter distribution functions, respectively.

For each one of the distribution functions that results from these methods, we calculated the values that correspond to the different molecular weight averages, most probable molecular weight (M_{mp}), and mean square deviation (λ) of the integral curve with respect to the accumulated fractions. The results of these calculations are shown in Table 2.

Let us consider now the adequacy of these different distribution functions to represent our fractionation data. No function can give the best fit of molecular weight averages and, at the same time, the minimum square deviation. The results of Table 2 show that the fit of the different functions tested is in the following order of goodness: when the molecular weight averages alone are considered

Method A > Schulz > Method B > Tung

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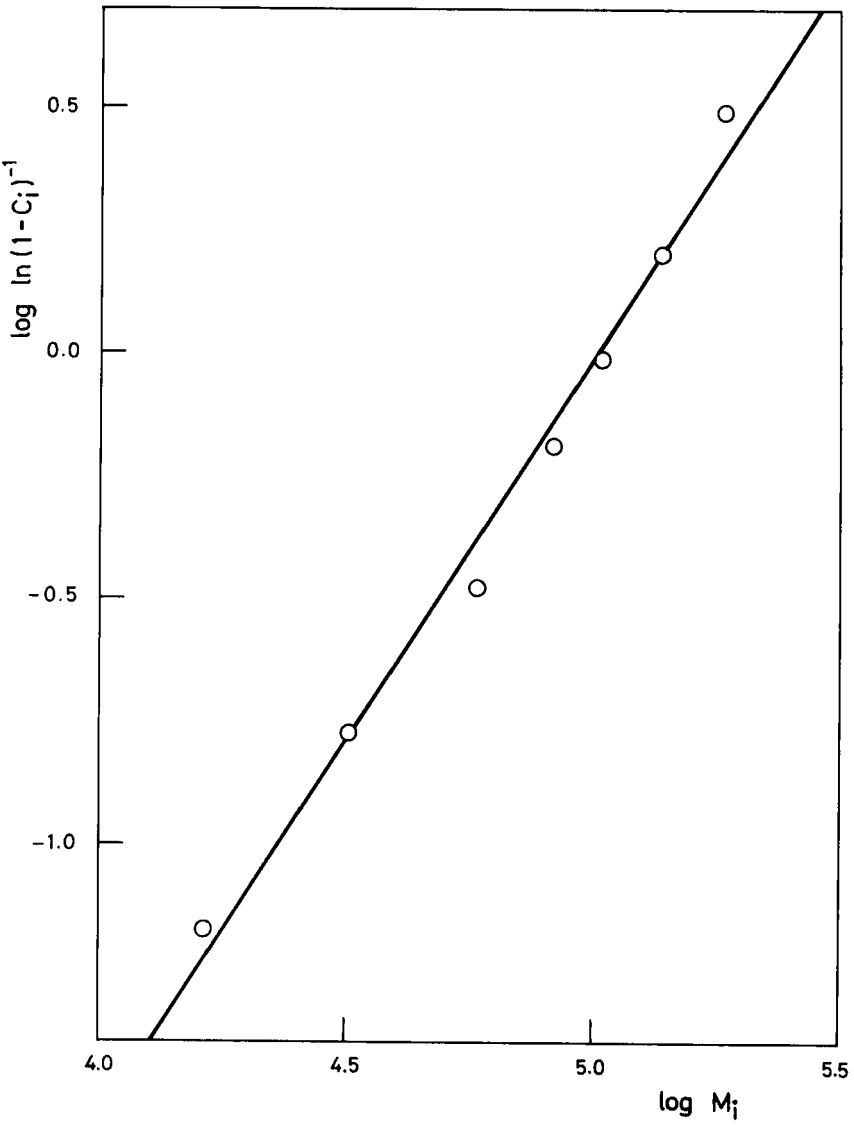

FIG. 1. Plot of the fractions (w_i) of Table 1 according to the method of Tung. (○) Accumulated fractions, $C_i = \frac{1}{2} w_i + \sum_{j=1}^{i-1} w_j$; (——) least squares linear fit.

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TABLE 2. Fit of Theoretical Distribution Functions to the Fractionated Dextran[a]

Distribution	$M \times 10^{-3}$					
	$\overline{M}_n$	$\overline{M}_w$	$\overline{M}_z$	$\overline{M}_{z+1}$	M_{mp}	$\lambda \times 10^3$
Tung	55.1	122	174	219	69	0.98
Schulz	54.0	90.3	127	173	54	1.62
Linear combination:						
Method A	54.0	90.3	117	134	55	1.51
Method B	54.0	90.3	108	54.9	68	0.44

[a]Values of the molecular weight averages, $\overline{M}_n$, $\overline{M}_w$, $\overline{M}_z$, $\overline{M}_{z+1}$, and of the most probable molecular weight (M_{mp}), calculated with each distribution; λ = mean square deviation between each integral distribution and the accumulated fractions.

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but, for the mean square deviation alone, it turns out to be

Method B > Tung > Method A > Schulz

Taking both fitting criteria as a whole, the function that best describes our dextran is the linear combination of Method A. It is plotted in Fig. 2.

For this function, the most probable value of molecular weight is larger than $\overline{M}_n$ (see Table 2). This behavior is contrary to that proposed by Granath [8] who described his data on dextran, obtained by an extraction technique, by means of the logarithmic function according to the method of Lansing and Kraemer [32]. This method applied to our precipitation data gives a poor fit, worse than that obtained with any of the four functions reported in Table 2.

This result apparently indicates that the distribution obtained depends on the fractionation technique used. The fact that the logarithmic form is favored by extraction and the exponential form (which is the dominant component of the linear combination) is favored by precipitation, points to the possible existence of some inconsistency in one of these two experimental techniques, or in both. In connection with this, it should be remembered that the asymmetric form of the

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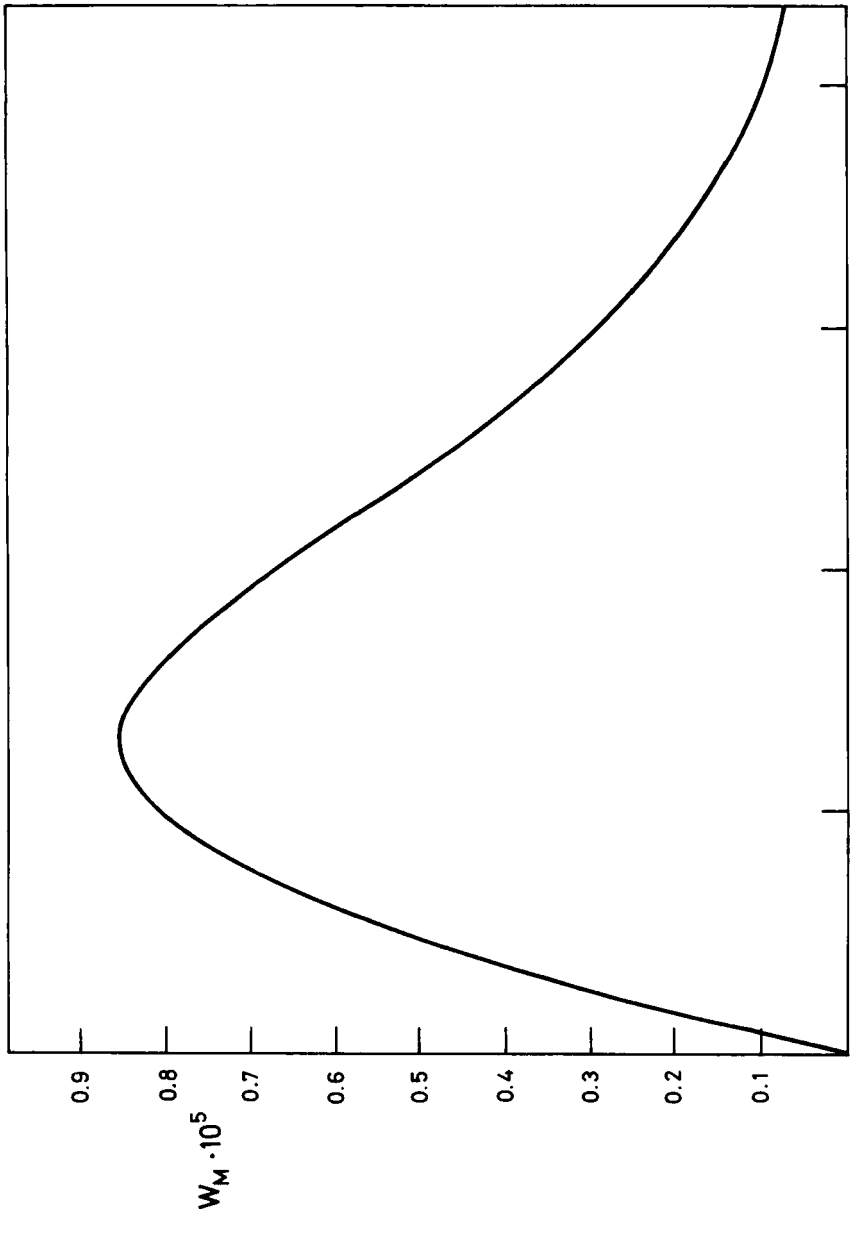

FIG. 2. Molecular weight distribution of clinic dextran (linear combination, Method A).

logarithmic function puts special emphasis on the region of low molecular weights, which is the one that is separated first in an extractive experiment. On the other hand, the functions of the exponential type are more uniformly centered around $\overline{M}_n$, and they put no special emphasis either on the first or on the last fractions that are being separated. Also, the technique of fractional precipitation that we have used to characterize our dextran seems well established enough, since its introduction for this material [33], to allow confidence in the results obtained.

Data obtained by GPC or sedimentation analysis are not comparable because of the serious problems that these techniques present in the determination of molecular weight distributions. In the case of GPC, the main difficulties are axial dispersion of the sample in the column, coexistence of adsorption (especially important in the case of polar systems such as dextran), and calibration (which must be done with standard samples of the same material to be fractionated and in identical experimental conditions). For dextran, the fractions that have been used are not narrow enough to be considered as standards to carry out a direct calibration of GPC columns. In the case of sedimentation in the ultracentrifuge, the analysis of dextran is hampered by the large deviations from ideality shown by the dextran-water system. Nonideal systems give only apparent distributions of the sedimentation coefficient, even at high dilution [16]. Under these circumstances, it is necessary to calculate the real distribution by extrapolation of the apparent boundary spreading of velocities to infinite dilution, thus introducing important inaccuracies in the final results.

We conclude that the molecular weight distribution of our clinic dextran is best represented by the linear combination of Eq. (2), which is plotted in Fig. 2.

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