

## RESEARCH PAPERS

### THE WEIGHT AVERAGE MOLECULAR WEIGHT OF POLYVINYLPIRROLIDONE PREPARATIONS AS DETERMINED BY LIGHT SCATTERING

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THE commonly accepted method for the characterisation of polyvinylpyrrolidone solutions used as plasma substitute is the measurement of the viscosity. The method is also applied to fractions separated from them by fractional precipitation, usually by the addition of acetone. From these measurements, reported in terms of intrinsic viscosity or  $K$  (Fikentscher)<sup>1</sup> values, figures, reputed to represent molecular weights, have been derived. Levy and Frank<sup>2</sup> have discussed these derivations and have listed a number of equations proposed by various investigators to relate intrinsic viscosity to molecular weight. The graphical representations of several such equations are presented in Figure 1. From these the interpretation may be made that an intrinsic viscosity of 0.2 dl./g. corresponds to a molecular weight between 15,000 and 43,000 while an intrinsic viscosity of 0.4 dl./g. corresponds to a molecular weight between 50,000 and 150,000. Obviously these interpretations must be treated with some reservation.

Since many of the *in vivo* characteristics of a particular plasma substitute depend on its molecular size it would seem desirable to characterise these materials by a procedure which measures molecular weight directly rather than by the interpretation of viscosity data. Light scattering photometry, which measures directly the weight average molecular weight of a polymer, has been used for this purpose on polyvinylpyrrolidone by several investigators<sup>2,5,7</sup>; but a detailed method has not been published. Since polyvinylpyrrolidone presents some problems in light scattering photometry, it appeared of interest to report the results of studies conducted in these laboratories.

#### EXPERIMENTAL

Seven commercial plasma substitutes from three sources, four dried polyvinylpyrrolidone fractions suitable for clinical use, and five special samples of molecular weight above and below the clinical ranges were studied. It was at once evident that the usual light scattering procedure, such as that applied to dextran plasma substitutes<sup>8</sup>, could not be applied directly to polyvinylpyrrolidone solutions, which exhibited fluorescence when exposed to blue light of 436 m $\mu$ , the wavelength commonly used. The procedure adopted was as follows.

Aliquots of an approximately 1.0 to 3.5 per cent (w/v) solution of polyvinylpyrrolidone in water were pipetted into 50-ml. volumetric flasks

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to produce, after dilution to the mark with optically-clear glass-distilled water, dilutions of approximately 1:10, 2:10, 3:10, 4:10, and 5:10. The higher concentration starting solutions were used with the lower molecular weight material. The carefully mixed dilutions were filtered under positive pressure through an ultra-fine sintered glass filter (nominal maximum pore size 1 to 2  $\mu$ ) into clean dissymmetry cells. Clear, glass-distilled, filtered water was used as the blank. Readings were taken in the Brice-Phoenix Light Scattering Photometer, series 1000, using blue light ( $\lambda$  436  $m\mu$ ). The procedure described by Brice, Nutting and Halwer<sup>9</sup>

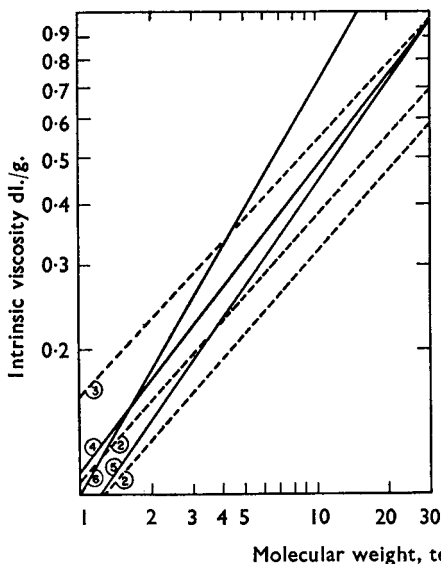


FIG. 1. Lines representing published relationships found between molecular weight and intrinsic viscosity of polyvinylpyrrolidone. The numbers refer to bibliographic references.

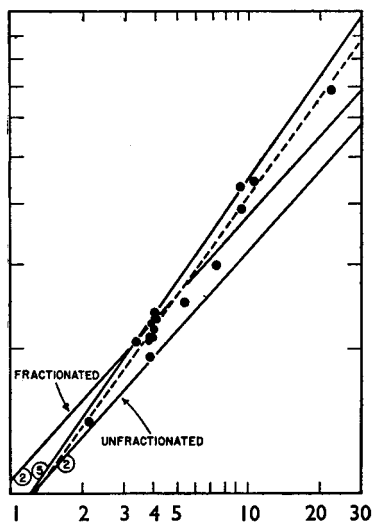


FIG. 2. Lines representing the relationship between molecular weight and intrinsic viscosity of polyvinylpyrrolidone found by Levy and Frank<sup>2</sup> and by Hengstenberg and Schuch<sup>5</sup>. The dashed line represents the line of best fit for the plotted data obtained in the present investigation.

was used to correct for fluorescence. In this procedure the unpolarised incident light is passed through the test solution, and the intensity of the polarised light scattered at 90° is compared with the intensity of the transmitted light for both horizontal and vertical vibrations. The depolarisation of the fluorescent light is measured by inserting a yellow filter (which transmits only fluorescent light) in the 90°-scattered light path and determining the proportion of horizontally to vertically polarised fluorescent light. This value is used to correct the true scattering ratio.

The intensity of the light scattered at 45° and 135° is determined to assess the dissymmetry of scattering. These values are corrected also for fluorescence. Under the conditions of these investigations, properly

clarified solutions of polyvinylpyrrolidone in the molecular weight range 20,000 to 100,000 regularly exhibited corrected dissymmetry ratios of less than 1.15 and usually less than 1.10.

The turbidity data obtained from the photometer readings were used to calculate the relation  $Hc/\tau$ .<sup>\*</sup> These values were plotted against the concentration  $c$  and the line of best fit was determined by eye and extrapolated to zero concentration. The line thus obtained usually showed some upward curvature as  $c$  increased. The intercept with the Y axis is the reciprocal of the weight average molecular weight. From the data shown in Table I, the coefficient of variation of the method was found to be less than  $\pm 4.3$  per cent. The 95 per cent confidence limits of the mean amounted to  $\pm 3.1$  per cent or less.

TABLE I  
REPRODUCIBILITY OF LIGHT SCATTERING MOLECULAR WEIGHT DETERMINATIONS

	Sample			
	G1	S37	G2	IBH
	Weight average molecular weight			
	22,900	33,300	56,500	77,000
	21,300	33,800	55,000	80,000
	21,400	33,400	53,500	79,000
	22,400	33,000	58,600	80,000
	22,700	32,000	55,000	74,000
	22,200	31,300	54,000	71,500
	22,000	33,000	55,100	80,000
		33,000	55,100	80,000
		31,700	58,800	80,000
		32,000	58,800	74,200
Mean .. .. .	22,130	32,650	55,660	76,990
Coefficient of variation .. .. .	2.75	2.55	4.27	4.00
Standard error of the mean as per cent .. .. .	1.04	0.81	1.35	1.27
95 per cent confidence limits of the mean as per cent .. .. .	2.45	1.84	3.06	2.88

As a check on the calibration of the photometer the Rayleigh ratio for freshly distilled benzene was measured at  $436 \mu\mu$  and found to be  $48.25 \times 10^{-6}$ . This is 99.8 per cent of the value reported by Brice, Halwer and Speiser<sup>11</sup> and 102.3 per cent of the value reported by Oster<sup>12</sup>.

Rather than use blue incident light which causes fluorescence, green light  $546 \mu\mu$  may be used. At this wavelength fluorescence is low in intensity and the corrections are small. At the same time the intensity of the scattered light is much reduced and the precision was found to be about the same as when blue incident light was used.

Some results obtained at the two wavelengths are shown in Table II. It will be noted that higher results were obtained using the longer wavelength. When the molecular weight found with green light was plotted against the molecular weight found with blue light, for the range of molecular sizes examined, the linear regression coefficient for the data

\* For the calculation of the value for  $H$ , the refractive index increment  $dn/dc$  was found to be 0.185 for aqueous solutions. This is larger than the 0.175 reported by Hengstenberg and Schuch<sup>6</sup> but agrees with the 0.185 found by Levy and Frank<sup>3</sup>. Where the solvent was methanol the value 0.197 was used for  $dn/dc$ . Solutions were analysed for polyvinylpyrrolidone content by the colorimetric iodine method of Thrower and Campbell<sup>10</sup> as modified for use in this laboratory along the lines suggested to us by Drs. Levy and Ferguson.

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was 1.247. This was significantly different from 1 ( $P = 0.01$ ). This finding confirms similar results reported by Hengstenberg and Schuch<sup>5</sup>.

Experiments also were conducted to compare the results obtained by light scattering photometry using methanol as solvent for the polyvinylpyrrolidone with those obtained using water. Where dry polymer samples

TABLE II

EFFECT OF INCIDENT LIGHT WAVELENGTH ON THE MOLECULAR WEIGHT DETERMINED BY LIGHT SCATTERING

Wavelength	Sample				
	G1	S37	G2	IBH	G4
	Weight average molecular weight				
436 m $\mu$	22,000	33,800	55,000	74,000	235,000
546 m $\mu$	25,200	40,000	63,500	85,000	290,000
Ratio	0.87	0.85	0.87	0.87	0.81

were used as the starting materials the results, in most instances, agreed fairly well regardless of the solvent. With the commercial solutions where the polyvinylpyrrolidone was precipitated by the addition of excess acetone, dried *in vacuo*, and redissolved in anhydrous methanol the results invariably were high compared with those obtained with water as

TABLE III

EFFECT OF SOLVENT ON THE MOLECULAR WEIGHT DETERMINED BY LIGHT SCATTERING

A. Dry powders									
Solvent									
Sample									
G1									
S37									
G2									
G4									
Weight average molecular weight									
Water	..	..	..	..	..	21,400	33,800	55,000	235,000
Methanol	..	..	..	..	..	22,700	34,800	58,700	232,000

B. Clinical solutions									
Solvent									
Sample									
M4									
M9									
S86									
S88									
Weight average molecular weight									
Water	..	..	..	..	..	40,000	39,200	41,000	38,500
Precipitated by acetone, redissolved in methanol	..	..	..	..	..	85,000	80,000	83,000	71,000
Precipitated by acetone, redissolved in water	..	..	..	..	..	41,500	—	—	39,600

the solvent. If the samples, after acetone precipitation and drying, were redissolved in water, the results agreed with those found with the original aqueous solution for direct analysis. Apparently some material, precipitated by acetone, failed to dissolve completely in methanol although it was readily soluble in water. This precipitate was in such a finely divided state that it passed through the ultra fine filter and caused high

turbidities. Illustrative results are presented in Table III. Intrinsic viscosities of the methanol solutions, unlike the light scattering measurements, were not affected by the treatment but were in the range to be expected from the relations reported by Levy and Frank<sup>2</sup>. This indicates that the treatment had not caused aggregation of the molecules.

It has been reported by Frank and Levy<sup>7</sup> that the weight average molecular weight of polyvinylpyrrolidone, measured in methanol, is not

TABLE IV  
EFFECT OF SALTS ON THE DETERMINATION OF THE MOLECULAR WEIGHT OF POLYVINYLPIRROLIDONE BY LIGHT SCATTERING

Solvent	Sample		
	G1	S37	IBH
	Weight average molecular weight		
Water .. .. .	21,400	33,800	74,000
0.9 per cent NaCl .. .. .	21,600	33,300	74,000
1.8 per cent NaCl .. .. .	—	33,000	71,000
3.6 per cent NaCl .. .. .	—	32,000	—
Mixed salts* .. .. .	—	33,000	80,000
Double strength mixed salts* .. .. .	—	33,000	74,200
Mean .. .. .	21,500	33,000	74,700

\* For composition see text.

identical with that measured in water. It has been noted too that polyvinylpyrrolidone in methanol fluoresces more strongly than does an aqueous solution of the same concentration. In view of these findings it has been found desirable to make the light scattering measurement on aqueous solutions.

Investigation was made of the possible effect of the presence of sodium chloride, and of other salts used in physiological solutions, in the test solutions of polyvinylpyrrolidone. Equal weights of the same dry polyvinylpyrrolidone sample were dissolved in water, in 0.9, 1.8, or 3.6 per cent (w/v) aqueous sodium chloride solutions, or in a physiological salts\* solution of usual or twice usual concentration. These starting solutions containing 1 to 3 per cent (w/v) polyvinylpyrrolidone were used to prepare the 5 dilutions in optically clear, glass-distilled water and these were subjected to filtration and light scattering measurements in the usual manner. The results of these analyses, presented in Table IV, indicate that the presence of these salts in the amounts indicated had no significant effect on the molecular weight determination by light scattering.

Frank and Levy<sup>7</sup> reported that 1M sodium chloride solution had little effect on the intrinsic viscosity of polyvinylpyrrolidone solutions. On the other hand, Jergensons<sup>13</sup> found the intrinsic viscosity to be reduced by the presence of 1.0M KCl and 0.25M MgCl<sub>2</sub> and to be slightly increased by

\* Usual concentration physiological salts solution.

NaCl	0.8 g.	MgCl <sub>2</sub> ·6H <sub>2</sub> O	0.0005 g.
K Cl	0.042 g.	Na HCO <sub>3</sub>	0.168 g.
CaCl <sub>2</sub> ·6H <sub>2</sub> O	0.05 g.	N HCl	1.171 ml.
		Water to make	100 ml.

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1.0M MgCl<sub>2</sub>. In Table V are presented data on the intrinsic viscosity† of the same solutions used to assess the effect of salts on light scattering measurements. Viscosity determinations were made as described previously for dextran<sup>8</sup>. It may be seen that there appeared to be no significant effect of salts on intrinsic viscosity measurements. On the basis of these experimental results it was considered unnecessary to free the commercial solutions of salts before performing viscosity and light scattering measurements.

TABLE V  
EFFECT OF SALTS ON THE INTRINSIC VISCOSITY OF POLYVINYLPIRROLIDONE

Solvent	Sample		
	G1	S37	IBH
	Intrinsic viscosity dl./g.		
Water .. .. .	0.142	0.210	0.284
0.9 per cent NaCl .. .. .	0.142	0.209	0.282
1.8 per cent NaCl .. .. .	—	0.208	0.286
3.6 per cent NaCl .. .. .	—	0.216	—
Physiological salts solution* .. .. .	—	0.207	0.284
Double strength physiological salts solution* .. .. .	—	0.211	—
Mean .. .. .	0.142	0.210	0.284

• For composition see text

As pointed out earlier, attempts to establish a reliable correlation of weight average molecular weight and intrinsic viscosity have not been too successful. It would appear that the viscosity of a particular sample of polyvinylpyrrolidone may bear a different relation to its weight average molecular weight than may be the case for another sample. Levy and Frank<sup>2</sup> have presented evidence which suggests that this is due to variations in homogeneity of the samples. In the lower ranges, application of the Levy and Frank formulae<sup>2</sup> for fractionated and unfractionated polyvinylpyrrolidone samples to the viscosity data assembled here yielded molecular weight values which bracketed most of the actual values obtained by light scattering. In the range above molecular weight 75,000 the data obtained here showed considerable divergence from the Levy and Frank relationships. The equation for the line of best fit over the entire range considered here, determined by least squares and converted to the usual form, was  $[\eta] = 1.575 \times 10^{-4} M^{0.68}$ . This line is shown in Figure 2 together with the lines representing the relationships found by Levy and Frank<sup>2</sup> for "fractionated" and "unfractionated" material and by Hengstenberg and Schuch<sup>5</sup>.

SUMMARY

1. A method for the determination of the weight average molecular weight of polyvinylpyrrolidone is described. Corrections for the effects of fluorescence are applied.

† "Intrinsic viscosity" here is in some instances more properly termed "apparent intrinsic viscosity" since water alone was used to calibrate the viscosity pipettes as would be the case if the analyst were unaware of the presence of salts.

2. Measurements are carried out in aqueous solution in preference to methanol solution.

3. The presence of physiological concentrations of salts such as sodium chloride in the solutions analysed did not significantly alter light scattering or intrinsic viscosity measurements.

4. A relation between intrinsic viscosity and weight average molecular weight is derived, but any interpretations of intrinsic viscosity in terms of molecular weight must be regarded as approximations.

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