THE PREPARATION OF TRIACETYL STARCH AND ITS MOLECULAR WEIGHT.

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Although it is quite necessary, in the study of starch, to get the solution of starch or of its derivatives, yet our knowledge of this field is far from being complete. The author has given a convenient method of preparing acetyl derivative of starch yielding a large quantity of it at a time, has examined its solubility in various solvents, and has estimated its molecular weight in several media by the cryoscopic and the ebullioscopic methods.

The Acetylation of Starch.

Acetylation of starch has already been carried out in various ways,⁽¹⁾ among which there are fairly available methods such as that with acetic anhydride and pyridine⁽²⁾ and that with acetic anhydride and sulphuric acid,⁽³⁾ but still they either take a long time (a few days or more) or are apt to give more or less depolymerised products. It is readily understood that starch cannot be expected to undergo mild acetylation without difficulty in the absence of suitable dispersing medium either for the starch or for the reaction product as in the case of the usual acetylating methods.

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(1) See M. Samec, "Kolloidchemie der Stärke," (1927), p. 436.

(2) M. Bergmann and E. Knehe, *Ann.*, 452 (1927), 141.

(3) F. Pregl. *Monatsh.* 22 (1901), 1049; H. Pringsheim and K. Wolfsohn, *Ber.*, 57 (1924), 887.

The author has succeeded in preparing acetyl starch quite easily, overcoming the above difficulty in the following way:

Fourty grams of starch(1) (air-dry, containing 7% of water) are mixed up with a saturated aqueous solution of zinc chloride (50 gr. of anhydrous salt in 23 gr. of water) to a *transparent sticky paste*, heating on the water bath, and 220 c.c. of acetic anhydride are gradually added, a part of which is at first converted by the water into acetic acid, which plays an important rôle as a nice solvent for the acetyl starch which is to be produced. As water often acts with acetic anhydride violently the reaction mixture is carefully warmed (sometimes it must be cooled) with constant stirring on the water bath, the temperature not far exceeding 70°. After the water is acted the gelatinized starch goes into solution quite markedly, the acetylation comes to the end within half an hour. Small lumps of unchanged starch are left, which can no more be attacked on further addition of acetic anhydride. This is centrifuged away and the somewhat turbid, viscous solution, after evaporating off some acetic acid, is poured in ice water. The acetylated product separates out as a flocky precipitate, while zinc chloride and others dissolve in the water. By repeatedly rubbing with fresh water the solid is reduced to a fine powder, which is nearly free from impurity. Yield 60 gr. of the well-dried substance, that is 90% of the theoretical amount.

For the purification the dried acetyl starch was dissolved in hot chloroform and alcohol was added until a turbidity appeared. When allowed to cool, the acetyl starch separated out as a white fine powder, which was filtered and washed with alcohol. Although the pure chloroform solution was left, crystals did not appear, but a thin film like that of collodium was formed on the wall of the vessel.

This acetylated starch is different in several points (temperature of decomposition, solubility, specific rotation, etc.) from any of those already described(2); when heated in a capillary tube, it sinters at 258° and slowly decomposes over 270°. It dissolves in acetic acid, chloroform (free from alcohol), pyridine, acetic anhydride, conc. sulphuric acid (remaining without a slight decomposition for 20 minutes), ethylene bromide, bromoform, and phenol, far more readily at higher temperatures. The solution is clear when dilute but more or less turbid when over 2–5 percent. It is tolerably

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(1) "Starch soluble Merck" is used. It is never "soluble" and swells up with hot water just like the potato starch washed with dilute alkali and dilute acid. The specific rotation (190°) and the content of ash (0.18%) is also the same.

(2) F. Pregl; M. Bergmann and E. Knehe, loc. cit. in which detailed descriptions on its properties, especially on its solubility are deficient.

soluble in acetone, acetic ether, and toluene, less in benzene and carbon tetrachloride, far less in petroleum ether, ether, and alcohols, and almost insoluble in water. In hot toluene it is fairly soluble; a beautiful fluorescence is observed when its solution is allowed to cool.

For the analysis the purified acetyl starch was again dissolved in a mixture of toluene and acetone on the water bath, precipitated with alcohol, and dried at 110° in vacuum over phosphorus pentoxide.

0.1930 gr. of subst. gave 0.3513 gr. CO_2 and 0.0988 gr. H_2O. (Found: $C=49.64$; $H=5.73$. $C_6H_7O_5(C_2H_3O)_3$ requires $C=49.98$; $H=5.60\%$)

The acetyl value was determined by saponifying the substance with a known amount of 1/4 n-NaOH, in warm acetone solution,⁽¹⁾ the excess of alkali being titrated with 1/10 n-HCl, using phenolphthalein as the indicator.

0.1660 and 0.2000 gr. of subst. required 6.93 and 8.37 c.c. of 1/4 n-NaOH respectively. (Found: $CH_3CO=44.8$; 44.9. $C_6H_7O_5(CH_3CO)_3$ requires $CH_3CO=44.8\%$)

Optical study was made in readily dissolving solvents:

$$[\alpha]_D^{28}=\frac{2.86°\times 100}{1.680\times 1}=170.2° \text{ (in chloroform)},$$

$$[\alpha]_D^{28}=\frac{2.72°\times 100}{1.695\times 1}=160.4° \text{ (in acetic acid)},$$

$$[\alpha]_D^{14}=\frac{2.35°\times 100}{1.471\times 1}=159.6° \text{ (in pyridine)}.$$

The Saponified Product of the Triacetyl Starch. Saponification was carried out most easily in the following way: dried triacetyl starch (2 gr.) was dissolved in hot toluene (40 c.c.) and sodium ethylate (0.6 gr. Na in 15 c.c. $C_2H_5\,OH$) was added. Reaction occurred immediately, the vessel being filled with a gelatinous product, which was dissolved on addition of water. The excess of alkali was neutralized with acetic acid. The liquid separated into two layers, namely, a layer of turbid aqueous solution and that of clear toluene, which was separated. The former solution was filtered, evaporated at 40° in vacuum to a small volume (35 c.c.) and poured in methyl alcohol (250 c.c.). The saponified product separated out as a white precipitate, which was filtered and washed with alcohol. It was again dissolved in water and precipitated with alcohol.

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(1) In fact, the substance was first dissolved in a small amount of boiling toluene, then acetone (50 c.c.) was added and warmed on the water bath.

It is an amorphous white somewhat hygroscopic powder, forming a clear solution with water when dilute, but an opaque solution when concentrated. It gives a pure indigo-blue colour with iodine and does not reduce Fehling's solution. For analysis it was dried at 110° and 1 mm. over phosphorus pentoxide.

0.2122 gr. of subst. gave 0.3430 gr. CO_2 and 0.1203 gr. H_2O. (Found: C=44.08; H=6.34. $C_6H_{10}O_5$ requires C=44.42; H=6.22%)

$$[\alpha]_D^{21}=\frac{1.98^\circ\times100}{1.046\times1}=189.3^\circ \text{ (in water).}$$

The original starch used for the acetylation has the specific rotation:

$$[\alpha]_D^{27}=\frac{1.76^\circ\times100}{0.926\times1}=190.0^\circ \text{ (in water).}$$

Though the saponified product of the acetyl starch is identical with the original starch in the specific rotation and the colouration with iodine, they differ in the following respect: the former dissolves in water, while the latter swells up with hot water, forming the paste.

These two starches cannot, however, be regarded as essentially different, because according to Samec the fraction of starch containing phosphate (amylopectin) forms the paste, while that free from phosphate (amylose) dissolves in water without forming the paste, thus the cause of forming the paste may be attributed to the component containing phosphate(1); and indeed, when the phosphate is removed, the amylopectin gives a homogenous, not opaque solution(2) and on the other hand, when phosphoric acid is introduced into the amylopectin which is deprived of its phosphate, there is formed a product similar to the original amylopectin.(3) Now, the present saponified product of the triacetyl starch has been found to contain no phosphate, so it is quite natural that it should be more soluble than the original starch containing phosphate. That the soluble saponified product may be essentially " soluble starch " is shown by the fact that its clear solution yields a light cloudy precipitate when left a few days, as is usually the case with the clear solution of amylose.

Finally, it must be mentioned that it was confirmed that during the process of acetylation the starch remains unchanged (in the specific rotation, the colouration with iodine, the behaviour towards water etc.) before the acetyl group is introduced, even under the treatments of gelatinization with zinc chloride solution and heating in acetic acid.

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(1) M. Samec and v. Hoefft, *Kolloidchem. Beih.*, 5 (1913), 141.

(2) M. Samec, *Kolloidchem. Beih.*, 6 (1914), 23.

(3) M. Samec and A. Mayer, *Comp. rend.*, 173 (1921), 321.

From these facts it may be said that the starch is not changed (essentially) by the treatments of the acetylation and the saponification.

The Reacetylation of the Saponified Product. Acetyl group was introduced again into the saponified product of the triacetyl starch by the same method, the acetylated product being purified as before. When heated in a capillary tube it sinters at 257° and gradually decomposes over 270°. The specific rotation is also the same as before:

$$[\alpha]_D^{28} = \frac{2.90° \times 100}{1.698 \times 1} = 170.6° \text{ (in chloroform)},$$

$$[\alpha]_D^{28} = \frac{2.86° \times 100}{1.785 \times 1} = 160.2° \text{ (in acetic acid)}.$$

It is concluded, therefore, that the saponified product of the acetyl starch can be brought back to the original acetyl starch by introducing the acetyl group without causing a change.

The Molecular Weight of the Triacetyl Starch.

Since Karrer published an idea that starch may be a polymerised product of a relatively small molecule such as maltose anhydride,(1) other polysaccharides naturally have come to be believed as similarly composed of such small elementary molecules. This idea was directly demonstrated with inulin by the cryoscopic study in liquid ammonia,(2) and with various polysaccharides further direct confirmations of this idea were brought forward also by the cryoscopic investigations of their acetyl derivatives. The conclusions are striking: The triacetyl derivative of cellulose,(3) lichenin(3)(8), inulin(4)(8), glycogen(5), salep-mannan,(6) amylose(8) in *acetic acid*, and that of amylopectin(8), amylose(7) in *phenol*, all may be considered quite unexpectedly to exist as a single molecule of triacetyl hexosan (mol. wt. 288).

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(1) P. Karrer and C. Nägeli, *Helv. Chim. Acta*, 4 (1921), 263; P. Karrer, *Naturw.*, 9 (1921), 399; P. Karrer, *Z. angew. Chem.*, 35 (1922), 85.

(2) L. Schmid and B. Becker, *Ber.*, 58 (1925), 1968; Reichlen and Nestle, *Ber.*, 59 (1926), 1159. From these experiments inulin is considered to exist as such a small molecule as fructose-disaccharide anhydride, but it was recently reported by L. Schmid and G. Bilowitzki (*Monatsch.*, 48 (1927), 163) that no elevation of the boiling point of piperidine is observed upon the addition of inulin.

(3) K. Hess and Schultze, *Ann.*, 448 (1926), 99.

(4) K. Hess and Stahn, *Ann.*, 455 (1927), 104.

(5) K. Hess and Stahn, *Ann.*, 455 (1927), 115.

(6) H. Pringsheim and G. Liss, *Ann.*, 460 (1928), 32.

(7) M. Bergmann and E. Knehe, *Ann.*, 452 (1927), 141.

(8) M. Bergmann, E. Knehe and v. Lippmann, *Ann.*, 458 (1927), 93.

Of these experiments it must, however, be remarked that the depressions of the freezing point giving such a small molecular weight, could be observed only at low concentrations (ca. 0.05—0.5%) and only when observations were carried out not immediately but several hours or several days after dissolving the substance. The investigators explain this phenomenon as due to the disassociation of the large molecule of polysaccharides, which proceeds quite slowly and only in dilute solutions.

Expecting to obtain similar results also in other media which are neutral and indifferent to the substance, the author has made cryoscopic studies of the triacetyl starch in ethylene bromide, bromoform and camphor, and also in acetic acid and phenol, and ebullioscopic studies in chloroform, acetone, and acetic acid. The result is that, in almost all solvents, the depressions of freezing point or the elevations of boiling point were exceedingly small at high or low concentrations. As a comparative study, the author has observed also the freezing point depression of the solvent caused by the acetyl derivative of some simple carbohydrates as glucose and saccharose, the results of which have confirmed that the triacetyl starch exists all the same as a huge, polymerised molecule compared with such simple carbohydrates, even in dilute solutions.

The Cryoscopy. The freezing vessel is so constructed that measurements may be carried out in high vacuum. The stirrer is made of platinum wire, to the top of which a ring of tin-plate is attached, and moved up and down by means of an electro-magnet.(1) The results of the cryoscopic studies of the triacetyl starch using ethylene bromide, bromoform, camphor, and acetic acid as solvent, are given in the following tables.

1. *Ethylene bromide.* Freezing point 9.5°. K=120.(2) It has proved to be an excellent cryoscopic solvent for the acetyl starch, owing to the fairly large dissolving power, the large cryoscopic constant and the ease of purification. It was purified by shaking with a soda solution, drying with calcium chloride, and distilling two times in the space shut off from the moist air. Boiling point 131.2°. After introducing the solvent or the substance, the freezing vessel was evacuated so as to expel the moist air. Evacuation has also the benefit of hastening the dissolving of the substance.

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(1) Ostwald-Luther, "Physiko-chemische Messungen," (1925), p. 332.

(2) The mean value of several observers'. See P. Walden, "Molekulargrössen von Elektrolyten in nichtwässerigen Lösungsmitteln," (1923), p. 208.

Triacetyl starch (gr.)	Ethylene bromide (gr.)	Conc. (%)	Depression (degree)	Mol. wt.	Remarks.	
					subst. dissolved at:	observed after: (hour)
0.0148	17.6	0.084	0.004	(2600)		1
0.0148	17.6	0.084	0.003	(3400)		3
0.0305	23.5	0.130	−0.005		60°	3
0.0305	23.5	0.130	0.000			21
0.0266	17.6	0.151	0.004	(4500)	80°	0.5
0.0718	20.8	0.345	0.010	4100	70°	4.5
0.0718	20.8	0.345	0.010	4100		22
0.0748	17.6	0.425	0.010	5100		1
0.1524	17.6	0.865	0.017	6200		3
0.174	19.4	0.897	0.019	5600		20
0.159	17.6	0.903	0.021	5100		1
0.181	19.3	0.938	0.021	5350	60°	6
0.181	19.3	0.938	0.022	5100		26
0.114	17.6	0.648	0.010	8050		0.3
0.463	17.6	2.63	0.051	6400		3
0.463	19.8	2.34	0.057	4900		3.5
0.463	19.8	2.34	0.048	5850		22
β-Pentaacetyl glucose (mol. wt. 390)						
0.151	17.6	0.859	0.251	410		
0.187	17.6	1.062	0.311	410		
0.189	19.8	0.954	0.272	420		
Octaacetyl saccharose (mol. wt. 678)						
0.055	17.6	0.313	0.063	600		
0.073	16.0	0.456	0.085	643		
0.163	17.6	0.926	0.170	652		
0.314	19.8	1.58	0.281	677		
0.489	19.8	2.48	0.422	702		

2. *Bromoform.* Freezing point 7°. K=144.

Triacetyl starch (gr.)	Bromoform (gr.)	Conc. (%)	Depression (degree)	Mol. wt.
0.120	19.4	0.620	0.017	5020
0.144	22.3	0.646	0.020	4650
0.321	22.3	1.44	0.041	5050
β-Pentaacetyl glucose (mol. wt. 390)				
0.148	19.5	0.759	0.276	408
Octaacetyl saccharose (mol. wt. 678)				
0.157	19.4	0.809	0.174	671

3. *Camphor*. Melting point 178°. K=400.

Triacetyl starch (gr.)	Camphor (gr.)	Conc. (%)	Depression (degree)	Mol. wt.
0.1140	0.671	17.0	3.5	1940
0.0674	0.400	16.5	3.1	2120
0.0316	0.553	5.70	1.5	1520

In spite of high concentrations the molecular weight has come out considerably small, about one-third of that found in other solvents. This is probably due to the disassociation of the large molecule caused by the high temperature.

4. *Acetic acid*. Freezing point 16°. K=39. Measurements were carried out in high vacuum, as Hess(1) and Bergmann(2) have recommended in the cryoscopy with the polysaccharide acetate in this solvent, in order to avoid irregular depressions which would occur otherwise.

Triacetyl starch (gr.)	Acetic acid (gr.)	Conc. (%)	Depression (degree)	Mol. wt.	Remarks. observed after: (hour)
0.0110	26.2	0.042	0.007	230	19
0.0110	26.2	0.042	0.010	160	72
0.0400	15.7	0.254	0.120	82	20
0.0400	15.7	0.254	−0.090		72
0.0870	21.0	0.414	0.080	195	21
0.0870	21.0	0.414	−0.050		72
0.0987	22.6	0.436	0.009	1900	1
0.0480	7.40	0.648	0.012	2100	0.5
0.284	24.1	1.13	0.072	610	22
0.284	24.1	1.13	0.008	5600	72
0.100	7.10	1.48	0.030	1910	0.5
β-Pentaacetyl glucose (mol. wt. 390)					
0.0747	7.40	1.09	0.100	393	

It is seen in the table that soon after dissolving the substance, depressions were quite small, then became larger and after more hours became again smaller and in some cases even elevations were observed. The pure

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(1) *Ann.*, **448** (1926), 99.

(2) *Ann.*, **458** (1927), 93.

solvent itself being sometimes found to change its freezing point with the time,(1) the author dare not attribute these changes of depression to the disassociation of the acetyl starch or to the reassociation of the elementary molecules thus disassociated.

5. *Phenol.* Freezing point 40°. K=79. Phenol is more difficult to manipulate than acetic acid; the freezing temperature even of pure phenol fell quite irregularly by 0.02° or more at every freezing with all precautions,(2) but no particular depression was observed due to the addition of a small amount (0.3%) of the triacetyl starch.

From the above studies it may be clearly pointed out that such solvents which are not indifferent but sensitive to moisture and air as acetic acid and phenol, quite unlike the neutral solvents, being very liable to cause too large (improper) depressions, are regarded to be no adequate cryoscopic solvent, especially for higher polysaccharides because depressions caused by the addition of them may be considerably small in any case.

The Ebullioscopy. The results of the ebullioscopic studies of the triacetyl starch using acetic acid and chloroform as solvent are given in the following tables. These solvents, being somewhat difficult to manipulate, and elevations of boiling point being quite small, the values of molecular weight are not very reliable, but it is clearly shown that the triacetyl starch has a molecular weight far larger than the (β-pentaacetyl) glucose.

1. *Acetic acid.* Boiling point 118°. K=25.

Triacetyl starch (gr.)	Acetic acid. (cc.)	Conc. (%)	Elevation (degree)	Mol. wt.
0.911	19.5	4.67	0.016	7100
0.911	22.0	4.14	0.017	6100
0.911	23.0	3.96	0.020	5000
β-Pentaacetyl glucose (mol. wt. 390)				
0.690	11.5	6.00	0.330	455
0.690	17.5	3.94	0.240	416
0.690	19.8	3.52	0.241	368

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(1) Therefore, in the above determinations the freezing point was also observed of the pure solvent in parallel with the solutions.

(2) Purified phenol with a definite boiling point was frozen in high vacuum.

2. *Chloroform.* Boiling point 61.2°. K=26.

Triacetyl starch (gr.)	Chloroform (cc.)	Conc. (%)	Elevation degree)	Mol. wt.
0.732	17	4.30	0.017	6900
0.732	1	3.48	0.014	6500
0.732	25	2.93	0.011	6900
0.732	26	2.81	0.013	5600
0.117	10	1.17	0.005	6100

3. *Acetone.* Boiling point 56°. K=22. No measurable elevation of the boiling point was observed on the addition of the triacetyl starch.

The results of the above cryoscopic and ebullioscopic investigations give a conclusion that *the triacetyl starch possesses, at least in some neutral solvents, a molecular weight as large as or more that of 18 times polymerised anhydro-glucose triacetate*⁽¹⁾ $[\{C_6H_7O_5(C_2H_3O)_3\}_{18}=288\times18=5184]$ *and does not seem to be disassociated to the elementary molecules merely by diluting or by leaving the solution,* as other investigators⁽²⁾ have observed by doing so.

However, in further more dilute solutions the complete disassociation may well take place, but that can no longer be confirmed with the present cryoscopic method.

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(1) This result is strongly in favour of A. Pictet's estimation that soluble starch (least polymerised) should be tri-hexahexosan (18 times polymerised anhydro-glucose) considering from the specific rotation of some lower polyhexosans. *Helv. Chim. Acta.*, 9 (1926), 33.

(2) K. Hess; M. Bergmann, loc. cit.