IV. THE COMPOSITION OF STARCH. PART I. PRECIPITATION BY COLLOIDAL IRON. PART II. PRECIPITATION BY IODINE AND ELECTROLYTES.

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THE experiments recorded in this paper deal with the effects produced by (a) colloidal iron and (b) iodine on a solution of potato starch in water. The starch solution was obtained by adding starch suspended in a small volume of cold water to the required volume of boiling water and continuing the boiling for one minute.

I. THE PRECIPITATION OF STARCH BY COLLOIDAL IRON.

There are considerable differences of opinion regarding the nature of starch granules, but, generally speaking, starch is now regarded as consisting of two substances. Nägeli who investigated the action of hydrochloric acid and amylolytic enzymes on starch proposed the names amylogranulose and amylocellulose to designate them. Amylogranulose, the chief constituent of starch, goes into solution under the action of hydrolytic agents and gives a blue colour with iodine. Amylocellulose on the other hand does not go into solution under these conditions and is not coloured blue by iodine. This work has been extended by Maquenne and Roux [1903, 1905] and Fernbach and Wolff [1904]. From their experimental results they deduce that starch granules consist of two substances, (a) amylose and (b) amylopecten. Amylose, the chief constituent of starch, is insoluble in cold water but completely soluble in water boiled under pressure. On cooling such a solution the soluble amylose tends to revert to the insoluble form. Amylopecten, on the other hand, is of an entirely different nature. It swells up without dissolving when heated with water and is not coloured blue with iodine.

The results recorded in the following pages indicate that the soluble portion of starch (amylogranulose) may be further differentiated into a series of fractions.

(i) *Precipitation by colloidal iron only.*

The investigations of Bottazzi and Victoroff [1910] on the electrical state of starch in solution indicate that starch is electrically neutral. This conclusion is not supported by the effects observed when colloidal iron is added

to a solution of starch. Under these conditions three well marked phases may be recognised: (i) a portion of the starch is precipitated by the colloidal iron only, (ii) a second portion of the starch is carried down with the colloidal iron when an electrolyte is added to the solution, and (iii) the filtrate from (ii) contains starch which is not precipitated by colloidal iron either in the presence or absence of electrolytes.

The amount of starch precipitated by colloidal iron only is shown in the following experiment.

Colloidal iron was added to 1 % starch in the following proportions:

Colloidal iron	Starch 1 %	H ₂ O
4 cc.	20 cc.	16 cc.
8 cc.	20 cc.	12 cc.

A gelatinous precipitate was produced in each case. This precipitate was allowed to settle, after which the amount of starch remaining in the clear fluid, and the amount of starch in the original solution, were determined. In both cases it was found that 80 % of the original starch was precipitated by the colloidal iron. The amount of starch precipitated was independent of the amount of iron added. The lower limit, giving the minimal amount of iron necessary for precipitation, was not determined. Precipitation presumably depends upon the neutralisation of the negative charge on the starch by the positive charge on the colloidal iron. It became of interest therefore to determine how far the precipitation limits of colloidal iron by electrolytes were influenced by the addition of a solution of starch to it.

The minimal quantity of potassium sulphate required to precipitate 1 cc. of colloidal iron is given in the following figures.

Colloidal iron	H ₂ O	<i>M</i> /10 K ₂ SO ₄	
1 cc.	8.95 cc.	0.05 cc.	No precipitation
	8.9	0.1	"
	8.8	0.2	"
	8.6	0.4	"
	8.5	0.5	Complete precipitation

With 0.4 cc. *M*/10 K₂SO₄ there was no formation of distinct granules, but there was an obvious change in the appearance of the solution viewed by partially reflected light.

The same experiment was repeated except that 5 cc. of starch replaced 5 cc. of added water, thus:

Colloidal iron	Starch 1 %	H ₂ O	<i>M</i> /10 K ₂ SO ₄	
1 cc.	5 cc.	4 cc.	0.0 cc.	No precipitation
		3.95	0.05	"
		3.9	0.1	"
		3.8	0.2	"
		3.6	0.4	Partial precipitation
		3.5	0.5	Complete
				"

In the first four tubes there was a precipitate of starch, but this precipitate was greatly augmented by precipitated iron in the fifth tube. In the sixth tube

complete precipitation of the iron had been produced. The partial precipitation of the colloidal iron in the presence of starch by 0.4 cc. K_2SO_4 ($M/10$) compared with the amount 0.5 cc. K_2SO_4 ($M/10$) required when no starch was present indicates that the starch had diminished to a small degree the quantity of electrolyte required for precipitation. The results show that the electric charge on starch in solution is negative, though small in comparison with the electropositive charge on colloidal iron.

(ii) *The precipitation of starch by colloidal iron in the presence of electrolytes.*

The amount of starch precipitated from solution by colloidal iron is increased by the presence of electrolytes. But however much colloidal iron may be used, and however much precipitating electrolyte may be added, some starch remains in the filtrate. The amount of starch *not* precipitated by colloidal iron in the presence of electrolytes was determined.

To 100 cc. of approximately 1 % starch, 20 cc. of colloidal iron were added. Five minutes later 1.2 cc. of K_2SO_4 (M) were added as the precipitating electrolyte. The resultant filtrate was water clear in appearance but gave a deep blue colour on the addition of iodine. The quantities of starch in the original solution and filtrate were determined by hydrolysis to dextrose. The results showed that 89 % of starch was precipitated whilst 11 % remained in solution. Now a previous experiment had shown that on the addition of colloidal iron only to the starch solution 80 % of the starch was precipitated. We must therefore conclude that 9 % of the original starch is carried down by colloidal iron precipitated by electrolytes. The results show that three types of starch must be recognised as present in a solution of potato starch in water: (α) starch precipitated from solution by colloidal iron only; (β) starch taken down with colloidal iron when precipitated by a divalent negative ion; (γ) starch not affected by colloidal iron in any way. The results given above show that a solution of potato starch contains 80 % of (α), 9 % of (β), and 11 % of (γ). It may be remarked that the first fraction (α) includes the insoluble constituent of starch (amylocellulose) since this insoluble constituent would be mechanically carried down by the soluble starch precipitated by the colloidal iron. On this assumption the starch granule consists of amylocellulose and amylogranulose (α), (β) and (γ), the terms (α), (β) and (γ) designating those varieties which are precipitated by (α) colloidal iron only, (β) colloidal iron in the presence of electrolytes, and (γ) not precipitated by colloidal iron.

This, however, does not exhaust the possibilities of the substances contained in the starch granule, since precipitation by iodine in the presence of electrolytes, the amount of iodine added being just short of that required for complete precipitation of the starch, gives a filtrate which is coloured brown by iodine. This colour is due to the presence of dextrin in the starch solution. The presence of dextrin might be due to its production when the starch is dissolved in boiling water, but against this hypothesis is the fact that glycogen dissolved in cold water gives similar results. The complete precipitation of

starch by equivalent quantities of iodine in the presence of electrolytes shows that the granule contains no *soluble* substances which do not react with iodine. It appears reasonable to conclude that the starch granule contains a large number of polymers of the general formula $(C_6H_{10}O_5)_n$ increasing in complexity from dextrin to cellulose, the dextrin and cellulose being present in small quantities whilst amylogranulose (α), (β) and (γ) form the bulk of the granule. Probably other methods of precipitation would differentiate the amylogranulose (α) into a series of fractions.

II. THE PRECIPITATION OF STARCH BY IODINE AND ELECTROLYTES.

When iodine dissolved in potassium iodide is added to a solution of starch in water two characteristic changes may be observed: (i) the solution assumes a deep blue colour, and (ii) the lyophilic emulsoïd colloid of starch is changed into a suspensoïd colloid of marked lyophobic character.

A considerable amount of experimental work has been done to determine the nature of the changes involved. Owing to the varying content of "starch iodide" the chemical aspect of the problem has given place to views in which the physical element predominates. Küster [1894] put forward the hypothesis that iodide of starch consists of a solid solution of iodine in starch, and explained in this way the varying content of the iodine in starch iodide with the iodine concentration of the solution with which it was in equilibrium. Harrison [1911] on the other hand considered that in the case of iodine and starch an adsorption compound was formed. The most recent work on this subject has been done by Barger and Field [1912] in their analyses of the phenomena observed in the formation of blue adsorption compounds of iodine with various substances. From their experimental work they conclude that on the addition of iodine to starch there is a considerable adsorption of iodine, that the presence of electrolytes (potassium iodide) is necessary to bring about this adsorption, and that the blue substances formed behave as negative suspension colloids which have been rendered lyophobic by the iodine adsorbed.

The electronegative nature of starch iodide was shown by the experiments of Padoa and Savaré [1906] in which starch iodide moved to the anode when placed in an electric field. The experiments of Barger and Field on the precipitation of starch iodide by cations of inorganic salts confirmed this deduction as to the electronegative nature of starch iodide and demonstrated that the addition of iodine to starch changed a lyophilic emulsoïd colloid (the starch solution) to a lyophobic suspensoïd colloid (starch iodide). The facts that starch dissolved in water forms an emulsoïd colloid and carries a small negative charge, that the reacting iodine dissolved in potassium iodide is presumably ionised and negatively charged, and that the resulting starch iodide forms a suspensoïd colloid and is also negatively charged, militate against the acceptance of a purely physical hypothesis for the explanation of those changes which occur when iodine is added to starch. The experiments recorded in the

following pages on the effects observed when iodine dissolved in potassium iodide is added to a solution of starch indicate that the iodine first reacts quantitatively with the starch producing a definite chemical compound and that the starch iodide thus formed is a lyophobic suspensoid colloid which adsorbs iodine from solution according to the recognised laws of adsorption.

(A) *The quantitative relation of iodine to starch.*

The following experiments indicate that on the addition of small quantities of iodine to starch a reaction takes place in accordance with the recognised laws of chemical action.

(i) *Varying iodine.* Varying quantities of iodine were added to 10 cc. of a 1 % solution of starch contained in a series of tubes, and the resulting starch iodide was precipitated from solution by a small quantity of magnesium sulphate. After an interval of five minutes the contents of the tubes were filtered and the filtrates were tested for excess of iodine or starch. The following figures show the results obtained in a typical experiment:

Starch 1 %	Iodine 1 %	H ₂ O	MgSO ₄ (M)	Filtrate
10 cc.	0.3 cc.	9.6 cc.	0.1 cc.	Excess of starch
10	0.4	9.5	0.1	" "
10	0.5	9.4	0.1	" "
10	0.6	9.3	0.1	" "
10	0.7	9.2	0.1	Nil
10	0.8	9.1	0.1	Excess of iodine
10	0.9	9.0	0.1	" "

An experiment done under these conditions shows that when iodine is added to starch, and the lyophobic starch iodide is precipitated from solution by a divalent ion, a point is reached at which the filtrate contains neither starch nor iodine. Previous to this point the filtrate contains an excess of starch, and after this point the filtrate contains an excess of iodine. It is difficult to postulate an hypothesis which would explain these results on the basis of physical adsorption. The occurrence of a definite point at which the filtrate contains neither starch nor iodine suggests that iodine reacts chemically with the starch, and that at this point there is a definite chemical equivalence of the starch and iodine. This deduction is strengthened by the experimental observations that the equivalent point is not affected by dilution, temperature, or precipitating electrolyte.

(ii) *Varying concentration of starch.* In the following experiment the quantity of starch, contained in a final volume of 20 cc., was varied from 10 cc. of a 1 % solution to 10 cc. of a 0.2 % solution. The same quantity of precipitating electrolyte (MgSO₄) was added in each case and the quantity of iodine required to produce a filtrate containing neither starch nor iodine determined:

1 % starch	H ₂ O	I 0.1 %	MgSO ₄ (M)	Filtrate
10 cc.	5.5 cc.	4.75 cc.	0.2 cc.	Excess iodine
10	5.75	4.5	0.2	No iodine

0.8 % starch	H ₂ O	0.1 % I	MgSO ₄ (M)	Filtrate
10 cc.	6.2 cc.	3.8 cc.	0.2 cc.	Excess iodine
10	6.4	3.6	0.2	No iodine
0.6 % starch	H ₂ O	1 0.1 %	MgSO ₄ (M)	Filtrate
10 cc.	7.2 cc.	2.8 cc.	0.2 cc.	Excess iodine
10	7.4	2.6	0.2	No iodine
0.4 % starch	H ₂ O	1 0.1 %	MgSO ₄ (M)	Filtrate
10 cc.	8.2 cc.	1.8 cc.	0.2 cc.	Excess iodine
10	8.4	1.6	0.2	No iodine
0.2 % starch	H ₂ O	1 0.1 %	MgSO ₄ (M)	Filtrate
10 cc.	9.0 cc.	1.0 cc.	0.2 cc.	Excess iodine
10	9.2	0.8	0.2	No iodine

The mean values obtained from the above results are as follows:

% starch	Cc. of iodine required	Iodine/starch
10 cc. of 1 %	4.625	4.6
10 cc. of 0.8 %	3.7	4.6
10 cc. of 0.6	2.7	4.5
10 cc. of 0.4	1.7	4.25
10 cc. of 0.2	0.9	4.5

The direct relation between the amount of starch and the quantity of iodine required to precipitate it in the presence of an electrolyte again indicates the chemical equivalence of the starch and iodine.

Similar experiments were made in which the precipitating electrolyte was varied from 0.2 cc. MgSO₄ (M/10) to 0.2 cc. MgSO₄ (M). There was no change in the point at which the filtrate contained neither starch nor iodine. Similarly varying the temperature at which the experiment was done from 15° to 40° did not influence the quantitative values of the reaction.

(iii) *The iodine equivalent of starch.* All these results indicate that when iodine is added to starch a quantitative reaction takes place between the starch and the ionised iodine. The iodine equivalent of starch was determined from an experiment in which the amounts of starch and iodine in the solutions used had been accurately determined.

It was found that when 10 cc. of a starch solution containing 0.087 g. of starch were treated with 0.0068 g. of iodine the filtrate obtained after precipitation by MgSO₄ contained neither starch nor iodine. Therefore, on the above hypothesis of the chemical interaction of starch and iodine,

0.0068 g. of I is equivalent to 0.087 g. of starch,
or 127 g. of I is equivalent to 1635 g. of starch.

Now the sum of the atomic weights of C₆H₁₀O₅ is 162; and the empirical formula for starch has been assumed to be (C₆H₁₀O₅)_n. Therefore the least value of n, assuming that one molecule of starch reacts with one atom of iodine, is 10.

The experimental results obtained on adding colloidal iron to starch show that starch contains a variety of polymers. The value n = 10 can therefore

be taken only as a mean value for a number of starch complexes in which n is continually varying.

(iv) *The iodine equivalent of starch granulose (γ)*. The iodine equivalent of starch granulose (γ) was determined in the way described in the previous experiment. The following results give the details of an experiment:

100 cc. of starch were precipitated by 20 cc. of colloidal iron and 1.2 cc. K_2SO_4 (M). 5 cc. of the resulting filtrate containing starch granulose (γ) were put into a series of tubes to which were added varying quantities of iodine and $MgSO_4$ ($M/10$) to make the volume 10 cc. The filtrates were tested with starch and iodine.

Starch granulose (γ)	H ₂ O	0.1 % I	MgSO ₄ ($M/10$)	Filtrate
5 cc.	1.9 cc.	3.0 cc.	0.1 cc.	Excess of iodine
5	2.9	2.0	0.1	" "
5	3.9	1.0	0.1	" "
5	4.1	0.8	0.1	Nil
5	4.3	0.6	0.1	Excess of starch

The original starch solution gave as equivalent quantities 5 cc. starch and 4 cc. iodine. The amount of starch granulose (γ) contained in the filtrate was 10.6 % of the total starch contained in the original solution.

Therefore the iodine equivalent of starch granulose (γ) compared with that of an equal quantity of the original starch was approximately as two to one. In other words if 1600 g. of the original starch were equivalent to a gram atom of iodine, approximately 800 g. of starch granulose (γ) were equivalent to the same quantity of iodine.

In the case of starch $(C_6H_{10}O_5)_{10}$ is equivalent to I and for starch granulose (γ) $(C_6H_{10}O_5)_5$ is equivalent to I.

(B) *The adsorption of iodine by starch iodide.*

In the foregoing experiments the amount of iodine added to the starch solution before the addition of the precipitating electrolyte was relatively small, and under these circumstances it has been shown that the reaction passes through a definite stage previous to which there is an excess of starch, and after which there is an excess of iodine, in the solution. This result is not compatible with the hypothesis that starch adsorbs iodine *ab initio*. The question however arises whether starch iodide when formed further reacts with an excess of iodine, contained in the solution, in a chemical or physical way. Substances containing a variable quantity of iodine in relation to the starch have been isolated and this fact suggests that a physical process enters into the reaction after the formation of the starch iodide compound. The following experiment illustrates the relation of the iodine contained in the filtrate and that added to the original starch solution when the quantity of added iodine is continuously varied.

Starch 1 %	Iodine 1 %	H ₂ O	MgSO ₄ (M)	Excess of I in filtrate	Iodine precipitated
25 cc.	1.5 cc.	22.5 cc.	1 cc.	(starch in filtrate)	1.5 cc.
25	2.0	22.0	1	0	2.0 (x)
25	2.5	21.5	1	0.15 cc.	2.37
25	5.0	19.0	1	0.89	4.11
25	7.5	16.5	1	2.8	4.7
25	10.0	14.0	1	5.03	4.97
25	12.5	11.5	1	6.9	5.6

The figures show that after the equivalent point (x) at which the filtrate contains neither starch nor iodine, the starch iodide precipitated contains a varying quantity of iodine, the amount of iodine in the "starch iodide" continually increasing as the iodine content of the original mixture increases. In fact the figures show that after the point (x) the precipitated "starch iodide" takes down iodine with it from solution according to the recognised laws of adsorption. Therefore in the action of iodine on starch two separate and distinct phases may be recognised.

(1) The iodine reacts with the starch in a quantitative manner, approximately 1 gram-atom of iodine being equivalent to 1600 g. of starch or 800 g. of starch granulose (γ).

(2) The lyophobic starch iodide thus formed when precipitated by electrolytes adsorbs iodine from solution according to the recognised laws of adsorption provided an amount of iodine is present in excess of that required for the first reaction.

SUMMARY.

1. Precipitation of starch by colloidal iron shows that starch granulose can be separated into three fractions, (α), (β) and (γ), forming 80 %, 9 % and 11 % respectively of the starch granulose. (α) is precipitated by colloidal iron only, (β) by colloidal iron and electrolytes, and (γ) is not precipitated by colloidal iron under any conditions.

2. Precipitation of starch by iodine and electrolytes shows (a) that starch contains an insoluble constituent which does not react with iodine (amylocellulose), (b) that all the soluble constituents of starch are precipitated by iodine in the presence of electrolytes, and (c) that the final fraction precipitated by iodine gives a brown colour with iodine.

3. The results (1) and (2) indicate that starch contains a variety of polymers varying in complexity from amylo-dextrin to amylocellulose, the relative quantities of dextrin and cellulose being small whilst the bulk of the granule is composed of amylogranulose (α).

4. Iodine reacts with starch in a quantitative manner forming starch iodide. Approximately 1600 g. of starch are equivalent to 127 g. of iodine, or $(C_6H_{10}O_5)_{10}$ is equivalent to a gram-atom of iodine. In the case of starch granulose (γ) approximately 800 g. react with 127 g. of iodine; or $(C_6H_{10}O_5)_5$ is equivalent to a gram-atom of iodine.

5. Starch iodide adsorbs iodine from solution so that after the equivalent point the amount of iodine contained in the precipitated starch iodide is a function of the amount of iodine contained in the original solution.

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