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## A Remark on the Refractive Index Increment of Dextran for the Molecular Weight Determination by Light Scattering

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In the course of a study on the macromolecular properties of dextran of Yugoslav production, the need had arisen for information on the value of refractive index increment,  $dn/dc$ , of dextran in aqueous solutions for the determination of molecular weights by light scattering technique. A thorough search through the literature has revealed an astonishingly great dispersion of the values of the refractive index increment of dextran at 546  $m\mu$  as determined by numerous authors.<sup>1-10, 21-23</sup> Moreover, in the data, given by Senti et al.<sup>6</sup> and Mariani and Ciferri,<sup>7</sup> we have noted an unusual change of refractive index increment with the wave length of the light used (larger  $dn/dc$  at longer wave-length). The values of refractive index increment of dextran as published by various authors are collected in Table I.

TABLE I  
*Refractive index increments of dextran*

Authors	Ref.	$dn/dc$ (cm. <sup>3</sup> g. <sup>-1</sup> )	
		436 $m\mu$	546 $m\mu$
Price et al.	1		0.14
Aron and Frank	2		0.151
Sedlaček	3		0.147
Booth and Gold	4		0.148
Frömbling and Patat	5		0.15
Senti et al.	6	0.151	0.154
Mariani and Ciferri	7	0.1526*	0.1534*
Graham et al.	8,9	0.151*	
Wales et al.	10	0.151	
Squire et al.	21	0.153	
Ogston and Woods	22		0.149
Bovey	23	0.151 — 0.154	

The use of the data for green mercury line in calculating the molecular weight from light scattering measurements may lead to very serious discrepancies since  $dn/dc$  enters as square term in the equation connecting the turbidity and molecular weight. As an illustrative example, we have calculated the weight-average molecular weight of a clinical dextran using the light

\* calculated from the value of Debye light scattering constant  $H$ .

scattering results of one of our measurements and some of the values of refractive index increment from Table I (the highest, the lowest, and the mean value for 546 m $\mu$ ; Table II).

TABLE II

*The evaluation of light scattering measurements on dextran for various refractive index increments (compare Table I).*

$$\lambda = 5.46 \times 10^{-5} \text{cm.}; (c/\tau)_{c=0} = 3.85 \text{ g. cm.}^{-2}; H = 32 \pi^3 n_0^2 (dn/dc)^2/3 \lambda^4 N$$

$dn/dc$ ( $\text{cm.}^3 \text{ g.}^{-1}$ )	$(dn/dc)^2$	$H \times 10^6$	$M \times 10^{-5}$
0.154	0.0237	2.60	0.997
0.149	0.0222	2.44	1.065
0.140	0.0196	2.15	1.207

Obviously, the results of the evaluation of light scattering data for dextran, using the values of  $dn/dc$  reported in the literature, cannot be accepted as satisfactory.

In a recent communication Jose and Biswas<sup>13</sup> have noted a variation of 25% in  $dn/dc$  for polymethyl methacrylate in several solvents, which may produce a deviation in molecular weight by about 50%. The authors emphasized the need that more experimental details of the determination of refractive index increment should be reported in communications than are usually made. It seems to us that many uncertainties in evaluation and comparison of light scattering data obtained on the same polymers in various laboratories can be partly accounted for by the use of improper values of refractive index increment. Although this point is not so critical in evaluation of light scattering measurements, as are calibration and correction factors, its importance should not be underestimated.

Owing to wide use of dextran for the preparation of colloidal infusion solutions (blood plasma volume expanders), its macromolecular properties are intensively studied. Light scattering measurements are very often used to determine the molecular weight of various fractions of dextran in clinical and non-clinical range. Moreover, some official specifications<sup>14, 15</sup> for clinical dextran recommended the light scattering technique to be used in the control of the molecular weight. Having this in mind, we have decided to draw the attention of the workers interested in this field of research to the facts discussed above. At the same time, it seemed to us that a short report on the measurements of refractive index increment of dextran at various wave lengths performed in our laboratory might be warranted.

The difference in refractive index ( $\Delta n$ ) between solution and solvent (or between two solutions of various concentrations) was measured by means of a Rayleigh-Haber-Löwe interferometer manufactured by Zeiss (ref. 16, p. 1226). The measurements were performed at 436, 546 and 578 m $\mu$  using AH-4 mercury vapour lamp and suitable Corning glass filters. The procedure followed in working with monochromatic light is described in ref. 16, p. 1230. The cell (two-compartment of 2.00 cm. thickness) with solutions and/or solvent (redistilled water) was thermostated to  $\pm 0.02^\circ\text{C}$  by means of water circulation from

a constant-temperature bath. The constancy of the temperature was satisfactory only if the temperature of the water bath and of the laboratory did not differ more than 1°C. Accordingly, we had to adjust the temperature of water bath to be close to that of the laboratory (24–25°C). Since  $dn/dc$  did not change more than 0.2% for one degree and the precision of our measurements was about 1%, this procedure can be accepted as reliable. Under such conditions the steady fringes were obtained and their positions could be precisely located. When working with interferometer it is essential to avoid ambiguity in the identification of the correct fringe (because of the possibility of the shift of interference band due to the difference in the dispersion between solution and solvent<sup>17</sup>). For this reason we have used a series of aqueous solutions of dextran of various concentrations (about 0.2–1%), and  $\Delta n$  was measured not only between solutions and water, but also between two solutions with small difference in concentration. In the concentration range investigated no shift of interference bands was noted. The practical importance of this finding for the determination of dextran concentration in solutions from measured  $\Delta n$  values is obvious. The whole procedure was checked by measuring  $\Delta n$  of aqueous solutions of sodium chloride. Close agreement with the values listed by Kruis<sup>18</sup> was found. The uncertainty in  $\Delta n$  was about  $\pm 1 \times 10^{-6}$ .

Dextran used was a sample of clinical dextran of Yugoslav production ( $M_w$  by light scattering about 100,000). Stock solutions were prepared by pouring the powdered dextran into boiling distilled water followed by a two-hours heating of the solution in a water bath at 100°C. In this way the complete dissolution of dextran was assured. This was proved by low dissymmetry of light scattered after pressure filtration through Selas 03 porous porcelain filter candles ( $I_{45}/I_{135} < 1.10$  as measured in an Oster-Aminco light scattering photometer<sup>19</sup> using semioctagonal cells). The concentrations of stock solutions were determined by evaporation of aliquot portions of the solution, and subsequent drying in a vacuum-oven over  $P_2O_5$  at 110°C and 50 mm. Hg to constant weight. The agreement between duplicate determinations was better than 1%. According to the results of Riddick et al.,<sup>14</sup> the samples of dextran dried in similar fashion (without  $P_2O_5$ ) retained about 0.3% of water.

Our results, including five independent series of measurements, are collected in Table III.

TABLE III

Column a:  $\Delta n$  measured between solution of concentration  $c$  and water.  
Column b:  $\Delta n$  obtained by summation of the  $\Delta n$  values measured between a pair of solutions of successive concentrations. Errors of the results are estimated from the standard deviation of slopes of straight lines  $\Delta n$  versus  $c$ .

$c \times 10^3$ (g. cm. <sup>-3</sup> )	$\Delta n \times 10^4$					
	436 m $\mu$		546 m $\mu$		578 m $\mu$	
	a	b	a	b	a	b
2.00	2.96	2.97	2.89	2.89	2.88	2.88
3.92	5.85	5.89	5.73	5.74	5.73	5.72
5.88	8.70	8.76	8.53	8.54	8.51	8.51
7.76	11.62	11.72	11.42	11.43	11.40	11.39
9.60	14.58	14.60	14.24	14.23	14.20	14.19
$dn/dc$ (cm. <sup>3</sup> g. <sup>-1</sup> )	0.1518 $\pm$ 0.0012		0.1481 $\pm$ 0.0013		0.1476 $\pm$ 0.0013	
% deviation	0.79		0.88		0.88	

It is worthwhile to note that our values for  $dn/dc$  of dextran are very close to the mean values of the data quoted in Table I, the mean values being 0.1517 for 436  $m\mu$  and 0.1494 for 546  $m\mu$ . As regards the change of  $dn/dc$  with the wave length, we have obtained the results in accord with those for other polymers (normal dispersion). To avoid the numerous citations from the literature, we wish to draw the attention only to the results of Witnauer, Senti and Stern<sup>20</sup> for amylopectin, a polysaccharide similar to dextran ( $dn/dc$  being 0.156 and 0.154 for 436 and 546  $m\mu$ , respectively).

It ought to be remarked that a similar situation is very probable to be found for other polymer-solvent systems. The need for a thorough comparison of the published data of  $dn/dc$  and a study of the possible sources of discrepancies is quite obvious.

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## IZVOD

**Inkrement indeksa loma dekstrana za određivanje molekularne težine pomoću rasipanja svjetla**

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Usporedbom podataka iz literature za inkrement indeksa loma,  $dn/dc$ , dekstrana u vodi (Tablica I), ustanovljene su znatne razlike za valnu duljinu od 546 m $\mu$ . Budući da  $dn/dc$  ulazi kao kvadratni član u jednadžbu, koja povezuje turbiditet i molekularnu težinu, ove razlike mogu izazvati velike pogreške prilikom izračunavanja molekularne težine (Tablica II). Neki su autori k tome našli i anomalnu disperziju dekstrana (veći  $dn/dc$  za veću valnu duljinu). Zbog velike važnosti određivanja molekularne težine dekstrana metodom rasipanja svjetlosti prilikom pripravljanja i kontroliranja koloidnih otopina za infuziju (nadomjestak plazme), autori su u ovoj bilješci prikazali, držeći na umu gore navedene činjenice, rezultate vlastitih određivanja inkrementa indeksa loma dekstrana (Tablica III). Pritom su dobivene ove vrijednosti za  $dn/dc$  (u cm<sup>3</sup> g<sup>-1</sup>): 0.1518  $\pm$  0.0012 za 436 m $\mu$ ; 0.1481  $\pm$  0.0013 za 546 m $\mu$ ; 0.1476  $\pm$  0.0013 za 578 m $\mu$ . Disperzija je, kako se vidi, normalna. Pogreška kod mjerenja manja je od 1%. Ove vrijednosti neznatno se razlikuju od srednjih vrijednosti podataka iz literature.

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