The Kinetics of Retrogradation of Amylose

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The retrogradation of amylose in solutions was investigated by determination of its concentration by iodometric titration (amperometric indication). It is deduced that the retrogradation is a two-step process: the formation of aggregates in the solution (process analogous to nucleation), and, after the phase has been changed, the further growth of aggregates by addition of molecules to their surface. It was ascertained that potato amylose is practically water insoluble at lower temperatures.

The term retrogradation of amylose refers to the phenomenon of instability of water solutions of amylose, *i.e.* transition of the water soluble amylose to its insoluble form. Retrogradation has been studied by several authors who found out dependences as follows: The rate of retrogradation of amylose depends upon its molecular weight; the higher is the molecular weight, the lower is the rate of retrogradation [1—4]. The retrogradation is not accompanied by the change in conformation of the particular glucose units of amylose and the formation of aggregates is probably due to the intramolecular hydrogen bonds [4, 5]. The rate of retrogradation depends upon the pH of the medium [6]; retrogradation does not proceed in a strong alkaline medium. This fact is rationalized by the polyelectrolytic character of amylose in a strong alkaline medium, where amylose bears a negative charge; as a result, its molecules are repelled [7, 8].

Kinetics of retrogradation in a determined range of concentration of solution was investigated [9] by light scattering method. It was found out that the retrogradation mechanism is in accordance with the *Smoluchowski* [10] coagulation theory. In spite of the effort the retrogradation of amylose has not been definitely cleared.

Our paper deals with the alteration of amylose in solutions during a longer period of time. The amperometric titration with iodine was found to be a convenient tool even for this range where the light scattering method failed.

Experimental

Potato amylose was a product of AVEBE (Holland). It was characterized after crystallization from *n*-butanol by the internal viscosity $[\eta] = 137$ ml/g (1 N-NaOH) and by the sorption of iodine (18.2%), determined amperometrically. Dry basis found 82.1%.

Amperometric titration and the device used

The device consists of two platinum electrodes, an accurate galvanometer and DC source.

The electrodes were made of platinum wire 0.8 mm in diameter, 10 mm long and 40 mm apart. Galvanometer „Multiflex" of maximum accuracy 4×10^{-9} A mm^{-1} was connected with the circuit in usual manner.

The electrodes were supplied with a constant voltage (about 30 mV) from a 1.4 V dry cell through resistance.

The sample of amylose solution was titrated after acidifying with 5 ml 1 N-HCl with a 0.01 N-I_2 under constant stirring. The titration agent was added in 0.1 ml portions and the galvanometer reading was done 30 sec. after each addition. The point of equivalency was established from the amperometric titration curve.

The amperometric titration curves of retrogradated suspensions of amylose reveal a deformed shape, the value of the electric current intensity rises in the horizontal segment of the curve and the slope is changed. Furthermore, a new slope of the line appears in the curve and titrations become virtually unsuitable for evaluation (Fig. 1). Consequently, the withdrawn samples were centrifuged and the clear supernatant was subjected to amperometric titration.

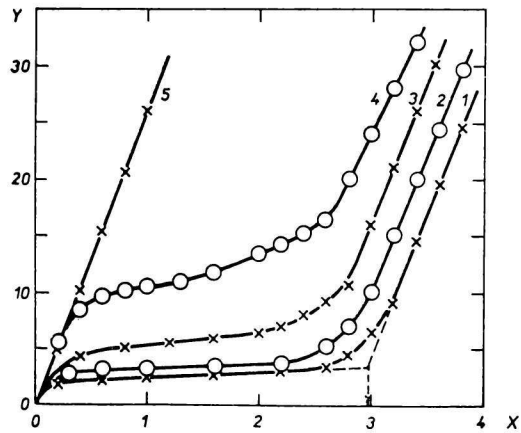

Fig. 1. Amperometric titration curves of uncentrifuged 0.1% amylose solution.
Abscissa x: 0.01 N-I_2 in mililiters.
Ordinate y: galvanometer reading in parts.
Curve *1.* 1th to 3rd day (graphically evaluated); *2.* after 6 days; *3.* after 9 days; *4.* after 12 days; *5.* blank test.

Investigation of retrogradation of amylose

Solutions of amylose (0.1, 0.2, 0.5 and 1.0%) were prepared by dissolving the proper amount of amylose in 150 ml 1 N-NaOH and adjusting pH to 5.2 by addition of 1 N-HCl and filling up 500 ml with distilled water. Solutions were kept cool in a refrigerator at +5°C, samples were withdrawn in chosen intervals, centrifuged and the clear supernatant was further analysed.

The process was modified in the case when the retrogradation was studied in the presence of a freshly retrogradated amylose; retrogradated amylose in proper amounts (expressed in Fig. 3 as a dry basis) prepared from a 5 days old 1% amylose solution kept at +5°C followed by a centrifugation was added to the solutions of amylose before distilled water was filled up. The freshly prepared amylose was used immediately for determination of concentration changes of amylose solutions and it was measured either as a suspension, or freeze-dried and weighed. Solutions with an addition of retrogradated amylose were stirred at +15°C and the concentration of amylose in solution was determined as in the previous case.

Results

Results obtained from the amperometric titration of the time dependences upon concentration changes of potato amylose in solution are given in Fig. 2 and 3.

The decrease of amylose concentration in particular, various concentrated, unstirred solutions kept at +5°C is given in Fig. 2. The course of these curves makes it evident that the beginning of the concentration decrease of amylose (the beginning of the precipitation of the insoluble amylose) and the rate of this decrease depends to a large extent on the initial concentration of amylose in solution, and, upon this reaction conditions the concentration of the solution does not attain zero, even during a longer time. The decrease of concentration of the unstirred solutions in the first stage of retrogradation is consistent with results given by *Loewus* and *Briggs* [3], who investigated retrogradation of stirred amylose by potentiometric titration in a short time period. In unstirred solutions, the concentration of the dissolved amylose does not change substantially once the sedimentation of the retrogradated amylose occured.

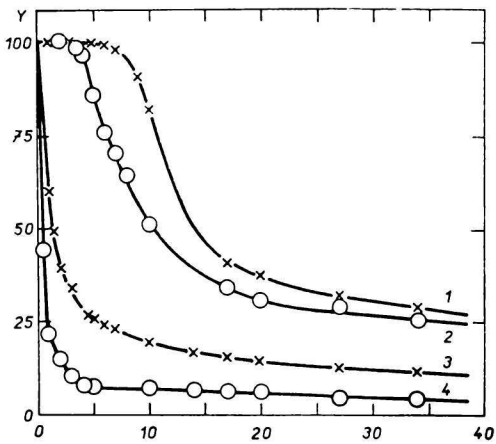

Fig. 2. Decrease of amylose concentration in unstirred solutions at +5°C. Abscissa x: time in days. Ordinate y: per cent of original amylose in the solution. Curve *1.* 0.1% solution; *2.* 0.2% solution; *3.* 0.5% solution; *4.* 1% solution.

The decrease of concentration of amylose, due to the addition of freshly prepared, or freeze-dried retrogradated amylose to the stirred solution at 15°C is plotted in Fig. 3. In this case one can see that the retrogradation starts immediately and it depends upon the amount of the solid amylose added and it is finished within a relatively short time. Evidence that the concentration of amylose in solution equals zero after the retrogradation had been attained is brought forward by the fact that no blue colour of the solution appeared after addition of iodine and that the amperometric titration curve is identical with this of the blank test. It follows that under this reaction conditions the potato amylose is virtually water insoluble.

In the case when hot-dried amylose was added to the mentioned solution the retrogradation course is similar to this where no addition took place (as shown in Fig. 2).

Discussion

It is known that the molecular weight of aggregates formed in diluted solution of amylose increases instantly after their preparation as it follows from the light scattering measurement [4, 6, 9]. This phenomenon is explained [9] in such a way that retrogradation is essentially coagulation in the sense of *Smoluchowski* theory [10].

The kinetics of retrogradation is markedly dependent upon the concentration of the solution as it can be seen from changes in concentration of amylose measured by iodometric titration with amperometric indication. Whereas both measurable decrease of concentration of dissolved amylose and change of the phase (visualized by the turbidity of solution) in 0.1 and 0.2% amylose solutions is observed after some days, in 0.5 and 1.0% solutions the decrease occurs practically immediately.

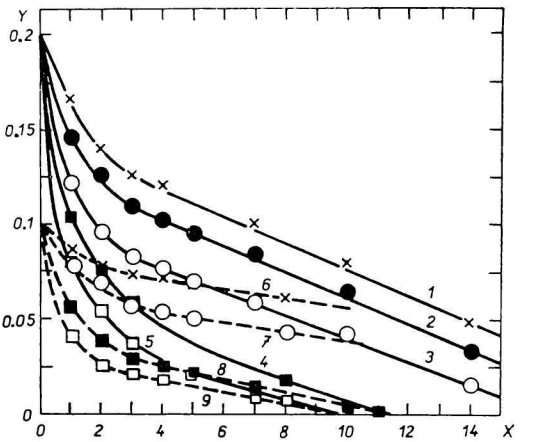

Fig. 3. Decrease of amylose concentration in stirred solutions — freshly retrogradated amylose being added — at +15°C (±2°C).
Abscissa *x*: time in days.
Ordinate *y*: concentration of the solution in per cent.
Curve *1.* and *6.* 1 g; *2.* and *7.* 2 g; *3.* 4 g; *4.* and *8.* 6 g; *5.* and *9.* 10 g; retrogradated amylose added.
The proper amount of the retrogradated amylose was added to 500 ml 0.2% and 0.1% amylose solution, respectively.

At the beginning of retrogradation, aggregation of amylose molecules takes place in diluted solutions without the estimated concentration having been decreased; as a result, an unchanged iodine sorption by amylose aggregates up to a definite critical size is observed. This size being exceeded a decrease of iodine sorption results together with alteration of the phase. The first stage of amylose retrogradation can be compared to nucleation reported by *La Mer* [11]. This explains both the great dependence of the first retrogradation stage of amylose upon its concentration, *i.e.* upon the distance between molecules in the solution and the substantial retardation of the process of retrogradation in unstirred solutions, where the formed solid phase sedimented on the bottom (Fig. 2).

After the phase change was achieved the second stage of retrogradation takes place, *i.e.* the growth of insoluble aggregates by addition of molecules from solution over the surface of aggregates formed. This second stage of retrogradation of amylose solutions (Fig. 3) depends upon the amount of the added retrogradated or freeze-dried amylose only. We do not report the numerical value of the rate constant of this process taking place in the boundary between phases because of the unknown active surface of the solid phase added.

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