COACERVATION OF STARCH

by

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INTRODUCTION

Coacervation is a colloidal phenomenon. In coacervation, a colloidal sol separates into two non-miscible parts, one richer in dispersed colloidal material than the other. The colloid-rich phase is called the coacervate; the colloid-poor phase is called the equilibrium liquid and has a low or negligible content of colloidal material. In a coacervate, the distribution of colloid particles is statistically uniform, as in the original sol, although their concentration has been increased. If the colloid particles are considered to be the dispersed phase, their state has not been changed in the coacervation process; and yet clearly a new phase boundary is formed between a layer rich in colloid and one poor in colloid. Another view of the situation in an aqueous system is that the coacervate can be regarded as a solution of water in the colloid (swelling) and the equilibrium liquid must then represent a solution of a small amount of colloid in water.

Coacervation has been observed in colloidal solutions both of electrolytes and of non-electrolytes. The former have been studied extensively by Bungenberg de Jong (5) and his collaborators, and the latter by a number of workers, notably Dobry (12). Coacervation has also been reported to occur under various types of conditions, including induced change of ionic charge on the dispersed material and the action of desolvation agents (9).

A coacervate may occur as droplets, myelin structures or a layer separated from the equilibrium liquid. The phenomenon was described in detail and named by Bungenberg de Jong and Kruyt (7, 8, 10).

The ability of a starch paste to undergo coacervation has been recognized for some time (18, 19, 22), yet but little attention has been given to the

conditions that bring about the phenomenon. There are, however, practical aspects that deserve consideration. If coacervation should occur in beater sizes, or in soups or sauces thickened with starch, for example, the useful application of the starch would be materially decreased. On the other hand, starch sponge, manufactured for use as a hemostatic agent in surgery, is an advantageous form of coacervated starch (4, 16, 20, 23), and other favorable instances may occur.

The purpose of the broad investigation of which the present study is a part is to define specific conditions required for the coacervation of starch and to develop a clearer understanding of the phenomenon.

REVIEW OF LITERATURE

The name "coacervation" was given by Bungenberg de Jong and Kruyt (8) to the phenomenon of limited solution observed with a large number of colloids and colloid mixtures. A coacervate is thus regarded as a colloidal solution which is immiscible with excess solvent.

Bungenberg de Jong (6) made an extensive series of investigations of instances of coacervation and attributed this phenomenon to the separation of highly solvated colloidal particles out of colloidal solution by the addition of some nonsolvent or precipitant. The original solvation was due to the electrostatic attraction for water of the charged colloidal particles. This idea was widely prevalent till 1940, since the recorded data on coacervation were confined mostly to electrically charged colloidal particles in aqueous solution, e.g., gelatin, gum arabic, starch, etc.

In 1942, Dobry (12) showed that coacervation can take place in non-aqueous media with high molecular weight substances, which are generally

molecular colloids. Thus, in precipitating cellulose acetate or polystyrene out of a good solvent by the addition of a suitable nonsolvent as precipitant, Dobry many times obtained separation of coacervates rather than granular precipitates.

Basu and Bhattacharya (3) also observed that in precipitating polymethyl acrylate out of methyl ethyl ketone by the addition of methyl alcohol a viscous liquidlike mass separated out, which on prolonged standing shrank and left a film of polymeric substance at the bottom of the container. Thus it became evident that an explanation based on the electrical charge of the particles should be revised in order to accommodate within its purview cases involving uncharged particles as well. They also suggested that coacervates consist of aggregates with a large amount of solvent trapped in the chains which are strongly coiled up in a poor solvent. Consequently, if the molecules can be made to precipitate in an extended configuration, no coacervation should take place and a solid product should be obtained.

Coacervation is discussed briefly from a molecular point of view by Bamford and Tompa (2), who suggested that coacervation is a type of phase separation that occurs in colloidal solutions under suitable conditions. The thermodynamic aspects of coacervation in systems of non-electrolytes are completely explained in terms of the current statistical theories of polymer solutions.

Dervichian (11) summarized that a coacervate is a phase and not a compound. He noted a tendency to use the term coacervate as an alternative to the term "complex" or "compound". In fact, the apparent separation of a coacervate is often due to the formation of a "complex" between the different constituents, leading to a diminution of solubility, although some reservations

should be made about the real significance of this "complex". This misuse of the term "coacervate" is as incorrect as if "precipitate" were used to mean "compound". In other words, coacervation is the separation of a colloidal system into two fluid optically isotropic phases. The coacervate is the more concentrated of these two phases and may, in some cases, contain nearly all of the dissolved colloid. The more dilute phase is the equilibrium liquid which may be composed, under optimal conditions, almost entirely of the solvent or solvents.

Conditions under which gelatin may be salted out into two liquid layers at 35°C were studied by McBain and Kellogg (21). The equilibrium governing the amounts and composition of the layers salted out with sodium chloride were found to accord with the requirements of the phase rule for the quaternary system gelatin-sodium chloride-hydrogen ion-water. It is evident from the work of these authors that in this case the term "coagulation", as ordinarily applied to the salting out protein, is definitely a misnomer. This was an instance of coacervation.

Frey-Wyssling (15) stated that, morphologically, coacervation shows many features which have their counterpart in phenomena occurring in cells. In the first place, vacuolization deserves mention. If, in a system consisting of equilibrium liquid and suspended coacervate droplets, the equilibrium is modified, as a result of changes in temperature or composition, in the direction of a further dehydration (heating, addition of more sensitizer), vacuoles appear in the droplets. These vacuoles represent separated equilibrium liquid which has remained inside the coacervate droplets. Probably vacuolization by dehydration is comparable with the formation of vacuoles in the cell, since, in that case too, liquid is being separated from the

plasma colloids.

Doi and Nikuni (13) found that potato starch and corn starch were recovered almost quantitatively in granular form when a suitable amount of gelatin was added to a hot clear dispersed starch solution and the whole was allowed to coagulate. Granulation seemed to begin on gel formation and to be completed within a few days. A large amount of warm water was added to the gelatinous gel and the resulting sol was centrifuged. The precipitated starch "granules" were obtained in almost quantitative yield. This, again, appears to be a case of coacervation which, in this instance, was followed by partial crystallization of the colloidal material as equilibrium liquid was progressively eliminated from the coacervate droplets.

MacMasters, et al. (18) reported that starch, under certain conditions, undergoes coacervation which is essentially a partial dehydration of the colloid. Starch sponge obtained by freezing a paste, sol, or gel, starch precipitated from the sol state by addition of alcohol, and the complex formed by treating starch with chloral hydrate were considered to be examples of coacervated starch. Fractionation of starch by butanol or other alcohols, nitroparaffins, fatty acids, soaps, and chloral hydrate may be initially effected by coacervation of the straight-chain component, amylose, with the fractionating agent. A high degree of orientation of amylopectin and glutinous or waxy starch molecules is evidenced by the strong birefringence of the coacervates of these materials obtained by freezing their pastes. The theory was advanced that starch granules may be formed as coacervate droplets within the plant cells.

The comparatively greater translucency and low degree of birefringence of starch granules observed in corn and wheat starches at early stages of

maturity of the grain lends credence to the view (18). Rhythmic crystallization, observed by others within some coacervates, would possibly explain the microscopic appearance of lamellation of starch granules in the mature plant. In fact, lamellation is then apparent, whereas often none can be observed in granules in the immature seed. Any growth of individual granules would probably result from the coalescence of coacervate droplets.

The work of Evans (14) supports these views. He made some observations on starch granules from corn kernels at different stages of maturity. The outlines of the granules from corn 15 days after silking are round and the granules are much smaller than those from more mature kernels. Evans found that the starch granules from corn increase in size as the kernel becomes more mature and that their outlines change from circular to polygonal. This change in shape is due to the packing of the granules as the kernel fills and becomes hardened. For this to occur, the granules must be more plastic than they are at maturity.

From Evans' studies, it is possible that starch granules may be formed as coacervate droplets within the plant cells and that either the apparent increase in their size is merely the illusion caused by the formation of many relatively much larger droplets after the first stage of starch deposition or is the result of fusion of small coacervate droplets to form larger ones.

MATERIALS AND METHODS

Commercially prepared starch from yellow dent field corn was used for most experiments. A few experiments were made with grain sorghum starch separated in the laboratory without the use of sulphur dioxide.

Method I Coacervation by Freezing

Starch sponges were prepared as follows: Starch samples were weighed and each placed in a 250-ml glass beaker. Sample weights of from 0.1 to 10.0 grams were used. Sufficient distilled water was added to each sample to make a total of 100 grams. The starch-water suspension was then heated in a water bath with gentle stirring by means of a glass rod to keep the starch in suspension until it was gelatinized.

When the suspension became opalescent, a drop was taken for microscopic observation to confirm that all of the starch was completely gelatinized. Heating was continued for thirty minutes after gelatinization was complete; during this period, the boiling water bath was covered. All pastes lost some water during heating which was not replaced as this is a normal loss and was considered to be relatively uniform in amount.

The prepared pastes were transferred to plastic beakers and frozen slowly in the freezing unit of an electric refrigerator at an average temperature of -2° to $-3^{\circ}C$. All pastes were left undisturbed in the freezing unit for at least 24 hours, except for one series from which samples were taken at various time intervals to observe the course of coacervation.

Frozen pastes, upon removal from the freezing unit, were allowed to stand at room temperature until completely thawed. Each was then examined microscopically. A stock solution of I_2KI was diluted to faint straw-color for use in staining the microscopic mounts. This facilitated the positive identification of starch without obscuring details.

Method II Coacervation by Action of Chloral Hydrate

Chloral hydrate, $CCl_3CH(OH)_2$, was used as a desolvating agent in these experiments.

Pastes of starch were made at from 0.1 to 10.0% concentration at the temperature of boiling water. While the paste was still hot, it was placed into a Waring Blendor, at low speed, and blended for 3 minutes in order to break down the starch granule sacs. Then 50 ml. portions of the paste were transferred individually into 250-ml beakers.

To each paste, 0.5 to 20.0 grams of chloral hydrate were added (dissolved in 50 ml. of boiling distilled water). Each paste was stirred with a glass rod, then set in a refrigerator (not freezer part) as soon as possible, to cool the paste rapidly and to help the coacervation to take place within a given period. The pastes then were removed from the refrigerator, and each was examined microscopically. A stock solution of I_2KI was diluted to faint straw-color for use in staining the microscopic mounts. This facilitated the identification of the coacervated droplets more clearly.

Method III Coacervation by Salting-out

Sodium chloride, potassium chloride, sodium carbonate, sodium bicarbonate, sodium sulfate and calcium chloride were used as salting-out agents in these experiments.

Pastes of corn starch were made at from 0.1 to 3.0% concentration, at the temperature of boiling water, and held for 30 minutes or more after the paste reached maximum temperature. A spatula was used for stirring the paste constantly and an effort was made to break the starch granule sacs. Or the

pastes of starch were made by heating to boiling, and while the paste was still hot, it was placed into a Waring Blendor, and blended at low speed for 3 minutes to break down the starch granule sacs completely.

To each 50 ml. aliquot of the paste, 50 ml. of a hot solution of one of the different salts were added. The concentrations used of each of these salts were 1.0M, 0.5M, 0.25M, and 0.125M.

While the salt was added, the paste was stirred with a glass rod. Then the paste was set in the storage part of a refrigerator (not freezer part) for 24 hours or more for coacervate formation. The pastes were then removed from the refrigerator and each was examined microscopically. A stock diluted solution of I_2KI was used in staining the microscopic mounts.

Method IV Coacervation upon Dehydration by Evaporation

This method was simply achieved by heating the paste carefully over a flame, to evaporate some water out of the paste. Then it was set in the storage part of a refrigerator for coacervate formation.

RESULTS AND DISCUSSION

Coacervation of Starch by Freezing

At all concentrations studied, coacervate forms developed during slow freezing. At original concentrations of less than 1.0% starch, coacervate droplets, myelin structures and small sponge pieces were predominant. Examples are shown in Fig. 1. The sheet of material evident there is typical of starch sponge. As the original starch concentration was increased, the spongy forms became more prevalent and thicker, as illustrated in Fig. 2. A frozen paste with original starch concentration of 4.5%, or greater, charac-

teristically became one spongy mass, upon thawing, with small amounts of other coacervate forms in the interstitial liquid.

Ungelatinized starch, similarly frozen and thawed as a control, remained unchanged, with no evidence of coacervation.

Coacervate forms were first found, in a 0.5% starch paste, just as ice crystals were beginning to appear. The first coacervate forms to appear were droplets and myelin structures. At this time, the granule sacs and colloidal starch that are characteristic of starch paste were also present, in the portion that was still liquid. Characteristic sponge forms (sheets and thick strands) made their appearance as freezing progressed further. Thin, smooth sheets and small strands were first evident. Later, the sheets looked thicker and had a rough, pebbly appearance.

All sheets showed some myelin projections (Fig. 3 and 4). This gave rise to the concept that the sheets and strands of the sponge are formed by the addition of more coacervated starch to myelin forms which arise in the early stages of freezing. Such an accretion would be comparable to the coalescence of coacervate droplets to form larger droplets or a layer, a phenomenon that has been widely reported by others to occur in various colloidal systems.

Upon the completion of freezing, practically all starch was in the coacervate form. No colloidal starch or free granule sacs could be found in the thawed material by the method used (1), whatever the initial concentration of the starch in the paste.

Coacervation was reversed by heating the mass of coacervated starch in its surrounding water, or by heating the squeezed sponge in fresh distilled water. The starch paste thus obtained was like the original paste, composed

Figure 1. Myelin structures, droplets and strands of starch sponge formed in 0.2% starch paste by freezing at -2° to -3°C. Magnification 100X.

Figure 2. Continuous sheets of starch sponge formed in 2.5% starch paste by freezing at -2^{o} to -3^{o}C. Magnification 100X.

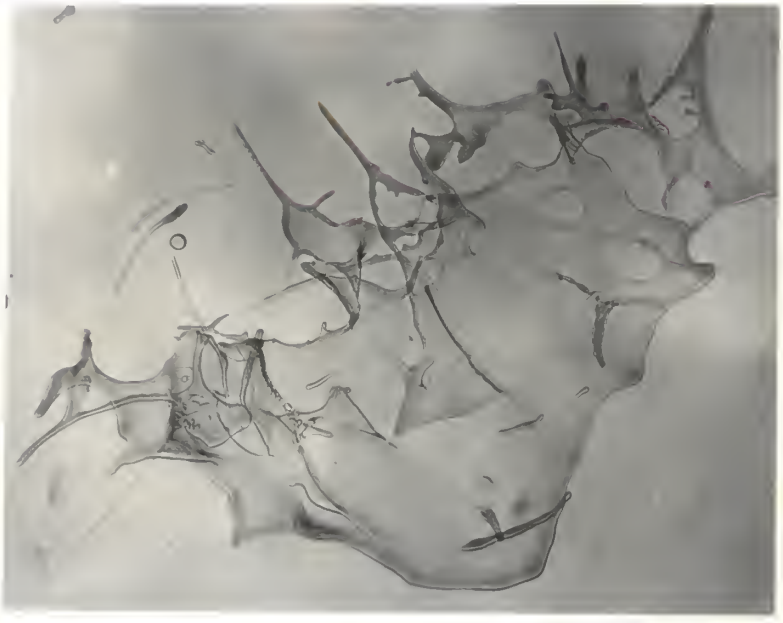

Figure 3. Myelin projections at edges of sheets of starch sponge formed in 0.5% starch paste by freezing at -2° to -3°C. Magnification 100X.

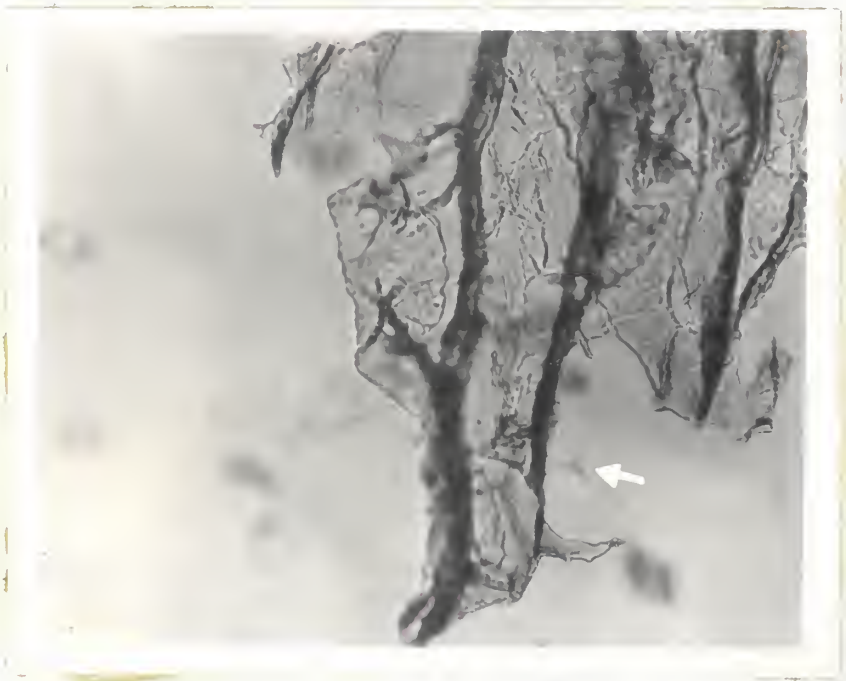

Figure 4. Arrow points to a myelin projection at the edge of a starch sponge formed in 5% starch paste by freezing at -2° to -3°C. Magnification 100X.

of swollen granule sacs and colloidal starch.

Because coacervation begins to occur as soon as freezing has started, it is postulated that this particular type of coacervation is caused by the removal of water, as ice, to yield a critical ratio of starch to water which induces the phenomenon. The process is similar in nature to that brought about by the addition of desolvating agents to a sol. In the latter case, however, the action is rapid throughout the sol, whereas the desolvation effected by ice formation is restricted to portions of the sol at one time and is thus only gradual in its influence on the whole.

Coacervation by Action of Chloral Hydrate

The starch which was used in this experiment was slightly acidified with sulphur dioxide during the corn refining process, so the pH of the paste was around 5.8. Therefore the starch was washed once with very dilute (0.03N) sodium hydroxide and then washed three times with distilled water to remove excess sodium hydroxide and dried. The neutralized washed starch was used for the experiment.

The presence of granule sacs is another factor which might affect the formation of coacervates. Therefore, the paste was blended in a Waring Blendor at low speed for 3 minutes to break the granule sacs.

At all starch concentrations studied, coacervate forms could be obtained by the action of chloral hydrate. In these experiments, chloral hydrate probably acted as a indirect desolvating agent.

It was found that coacervate droplets were formed chiefly within a certain range of concentration of chloral hydrate to starch paste. Examples are shown in Fig. 5, 6, and 7. There were only coacervate droplets; no

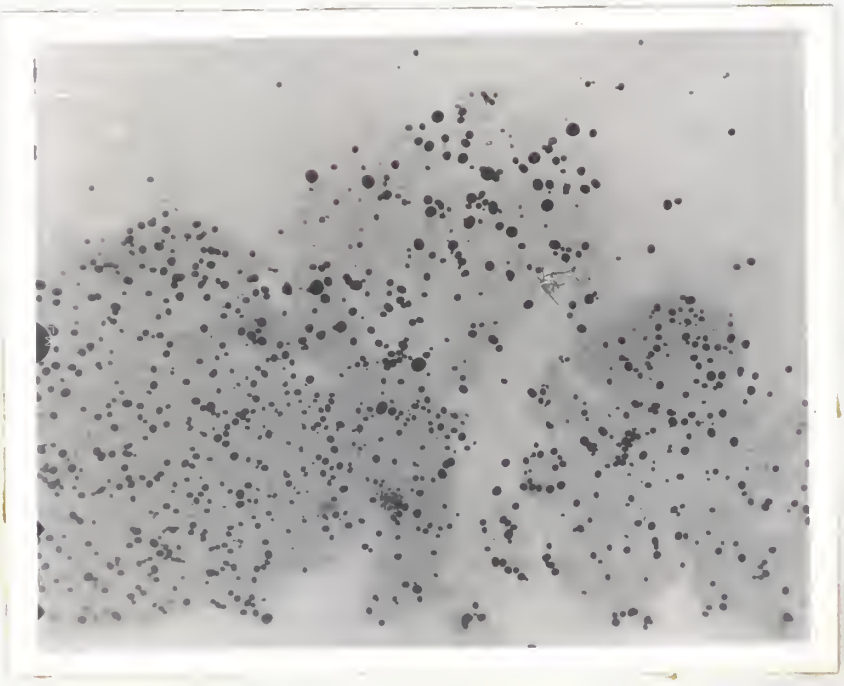

Figure 5. Coacervate droplets formed in 50 ml. of 1% starch paste by the addition of 4 grams of chloral hydrate in 50 ml. aqueous solution at pH 6.5. Stained by I2KI. Magnification 75X.

Figure 6. Coacervate droplets formed in 50 ml. of 1% starch paste by the addition of 2 grams of chloral hydrate in 50 ml. aqueous solution at pH 6.5. Stained by I_2KI. Magnification 475X.

Figure 7. Coacervate droplets formed in 50 ml. of 1% starch paste by the addition of 8 grams of chloral hydrate in 50 ml. aqueous solution at pH 6.5. Stained by I_2KI. Magnification 475X.

myelin structures and sheets were found.

Only when 50 ml. of 1% starch paste at pH 6.5, and 2 to 8 grams of chloral hydrate (50 ml. solution) were combined, were large numbers of coacervate droplets formed. This was confirmed by using a grain sorghum starch, separated by a laboratory method which utilized a water steep, i.e., no sulphur dioxide was used. At the other concentrations of starch and of chloral hydrate used, only a few or no droplets were obtained.

It was found that the higher concentrations of chloral hydrate added to the paste gave only retrogradation and a crystalline amylose complex was formed. At lower concentrations of chloral hydrate added to the pastes, no coacervation took place nor did retrogradation become evident. It is thought that when starch paste is dehydrated too much, so as to induce retrogradation, or when dehydration is not enough, coacervation can not take place. Thus, coacervates were obtained only at intermediate ratios of chloral hydrate to starch paste.

The pH of the mixture of starch paste and chloral hydrate is one of the important factors which determines the formation of a coacervate. For instance, most coacervation took place at pH around 6.5; below pH 6.0 or above pH 7.0, no coacervation took place. This may also explain why, at higher or lower concentrations of chloral hydrate added to the pastes, no coacervate could be obtained. According to Koets (17), amylose in water is weakly, negatively charged. This charge is probably due, in part, to the ionization of OH groups of the constituent glucose molecules. Chloral hydrate in water is apparently dissociated to give H^{+} ions, as it gives an acidic reaction. Therefore, pH determines the effective attraction of the charged particles, and the water content of coacervates must be also a

function of the pH of the medium. So the fact that coacervates can be obtained by using chloral hydrate may be explained by indirect desolvation.

The general explanation of dehydration of colloids advanced by Frey-Wyssling (15) appears to be applicable to coacervation of starch by chloral hydrate. It may, for this special case, be stated as follows: If water is withdrawn from the diffuse solvation layer, the difference between bound and freely moving dipoles becomes noticeable. The water layer around the colloidal starch particle acquires a surface and if two such dehydrated particles meet, the surface energy which tends towards a minimum value will cause the surrounding water layers to unite. The colloidal starch particles, however, can not come into direct contact with each other because of their solvation layers. But they no longer possess separate layers, for these have all united into a liquid sphere. If the number of colloidal starch particles united in this way becomes so large that they form a microscopically visible conglomeration, as flocculates, several of these can further cluster into droplets and finally into a liquid layer. Thus coacervates are liquids rich in colloid which have been separated by means of dehydration. In other words, an agent such as chloral hydrate disturbs the stability of the sol and increases tendency to separate.

Coacervation by Salting-out

Simple coacervation is chiefly the result of direct partial dehydration, even if there is also a very weak indirect desolvation. The partial dehydration of colloid particles can be bought about in various way; in these experiments, salting-out agents were used.

At the beginning of these experiments, negative results were obtained

by use of salts at concentrations of from 0.001 to 0.01M added to a 1% paste for inducing coacervation of starch. It may be that the concentrations of salts were too low. It was thought that it might be possible to obtain coacervation, however, by the use of higher concentrations of salt, similarly to the salting out of protein from aqueous solution in the presence of very high concentrations of neutral salts.

The use potassium chloride or sodium chloride always gave a fine sheet-like material at 0.125 to 1.0M concentration (Fig. 8 and 9). It was at first thought that this sheet-like material was a surface film from the upper surface of the starch paste. But after several tests, it was found that films formed from the paste to which potassium chloride or sodium chloride had been added were different than surface films. No films could be found in the control. Therefore, several controlled experiments were made, and it was found that the sheet-like material was a different morphological aspect of the coacervate form.

By use of sodium carbonate or sodium bicarbonate, very few coacervate droplets and little sheet-like material were found, and these only in 50 ml. of 1% to 3% starch paste to which had been added 50 ml. of 0.25M to 1.00M sodium carbonate or sodium bicarbonate solution. An example is shown in Fig. 10. Under other conditions, these salts failed to cause coacervation; the pH, temperature, concentration, etc., of the mixture of the paste and salt may also be factors influencing coacervation of starch.

When either sodium sulfate or calcium chloride was used under the conditions given, no coacervation was obtained.

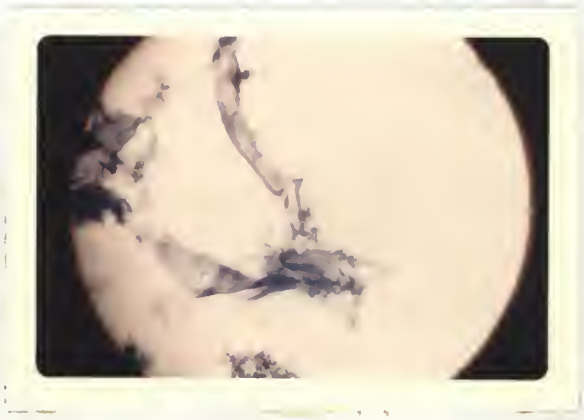

Figure 8. Sheet-like coacervate formed in 50 ml. of 1% starch paste by the addition of 50 ml. of 1M KCl solution. Stained by I_2KI. Magnification 75X.

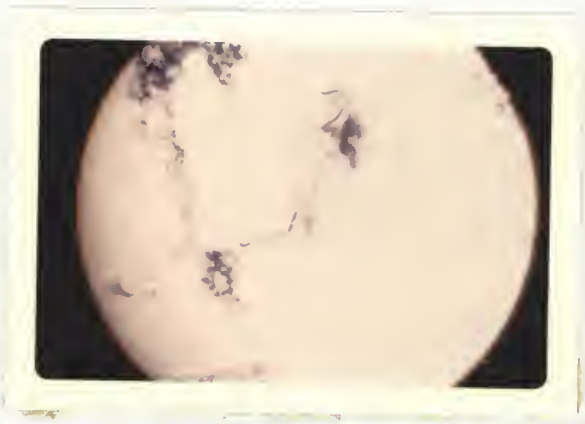

Figure 9. Sheet-like coacervate formed in 50 ml. of 1% starch paste by the addition of 50 ml. of 1M NaCl solution. Stained by I_2KI. Magnification 75X.

Figure 10. Coacervate droplets formed in 50 ml. of 1% starch paste by the addition of 50 ml. of 1M $NaHCO_3$ solution. Stained by I_2KI. Magnification 475X.

Figure 11. Coacervate droplet formed upon evaporation of a 1% starch paste. Stained by I_2KI. Magnification 475X.

Coacervation upon Dehydration by Evaporation

It is interesting that coacervates could be formed by a direct dehydration method. The resulting paste appeared like a gel. After refrigeration, only coacervate droplets were found (Fig. 11); these were very similar to droplets obtained by freezing a paste. Possibly this indicates that a gel can form coacervates, as well as a paste. Or it may be that the coacervate droplets formed during the evaporation.

SUMMARY AND CONCLUSION

By slowly freezing a corn starch paste or gel, of initial starch concentration within the range of 0.1 to 10.0%, coacervation of starch results. Droplets and myelin structures are the most common coacervate forms at the lower of these concentrations. At concentrations of 4.5%, and greater, a continuous mass of sheets and strands, forming starch sponge, with small amounts of droplets and myelin forms in the interstices, is obtained. All, or practically all, of the starch is in the coacervate after freezing and thawing. Granule sacs are apparently held as inclusions in the coacervate. On the basis of experimental observations, the concept has been developed that coacervation by freezing is the result of dehydration of the paste, by ice formation, to the point where a starch-water relationship critical for coacervate formation is obtained.

It was found that coacervation by action of chloral hydrate is simply an indirect desolvation of colloidal starch particles. Droplets were the only coacervate forms occurring in pastes upon addition of chloral hydrate. Retrogradation took place at the higher concentrations of chloral hydrate

added to the pastes and, at the lower concentrations of chloral hydrate added to the pastes, no coacervate was obtained. Thus, coacervation was obtained only at intermediate ratios of chloral hydrate to starch paste, and this only under proper pH conditions. For instance, large numbers of coacervate droplets were formed only when 50 ml. of 1% starch paste at pH 6.5, and 50 ml. of solution containing 2 to 8 grams of chloral hydrate were combined. At pH below 6.0 or above 7.0, no coacervation took place. The pH might determine the effective attractions between the negatively charged colloidal starch particles and the dissociated charges of chloral hydrate, and so induce the indirect desolvation of the system.

A fine sheet-like material, an aspect of the coacervate form, was always found to result from addition of 50 ml. of 0.25 to 1.0M potassium chloride or sodium chloride solution to 50 ml. of 1% starch paste. Also coacervate droplets and sheet-like material were formed by use of 0.25M to 1.0M sodium carbonate or sodium bicarbonate added to 1 to 3% starch paste, under proper conditions. But no coacervate was obtained by use of either sodium sulfate or calcium chloride under the conditions used. Coacervation by salting-out is the result of indirect desolvation of the paste, by electrically charged salts, to neutralize some of the charges on the colloidal particles.

Coacervation by dehydration upon evaporation is the result of direct desolvation of the paste, by removal of water from the system by mechanical evaporation. The result is a critical ratio of starch to water which induces coacervation.

SUGGESTIONS FOR FURTHER RESEARCH

Further study upon the subject of coacervation of starch is needed to gain more knowledge of the structure of boundary layers of starch in the coacervate forms. These studies should also be extended to include the inner structure of coacervate systems. Since, in biological objects, the coacervate has a submicroscopic gel structure, therefore, apart from a knowledge of boundary structure, there is need of deeper insight into the inner structure of colloid particles and coacervate flocculates. In addition to this, the separation and industrial significance of coacervates of starch should be studied. Of especial significance in this respect would be the precise definition of the starch-water ratio at which coacervation takes place during evaporation.

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COACERVATION OF STARCH

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Coacervation is a colloidal phenomenon in which a sol separates into two phases. One, called the coacervate, is rich in the dispersed material, while the other, termed the equilibrium liquid, is usually very poor in dispersed material. The coacervate may occur as droplets or myelin structures which tend to sink in the equilibrium liquid, or it may separate as a continuous layer below the equilibrium liquid.

The concept has been developed that coacervation by freezing results from desolvation of the colloid. Ice formation apparently removes water to the point where a starch-water relationship critical for coacervate formation is obtained. Droplets and myelin structures are the most common coacervate forms at the lower of these concentrations of starch. At concentrations of 4.5%, and greater, a continuous mass of sheets and strands, forming starch sponge, with small amounts of droplets and myelin forms in the interstices, is obtained.

Coacervation by the action of chloral hydrate is simply an indirect desolvation of colloidal starch particles. Coacervate droplets were obtained only at intermediate ratios of chloral hydrate to starch paste, and this only under proper pH conditions. For instance, large numbers of coacervate droplets were formed only when 50 ml. of 1% starch paste at pH 6.5, and 50 ml. of water containing 2 to 8 grams of chloral hydrate were combined.

A different morphological aspect of the coacervate form was obtained by salting-out agents. A fine sheet-like material was always found to result from addition of 50 ml. of 0.25 to 1.0M potassium chloride or sodium chloride solution to 50 ml. of 1% starch paste. Also coacervate droplets and sheet-like material were formed by use of 0.25 to 1.0M sodium carbonate or sodium bicarbonate added to 1 - 3% starch paste, under proper conditions.

No coacervate was obtained by use of either sodium sulfate or calcium chloride under the conditions used. Coacervation by salting out is the result of indirect partial desolvation of the paste, by electrically charged salts.

Coacervation by dehydration upon evaporation is the result of direct dehydration of the paste, by removal of water from the system by mechanical evaporation. The result is a critical ratio of starch to water which induces coacervation.