

Investigations on the Osmotic Pressure of Aqueous Solutions of Dextran Glycerol Glycoside and of Potassium Hyaluronate

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A preparation of dextran giving a constant colloid osmotic pressure and having a molecular weight of 1.2×10^6 is prepared. It is shown that the ratio between the weight concentration (c) and the osmotic pressure (p) is fairly constant in the case of dilute solutions of the electrically neutral dextran; in case of dilute solutions of the electrically charged hyaluronate the value of this ratio decreases with increasing concentration. In respect to hyaluronate it is found that the relation between pressure and concentration can be given by the following expression: $c/p = A(1-Bc)$, where the constant B is independent of the molecular weight.

Although blood plasma substitutes, artificial "plasma expanders", were tried during the first world war, it was only during the second world war that their use developed. It seems to be a fact that dextrans surpass all plasma substitutes used so far, as they afford the most marked and most prolonged increase in circulating blood volume. Dextran which is a polymer of glucose joined chiefly through α -1,6-glycosidic linkages, is derived from the fermentation of sucrose by various strains of *Leuconostoc mesenteroides*. The native dextran has a molecular weight of several millions, and has a harmful effect when injected into the animal organism. When this so-called crude dextran is degraded, *e.g.* by acid hydrolysis, to a molecular size corresponding approximately to plasma proteins it becomes usable as "clinical dextran". If the partial depolymerization is carried out in glycerol solution (Hultin and Nordström¹) the biologically active free aldehyde groups, often giving rise to allergic manifestations, are inactivated. By this process dextran glycerol glycoside is formed. Such a preparation is commercially available as "Infudex" or "Plasmodex *". The results of some osmotic investigations on such a preparation are reported in the present paper.

* This commercial preparation was kindly placed at our disposal by Roskilde Medical Company, Roskilde, Denmark.

EXPERIMENTS ON DEXTRAN

The colloid osmotic pressures were measured by means of the inverted microsmometer described by Christiansen and Jensen². The solution of collodion used for the preparation of semipermeable membranes was made in the following way: 4 g of completely dry pyroxylin (Parlodion "Mallinckrodt") was dissolved in a mixture of 75 ml of absolute alcohol and 25 ml of anhydrous ether; to this mixture were added 5 ml of ethylene glycol freshly distilled *in vacuo*.

The first measurements were performed on an 1 % solution of "Plasmodex", the osmotic pressure of which was estimated at about 5.4 cm water pressure. It was observed, however, that the pressure was decreasing with time indicating that this preparation of dextran contained low molecular components capable of permeating the membrane employed. After 15 h the "equilibrium length" of the first mentioned solution had fallen to 4.5 cm; the result of this measurement is in Table 1.

In order to determine the proportion of low molecular components contained in this preparation of dextran the aqueous solution of the preparation was dialyzed for 24 h against running tap water in a tube of cellophane at room temperature. Then the dialyzed solution was distilled *in vacuo*, the temperature of the water being 40–45°C, and freeze-dried. The yield was 96 % of the starting material. Osmotic pressure measurements performed on this preparation showed, that a fraction of the dextran molecules was still permeating the collodion membrane and that the osmotic pressure was slightly lower than that of the nondialyzed preparation of dextran at the same concentration.

Then the dextran was dialyzed in a test-tube shaped membrane of collodion prepared in exactly the same way as the membranes used for the osmotic pressure measurements. The dialysis lasted for 6 days, the outer solution (distilled water) being replaced twice every 24 h, whereafter the solution was distilled *in vacuo* and freeze-dried. Yield: 40 % of the starting material. The osmotic pressure of a 0.6 % solution of this preparation was found to be about 2.6 cm water column. But even now some dextran molecules were permeating,

Table 1. Osmotic pressure of an 1 % solution of dextran. h = height of air column. V = rate of movement of the upper meniscus of the air column in micrometer divisions in 10 min. For details *cf.* Christiansen and Jensen².

h in cm	V in 10 min.
4.89	+0.18
3.80	-0.30
5.29	+0.38
6.27	+1.20
4.10	-0.18
5.73	+0.55

Equilibrium length: 4.5 cm

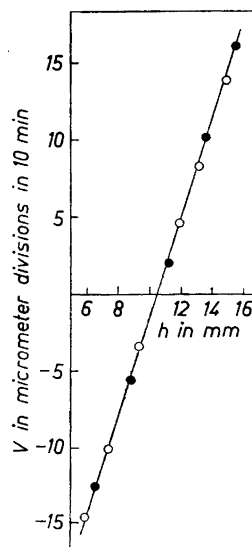


Fig. 1. Osmotic pressure of an aqueous solution of dextran. The graph shows the relation between height of the air column (abscissae), and the rate of movement of the upper meniscus of the air column (ordinates) in micrometer divisions in 10 min. Concentration of dextran: 5.20 mg in 1 ml of water. Equilibrium length: 10.5 mm water column. The measurements represented by the black spots are performed 24 h after the measurements represented by the circles.

although to a smaller degree than in the preceding experiment. Therefore another attempt was made to get rid of the low-molecular components by means of precipitation with acetone. 60 ml of acetone were added to 100 ml of a 10 % aqueous solution of dextran, which resulted in the appearance of a two-phase system. The lower layer was yellow and consisted predominantly of water and dextran, while the upper layer was white and consisted predominantly of acetone and little dextran. The upper layer was decanted, whereafter the lower layer was treated with acetone; by doing so the dextran was precipitated. The filtrate was washed several times with acetone and dried for 24 h in a vacuum desiccator containing phosphorus pentoxide. After dialysis in a collodion tube this preparation showed a constant osmotic pressure. The pressure of a 0.52 % aqueous solution was measured at 1.05 cm water column corresponding to a molecular weight of this preparation of 1.2×10^5 . The result of these measurements are given in Fig. 1, the black spots corresponding to measurements performed 24 h after the measurements indicated by circles.

In order to investigate the validity of the well known limiting law of van't Hoff: $\lim_{c \rightarrow 0} (p/c) = RT$, where p is the difference in hydrostatic pressure between the inner and outer solutions at equilibrium, and c is the concentration of the solute in moles per liter of solution, we determined the osmotic pressure of dextran solutions at different concentrations of dextran. The results of these measurements are in Table 2. Evidently van't Hoff's law is valid at the lower concentrations, while at concentrations from about 2 g/100 ml a slight decrease of the ratio c/p is observed. Our results agree closely with those obtained by Mariani *et al.*³ Further our results are in fair agreement with those obtained by Autio⁴ who determined the osmotic pressure of solutions of dextran ("Macrodex") by means of the Holm-Jensen osmometer.

Table 2. The relation between concentration (c) and colloid osmotic pressure (p) of aqueous solutions of dextran.

c mg/ml	p cm	c/p
5.19	1.05	4.94
19.40	2.07	5.31
31.51	6.45	4.89
40.00	8.80	4.55

EXPERIMENTS ON POTASSIUM HYALURONATE

Using osmotic measurements, Christiansen and Jensen^{2,5} estimated the molecular weight of potassium hyaluronate isolated from human umbilical cords and from vitreous bodies of cattle at 5×10^5 and 2.7×10^5 , respectively. These determinations were performed on solutions which contained about 10 mg of hyaluronate per 1 ml of solution.

Molecular weight calculations from osmotic pressure data were made by means of the equation: $\frac{246.8 \times 10^5 \times c}{p}$ where c = concentration of dry substance in g per 1 ml of solution and p = osmotic pressure in cm water pressure. All measurements were performed at 20°C.

It has long been known, however, that this equation is not valid for electrically charged thread-shaped molecules at concentrations where experiments could be performed. The ratio p/c , the so-called reduced osmotic pressure, usually increases with the concentration so that its limiting value must be determined by some sort of extrapolation. To perform this extrapolation we plotted p/c against c , but this yielded a curve with a distinct bend upwards. When, however, we plotted c/p against c we got curves which were very nearly straight lines with a downward slope.

Four preparations of different molecular weight were used, three of them having relatively small and one a relatively high molecular weight. The measurements of the colloid osmotic pressure were determined at four or five different concentrations of hyaluronate. The molecular weights calculated from the pressures obtained from extrapolation to zero concentration (the so-called reduced molecular weight) appeared to be about one and a half as big as the uncorrected values obtained from measurements performed on solutions containing about 10 mg of hyaluronate in 1 ml of solution.

Attempts at finding an adequate relation between the colloid osmotic pressure of the solution (or the molecular weight) and the concentration of the solute resulted in the following empirical expression: $c/p = A(1 - Bc)$. Here c is the weight concentration (mg/ml); p is the colloid osmotic pressure in cm water pressure; A is a quantity which is constant for a given preparation, but proportional to the molecular weight; B is a constant for all the employed preparations (independent of the molecular weight). The values of A and AB were obtained graphically by plotting c/p against c . The results of the

Table 3. The osmotic pressure of some preparations of potassium hyaluronate and some calculated quantities showing that the ratio between concentration and pressure is equal to $A(1-Bc)$ where A and B are constants (For details see the text).

Prep.	<i>c</i> Conc. in mg/ml	<i>p</i> Osmo- tic pres- sure in cm	<i>c/p</i> expt.	A From graph	AB From graph	$\frac{AB}{A} = B$	$A(1-B_{AV}c)$ $= (c/p)_{calc.}$	$(c/p)_{expt.} - (c/p)_{calc.}$
I	16.00	7.9	2.03	5.1	0.190	0.0372	2.02	+0.01
	13.00	4.9	2.65				2.60	+0.05
	11.00	3.7	2.97				2.98	-0.01
	7.00	1.85	3.78				3.75	+0.03
	5.00	1.29	3.87				4.14	-0.27
II	16.00	6.5	2.46	5.8	0.215	0.0371	2.30	+0.16
	13.00	4.2	3.10				2.96	+0.14
	11.18	3.2	3.49				3.36	+0.13
	5.00	1.00	4.72				4.70	+0.02
III	7.51	1.85	4.06	6.0	0.219	0.0365	4.30	-0.24
	5.10	1.10	4.64				4.85	-0.21
	3.53	0.71	4.97				5.26	-0.29
	2.56	0.49	5.22				5.42	-0.20
IV	19.99	3.49	5.72	38.5	1.595	0.0401	9.47	-3.75
	16.00	1.20	13.33				15.28	-1.95
	13.78	0.85	16.21				14.48	-2.27
	10.21	0.45	22.69				23.68	-0.99
	7.48	0.28	26.71				27.64	-0.93

$$B_{AV} = 0.0377$$

measurements and the values of A, AB and B ($B = \frac{AB}{A}$) are in Table 3. It is seen that the agreement between the measured values of c/p and $A(1 - B_{AV}c)$ is reasonably good. The most pronounced deviation is found in series IV at the highest concentration of hyaluronate, at which the measurements are difficult on account of the high viscosity of the solution. It is to be noted that the value used for B in the calculations is the average value of the individual B's of the four experiments. If we, in series IV, make use of the method of least squares giving equal weight to the individual experimental points we find the corrected values of A and AB to be 39.4 and 1.675, respectively. Using these values the differences between $(c/p)_{expt}$ and $(c/p)_{calc}$ are: -0.24; 0.58; -0.14; 0.34 and -0.18, respectively.

From the results obtained from the experiments performed on dextran and on hyaluronate the difference in osmotic behaviour between solutions of electrically neutral and electrically charged substances is obvious from the fact that the values of c/p do not vary with the concentration of solute in the case of dextran, but do vary in the case of hyaluronate.

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