XLV.—*The Determination of the Molecular Weights of the Carbohydrates.*

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BUT very little is known with certainty, at the present time, about the true molecular composition of the majority of the carbohydrates. The vapour-density method is obviously inapplicable to them, and chemists have had, for the most part, to be satisfied with a determination of the *minimum* size of their molecules, from a study of their somewhat indefinite metallic derivatives, and of their acetyl-compounds.

Musculus and Meyer (*Bull. Soc. Chim.* [2], 35, 370) have attempted to determine the relative size of the molecules by observing the rate of diffusion of the different members of the group, but, although the results are of interest, they do not go far in determining the particular point we have before us.

In the case of *dextrose* and *levulose*, there is a considerable amount of positive evidence that their molecular composition is expressed by the formula $C_6H_{12}O_6$. This is shown by their intimate relation to the hexahydric alcohol *mannitol*, and by the recent researches of Kiliani on their cyanhydrins, which yield hydroxy-acids containing seven atoms of carbon.

With regard to the higher carbohydrates, the knowledge we possess as to their molecular weight is entirely indirect. In the case of *starch* and the *dextrins*, we are quite sure, from the way in which they break up under the action of diastase and dilute acid, that their molecular structure must be very complex, and we are able to learn something about the *relative* size of the molecules, but that is all.

Within the last few weeks, Victor Meyer (*Ber.*, 1888, 556) and Auwers (*Ber.*, 1888, 860) have directed attention to a new method for determining molecular weights, which is applicable to all organic substances, and is of special value where a determination of vapour-density is impossible. This method was devised by Raoult (*Ann. Chim. Phys.* [5], 28, 133, 1883; *id.* [6], 2, 66—124, 1884; *id.* [6], 4, 401, 1885; *id.* [6], 8, 289 and 317, 1886; *Compt. rend.*, 94, 1517, 1882; 101, 1056, 1885; 102, 1307, 1886), and is the outcome of his elaborate investigation into the laws governing the freezing point of dilute solutions, and it is certainly a remarkable fact that these important researches should, up to recently, have received so small a share of attention from chemists.

Blagden, as early as 1788 (*Phil. Trans.*, **78**, 277), established the fact, with regard to inorganic salts, that the lowering of the freezing point of their aqueous solutions is proportional to the weight of substance dissolved in a constant weight of water. De Coppet (*Ann. Chim. Phys.* [4], **23**, 25—26), in 1871–72, clearly pointed out that when this lowering of the freezing point is worked out for a determinate quantity of the substance dissolved in 100 grams of water, the result, which he terms the “*coefficient of depression*,” is constant for the same substance, and that the coefficients for different substances bear a simple relation to their molecular weights. It was left for Raoult to show clearly what those relations are, and to extend the investigation to organic substances, and to other solvents besides water.

Briefly stated, Raoult's generalisations are as follow :—When certain quantities of the same substance are successively dissolved in a solvent on which it has no chemical action, there is a progressive lowering of the point of congelation of the solution, and this lowering is *proportional to the weight of the substance dissolved in a constant weight of the solvent*; in other words, the lowering of congelation is dependent solely on the respective masses of the substance and solvent, and is independent of the temperature.

If the observed depression of the point of congelation of a solution be taken as C, and the weight in grams of the anhydrous substance in 100 grams of the solvent as P, then, when the substance exists in solution in the anhydrous state, the quotient $\frac{C}{P}$, which we will represent by A, and which Raoult terms “gross coefficient of depression” (*coefficient d'abaissement brut*), is the lowering of congelation produced by 1 gram of substance in 100 grams of solvent. If we multiply this coefficient, $\frac{C}{P} = A$, by the molecular weight of the body dissolved, we obtain the depression which would be produced if 1 mol.* of the substance were dissolved in 100 grams of the solvent. This is the “true molecular depression” (*abaissement moléculaire vrai*) which is represented by T.

We have then—

$$T = \frac{C}{P} \times M.$$

This formula Raoult finds to be sensibly correct, even for substances which do not exist in solution in the anhydrous form, provided we take solutions so dilute that the observed lowering of congelation C is about 1° C.

* By “1 mol. of substance” dissolved in 100 grams of water, Raoult means of course a weight of the substance in grams equal to its molecular weight.

T is a quantity varying with the nature of the solvent, *but with the same solvent remaining constant for numerous groups of compounds*, and it may consequently be considered as a known quantity. On the other hand, the *coefficient* of depression $\frac{C}{P}$ or A can be obtained experimentally, so that if the molecular weight M of the substance is unknown, it can be calculated by the formula—

$$M = \frac{T}{A},$$

and from amongst the possible molecular weights of the substance under experiment, we take that which approaches nearest to $\frac{T}{A}$.

In carrying out this method, any liquid may be used as a solvent, provided it is capable of solidifying at a definite temperature. It suffices merely to know the value of T for that particular solvent, and for certain groups of bodies analogous to the one under experiment. The solvents which Raoult recommends in his latest paper (*Ann. Chim. Phys.* [6], 8, 317, 1886) are water, acetic acid, and benzene.

For *inorganic salts*, the values of T for water have been found by Raoult to be six in number, corresponding to certain well-defined groups of salts: with benzene, the values of T are reduced to two, and for acetic acid T has a constant value for all inorganic compounds. With *organic* compounds, with but very few exceptions, the respective values of T for the solvents mentioned above remain constant; they are as follows:—

	T.
Water	19
Acetic acid	39
Benzene	49

Raoult has examined a large number of organic substances, the molecular weights of which have been put beyond doubt by determinations of their vapour-densities, and the results illustrate in a remarkable manner the accuracy and general application of his method. In determining the value of T for water, Raoult has recorded in his tables numbers which he obtained for cane-sugar, invert-sugar, milk-sugar, and mannitol, and which indicate that the commonly received formulæ for these substances express their molecular composition, but he does not call any special attention to these experiments, nor does he appear to have examined any other of the carbohydrates. It seemed therefore a matter of interest, in the present state of our knowledge of this important group of substances, to submit its various members to a method which promises, in the

near future, to throw much light on the molecular size of non-volatile organic compounds.

Owing to the comparative ease with which most of the carbohydrates, with the exception of dextrose and levulose, are hydrolysed, the employment of acetic acid as a solvent was impracticable, and our choice was necessarily limited to that of water. With the exception of oxalic acid and the amines, all the bodies examined by Raoult in aqueous solution give normal results.

The method of experiment we have adopted is extremely simple, and is essentially the same as that described by Auwers (*Ber.*, 1888, 712), with the exception that when working with aqueous solutions, it is not necessary to take any precautions to exclude the air. A solution of the carbohydrate is prepared, containing a known weight of the substance in 100 c.c. of the liquid. About 120 c.c. of this are introduced into a thin beaker of about 400 c.c. capacity, closed with an india-rubber plug with three perforations, through one of which a small glass stirrer passes, and through the second a thermometer graduated to $\frac{1}{20}$ th of a degree C. This is viewed through a telescope, and since the scale is an open one, there is no difficulty in taking readings to $\frac{1}{200}$ th of a degree. The beaker is immersed in a mixture of ice and brine at a temperature from 2° to 3° below the freezing point of the solution, which is allowed to fall in temperature from 0.5° to 1° below its point of congelation; this it will readily do without the formation of ice. Freezing is now brought about by dropping into the beaker, through the third aperture in the plug, a very small fragment of ice from a little of the same solution which has been previously frozen in a test-tube. The liquid is stirred briskly, and as freezing commences the thermometer rises very rapidly, and, in a few seconds becomes stationary at the true freezing point of the solution, the concentration being always so arranged that the observed depression is never more than 1° to 2° below zero.

If we take

- C = observed depression of freezing point,
 x = grams of substance in 100 c.c. of solution,
 y = grams of water in 100 c.c. of solution,

then the "coefficient of depression" is expressed by the equation

$$\frac{C \times y}{x \times 100} = \frac{C}{P} = A,$$

and the molecular weight M of the substance by

$$M = \frac{19}{A}.$$

As the molecular composition of dextrose may be looked upon as fairly well established, it will be well, in the first place, to show how far Raoult's method, when applied to this substance, bears out the generally accepted formula $C_6H_{12}O_6$.

In all the experiments which follow,

Column E gives the observed temperature.

„ C „ the depression of freezing point, *corrected*.*

„ A „ the "coefficient of depression."

„ M „ the molecular weight deduced from experiment.

Dextrose, $C_6H_{12}O_6$. M = 180.

Freezing point of water used $0^{\circ}000$.

Strength of solution, 12.616 grams dextrose in 92.25 grams water.

E.	C.	A.	M.
-1.450°	1.450°	0.106	179
-1.450	1.450	0.106	179
-1.450	1.450	0.106	179

Strength of solution, 8.3704 grams dextrose in 94.86 grams water.

E.	C.	A.	M.
-0.945°	0.945°	0.107	177
-0.940	0.940	0.106	179
-0.940	0.940	0.106	179

Strength of solution, 4.1140 grams dextrose in 97.47 grams water.

E.	C.	A.	M.
-0.445°	0.445°	0.103	184
-0.445	0.445	0.103	184
-0.440	0.440	0.104	182

Calculated for $C_6H_{12}O_6$.		Found (Mean).	
A =	0.106	A =	0.1052
M =	180.0	M =	180.2

These experiments with dextrose show very clearly the concordant nature of the results obtained by this method, even when the solutions vary considerably in density.

If dextrose had been a previously unknown substance, whose relations and derivatives had not been studied, and for which only

* The correction here applied is the difference between the observed freezing point of the solution and the freezing point of water determined in the same apparatus, and under exactly similar conditions.

the empirical formula CH_2O had been determined by combustion, we should have had no hesitation in selecting from among the possible molecular weights, 30, 60, 90, 120, 150, 180, 210, &c., the one which corresponds most nearly with the observed value of $M = 180$, and with the formula $\text{C}_6\text{H}_{12}\text{O}_6$.

Dextrose exhibits in a pre-eminent degree the phenomenon of *birotation*; the action on polarised light of a freshly-prepared solution of crystallised dextrose being double that of the same solution after standing for some hours. The phenomenon of *birotation* has never received any physical explanation, and as it seemed to us possible that it might be in some way intimately connected with the size of the molecule in solution, we submitted to Raoult's method freshly prepared solutions in which the amount of *birotary* carbohydrate was concurrently estimated by the polariscope.

Freezing point of water used, $+0.025^\circ$.

Strength of solution, 10.013 grams dextrose in 93.85 grams water.

E.	C.	A.	M.
-1.115°	1.140°	0.106	180

$[\alpha]_j$ at time of experiment $105.6^\circ = 72.6$ per cent. *birotary dextrose*.

E.	C.	A.	M.
-1.115°	1.140°	0.106	180

$[\alpha]_j$ at time of second experiment $97.2^\circ = 58.6$ per cent. *birotary dextrose*.

Calculated for $\text{C}_6\text{H}_{12}\text{O}_6$.		Found.	
A =	0.106	A =	0.106
M =	180.0	M =	180.0

It is clear from the above, that whatever may be the cause of *birotation* it is certainly not to be attributed to a condensation of the molecule.

Cane-sugar. $\text{C}_{12}\text{H}_{22}\text{O}_{11}$. $M = 342$.

Freezing point of water used, 0.000 .

Strength of solution, 13.052 grams in 91.98 grams water.

E.	C.	A.	M.
-0.825°	0.825°	0.059	322
-0.835	0.835	0.058	328
-0.835	0.835	0.058	328

Strength of solution, 10·1410 grams sugar in 93·77 grams water.

E.	C.	A.	M.
—0·600°	0·600°	0·055	345
—0·600	0·600	0·055	345
—0·600	0·600	0·055	345

Strength of solution, 8·2580 grams sugar in 94·93 grams water.

E.	C.	A.	M.
—0·500°	0·500°	0·057	333
—0·490	0·490	0·056	340
—0·490	0·490	0·056	340

Strength of solution, 6·064 grams sugar in 96·28 grams water.

E.	C.	A.	M.
—0·355°	0·355°	0·056	340
—0·355	0·355	0·056	340
—0·350	0·350	0·055	345

Calculated for $C_{12}H_{22}O_{11}$.		Found.	
A =	0·0555	A =	0·0562
M =	342·0	M =	337·5

The lowest possible empirical formula, $C_{12}H_{22}O_{11}$, evidently represents the molecule of cane-sugar in solution, and our results consequently do not bear out a suggestion which Winter appears to make in his recent paper on levulose (*Annalen*, **244**, 1888, 308) that the molecule of cane-sugar is more complex than this.

Inverted Cane-sugar.

Since the molecular weight of cane-sugar is 342, and that of dextrose 180, it seemed almost certain that Raoult's method applied to invert-sugar would yield a value for M of 180, and that the value of A would consequently be approximately doubled during the process of inversion.

The experiment was made by determining the depression of the freezing point of the *same solution* of cane-sugar, both before and after inversion, which was brought about by the addition of a little *invertase*.*

* Invertase is readily prepared by triturating fresh solid yeast with fine sand, digesting the pasty mass with water at the ordinary temperature for a few hours, filtering, and precipitating the filtrate with alcohol of about 80 per cent. The precipitate is well washed with alcohol, dehydrated with absolute alcohol, and dried over sulphuric acid in a vacuum. As thus prepared, *invertase* is a white, friable substance, completely soluble in water, and readily inverting several hundred times its own weight of cane-sugar.

The results were as follows :—

Cane-sugar Solution before Inversion.

Freezing point of water, $+0.025^{\circ}$.

Strength of solution, 4.9818 grams sugar in 96.94 grams water.

E.	C.	A.	M.
-0.270°	0.295°	0.058	328
-0.270	0.295	0.058	328
-0.275	0.300	0.058	328

Calculated for $C_{12}H_{22}O_{11}$.		Found (mean).	
A =	0.0555	A =	0.058
M =	342.0	M =	328.0

The above solution was completely inverted with 0.030 gram of invertase, the volume being maintained constant; it gave

After Inversion.

Freezing point of water $+0.025^{\circ}$.

Strength of solution, 5.344 grams sugars in 96.72 grams water.

E.	C.	A.	M.
-0.585°	0.610°	0.110	173
-0.580	0.605	0.109	175
-0.580	0.605	0.109	175

Calculated for $C_6H_{12}O_6$.		Found (mean).	
A =	0.106	A =	0.1093
M =	180.0	M =	174.3

This experiment may be taken as a proof that the value of M for levulose, like that for dextrose, is 180.

Maltose. $C_{12}H_{22}O_{11}$. M = 342.

That this is a saccharose having the same elementary percentage composition as cane-sugar there can be but little doubt, but whether these two compounds are metameric or polymeric is still open to question. The metallic derivatives and acetyl compounds of maltose tend to show that the simplest possible formula, $C_{12}H_{22}O_{11}$, also expresses its molecular composition, but Herzfeld has recently questioned this (*Annalen*, **220**, 1883, 220) and is inclined, from his

experiments on the behaviour of maltose towards Fehling's solution, to assign to it a molecular formula at least three times as large. To put this matter to the test of direct experiment, the following determinations were made by Raoult's method:—

Freezing point of water, $+0.030$.

Strength of solution, 15.785 grams maltose in 90.40 grams water.

E.	C.	A.	M.
-1.010°	1.040°	0.059	322
-1.000	1.030	0.059	322
-1.005	1.035	0.059	322

Strength of solution, 10.499 grams maltose in 93.68 grams water.

E.	C.	A.	M.
-0.635°	0.665°	0.059	322
-0.635	0.665	0.059	322
-0.635	0.665	0.059	322

Strength of solution, 5.124 grams maltose in 96.89 grams water.

E.	C.	A.	M.
-0.285°	0.315°	0.059	322
-0.285	0.315	0.059	322
-0.285	0.315	0.059	322

Calculated for $C_{12}H_{22}O_{11}$.		Found (mean).	
A =	0.0555	A =	0.059
M =	342.0	M =	322.0

These experiments prove beyond doubt that the molecules of cane-sugar and maltose, when in solution, are of equal size, and that these substances are therefore metameric, not polymeric. We must consequently attribute the difference in their properties to the different arrangement of atoms in the molecule.

Milk-sugar, $C_{12}H_{22}O_{11}$. M = 342.

Freezing point of water, $+0.030^{\circ}$.

Strength of solution, 10.263 grams in 93.59 grams water.

E.	C.	A.	M.
-0.580°	0.610°	0.055	345
-0.585	0.615	0.055	345
-0.585	0.615	0.055	345

Calculated for $C_{12}H_{22}O_{11}$.		Found.	
A =	0.0555	A =	0.055
M =	342.0	M =	345.0

These results indicate that the usually accepted formula for milk-sugar expresses its molecular composition, and that the suggestion made by Herzfeld (*Annalen*, **220**, 1883, 222) that the true formula is a multiple of $C_{12}H_{22}O_{11}$, is not correct, but that the three sugars of the saccharose group, cane-sugar, maltose, and milk-sugar, have the same molecular weight.

Arabinose, $C_5H_{10}O_5$. $M = 150$.

This sugar, a product of the action of dilute acid on gum-arabic, was formerly considered to have the formula $C_6H_{12}O_6$. Recent researches of Kiliani (*Ber.*, **20**, 1887, 343 and 2710) have, however, established the fact that it is a compound with only 5 atoms of carbon, and that it is represented by the formula $C_5H_{10}O_5$.

We have submitted this substance to Raoult's method, with the following results, having first convinced ourselves of its purity by a determination of its optical activity :—

Freezing point of water, $+0.030^\circ$.

Strength of solution, 4.1355 grams in 97.45 grams water.

E.	C.	A.	M.
-0.510°	0.540°	0.127	149.6
-0.505	0.535	0.126	150.7
-0.505	0.535	0.126	150.7

Calculated for $C_5H_{10}O_5$.		Found (mean).	
A =	0.126	A =	0.1263
M =	150.0	M =	150.3

These results fully confirm Kiliani's conclusions.

Raffinose.

This sugar, which occurs in the molasses of beet-sugar, and in the seeds of various plants, has been the subject of considerable investigation. It crystallises in well-defined needles or prisms, and from analyses the formula $C_{18}H_{32}O_{16} \cdot 5H_2O$, or a multiple of this, has been deduced, preference being given to a molecule represented by double this formula.

We are indebted to Dr. Griess for a specimen of pure crystalline raffinose, as well as that of the pure arabinose mentioned above.

The substance had an optical activity of $[\alpha]_j$ 116.6°, or $[\alpha]_D$ 104.6, which agrees exactly with the numbers given by Tollens for hydrated raffinose.

Freezing point of water, $+0.030^\circ$.

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Strength of solution, 8.2225 grams hydrated substance in 94.51 grams water.

E.	C.	A.	M.
-0.280°	0.310°	0.0356	533
-0.280	0.310	0.0356	533
-0.290	0.320	0.0367	518

Calculated for $C_{18}H_{32}O_{16}, 5H_2O$.		Found (mean).	
A =	0.032	A =	0.036
M =	594.0	M =	528.0

Our results indicate that the molecule of raffinose approximates to the above formula, and that it is not a multiple of this.

Mannitol, $C_6H_{14}O_6$. M = 182.

Although this substance is not strictly included in the ordinarily accepted definition of a carbohydrate, yet its relations to the group are of so intimate a nature, and the size and constitution of its molecule have been so accurately determined by its reactions, that we have considered it well, as a further proof of the accuracy of Raoult's method when applied to compounds of this class, to include the results of its examination.

Freezing point of water, +0.030°.

Strength of solution, 7.5382 grams in 95.09 grams water.

E.	C.	A.	M.
-0.805°	0.835°	0.105	181
-0.805	0.835	0.105	181
-0.805	0.835	0.105	181

Calculated for $C_6H_{14}O_6$.		Found.	
A =	0.104	A =	0.105
M =	182.0	M =	181.0

The application of this new method to starch, and to the non-crystallisable products of its transformation, soluble starch, the dextrins and malto-dextrin, seemed full of promise, since chemists are still divided in their opinions as to the true nature of these compounds, and as to whether the differences in the properties of the dextrins are such as to justify the view that they are polymeric, or, on the other hand, compounds having the same molecular weight, but differing in constitution.

Certain difficulties have, however, arisen at this stage of our inquiry, owing to the very high molecular weight which these substances evidently possess. As a result of this, the freezing point of even very strong solutions is depressed to such a small extent as to render it necessary, before we can assign any approximately accurate numerical value to our results, to determine the limits of error of the method, which manifestly increase with the molecular weight of the substance. We have, however, convinced ourselves that the molecular complexity of these compounds is very great indeed, and we hope to lay certain results before the Society at an early date.