LIGHT-SCATTERING, INTRINSIC VISCOSITY, AND GOLD NUMBER RELATIONSHIPS FOR SOME DEXTRAN FRACTIONS

M. K. HAMDY, E. GARDNER, Q. VAN WINKLE, AND G. L. STAHLY
Departments of Bacteriology and *Chemistry, The Ohio State University, Columbus, Ohio*

The usefulness of clinical dextran is dependent upon the size of its molecules, which can be decreased by degradation or obtained directly by controlled fermentation as reported by Grönwall and Ingelman (1945), Stacey and Pautard (1952), Ingelman (1948), Whiteside-Carlson (1952), Wolf *et al.* (1953, 1954), Stacey (1951), Lockwood *et al.* (1951), Hamdy *et al.* (1956), Nadel *et al.* (1953), Spendlove (1953), Koepsell *et al.* (1953), Hehre (1953), and Tsuchiya *et al.* (1952, 1953, 1955).

Several physico-chemical experiments on dextran have been reported in the literature to establish the molecular properties of this polysaccharide (John *et al.*, 1954). Ingelman and Halling (1949) used viscosity measurements, ultracentrifugation, diffusion and adsorption experiments to characterize some dextran fractions with respect to their molecular weights. They established the following linear relationship between intrinsic viscosity and the molecular weight (M) for some partially acid-hydrolyzed dextran fractions in the intervals $40{,}000 < M < 300{,}000$:

$$[\eta] = 8.2 \times 10^{-7}\, M + 0.18$$

Wales *et al.* (1953), studying the intrinsic viscosity-molecular weight relationship for acid-hydrolyzed dextran produced by *Leuconostoc mesenteroides* B–512, used the sedimentation equilibrium method in order to obtain a measure of the polydispersity of the fractions as well as a weight-average molecular weight. They established the following relationship between the intrinsic viscosity $[\eta]$ and the weight-average molecular weight for some dextran fractions in the range of $20{,}000 < M < 250{,}000$:

$$[\eta] = 10^{-3} \times M^{1/2}$$

Wolff *et al.* (1954) established the following relations between the inherent viscosities and the molecular weight (M_n and M_w):

$$[\eta] = 8.85 \times 10^{-4}\, M_n^{0.528}$$

and

$$[\eta] = 2.03 \times 10^{-3}\, M_w^{0.431}$$

Physico-chemical studies involving measurements of molecular weight using intrinsic viscosity and light-scattering measurements are presented here for dextran fractions and subfractions produced through acid, enzyme and ultrasound degradation of high molecular weight dextran produced by *Leuconostoc mesenteroides* B–512 and 683. The protective characteristic of these dextran fractions was also investigated using gold number measurements. This gold number is the weight of the protective substance (in milligrams) which is just insufficient to prevent a change in color of 10 ml of a red gold solution when 1 ml of 10 percent sodium chloride is added to it. The relation between the gold number of some dextran fractions and their molecular weight as measured by the intrinsic viscosity was also investigated and correlated.

MATERIAL AND METHODS

Production, Degradation and Fractionation of Dextran

Leuconostoc mesenteroides B–512 and 683 cultures were used for the production of dextran using the following sterile medium (pH 7.2): sucrose 150.0 gm, acid hydrolyzed casein 5.0 gm, K_2HPO_4 5.0 gm, yeast extract 1.0 gm, NaCl 2.0 gm, and $MgSO_4$ 0.022 gm per liter of distilled water.

After inoculation and incubation of the culture medium at 25°C, the dextran polymer was precipitated after clarification, effected by filtration through bacterial filters, using 99 percent isopropyl alcohol (Hamdy *et al.*, 1954). Degradation of the high molecular weight dextran, achieved through acid, enzyme and ultrasound, was followed by fractionation experiments of the degraded polymer as previously described (Hamdy *et al.*, 1956).

Measurements of dextran concentrations.—Dextran concentrations used in this study, except for gold number, were determined with the B–S differential refractometer.

Intrinsic viscosity measurements.—The intrinsic viscosities of the dextran fractions and subfractions were determined using a Fenske modified Ostwald viscosimeter No. 50, and concentrations between 0.2 and 0.6 gm/dL. at 25°C ± 0.05.

Light-scattering.—The light-scattering studies for molecular weight measurements were made using the B–S light-scattering photometer designed by Brice *et al.*

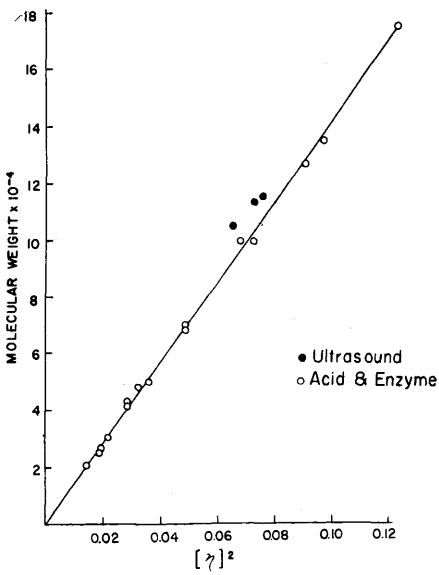

FIGURE 1. Relationship of the weight-average molecular weights to the square of the intrinsic viscosities of the dextran samples.

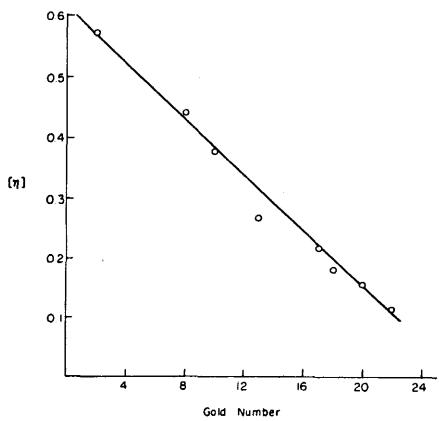

FIGURE 2. The relationship between the intrinsic viscosities and the gold number values of various dextran fractions.

(1950). Slight amounts of dust particles scatter light considerably and give rise to erroneous results. Consequently, the dextran solution was filtered through an ultrafine sintered glass filter into a dissymmetry cell and light-scattering measurements were made at 0, 45, 90, and 135 degrees for both blue and green light to obtain values for both turbidity and dissymmetry, Z ($Z = i\ 45°/i\ 135°$). No correction for depolarization of the scattered light was made in this study. Dissymmetry values were in the range of 1.1 to 1.2 for the samples investigated.

Gold number.—For the determination of the gold number, the dextran sample was first placed in a 56°C oven and dried to constant weight. Aqueous solutions of 1, 2, and 4 percent of the dried dextran were made. The range of the protective colloid of each concentration was determined using 0.01, 0.1 and 1.0 ml of the

[1]Prepared according to the formula of the United States Army Dept. Technical Manual 8-227 method for preparation of colloidal gold. (Chlorauric acid was reduced with formaldehyde.)

aqueous solution, added to test tubes containing 10 ml of gold hydrosol.[1] The tubes were immediately shaken for 3 minutes and 1 ml of a 10 percent aqueous solution of sodium chloride was added to the mixture while stirring. The gold number, which is the weight of the dextran in milligrams which will just fail to prevent a change in color from red to violet, was then calculated.

RESULTS AND DISCUSSION

Light Scattering-intrinsic Viscosity Relationship

Twenty samples of dextran were used in this study. They were fractions and subfractions of samples previously depolymerized by different procedures, namely: acid, enzyme and ultrasound (Hamdy *et al.*, 1956). The intrinsic viscosities of these twenty samples and their weight-average molecular weight as measured by the light-scattering are recorded in table 1. When the molecular weight of these samples were plotted against the square of their intrinsic viscosity (fig. 1), the data conformed to a straight line, over a molecular weight range of 20,000 to

TABLE 1

Intrinsic viscosity values and the corresponding weight-average molecular weight of some acid, enzyme and ultrasound degraded dextran fractions and subfractions

Type of Degradation	Strain No.	Sample No.	$[\eta]$	$[\eta]^2$	Molecular weight[1]
Acid	683	F_2, b	0.360	0.1296	179,000
		F_2, c	0.310	0.0961	135,000
		F_3, c	0.280	0.0676	100,000
		F_4, c	0.220	0.0484	69,000
		F_5, d	0.190	0.0391	45,000
		F_5, g	0.170	0.0289	43,000
		F_6, f	0.140	0.0196	27,500
	B–512	G_1, b	0.300	0.0900	127,000
		G_1, c	0.220	0.0484	70,000
		G_3, a	0.170	0.0280	42,000
		G_3, b	0.150	0.0225	31,500
		G_4, c	0.140	0.0196	27,000
		G_3, c	0.120	0.0144	21,000
Enzyme	B–512	E_1, b	0.580	0.3364	500,000
		E_1, d	0.350	0.1225	175,000
		E_3	0.270	0.0729	100,000
		E_4	0.180	0.0324	48,000
Ultrasound	B–512	U_1, c	0.275	0.0756	116,000
		U_2, a	0.270	0.0729	114,000
		U_3	0.250	0.0625	105,000

[1]Weight-average molecular weight measured by light-scattering.

180,000. The slope of this curve was calculated and the equation representing the relation between intrinsic viscosity and molecular weight was found to be:

$$M = 1.42 \times 10^6 \times [\eta]^2$$

$$[\eta] = 8.39 \times 10^{-4}\ M^{1/2}$$

All the M values of these different fractions of dextran 683 and B–512, followed this relationship except those of the ultrasound depolymerized samples, which showed lower intrinsic viscosities for comparable molecular weights. It appears that the ultrasound fractions and subfractions have intrinsic viscosities which are lower for a given molecular weight than provided for by the above equation. This may throw some further light on the mechanism of the degradation of dextran by the ultrasound (Hamdy *et al.*, 1956). The starting material before depolymerization is in all likelihood made up of large rodlike structures which are

essentially bundles of dextran chains held together in a compact arrangement. It is believed that acid hydrolysis and enzyme degradation (Hamdy *et al.*, 1954) cause these chains first to unfold and then to break along the glycosidic linkage, while ultrasound does not unfold the chain but fractures the bundle of chains at bonds other than the glycosidic linkages probably carbon to carbon bonds and results in compact fragments which would exhibit lower intrinsic viscosities than the chainlike structures of acid and enzyme hydrolyzed dextran.

The relationship obtained in this investigation for $[\eta]$ vs M gives intrinsic viscosities which are lower than those given by the relationship of Wolff *et al.* (1954) by 10 percent in the 100,000 molecular weight range and by 20 percent in the 20,000 molecular weight range. A 10 percent difference is within the experimental error of the light-scattering measurements.

TABLE 2

The gold number-intrinsic viscosity relation of dextran B–512

Sample	$[\eta]$	*Gold number* mg
Acid hydrolyzed	0.135	22
Ultrasound	0.160	20
Ultrasound	0.200	18
Acid hydrolyzed	0.220	17
Acid hydrolyzed	0.270	13
Acid hydrolyzed	0.380	10
Acid hydrolyzed	0.440	8
Enzyme	0.560	2

Gold Number and Intrinsic Viscosity Relationship

The protective action of various dextran samples having different intrinsic viscosities (molecular weights) was studied. The relation between the gold number and the degree of polydispersity of some dextran fractions and subfractions was also investigated. Approximately 400 separate gold number determinations were made on 60 dextran samples of different intrinsic viscosities representing first, second and third fractionations of acid, enzyme and ultrasound degraded dextran B–512 and 683.

The gold number and intrinsic viscosity of some homogeneous dextran subfractions produced by *L. mesenteroides* B–512 are recorded in table 2 and in figure 2. While it was relatively easy to differentiate between samples of widely different intrinsic viscosities (or molecular weights) by means of gold number measurements, gold number values for samples with intrinsic viscosities which differ by only a few percent were variable and did not correlate well with the intrinsic viscosities.

It was also observed that the more homogenous the dextran, the higher the gold number, while a heterogenous sample of approximately the same intrinsic viscosity had a lower gold number; *i.e.*, a high protective action. Jirgensons (1951) studied the protective action of various fractions of polyvinylpyrrolidone (PVP) on sliver sols and found that the lower molecular fractions of PVP had a greater protective power than the very high molecular fractions.

SUMMARY

Twenty different samples of fairly homogenous type dextrans resulting from three different procedures of degradation were used for molecular weight measurements. Using the light-scattering measurements, the relation between the

intrinsic viscosity of the samples and their weight-average molecular weight was found to be a straight line function in the range between 20,000 and 180,000:

$$M = 1.42 \times 10^6 \times [\eta]^2$$

or

$$[\eta] = 8.39 \times 10^{-4} \, M^{1/2}$$

All the weight-average molecular weight values of these different samples followed this relationship except those of ultrasonically depolymerized dextran which fall just a little above the curve. This may be due to a fundamental difference in the flexibility and configuration of the dextran molecule. The protective action as measured by gold number of 60 dextran fractions was investigated. No close correlation between gold number values and either molecular weight or the degree of polydispersity was found.

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