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Physicochemical Studies of Dextran. I. Characterization of Clinical Samples

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Physicochemical characterization of clinical dextran samples of Yugoslav production was made and the results were compared with the specifications of clinical dextrans produced in other countries. Measurements of optical rotation, density, viscosity and light scattering were undertaken, and the kinetics of the alkaline iodine oxidation was analysed. The experiments were performed on unfractionated clinical samples and both low and high 10% fractions. For the purposes of routine determinations of molecular weight a relation between $[\eta]$ and $\bar{M}_w$ was given. It is valid for unfractionated clinical samples produced in the same way and having $\bar{M}_w$ in the range from 65,000 to 95,000. From our data it could be concluded that Yugoslav clinical dextran belongs to the group of clinical dextrans with lower molecular weight (Swedish and U. S. dextran).

INTRODUCTION

In the past 20 years clinical dextran solutions have been used with much success as *plasma expanders*[1,2]. After the pioneering work of the Swedish investigators Grönwall and Ingelman, resulting in the industrial production of clinical dextran, in many countries extensive studies of dextran properties have been made and the production of dextran has been started. Similar efforts have also been made in Yugoslavia[3].

Clinical dextran is a product of hydrolytic cleavage of native dextran synthesized by microbial fermentation of sucrose. Chemically it is a polyglucose with different chain branching and a very broad distribution of molecular weights. The properties of dextran depend on the production procedure, *i.e.* on the type of microorganisms, on the conditions of fermentation and on the processes of hydrolysis and fractionation[1,2,4]. In view of this, a direct correlation of the properties of dextrans of different origin may not be permissible. For this reason it was of interest to undertake a study of the physicochemical properties of clinical dextran of Yugoslav production. This work was limited to investigating those properties that are necessary in characterizing the dextran in terms of molecular size, *i.e.* light scattering,

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viscosity, fractionation, and alkaline iodine oxidation. In addition, certain analytical data (density, optical rotation) were also obtained.

EXPERIMENTAL

Materials

The samples of clinical dextran were produced by the Dextran Research Laboratory, Novi Sad, Yugoslavia (manufacturer's code PVTD). This dextran was produced by a strain of *Leuconostoc mesenteroides* (Strain No. V)[3]. The samples are used as obtained from the manufacturer without further purification.

All the samples were stored at + 2° C in glass bottles with ground glass stoppers. Under such conditions the samples contained about 7.5% by weight of water as determined by drying in vacuum at 110° C in the presence of phosphorus pentoxide. The mean value of several determinations of ash was 0.044% of inorganic residue in the original sample. This was obtained by combustion of 1—2 g. of the dried sample. Nitrogen was determined by the micro-Kjeldahl method. To 1 g. of dextran sample 10 ml. of a 30% solution of hydrogen peroxide was added to destroy carbohydrate, and the solution was kept on a water bath until all liquid evaporated. Then the dry residue was digested with sulfuric acid and catalyst in the usual way. The mean values of several determinations of two typical samples were 0.0025 and 0.0028 g. of nitrogen per 100 g. of dextran respectively.

Solutions

Dextran PVTD is only slightly soluble in cold water, in contrast to some other types of dextran described in literature[5–8]. As the most convenient way of preparing solutions without aggregates the following procedure was adopted: Small portions of powdered dextran were added into the boiling redistilled water. Then the solution was kept in a water bath at the temperature of boiling for 2 hours. After cooling the solution was filtered into a volumetric flask, water was added to the mark and the solution stored in a refrigerator to prevent the growth of microorganisms. The amount of solution prepared was usually such as to allow experimentation for several days. During this time microorganisms did not appear. If the 2-hour boiling was omitted, the solution was remarkably turbid indicating incomplete dissolving of the polymer.

The concentration of dextran solutions was determined gravimetrically. Aliquots of the solution were evaporated on a water bath and the residue was dried in a vacuum oven at 110° C in the presence of phosphorus pentoxide for 16—24 hours. Since the impurities of inorganic and proteinic nature can be neglected in view of the above given data, and since the water residue in the samples dried under similar conditions but without phosphorus pentoxide[9] does not exceed 0.35% by weight, this appears to be the best and most reliable method for the determination of concentrations. The relative error of the concentration data depended upon the volume of the aliquots and varied in routine work from 0.5 to 1.0%, satisfying the requirements of other experimental methods.

Methods

a) Optical rotation. — Measurements of optical rotation were made by means of a Keston Standard Model D polarimetric unit attached to a Beckman Model DU spectrophotometer. The device was calibrated with standard sucrose solutions for several wavelengths at room temperature, and the angles of rotation α were determined using the formula[10]:

$$\alpha = K \tanh (0.576\ D) \qquad (1)$$

where K is the calibration constant, and D is the apparent optical density obtained as the spectrophotometer reading.

b) Viscosity. — Viscosity was measured by use of a Cannon-Ubbelohde dilution viscometer at (25.00 ± 0.01)° C. The kinetic energy correction was determined by measuring the flow time of redistilled water at 20, 25 and 30° C.

Densities of dextran solutions relative to water were determined using a flask pycnometer[11] at (25.00 ± 0.02)° C.

c) Light scattering. — For light scattering measurements an improved Oster--Aminco photometer[12] was used. To achieve greater stability of both the electronic part and the light source the photometer was connected to a »Philips« Model PE 4222 electronic voltage stabilizer. The measurements were carried out as before[13], and the photometer was calibrated with »Ludox« colloidal silica and benzene[13, 14]. The outer back face of the semioctagonal cells was blackened to eliminate stray reflections of light on cell walls. Dextran solutions were filtered through Millipore HA filters, and a twofold filtration usually assured good optical conditions for light scattering work. The water used in these experiments was obtained by redistillation from a special apparatus, similar to the one described in literature[15], which produces water as dust-free as possible.

d) Fractionation. — Separation of extreme fractions (low and high 10% fractions) was performed by using the fractional precipitation method. The precipitation was carried out by the addition of absolute ethanol to 6% water solutions of dextran at constant temperature of $(25.00 \pm 0.02)^0$ C.

The procedure tried first was a modified fractional precipitation developed by Weissberg[16]. From a dextran solubility curve obtained by stepwise addition of ethanol and further determination of the amount of precipitated polymer it was possible to obtain information on the amount of ethanol required for the precipitation of extreme fractions. First 10% of the high fractions were precipitated and this high molecular weight fraction was separated by centrifugation Then ethanol was added to precipitate the further 80% of the polymer and after centrifugation the remaining 10% of the polymer with low molecular weight molecules were isolated.

In order to avoid centrifugation, which led to a loss of polymer material, part of the fractionation work was carried out in an apparatus described by Hall[17].

e) Alkaline iodine oxidation. — The possibility of clarifying some points in the procedure of Lacko and Málek[31, 32] for the determination of number-average molecular weights, $\overline{M}_n$, of dextran was investigated. The method is based on the determination of the aldehyde groups content of dextran (end groups) by means of measuring the amount of iodine consumed by an alkaline dextran solution (the so-called hypoiodite method). Essentially the procedure of Lacko and Málek was followed with modifications that will be described under Results.

RESULTS

Measurements of optical rotation were made for several wavelengths (lines of the sodium vapor and mercury arc) in the concentration range 0.2—1.2 g. of dextran per 100 ml. of solution at room temperature. Table I gives data on specific rotations [α] at several wavelengths for two different dextran samples.

TABLE I

Specific Rotation [α] *at Several Wavelengths of Light in Vacuum*

	λ_0 (mμ)			
Sample	589	578	546	436
II	$+194\pm2^0$	$+209\pm5^0$	$+232\pm7^0$	$+382\pm5^0$
III	$+195\pm3^0$	$+212\pm3^0$	$+228\pm5^0$	$+379\pm5^0$

Limiting viscosity numbers (or intrinsic viscosities) [η] were determined by extrapolation of functions η_{rd} or $\ln \eta_r/C$ *vs.* concentration to zero concentration. Here η_{rd} is the viscosity number defined as $\eta_{rd} = \eta_{sp}/C$, where $\eta_{sp} = \eta_r - 1$, $\eta_r = \eta/\eta_0$, η is the viscosity of solution, η_0 the viscosity of solvent, and C is the concentration in g. per 100 ml. of solution. Both plots gave straight lines allowing simple extrapolation procedure and intercepting in the same point within the experimental error. The unit of [η] is $g.^{-1}dl.$ $(=g.^{-1} \cdot 100$ ml.). Fig. 1 shows a typical plot of η_{rd} or $\ln \eta_r/C$ *vs.* C.

The densities necessary for calculation of viscosity ratios were determined in solutions at several dextran concentrations. If density is expressed as relative density, ϱ_4^{25}, the relation, calculated by the least squares method, obtains the form

$$\varrho_4^{25} = 0.99707 + 0.00395\ C. \qquad (2)$$

Weight average molecular weights, $\overline{M}_W$, were evaluated from light scattering data using the well known Debye formula[19]

$$\overline{M}_W = \frac{1}{H\,(c/\tau)_0} \qquad (3)$$

where $H = (32\,\pi^3/3N_A\,\lambda_0^4)\,n_0^2\,(dn/dc)^2$, N_A is Avogadro's number, n_0 the refractive index of the solvent, dn/dc the refractive index increment, τ the excess turbidity (turbidity of the solution diminished by the turbidity of the

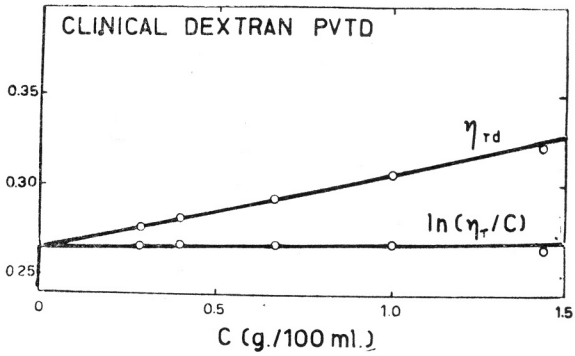

Fig. 1. Typical plots of η_{rd} and ln η_r/C *vs.* C. Extrapolation to zero concentration gives $[\eta]$ in $g.^{-1}$ dl.

solvent) and c is the concentration in grams of the polymer per gram of solution. The index zero denotes c/τ-values extrapolated to zero concentration. Extrapolations were undertaken on c/I'_{90} versus c plots where I'_{90} is the galvanometer reading at the detector position at 90°. After dividing $(c/I'_{90})_c$ by an appropriate calibration constant[13] C' values of $(c/\tau)_0$ were obtained. A typical plot of c/τ *vs.* c for wavelengths 546 and 436 mμ is shown in Fig. 2.

Corrections for dissymmetry were not necessary because the molecular weights lay in the range where theoretical dissymmetries practically did not differ from unity. In experimental work it was very difficult to obtain dissymmetries lower than 1.05 even if the solutions were very carefully clarified. The values of dissymmetry varied from cell to cell indicating the existence of stray reflections on the cell walls which could not be completely eliminated by blackening the back face of the cells.

The values of H were calculated using our previous data[20] on dn/dc of dextran obtained with a Rayleigh-Haber-Löwe interferometer. These data were verified recently on a Brice-Phoenix visual differential refractometer, and the same results were obtained within the experimental error. The values

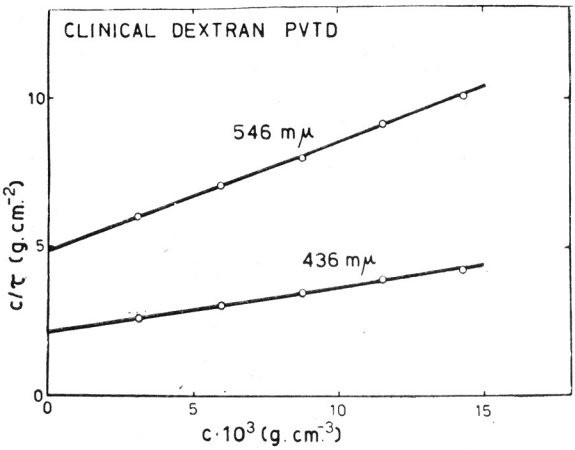

Fig. 2. Typical plots of c/τ *vs.* c for wavelengths 546 and 436 mμ.

of $H = 2.41 \cdot 10^{-6}$ and $6.30 \cdot 10^{-6}$ for 546 and 436 mμ, respectively, were obtained. The differences between the molecular weights obtained at 546 and 436 mμ were usually not greater than 5%, but never exceeded 10%, *i.e.* they lay within the limits of the experimental error.

A summary of data on optical rotation, molecular weights from alkaline iodine oxidation and light scattering, and viscosity of three samples of clinical dextran is given in Table II. These samples were of different date of pro-

TABLE II

Specific Rotations at Sodium D-Line, $[\alpha]_D$, *Molecular Weights Obtained by Alkaline Iodine Oxidation,* $\overline{M}_n$, *and Light Scattering,* $\overline{M}_w$, *and Limiting Viscosity Numbers,* $[\eta]$, *for Several Samples of Clinical Dextran*

Sample	$[\alpha]_D$	$\overline{M}_n$	$\overline{M}_w$	$[\eta]$ (dl. g.$^{-1}$)
I	—	43,800	88,200	0,265
II	$+ 194 \pm 2^0$	57,200	$83,300 \pm 4,000$	$0,263 \pm 0,004$
III[a]	$+ 195 \pm 3^0$	48,000	$86,000 \pm 2,200$	$0,248 \pm 0,003$

a) Manufacturers No. 14/1961.

duction, samples I and II were produced in the same way, the production procedure of sample III was somewhat different[21]. Where a greater number of data was available, a statistical analysis was undertaken. The experimental error was estimated as standard deviation.

In the specifications of clinical dextran it is usual to give also molecular weights of extreme 10% fractions of the whole sample. From the solubility curve of dextran in the mixed water-ethanol system given in Fig. 3 it follows that the high 10% fraction was precipitated at the ethanol concentration of 37.8—37.9% by volume at 25.00° C if the initial concentration of dextran before fractionation was 6.0 — 6.2 g. per 100 ml. of solution. The low 10% fraction was precipitated at 44.6—44.8% of ethanol at the same temperature and the same concentration of dextran. Determinations of $\overline{M}_W$ of these fractions by light scattering gave data listed in Table III. The fractions did not contain exactly 10% of polymer material, especially in the case of high fractions. It is obvious from the shape of the solubility curve, which is very

steep in the high fraction region, that it would be extremely difficult to achieve everytime cut-off at exactly 10% of the polymer. A flatter solubility curve can be obtained by decreasing the whole polymer concentration, but this would require large volumes of solution in order to obtain the same quantity of the fractionated polymer. Riddick *et al.*[9] showed that precipitation from more dilute solutions did not apparently cause differences in molecular weight of fractions in comparison with the precipitation from more concentrated solutions. This justifies our choice of fractionating more concentrated solutions.

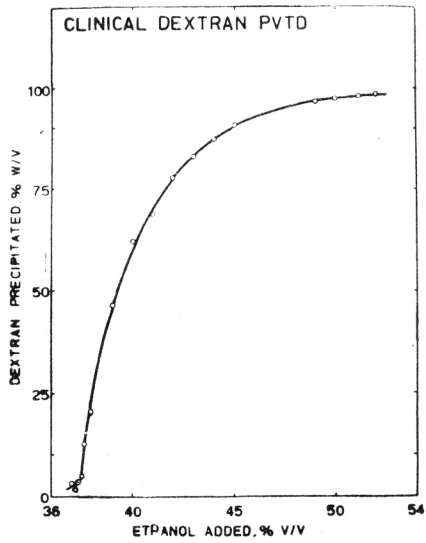

Fig. 3. Solubility curve of dextran in the mixed water — ethanol system at 25.00° C.

TABLE III

Molecular Weights of Low and High 10% Fractions of Several Samples of Clinical Dextran

Sample	Low Fraction		High Fraction	
	% by weight	$\overline{M}_W$	% by weight	$\overline{M}_W$
I[a]	9.6	28,500	10.8	294,000
II[a]	14.3	29,500	11.6	240,000
III[b]	10.0	22,000	10.0	156,000

a) Obtained by the fractionation of 10% low and high fractions
b) Obtained from the fractionation curve[22]

In order to check the uniformity in physicochemical characteristics of successive batches from current production of dextran, prepared under identical conditions, we measured $\overline{M}_W$ and $[\eta]$ in a series of dextran samples produced during a certain period of time. These data are presented in Table IV.

Since in the procedure of Lacko and Málek the amounts of dextran needed for alkaline iodine oxidation were very large, requiring several grams of dextran for one molecular weight determination, we first attempted to establish whether it would be possible to reduce the quantity of dextran needed in a run. This point is of particular importance if one wants to apply this

method of $\overline{M}_n$ determination to a series of fractions, the supply of which is usually rather limited. One typical run is represented in Fig. 4. The way of expressing the results and the notation are those of Lacko and Málek. $M_P = 200\ G/(T_0 - T)$, and $P = T_0/(T_0 - T)$, where G is the amount of dextran (in mg.), and T_0 and T stand for the number of ml. of $Na_2S_2O_3$ solution consumed

TABLE IV

$\overline{M}_w$ and [η] of Dextran Batches from the Same Production

Sample	$\overline{M}_w$	[η] (dl. g.$^{-1}$)
413/414	89,900	0.272
415/416	93,800	0.277
417/418	69,800	0.247
419/420	78,800	0.263
421/422	76,400	0.262
423/424	70,500	0.254

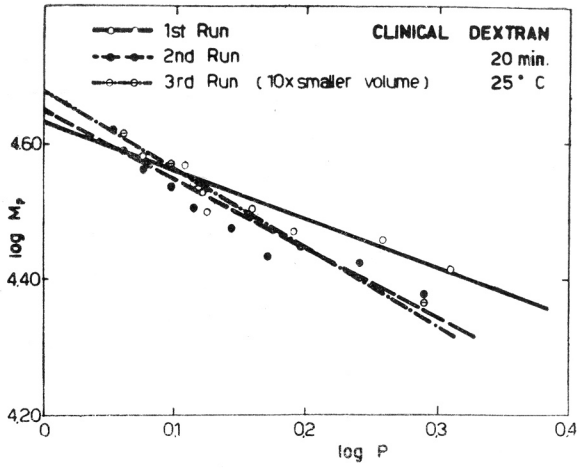

Fig. 4. Typical plots of log M_P *vs.* log P obtained from three runs in the same clinical dextran sample; reaction time: 20 min.

in the titration of iodine in a mixture without dextran present (blank, T_0) and with dextran in the mixture (T). The results refer to three independent runs with the same sample of dextran (Sample I). In two runs the conditions were identical, following the procedure of Lacko and Málek rather faithfully. In the third run the amount of dextran in the titration was decreased ten times with respect to the first two runs. Although there were rather great differences between the intercepts on the (log M_P)-axis, the data apparently gather about the same straight line taken as a first approximation for the functional dependence of M_P *vs.* P. In our work the dispersion of extrapolated $\overline{M}_n$ data (M_P at P = 1; see the papers by Lacko and Málek[31,32]) varied in the range of 10 to 20%.

The reaction time recommended by Lacko and Málek was 20 minutes (the same is valid for the data in Fig. 4). Since they did not give any reason for choosing this particular time at which the oxidation reaction was stopped (by acidifying the solution), we have conducted a few kinetic runs, in which the consumption of iodine [expressed in terms of $T_0/(T_0 - T)$ G] was

measured as a function of time. For the run represented in Fig. 5, 1.5 ml. of 4.57% stock dextran solution (= 0.0685 g.) were diluted to 4.0 ml. with water, and 1.0 ml. each of 0.01 *N* I_2 and 1.0 ml. 0.1 *N* NaOH were added. The mixture was kept in a small Erlenmeyer flask with a ground glass stopper at

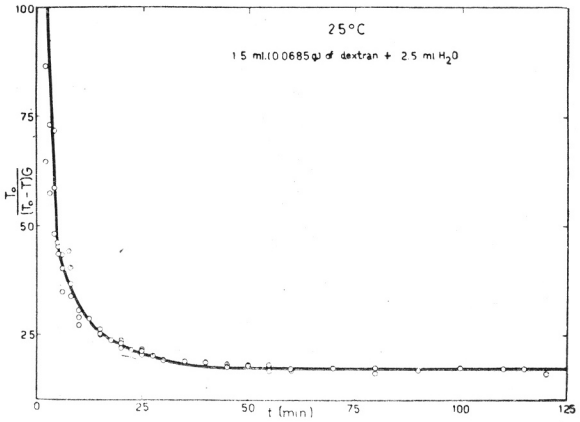

Fig. 5. Time dependence of $T_0/(T_0 - T)$ G.

(25.0 ± 0.1)°C in a water-bath thermostat. The reaction was stopped after a predetermined time by adding 0.5 ml. of 1.0 *N* H_2SO_4, and the unconsumed iodine titrated by means of a 1 ml. burette with 0.01 *N* $Na_2S_2O_3$ and with starch as indicator. For every reaction time (from 1 minute to 2 hours) a separate solution was used. The time of the reaction was taken as the time elapsed from the moment of delivery of the last drop of NaOH until the first drop

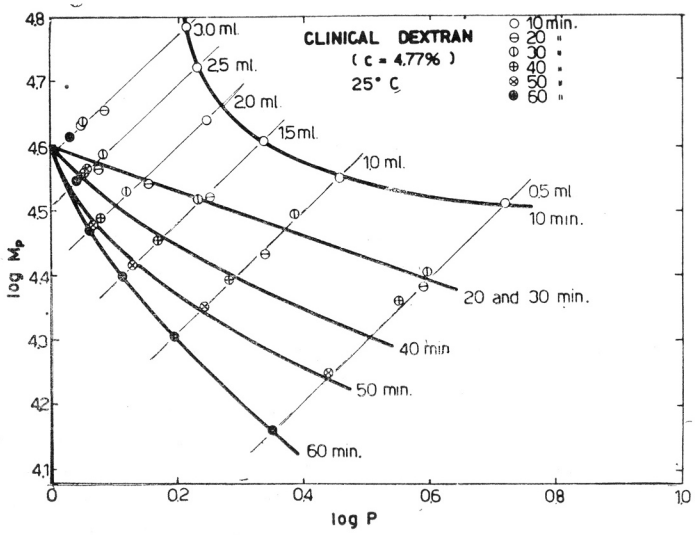

Fig. 6. Plots of log M_P *vs.* log P for different reaction times

of H_2SO_4 was added. The points in the diagram refer to several independent runs under identical conditions. There seems to be no systematic difference among them. These kinetic experiments were performed with varying amounts of dextran in solution, and the results are plotted in Fig. 6 in a manner analogous to that of Fig. 4. By inspecting the results of Figs. 5 and 6, one arrives at the following conclusions: (1) The data corresponding to 20 or 30 minutes as the reaction time should be preferred since they are leading to straight lines in the log M_P *versus* log P plots. (2) One should avoid the conditions leading to low values of P, *i.e.*, the use of too large amounts of dextran, or to small values of T compared to T_0 (the blank value). At small values of P, the points appear to deviate from the straight line even for 20 minutes. The same deviations at small P are noticeable in some of the results of Lacko and Málek. (3) The points corresponding to different times of reaction for the same amount of dextran appear to be on a straight line in the log M_P *versus* log P plot. Although at the present we do not know how significant this observation might be, it seems to be desirable to explore this point in more detail, particulary in terms of an analysis of the kinetics of the reaction under discussion.

DISCUSSION

Many authors have determined specific rotations of dextran in order to employ this simple method for the determination of dextran concentration in solutions. In Table V data from literature are collected. From data in Tables I and V it is evident that the specific rotation of dextran varies for the samples obtained by different strains of microorganisms, as observed earlier[4,18], although the differences could probably be, in part, explained by the uncertainties in the determination of concentration.

In the literature there are two relations for the density of dextran solutions *vs.* concentration. Snyder *et al.*[18] reported the relation

$$\varrho^{20} = 0.99717 + 0.00398\ p + 0.000016\ p^2 \qquad (4)$$

where p is the concentration in g. per 100 g. of solution, and Graham[23] gives the expression

$$\varrho^{25} = 0.9976 + 0.00393\ C. \qquad (5)$$

Since the densities at zero concentration are densities of pure water, the intercepts of both relations differ from the exact value of water by 0.1% and 0.05% in the case of relations (4) and (5) respectively. Our relation (2) gives much closer values for the density of water, but both relations (4) and (5) are useful for viscometric work. In our viscosity experiments the precision of time measurement amounted to 0.1%, so that a similar error in density can be tolerated.

For the purpose of clinical use, dextran samples must meet specifications which require that some properties, as molecular weight and polydispersity, are constant within certain limits. In several countries such specifications are issued, and are collected in Table VI.[1,2] Additional specifications refer to the purity requirements, *e.g.* the content of ash should be lower than 0.8% and the content of nitrogen lower than 0.015%. In Table VII we collected for the purpose of comparison with our results the properties of those commercially

produced clinical dextrans for which it was possible to find the data in the literature.

TABLE V

Specific Rotations of Dextran in Water

Authors	Ref.	Production and Strain	Temperature °C	[α] degrees (+) 589 mμ	[α] degrees (+) 546 mμ	Concentration Range g./100 ml.
Ingelman	26	Swedish	20	194		
Snyder *et al.*	18	U.S.A., Different	20	194.6 - 211.0		1
Snyder *et al.*	18	U.S.A., NRRL B-512	20	192±2	235±1	
Senti *et al.*	7	U.S.A., NRRL B-512	25	200		
Wolff *et al.*	27	U.S.A., NRRL B-512	25	199±1		
Wolff *et al.*	28	U.S.A., NRRL B-1254	25	198		
Wolff *et al.*	28	U.S.A., NRRL B-742	25	210		
Granath	25	Swedish, NRRL B-512 Pharmacia	20	199		0.5—1.0
Granath	25	German, A-179	20	206.7		0.5—1.0
Riddick *et al.*	9	U.S.A., NRRL B-512	25		235.67	0.7—15.1
Czechowska *et al.*	29	Polish		184.2 - 195.2		6
Chernyak and Polushina	30	U.S.S.R.,	20	199.3± ±1.8		
Vavra and Vavra	3	Yugoslav, No. V	20	198.0± ±0.5		

TABLE VI

American, British and Swedish Physicochemical Specifications for Clinical Dextran

	U.S.A.[a]	Great Britain[b]	Sweden[c]
Whole polymer, $\overline{M}_w$	75,000 ± 15,000		80,000 ± 10,000
10% high fractions, $\overline{M}_w$	$\leqslant$ 200,000		$\leqslant$ 200,000
10% low fractions, $\overline{M}_w$	$\geqslant$ 25,000		$\geqslant$ 25,000
Whole polymer, [η]	0.255 ± 0.035[d]	0.32 ± 0.03[e]	0.24 — 0.27[f]
10% high fractions, [η]		< 0.53[e]	

a) U. S. Military Medical Purchase Description, 1954; quoted by Squire *et. al.*, ref. 1, p. 57.
b) Ministry of Health Specification, 1954; quoted by Squire *et. al.*, ref. 1, p. 57.
c) Quoted by Grönwall, ref. 2 p. 50.
d) [η] in dol. g.$^{-1}$ at 25° C.
e) [η] in dl. g.$^{-1}$ at 37° C.
f) Temperature not specified.

TABLE VII

Physicochemical Properties of Several Clinical Dextrans

	British (1953) (a)	British (1954) (b)	British »Intradex« (c)	Swedish »Macrodex« (d)	American (1952) (e)	American (1954) (f)	American »Expandex« (g)
Whole polymer, $\overline{M}_w$	—	170,000—250,000	135,000	82,000	75,000 ± 25,000	70,000—80,000	77,000
Whole polymer, $\overline{M}_n$	90,000	80,000—90,000	—	—	—	50,000—60,000	—
10% high fractions, $\overline{M}_w$	—	—	300,000	165,000	—	—	168,400
10% low fractions, $\overline{M}_w$	—	—	30,000	27,400	—	—	28,400
Whole polymer, [η]	0,32—0,37	0,32—0,37	—	0,260	0,18—0,28	0,18—0,28	0,219
10% high fractions, [η]	—	—	—	0,355	—	—	—
10% low fractions, [η]	—	—	—	0,167	—	—	—
Specific rotation, [α]	202—208°	198—202°	—	—	198—200°	195—198°	—
Degree of branching[(h)]	0.1—0.2	0.05—0.1	—	—	0.05	0.05	—

(a), (b), (e), (f) Cited by Squire *et. al.*, ref. 1, p. 20
(c), (d), (g) Cited by Jones and Wilkie, ref. 33
(c) Produced by Glaxo (Canada) Ltd., and Glaxo Ltd., England.
(d) Produced by Pharmacia Ltd., Uppsala.
(g) Produced by Commercial Solvents Corp., Terre Haute, Indiana.
(h) Defined as the fraction of non-α(1,6)—glucosidic linkages.

From our results obtained with the samples produced in the same way (Tables II, III and IV) with the exception of sample III (No 14/1961), it can be seen that $\overline{M}_n$ for the whole polymer varies in the range from 43,000 to 58,000, $\overline{M}_W$ from 70,000 to 94,000 and [η] from 0.24 to 0.28 dl.g.$^{-1}$, so that the dextran of Yugoslav production has characteristics similar to those of the group of clinical dextrans with lower molecular weight (Swedish and U. S. dextran). However, Yugoslav dextran is more polydisperse, since the $\overline{M}_W$ in the high 10% fractions is higher than listed in the Swedish and U. S. specifications.

Sample III was produced in a different way involving the cutting off of higher molecular weight fractions. In this way a sample was obtained with a distribution shifted to lower molecular weights, as the data in Table III demonstrate.

By using the method of periodate oxidation Pavlović and Trpinac[24] found that Yugoslav clinical dextran contained 94.20% of α (1,6) linkages (the average value of all determinations carried out by two methods). This indicates that Yugoslav dextran belongs to the group of dextrans with a small degree of branching[25].

Lacko and Málek compared the number-average molecular weights, $\overline{M}_n$, for several dextran fractions, as determined by means of osmotic pressure measurements and the hypoiodite method. Good agreement was obtained in the molecular weight range from 21,000 to 50,000 ($\overline{M}_n$). Since molecular weight determination by means of osmotic pressure measurements is a tedious and

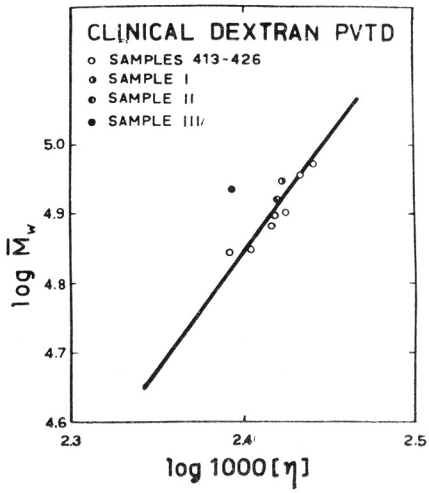

Fig. 7. Plot of log M_w *vs.* log 1000 [η] for different samples of clinical dextran.

time-consuming technique fraught with many pitfalls, the hypoiodite method seems to be preferable one when the degree of branching is low (otherwise the method will give too high an aldehyde content due to the presence of end-groups in the branch chains), although its precision is not high.

It is of great practical value to correlate the data of $\overline{M}_W$ and [η] obtained with several unfractionated samples from current production, since this facili-

tates routine molecular weight determinations. Plotting the data given in Tables II and IV on a log $\overline{M}_W$ *vs.* log 1000 [η] diagram a linear correlation is obtained as shown in Fig. 7. The point corresponding to sample III deviates from this line, which can probably be explained by the absence of higher molecular weight fractions and the presence of lower molecular weight fractions (possibly up to glucose units) as indicated by the results in Tables II and III. By analysing the deviations of data from the best line obtained by the least squares methods the coefficients of variation amounting to 4.7% in the case of $\overline{M}_W$ and 1.4% in the case of [η] were obtained. This is in good agreement with the coefficients of variation of sample II in Table II which have the same molecular weight distribution. The relation holding for the unfractionated clinical dextran of Yugoslav production (with the molecular weight distribution like sample II) has the form

$$[\eta] = 9.37 \cdot 10^{-3} \overline{M}_W^{0.295}. \qquad (6)$$

This relation must not be mistaken for the Mark-Houwink relation obtained on polymer fractions and can be used only in routine molecular weight determinations of clinical samples produced in the same way and having $\overline{M}_W$ in the range from 65,000 to 95,000.

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REFERENCES

1. J. R. Squire, J. P. Bull, W. d'A. Maycock, and C. R. Ricketts, *Dextran: Its Properties and Use in Medicine.* Blackwell Sci. Publ. Oxford 1955.
2. A. Grönwall, *Dextran and Its Use in Colloidal Infusion Solutions.* Almqvist & Wiksell Stockholm-New York-Oxford 1957.
3. I. Vavra and A. Vavra, *Bull-soc. chim. Beograd* **23—24** (1958/59) 285.
4. A. Jeanes, W. C. Haynes, C. A. Wilham, J. C. Rankin, E. H. Melvin, M. J. Austin, J. E. Cluskey, B. E. Fisher, H. M. Tsuchiya. and C. E. Rist, *J. Am. Chem Soc.* **76** (1954) 5041.
5. G. C. Booth and V. Gold, *J. Chem. Soc.* **1956**, 3380.
6. B. Sedlaček, *Collection Czechoslov. Chem. Commun.* **20** (1955) 278.
7. F. R. Senti, N. N. Hellman, N. H. Ludvig, G. E. Babcock, R. Tobin, C. A. Glass, and B. L. Lamberts, *J. Polymer Sci.* **17** (1955) 527.
8. K. Frömbling and F. Patat, *Makromolekulare Chem.* **25** (1957) 41.
9. J. A. Riddick, E. E. Toops, Jr., R. L. Wieman, and R. H. Cundiff, *Anal. Chem.* **26** (1954) 1149
10. P. M. Gallop, *Rev. Sci. Instr.* **28** (1957) 209.
11. N. Bauer, *Determination of Density,* in A. Weissberger, *Physical Methods of Organic Chemistry,* 3rd Edition, Part I, Interscience Publ., New York 1959, p. 149, Fig. 1 D.
12. Gj. Deželić and J. P. Kratohvil, *J. Colloid Sci.* **16** (1961) 561.
13. Gj. Deželić and J. P. Kratohvil, *Kolloid-Z.* **173** (1960) 38.
14. Gj. Deželić, *Croat. Chem. Acta* **33** (1961) 99.
15. F. P. Price, W. B. Bellamy, and E. J. Lawton, *J. Phys. Chem.* **58** (1954) 821.
16. A. Weissberg, quoted by S. V. R. Mastrangelo *et al., Anal. Chem.* **27** (1955) 262.
17. R. W. Hall, *The Fractionation of High Polymers,* in P. W. Allen, *Tehniques of Polymer Characterization,* Butterworths Scientific Publ., London 1959, p. 29.

18. C. F. Snyder, H. S. Isbell, M. R. Dryden, and N. B. Holt, *J. Research Natn. Bur. Standards* **53** (1954) 131.
19. P. Debye, *J. Phys. & Colloid Chem.* **51** (1947) 18.
20. M. Zebec, Gj. Deželić, J. P. Kratohvil, and K. F. Schulz, *Croat. Chem. Acta* **30** (1958) 251.
21. Private communication of Mrs. A. Vavra.
22. M. Zebec *et al.* (to be published).
23. W. D. Graham, *Canad. J. Technol.* **34** (1956) 83.
24. V. Pavlović and P. Trpinac, *Arhiv farm.* **12** (1962) 221.
25. K. A. Granath, *J. Colloid Sci.* **13** (1958) 308.
26. B. Ingelman, *Uppsala Läkarefören. Förhandl.* **54** (1949) 107.
27. I. A. Wolff, C. L. Mehltretter, R. L. Mellies, P. R. Watson, B. T. Hofreiter, P. L. Patrick, and C. E. Rist, *Ind. Eng. Chem.* **46** (1954) 370.
28. I. A. Wolff, R. L. Mellies, R. L. Lohmar, N. N. Hellman, S. P. Rogovin, P. R. Watson, J. W. Sloan, B. T. Hofreiter, B. E. Fisher, and C. E. Rist, *Ind. Eng. Chem.* **46** (1954) 2605.
29. Z. Czechowska, J. Dubrowski, A. Hausman, E. Kostrzewska, J. Krysiak, K. Murawski, J. Panasewicz, and K. Zakrzewski, *Pol. Arch. Med. Wewn.* **24** (1954) 1.
30. V. Ya. Chernyak and T. V. Polushina, *Med. Prom. SSSR* **15** (1961) 39.
31. L. Lacko and J. Málek, *Chem listy* **51** (1957) 47.
32. L. Lacko and J. Málek, *Collection Czechoslov. Chem. Commun.* **22** (1957) 1106.
33. J. K. N. Jones and K. C. B. Wilkie, *Canad. J. Biochem. Physiol.* **37** (1959) 377.

IZVOD

Fizičko-kemijske studije dekstrana. I. Karakterizacija kliničkih uzoraka

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Vršena je fizičko-kemijska karakterizacija kliničkog dekstrana jugoslavenske proizvodnje i rezultati su uspoređeni sa specifikacijama za kliničke dekstrane proizvedene u drugim zemljama. Vršena su mjerenja optičke rotacije, gustoće, viskoziteta i rasipanja svjetlosti, i analiza kinetike oksidacije jodom u lužnatu mediju. Eksperimenti su vršeni s nefrakcioniranim kliničkim uzorcima, niskim i visokim (10% od ukupnog polimera) frakcijama. U svrhe rutinskog određivanja molekularne težine određena je relacija između $[\eta]$ i $\overline{M}_w$ koja vrijedi za nefrakcionirane kliničke uzorke proizvedene na isti način s $\overline{M}_w$ između 65.000 i 95.000. Iz naših podataka moglo se zaključiti da jugoslavenski klinički dekstran spada u grupu kliničkih dekstrana niže molekularne težine (švedski i američki dekstran).

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