150. *The Osmotic Pressure of Solutions of Polysaccharide Derivatives. Part II. The Osmotic Pressure of Derivatives of Lichenin, Inulin, Glycogen, Starch, and Starch Dextrin.*

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The osmotic pressure–concentration relationship has been investigated, and in every case deviations from the van 't Hoff ideal were observed, the osmotic pressure always increasing with concentration more rapidly than demanded by the law. The magnitude of the deviation depends on the solvent; it is always greater for

chloroform than for carbon tetrachloride. Wo. Ostwald's " general solvatation equation " has been shown to offer a satisfactory mathematical representation of the relationship. Being free from " Donnan " membrane effects, the results are well adapted to the study of deviations from the ideal-solution laws. By extrapolating the Π/c–c curves to zero concentration, molecular or particle weights have been deduced. The limiting value of Π/c at zero concentration is independent of the solvent. By comparison with the size of the minimum chain lengths as determined by Haworth's end-group assay method, the particle weights of the polysaccharides are usually several times that of the fundamental chains. In the case of inulin, and possibly lichenin, the physical and the chemical units are identical.

By using the osmometer described in Part I (preceding paper), direct osmotic-pressure measurements have been made on a number of methylated and acetylated polysaccharides with particle weights ranging from 3000 to 3,500,000. The results obtained are summarised in Table I.

Table I.

Substance.	Solvent.	Particle weight by osmotic pressure.	Chain length (hexose units) by osmotic pressure.	Chain length (hexose units) by chemical assay.
Methylated lichenin (A)	CCl_4	10,700	52	80 *ca.*
,, ,, (B)	CCl_4	13,900	68	—
,, ,, ,, ...	$CHCl_3$	13,900	68	—
Acetylated lichenin (A)	$CHCl_3$	118,000	410	—
,, ,, (B)	$CHCl_3$	36,500	127	—
Directly methylated lichenin	$CHCl_3$	33,100	162	—
Acetylated inulin	$CHCl_3$	8880	30	—
Methylated inulin	$CHCl_3$	6210	30	30
Methylated glycogen :				
From rabbit liver	$CHCl_3$	830,000	4100	—
,, ,, ,, ...	CCl_4	620,000	3000	18
,, ,, ,, ...	$CH_3{\cdot}NO_2$	620,000	3000	—
,, dogfish liver	$CHCl_3$	620,000	3000	12
,, haddock liver	$CHCl_3$	620,000	3000	12
,, hake fish liver	$CHCl_3$	273,000	1340	12
Acetylated glycogen :				
From rabbit liver	$CHCl_3$	2,500,000	8700	—
,, dogfish liver	$CHCl_3$	3,500,000	12,000	—
,, haddock liver	$CHCl_3$	1,300,000	4500	—
,, hake fish liver	$CHCl_3$	1,900,000	6600	—
Methylated potato starch (A)	CCl_4	124,000	610	24
,, ,, ,, ,, ...	$CHCl_3$	124,000	610	—
,, ,, ,, (B)	$CHCl_3$	71,000	350	24
,, " soluble " starch	CCl_4	28,200	138	24
,, maize starch	$CHCl_3$	38,200	187	24
,, starch dextrin (C)	CCl_4	33,200	163	12
,, ,, ,, (A)	CCl_4	4520	22	12
,, ,, ,, (B)	CCl_4	2910	14	9

The Relation between Osmotic Pressure and Concentration.—A knowledge of the general form of the specific osmotic pressure–concentration curve is essential in the extrapolation to zero concentration to arrive at a value for the particle weight. All the results obtained in this investigation suggest that the Π/c–c values lie on smooth curves. In those cases where measurements were extended to low concentrations, no marked tendency to deviate from the smooth curve was observed. Irregular values falling away from the curve were likely to occur if too short a lapse of time were allowed before making measurements, and also if the solutions were not stirred.

The direct osmotic-pressure measurements of Morse and Frazer and of Berkeley and Hartley on cane-sugar solutions showed that, if V, in the ideal van 't Hoff relationship $\Pi V = \boldsymbol{R}T$, be taken as the volume of solvent instead of the total volume of solution, the experimental results are more in accord with this simple equation. Although concentrations have been expressed in terms of solvent throughout this paper, deviations, often of considerable magnitude, occur in every case. Sackur's equation (*Z. physikal. Chem.*, 1910, **70**, 477) in the form $\Pi(V - b) = \boldsymbol{R}T$ allowed for a larger volume correction. Adair (*Biochem. J.*, 1930, **24**, 1864) has shown that this type of equation satisfactorily

expresses the osmotic pressure of hæmoglobin up to concentrations of 20%. When the equation is applied to our measurements, however, agreement is observed only over the "near-dilute" range of concentration, the calculated values being greater than the experimental ones at higher concentrations. A better representation of our results is given by the equation suggested by Wo. Ostwald, *viz.*, $\Pi = ac + bc^n$, where a, b, and n are constants to be determined for each particular case. This expresses the total pressure Π as the sum of two quantities: (i) the true van 't Hoff osmotic pressure $\Pi_v = ac$, and (ii) the so-called "swelling pressure" $\Pi_Q = bc^n$. The constants have been determined according to the method of Ostwald's later paper (*Kolloid-Z.*, 1929, **49**, 60). The excellent agreement between calculated and experimental values over a wide range of concentration is shown for the case of methylated lichenin (Table II). Here, c is in g. per 100 c.c. of solvent and Π is expressed in atmospheres; n has a value a little over 2 in all the specimens examined. Similar values have been found by Wo. Ostwald for substances as widely different as rubber and hæmoglobin.

TABLE II.

Methylated lichenin (B) in carbon tetrachloride.

$$\Pi = 0{\cdot}0172c + 0{\cdot}00272c^{2{\cdot}17}.$$

c.	$\Pi_v = ac$.	$\Pi_Q = bc^n$.	Π (calc.).	Π (obs.).	Error, %.	c.	$\Pi_v = ac$.	$\Pi_Q = bc^n$.	Π (calc.).	Π (obs.).	Error, %.
0·743	0·0128	0·0014	0·0142	0·0142	0·0	0·499	0·0086	0·0006	0·0092	0·0092	+0·1
1·577	0·0271	0·0073	0·0345	0·0349	+1·4	0·317	0·0055	0·0002	0·0057	0·0059	+4·7
2·690	0·0464	0·0233	0·0696	0·0693	−0·5	1·195	0·0206	0·0040	0·0025	0·0244	−0·6
5·992	0·1030	0·1325	0·2355	0·2403	+2·0	0·171	0·0029	0·0001	0·0030	0·0030	0·0
3·827	0·0659	0·0498	0·1157	0·1169	+1·0						

Deduction of Particle Weight from the Osmotic-pressure Measurements.—Since considerable deviations from the van 't Hoff ideal relationship often occur with substances of high molecular weight, and may be expected in the polysaccharide substances where the structures often deviate from the spherical form, the molecular or particle weight M calculated from the equation $\Pi = \boldsymbol{R}Tc/M$ will depend on the concentration. It becomes necessary, therefore, to make measurements at various concentrations in order to extrapolate back to zero concentration, where the ideal conditions may be assumed to hold. The Π/c ratio, or specific osmotic pressure, is most conveniently extrapolated from the Π/c–c curve. The intercept on the Π/c axis gives directly $(\Pi/c)_0$ or limit $(c \to 0)\Pi/c$, from which the particle weight is found by substitution in the above equation, *i.e.*, $M = 248{,}000c/\Pi$ at 20°, c being in g./100 c.c., and the osmotic pressure, Π, in cm. of water.

Our measurements on a number of polysaccharide derivatives in organic solvents support the validity of the procedure of drawing the best smooth curve through the experimental points.

The Particle Size of Polysaccharides.—From the measurements given in this paper, it appears that aggregation brought about by the union of basal chain units almost invariably occurs in the polysaccharide group, the osmotic pressures being usually lower, sometimes equal to, but never higher (at low concentrations) than those calculated from molecular weights based on the chemical assay values. In every case so far examined, the value of Π/c has been found to increase as c increases, in contrast to the constant Π/c value in an ideal solution. Further, it is seen that the rate of increase of Π/c with c increases as c as raised up to the highest concentrations examined. There is thus good reason to believe that in those cases of abnormally low osmotic activity in which the particles consist of aggregates of the repeating unit of the basal chains, an increase in the degree of aggregation with increasing concentration never occurs. In contrast to this behaviour, we may quote the direct osmotic-pressure measurements (Grollman and Frazer, *J. Amer. Chem. Soc.*, 1923, **45**, 1707) of aqueous phenol in which a decrease in Π/c with c was observed.

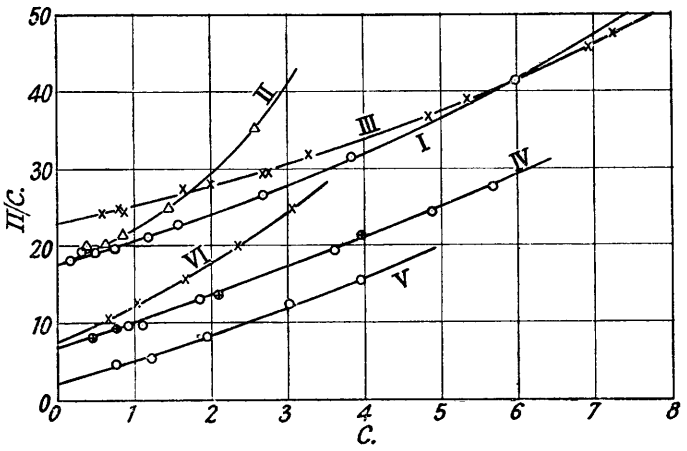
The forces responsible for the particle sizes of polysaccharide derivatives are therefore of a more permanent nature than those normally encountered in associated organic

substances. In further support of this view may be mentioned the constant particle weight found for a polysaccharide derivative obtained from different sources and by independent methods (*e.g.*, methylated glycogen) and also the constancy of the limiting osmotic pressure at low concentrations independently of the solvent used. The micelles formed by dyes, soap, etc., are usually variable in size, depending on the concentration, solvent, temperature, and past history, and usually occur in equilibrium with the simple chemical molecules.

Lichenin (Fig. 1). The acetylated and methylated lichenins examined here were prepared by Haworth and Michael (unpublished work).

Acetylated lichenin. Two specimens, A (*M*, 118,000) and B (*M*, 36,500), the latter being prepared a year after the former, were tested in chloroform (curves V and IV). The considerable difference in the particle weights of the acetates which were prepared under similar conditions might suggest that the enhanced aggregation of A might be due to its greater age. However, osmotic-pressure measurements on the acetate B, after a further 9 months' keeping, gave values which conformed closely to the original curve, and it is therefore evident that age cannot account for the disparity between the two acetates.

FIG. 1.



Lichenin derivatives.
II is in cm. of water. *c is in grams per* 100 *c.c. of solvent.*

Methylated lichenin. Two specimens of methylated lichenin prepared from the lichenin acetates A and B were investigated in carbon tetrachloride and in chloroform solution. A fully swollen membrane (0/100) proved to be perfectly semipermeable. This is supported by the low concentration ($c_s = 0{\cdot}006\%$) in the solvent chamber after experiments extending over 3 weeks. The osmotic pressures developed are of a convenient order of magnitude for accurate measurement, even at low concentrations, and the specific osmotic pressure–concentration relationship has been examined in some detail. It is seen that the experimental points in Fig. 1 are well represented by smooth curves down to low concentrations, particularly curves I, II, and (for the acetate) IV, there being no evidence of any abrupt rise or fall in the value of Π/c. Deviations from the van 't Hoff law are greater in chloroform than in carbon tetrachloride, but both curves (I and II) converge to the same limit at zero concentration, leading to the same value for the molecular weight (13,900) in the two solvents.

A methylated lichenin prepared by direct methylation of the polysaccharide (curve VI, chloroform as solvent) had a much higher particle weight (33,100) than those derived through the acetate.

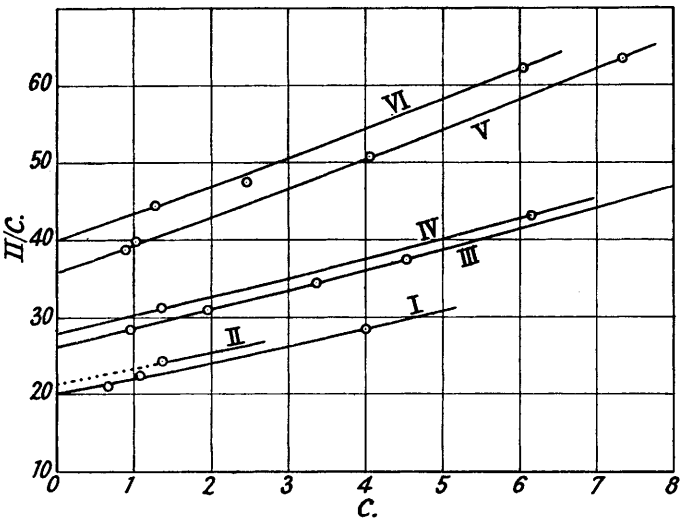
Inulin (Fig. 2). The inulin acetate and the corresponding methylated inulin prepared by Haworth, Hirst, and Isherwood (J., 1937, 782) were examined in chloroform solution. A chemical end-group assay of the methylated compound indicated a chain length of 30

fructose units. As might be expected from the relatively short chain length, a 0/100 (fully swollen) membrane proved to be imperfectly semipermeable, and the effect of membrane permeability on the osmotic pressure has been studied in some detail.

Inulin acetate. As seen from the concentration in the solvent chamber at the end of the series I, considerable diffusion occurs through a 0/100 membrane (curve I). During a determination a slightly high initial pressure dropped in a few hours to a steady value. Agitation of the solution did not appreciably alter these steady pressures. A 20/80 membrane produced only slightly higher pressures (curve II). A 50/50 membrane (curve III) showed a considerable increase in pressure level. In this case the readings conformed accurately to a smooth curve, no perceptible drop from the initial values was observed, and the solvent chamber concentration showed that diffusion through the membrane had been markedly reduced. Measurements in which a membrane (70/30) of still lower permeability was used showed a slight further increase in the pressure level (curve IV).

Methylated inulin. This specimen was prepared from the acetate just examined. The permeability of a 0/100 membrane to a chloroform solution was at once evident from the

FIG. 2.



Inulin derivatives.

Π is in cm. of water. *c is in grams per* 100 *c.c. of solvent.*

steady diminution in the " apparent " osmotic pressure with time. The " apparent " osmotic pressure was increased by stirring the solution. Measurements were carried out by using a 50/50 and a 70/30 membrane—curves V and VI (Fig. 2) respectively. No perceptible differences between the values with and without stirring could be observed in either series. The $(\Pi/c)_0$ value corresponded to 30 hexose units, which again represents a maximum for the mean particle weight.

Inulin itself is not sufficiently soluble in cold water to be examined by the osmotic method, but ebullioscopic determinations on a solution (7%) of inulin in water supported the work of Drew and Haworth (J., 1928, 2690), pointing to a value of 3500 for the molecular weight of the polysaccharide. A similar value was obtained for methylated inulin in chloroform solution (6%). There is thus good evidence that the physical units of inulin and of its acetylated and methylated derivatives have an average chain of about 30 hexose units in agreement with the end-group assay.

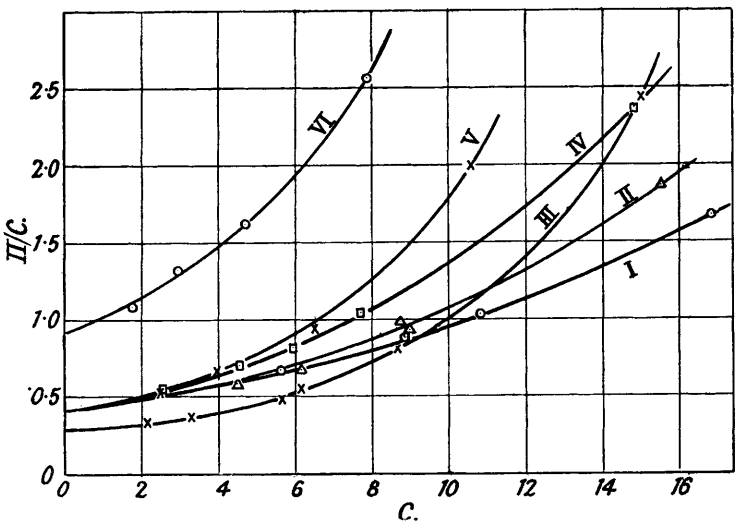
Methylated glycogen from rabbit liver. The specimen examined was prepared by Haworth, Hirst, and Isherwood (J., 1937, 577), and shown by the end-group method to have a chain length of 18 glucose units. Measurements were made in carbon tetrachloride solution against a 0/100 membrane. The difficulty of carrying out measurements at low concentrations owing to the minuteness of the osmotic pressures incurs some assumption

regarding the Π/c–c relationship in extrapolating a value for Π/c at infinite dilution. The extrapolation from the curve through the experimental points is considered justified, however, by the various results given in this paper. It should be noted that the scales of the axes in the various diagrams in this paper are different. The results are shown in curve I (Fig. 3), from which the value 620,000 (equivalent to 3000 glucose units) is obtained for the extrapolated particle weight. This value is of the same order as those obtained by Oakley and Young (*Biochem. J.*, 1936, **30**, 868).

The permanency of these large aggregates has been tested by making measurements on the same specimen of methylated glycogen in nitromethane, a solvent which has a dielectric constant 17 times that of carbon tetrachloride. The results shown in curve II (Fig. 3) point to a $(\Pi/c)_0$ value identical with that extrapolated from the measurements in carbon tetrachloride.

It was possible to increase the permeability of the membrane and still maintain its semipermeable nature towards these large aggregates, thereby facilitating the accurate measurement of low pressures. A membrane was prepared by treating "Viscacelle"

FIG. 3.



Methylated glycogen.
II is in cm. of water. *c is in grams per* 100 *c.c. of solvent.*

with 10% sodium hydroxide solution as the swelling medium (see Morton, *Trans. Faraday Soc.*, 1935, **31**, 262). Its permeability to chloroform was about 12 times that obtained when water is used as the swelling agent. Measurements were made in chloroform on a specimen of methylated rabbit-liver glycogen. The pressures showed no tendency to drop with time. Measurements on the same specimen against a 0/100 membrane showed slightly higher pressures, probably due to small amounts of substances of lower particle weight.

Glycogen from fish liver. Specimens of methylated and acetylated glycogen from the livers of the dogfish, hake, and haddock were prepared by Haworth, Hirst, and Smith (unpublished work). The chemical assay indicated a chain length of 12 hexose units in each case. The osmotic pressures have been measured in chloroform solution against a 0/100 membrane, and the results obtained for methylated glycogen from dogfish, hake, and haddock are shown in curves IV, V, and VI (Fig. 3) respectively.

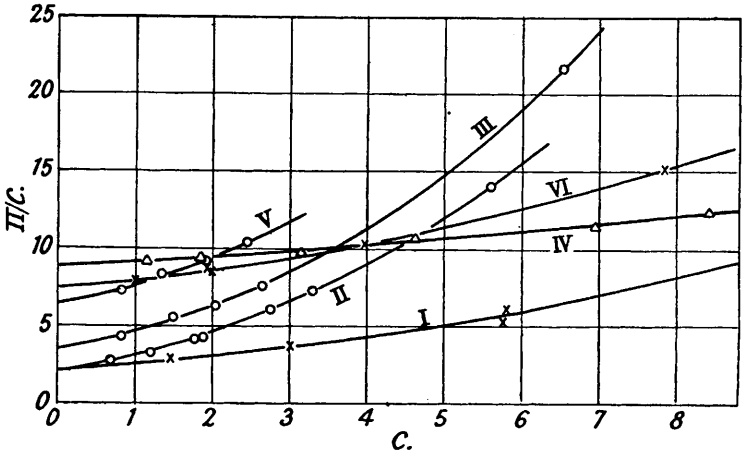
Glycogen acetates. Four glycogen acetates from which the methylated glycogen specimens tested above were prepared have been examined in chloroform solution against a 0/100 membrane. Only a brief examination has been made owing to the smallness of the pressures developed, which place these substances outside the range of applicability

of the present osmometer. A rough extrapolation has been attempted in each case, and the approximate particle weight of each specimen is given in the experimental section. The measurements on the four glycogen acetates and on the methylated glycogen of haddock and hake liver were all obtained in one continuous series using the same membrane. At the end of the series, the concentration in the solvent chamber was 0·017%, and as the permeability of the membrane has decreased by only 15% during the period (6 weeks), it is clear that Herzog's criticism (*Z. physikal. Chem.*, Bodenstein Festband, 1931, 239) regarding the unsuitability of " Cellophane " for osmotic-pressure determinations is not supported in this instance.

Ebullioscopic determinations on solutions (5%) of glycogen in water and of methylated glycogen in chloroform produced no measurable elevation in boiling point, and were thus in accord with the very low osmotic pressure results.

With particles of this order of magnitude, well-defined optical properties may be expected. Chloroform solutions of the glycogen acetates are noticeably opalescent and show a Tyndall beam. The methylated glycogen exhibits the same phenomenon, though to a lesser degree. The ultramicroscope revealed no definite particles but only a diffuse

FIG. 4.



Starch derivatives.

II is in cm. of water. *c is in grams per* 100 *c.c. of solvent.*

beam, probably owing to the small refractive-index difference between particles and solvent, which would be further diminished by solvation.

Starch (Fig. 4). Osmotic pressure measurements have been carried out on four different methylated starches and on a starch acetate.

Methylated potato starch A. This was prepared by Baird, Haworth, and Hirst (J., 1935, 1201, specimen No. 8, p. 1205) from starch acetate obtained by the Barnett process. The results of measurements in chloroform and carbon tetrachloride are shown in Fig. 4, curves I and II respectively, and both series of measurements point to the same $(\Pi/c)_0$ value (M = 124,000).

Methylated potato starch B. This was the specimen No. 6 prepared by Baird, Haworth, and Hirst (*loc. cit.*) from starch acetate obtained by the use of pyridine and acetic anhydride. The results for a chloroform solution are shown in curve III (M = 71,000).

Methylated " soluble " starch. This starch was shown by the end-group method to have the same chain length (*ca.* 25 units) as untreated potato starch (Haworth, Kitchen, and Peat, unpublished work). Osmotic-pressure measurements in carbon tetrachloride indicate a particle weight of 28,200 (*ca.* 40 glucose units) (see curve IV).

Methylated maize starch. This specimen had been methylated 18 times and showed OMe, 45·7% (Averill, unpublished work). Measurements in chloroform indicated a particle size of 38,200 (curve V).

These determinations on starch derivatives point to a wide range of possible values

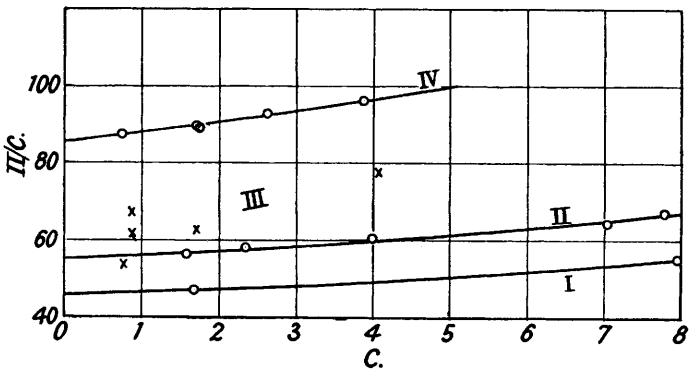
for the size of the physical units. Similar observations have been made on starch itself, *e.g.*, Samec's extensive osmotic-pressure measurements give values of 10,000 and upwards. Lamm's ultracentrifuge investigation showed the polydisperse nature of certain preparations of starch and a variable molecular weight according to the previous history (*Kolloid-Z.*, 1934, **69**, 44).

Starch dextrins. The methylated derivatives of three different breakdown products of potato starch, having chains of 17, 12, and 9 hexose units according to the end-group assay, have been examined in carbon tetrachloride.

Methylated starch dextrin C. The osmotic-pressure measurements, Fig. 4 (curve VI), were carried out on the specimen prepared by Haworth, Hirst, and Waine (J., 1935, 1299) by the breakdown of potato starch with barley amylase. At the time this was thought to be the dextrin usually designated α-amylodextrin, but subsequently Haworth, Hirst, Kitchen, and Peat (J., 1937, 791) gave reasons for supposing that the true α-amylodextrin has a chain length of 11—12 glucose units. Osmotic-pressure determination gave a particle weight of 33,200 (163 glucose units).

Dextrins A and B. These were prepared by Dr. L. N. Owen by the method of Haworth, Hirst, and Plant (J., 1935, 1214) by the glycerol hydrolysis of potato starch. Two acetates were obtained by a fractional precipitation of the acetylated dextrin, and from these were obtained methylated dextrin A (chemical assay: 12 hexose units) and

FIG. 5.



Methylated dextrins.

II is in cm. of water. *c is in grams per* 100 *c.c. of solvent.*

methylated dextrin B (chemical assay: 9 hexose units); osmotic pressure measurements (Fig. 5) gave particle weights of 22 and 14 hexose units respectively. They are the smallest particle weights in organic solvents determined with any osmometer up to the present. Bubble formation in the solvent chamber was very prone to occur in these measurements owing to the high pressures exhibited. The uniformity of the results obtained when using a 50/50 membrane, as also the low solvent chamber concentrations in both cases, show that such a membrane must be closely approaching perfect semi-permeability towards both specimens.

EXPERIMENTAL.

All osmotic-pressure measurements were made at 20·00°±0·02°, "Viscacelle 600" being used as the membrane. Concentrations are expressed as g. per 100 c.c. of solvent. Pressures are in cm. of water. The values in each series are given in the order in which they were determined. The concentration of dissolved solid in the solvent chamber at the end of a series of measurements is represented by c_s. A "70/30 membrane" indicates that a swelling medium of 70 c.c. of water and 30 c.c. of alcohol was used in its preparation. Particle weights are given as the usual molecular weight M, and in terms of hexose units (h.u.). The result for methylated lichenin B in carbon tetrachloride is calculated as follows:

$$(\Pi/c)_0 = \text{limit } (c \longrightarrow 0)\Pi/c = 17{\cdot}8 \text{ cm. of water;}$$

$$M \text{ (at } 20°) = \frac{RT}{(\Pi/c)_0} = \frac{22{,}400 \times 0{\cdot}1035 \times 293}{17{\cdot}8 \times 273} = 248{,}000c/\Pi = 13{,}900$$

or 13,900/204 = 68 hexose units (h.u.), 204 being the factor for a trimethyl hexose, and 882 for a triacetyl hexose.

Lichenin (Fig. 1).—(I) Methylated lichenin (B) in CCl_4; 0/100 membrane.

Series (a) (May 1935):

c.	Π.	Π/*c.*	*c.*	Π.	Π/*c.*	*c.*	Π.	Π/*c.*	*c.*	Π.	Π/*c.*
0·743	14·7	19·8	1·577	36·1	22·9	2·690	71·6	26·6	5·992	248·4	41·5
3·827	120·8	31·6									

$c_s = 0{\cdot}015\%$.

Series (b) (June 1935):

0·499	9·5	19·0	0·317	6·15	19·4	1·195	25·2	21·1	0·171	3·1	18·1

$(\Pi/c)_0 = 17{\cdot}8$; $M = 13{,}900$ (68 h.u.).

(II) Methylated lichenin (B) in $CHCl_3$; 0/100 membrane. (Oct. 1936.)

0·382	7·6	19·9	0·837	18·0	21·5	1·462	36·5	25·0	0·619	12·6	20·4
2·580	90·8	35·2									

$(\Pi/c)_0 = 17{\cdot}8$; $M = 13{,}900$ (68 h.u.).

(III) Methylated lichenin (A) in CCl_4; 0/100 membrane.

Series (a) (March 1935):

1·986	55·8	28·1	3·270	104·3	31·9						

Series (b) (April 1935):

0·797	19·8	24·8	1·620	44·4	27·4	4·832	178·0	36·8			

Series (c) (June 1935):

0·882	21·8	24·7	0·573	13·9	24·3	2·668	78·8	29·5	2·742	81·3	29·6
6·926	317·5	45·8	7·257	347·3	47·9	5·326	208·0	39·1			

$c_s = 0{\cdot}006\%$.

$(\Pi/c)_0 = 23{\cdot}1$; $M = 10{,}700$ (52 h.u.).

(IV) Lichenin acetate (B) in $CHCl_3$; 0/100 membrane. (June 1935.)

0·914	8·9	9·74	1·837	23·9	13·01	3·603	69·9	19·40	4·876	119·4	24·49
5·674	157·4	27·74	1·102	10·9	9·89						

$(\Pi/c)_0 = 6{\cdot}8$; $M = 36{,}500$ (127 h.u.).

(V) Lichenin acetate (A) in $CHCl_3$; 0/100 membrane. (July 1935.)

0·755	3·65	4·83	1·935	15·85	8·19	3·007	37·4	12·44	3·939	65·2	16·55
1·206	6·6	5·47									

$c_s = 0{\cdot}005\%$.

$(\Pi/c)_0 = 2{\cdot}1$; $M = 118{,}000$ (410 h.u.).

(VI) Lichenin acetate (B) in $CHCl_3$; 0/100 membrane. (April 1936.)

1·002	10·1	10·08	2·088	28·7	13·75	0·749	7·05	9·41	0·448	3·6	8·04
3·960	84·5	21·34									

(VII) Methylated lichenin (direct methylation) in $CHCl_3$; 0/100 membrane.

1·017	12·8	12·59	2·329	46·5	19·97	3·044	75·8	24·90	0·668	7·1	10·63
1·662	26·4	15·88									

$(\Pi/c)_0 = 7{\cdot}5$; $M = 33{,}100$ (162 h.u.).

Inulin (Fig. 2).—Inulin acetate in $CHCl_3$.

(I) 0/100 Membrane.

1·086	24·4	22·47	0·666	14·0	21·02	3·993	113·7	30·98

$c_s = 0{\cdot}079\%$.

(II) 20/80 Membrane.

0·696	15·0	21·55	1·369	33·4	24·40

$c_s = 0{\cdot}034\%$.

(III) 50/50 Membrane.

0·940	26·7	28·40	1·936	60·0	30·99	3·370	116·2	34·48	4·524	170·0	37·58
9·456	438·8	46·40									

$c_s = 0{\cdot}021\%$.

(IV) 70/30 Membrane.

1·354 42·2 31·17 6·151 265·0 43·08

$(\Pi/c)_0 = 28·0$; $M = 8860$ (30·8 h.u.).

Methylated Inulin in Chloroform.—(V) 50/50 Membrane.

1·011 40·3 39·86 4·049 205·6 50·78 7·340 466·0 63·49 0·880 34·0 38·64

$c_s = 0·028\%$.

(VI) 70/30 Membrane.

1·278 56·8 44·44 6·047 376·4 62·25 2·462 117·0 47·52

$c_s = 0·009\%$.

$(\Pi/c)_0 = 40·0$; $M = 6200$ (30·4 h.u.).

Glycogen (Fig. 3).—Methylated glycogen from rabbit liver.

I. In CCl_4; 0/100 membrane.

Series (a) :

8·82 7·8 0·884 5·60 3·7 0·661

$c_s = 0·001\%$.

Series (b) :

16·83 28·2 1·676 10·80 11·1 1·028

$c_s = 0·004\%$.

$(\Pi/c)_0 = 0·40$; $M = 620,000$ (3000 h.u. .

(II) In nitromethane; 0/100 membrane.

8·98 8·3 0·924 4·45 2·6 0·584 15·56 29·0 1·864 6·14 4·1 0·668
8·72 8·5 0·975

$(\Pi/c)_0 = 0·40$; $M = 620,000$ (3000 h.u.).

(III) In $CHCl_3$; 10% NaOH membrane.

2·033 0·68 0·334 3·250 1·19 0·366 5·637 2·71 0·481 6·127 3·37 0·550
8·606 6·90 0·802 15·01 36·6 2·44

$c_s = 0·006\%$.

$(\Pi/c)_0 = 0·30$; $M = 830,000$ (4100 h.u.).

Methylated glycogen from fish liver in $CHCl_3$; 0/100 membrane.

(IV) Dogfish.

14·83 35·0 2·36 5·98 4·85 0·81 2·55 1·4 0·55 4·55 3·2 0·70
7·69 8·0 1·04

$c_s = 0·009\%$.

$(\Pi/c)_0 = 0·40$; $M = 620,000$ (3000 h.u).

(V) Haddock.

10·55 21·1 2·00 6·53 6·0 0·92 3·96 2·6 0·66 2·43 1·3 0·53

$(\Pi/c)_0 = 0·40$; $M = 620,000$ (3000 h.u.).

(VI) Hake fish.

15·53 143·0 9·21 12·31 63·3 5·14 9·85 34·6 3·51 7·85 20·2 2·57
4·69 7·6 1·62 2·95 3·9 1·32 1·78 1·9 1·07

$(\Pi/c)_0 = 0·91$; $M = 273,000$ (1340 h.u.).

Liver Glycogen Acetates in Chloroform.—0/100 Membrane.

Dogfish.

16·75 5·6 0·334 9·24 1·35 0·146 5·84 0·6 0·103

$(\Pi/c)_0 = 0·07$; $M = 3,500,000$ (12,000 h.u.).

Haddock.

8·94 4·0 0·447 5·32 1·65 0·310

$(\Pi/c)_0 = 0·19$; $M = 1,300,000$ (4500 h.u.).

Hake fish.

13·95	6·8	0·487	8·2	2·1	0·256	5·1	1·0	0·196	3·13	0·5	0·160

$(\Pi/c)_0 = 0·13$; $M = 1{,}900{,}000$ (6600 h.u.).

Rabbit.

14·70	6·2	0·422	11·31	3·2	0·283	8·89	1·9	0·214

$(\Pi/c)_0 = 0·1$; $M = 2{,}500{,}000$ (8700 h.u.).

Starch (Fig. 4).—Methylated potato starch A; 0/100 membrane.

(I) In CCl_4:

1·46	4·1	2·81	5·75	30·5	5·30	10·81	138·5	12·81	5·80	35·2	6·07
3·01	11·2	3·72									

$c_s = 0·000\%$.

$(\Pi/c)_0 = 2·0$; $M = 124{,}000$ (610 h.u.).

(II) In $CHCl_3$:

1·87	8·0	4·28	3·30	24·3	7·36	1·20	3·9	3·25	2·75	16·6	6·04
1·78	7·3	4·10	0·68	1·9	2·79	5·60	79·0	14·11			

$(\Pi/c)_0 = 2·0$; $M = 124{,}000$ (610 h.u.).

(III) Methylated potato starch B in $CHCl_3$; 0/100 membrane.

1·504	8·3	5·52	2·018	12·8	6·34	2·630	20·0	7·60	6·530	141·5	21·67

$c_s = 0·004\%$.

0·803	3·5	4·36									

$(\Pi/c)_0 = 3·5$; $M = 71{,}000$ (350 h.u.).

(IV) Methylated " soluble " starch in CCl_4; 0/100 membrane.

1·836	17·3	9·42	1·155	10·6	9·18	4·615	49·3	10·68	6·939	79·8	11·50
3·152	31·0	9·84	8·420	104·7	12·43						

$c_s = 0·000\%$.

$(\Pi/c)_0 = 8·8$; $M = 28{,}200$ (138 h.u.).

(V) Methylated maize starch in $CHCl_3$; 0/100 membrane.

1·332	11·2	8·41	1·909	17·6	9·22	2·444	25·6	10·47	0·650	4·7	7·23

$c_s = 0·016\%$.

$(\Pi/c)_0 = 6·5$; $M = 38{,}200$ (187 h.u.).

Starch Dextrins.—Methylated starch dextrin C in CCl_4; 0/100 membrane (Fig. 4, Curve VI).

1·99	16·9	8·49	1·00	7·9	7·90	3·96	40·7	10·28	1·92	16·7	8·70
7·82	118·3	15·13									

$(\Pi/c)_0 = 7·5$; $M = 33{,}100$ (162 h.u.).

Methylated dextrin A in CCl_4 (Fig. 5).

(I) 0/100 Membrane.

1·679	79·3	47·23	7·938	438·0	55·18

$c_s = 0·019\%$.

(II) 50/50 Membrane.

Series (a):

1·591	89·5	56·25	7·778	522·0	67·11

$c_s = 0·008\%$.

Series (b):

2·326	135·1	58·08

Series (c):

7·018	451·3	64·31	3·993	241·6	60·51

$c_s = 0·002\%$.

$(\Pi/c)_0 = 55·0$; $M = 4510$ (22·1 h.u.).

Methylated dextrin B in CCl_4 (Fig. 5).

(III) 0/100 Membrane (curve not drawn).

0·856	57·6*	67·29	4·060	316	77·8	1·700	106·0	62·35	0·754	40·5	53·71
,,	52·6†	61·45									

$c_s = 0{\cdot}044\%$.

* Solution stirred. † Solution stagnant.

(IV) 50/50 Membrane.

3·868	372	96·17	1·710	153·3	89·77	0·743	65	87·48	1·747	156	89·30
2·620	243	92·75									

$c_s = 0{\cdot}003\%$.

$(\Pi/c)_0 = 85{\cdot}5$; $M = 2900$ (14·2 h.u.).

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