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Study on the Retrogradation of Starch. I. Particle Size and its Distribution of Amylose Retrograded from Aqueous Solutions

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SUMMARY

The morphological features of amylose precipitates, especially the particle size and size distribution, during retrogradation have been investigated using scanning electron microscopy. It was found that the retrograded amyloses consisted of particles with a surprising uniformity in size, and the volume of each particle increased proportionally with the degree of retrogradation. These facts suggest that the retrogradation of amylose proceeds via a nucleation process, which is complete within a short time after the onset of retrogradation; this is then followed by growth of the nuclei. It was also found that, at a given percentage of retrogradation, the particle volume increased with increasing concentration of KCl in the solution. It was, therefore, considered that the reduction in the rate of retrogradation induced by KCl may be attributed to a decrease in the number of nuclei produced in the initial stage.

INTRODUCTION

Many papers have been published on the retrogradation of gelatinised starch (Collison, 1968; Hizukuri, 1977). However, the mechanism of retrogradation is not completely understood. It has been shown that the retrogradation is basically a crystallisation process, and can be detected to some extent by changes in the X-ray diffraction pattern (Katz, 1930; Hizukuri *et al.*, 1971). Thus, the retrogradation process

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has been analysed using equations such as the Avrami equations that can be applied to the crystallisation of polymers (Colwell *et al.*, 1969; Ciacco & Fernandes, 1979). However, it has been often pointed out that the retrogradation process is more complicated than crystallisation (Kuge, 1977).

The fact that the amylose component is primarily responsible for the retrogradation of starch has been shown (Schoch & French, 1947; Schoch, 1967). This paper deals with the retrogradation of amylose in aqueous solutions. Molecular aggregation of amylose in such solvents has been studied by various methods (Whistler & Jornson, 1948; Paschall & Foster, 1952; Babor & Kaláč, 1969; Phannemüller *et al.*, 1971; Ohnishi & Hiromi, 1981). In this study, changes in the morphological features, such as particle size, size distribution and shape of amylose precipitates during the retrogradation were investigated by scanning electron microscopy.

EXPERIMENTAL

Amylose

The amylose was 'Avebe amylose', provided by Nichiden Kagaku Co. Ltd, Osaka. This material was purified by recrystallisation several times with butanol from aqueous solution. The crystalline residue was washed three times with ethanol, twice with ether to remove the butanol and dried for 2 days *in vacuo* over P_2O_5 at 70°C. The viscosity-average molecular weight, $\bar{M}_v$, was found by viscosity measurements in dimethyl sulphoxide (Kitamura *et al.*, 1982) to be $3{\cdot}9 \times 10^5$.

Retrogradation of amylose

Aqueous amylose solutions were prepared by dissolving the amylose in 1 N KOH overnight at 5°C, followed by dilution with distilled water and neutralisation with 1 N HCl. The pH of the solutions were adjusted to 4·5 by adding 1 M Na-acetate buffer solution. The final concentrations of amylose and buffer were 0·4% (w/v) and 0·04 M, respectively. The concentrations of KCl were controlled by appropriately changing the amount of 1 N KOH used to dissolve the amylose. No detectable change in molecular weight of the amylose was observed through the use of

this procedure when samples were examined by gel permeation chromatography.

The aqueous amylose solutions were placed in a refrigerator at $5 \pm 1°C$. After an appropriate period, the solution was centrifuged for 20 min at 4000 rpm and the retrograded amylose collected. The precipitate was washed with cold water, dehydrated with ethanol, washed with ether and dried *in vacuo* at room temperature. The samples thus prepared were submitted to study by scanning electron microscopy. The concentration of amylose remaining in the supernatant was determined by the phenol-sulphuric acid method (Hodge & Hofreiter, 1962). The percentage of the amylose retrograded was calculated from this and initial concentrations of amylose in the solution.

Scanning electron microscopy

The retrograded amyloses were mounted on double-sided adhesive tape attached to specimen stubs. These were coated with 60–100 Å layer of gold by ion sputtering using an Ion Coater IB-3 from Eiko Engineering Co. Ltd, Japan. The coated specimens were observed with an Hitachi scanning electron microscope (type S-310) at 4·2–4·6 kV accelerating potential and photographed on Kodak tri-X pan film.

Particle-size distribution

Several photomicrographs were taken for each retrograded amylose sample. The diameters of retrograded amylose particles in photomicrographs were measured manually with a rule, and classified into subgroups with size differences of 0·125 μm interval to obtain the histograms. The diameter of the particle was defined as the mean length of the axes perpendicular to each other of the spherical particle. The diameters of more than 200 particles in several photomicrographs of each sample were measured. The average particle size from individual fields usually agreed within 5%.

RESULTS

Figure 1 shows the time courses of retrogradation of amylose in an aqueous buffer solution (pH 4·5) having various concentrations of KCl.

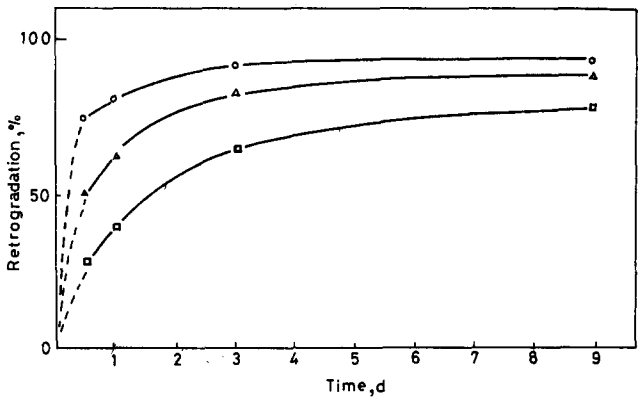

Fig. 1. Time courses of the retrogradation of amylose in 0·04 M acetate buffer solutions (pH 4·5) containing various concentrations of KCl at 5°C. KCl concentration: 0·1 M (○), 0·3 M (△), 0·5 M (□).

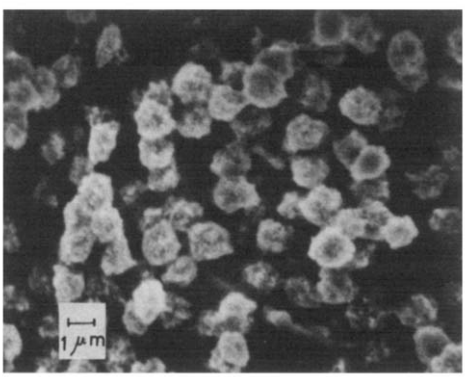

Fig. 2. Amylose particles retrograded from 0·1 M KCl aqueous solution, 1 day after the onset of retrogradation. Degree of retrogradation is about 80%.

The rate of retrogradation is markedly dependent on the salt concentration. Increasing the concentration of KCl leads to a decrease in the rate of retrogradation, which agrees with previous observations (Kuge, 1977).

Figures 2 and 3 show typical retrograded amylose particles as seen using scanning electron microscopy. The surfaces of the particles are not smooth, but the shapes as a whole may be approximately spherical.

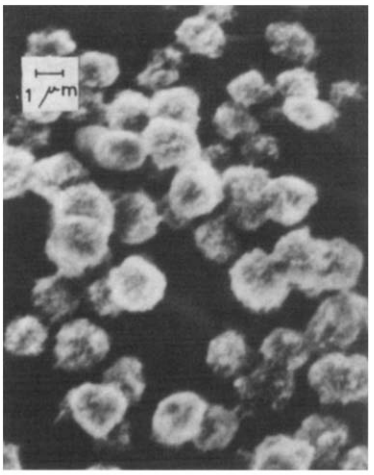

Fig. 3. Amylose particles retrograded from 0·5 M KCl aqueous solution, 9 days after the onset of retrogradation. Degree of retrogradation is about 80%.

It is evident that the particles for every sample have similar morphological features except for size.

The changes in the particle size and particle-size distribution during the period of retrogradation was also investigated. Figure 4 shows histograms of size distribution for the amylose particles. These histograms demonstrate that every amylose sample has a similar monomodal distribution with relatively little spread. Figure 4 also shows that the number-average particle diameter, $\bar{D}_n$, indicated by arrows, increases with the period of retrogradation. These results are numerically summarised in Table 1. The average value, $\bar{V}_n$, in Table 1 is defined as follows:

$$\bar{V}_n = \frac{\Sigma N_i D_i^3}{\Sigma N_i}$$

where N_i and D_i are the number of particles in each sub-group, i, and the specified diameter of sub-group, i, of the histogram, respectively. Figure 5 shows plots of $\bar{V}_n$ versus percentage retrogradation. It is clear that all of the plots are approximately linear and have a common intercept on the ordinate close to zero. This result indicates that the particle volume increases proportionally with increasing percentage of

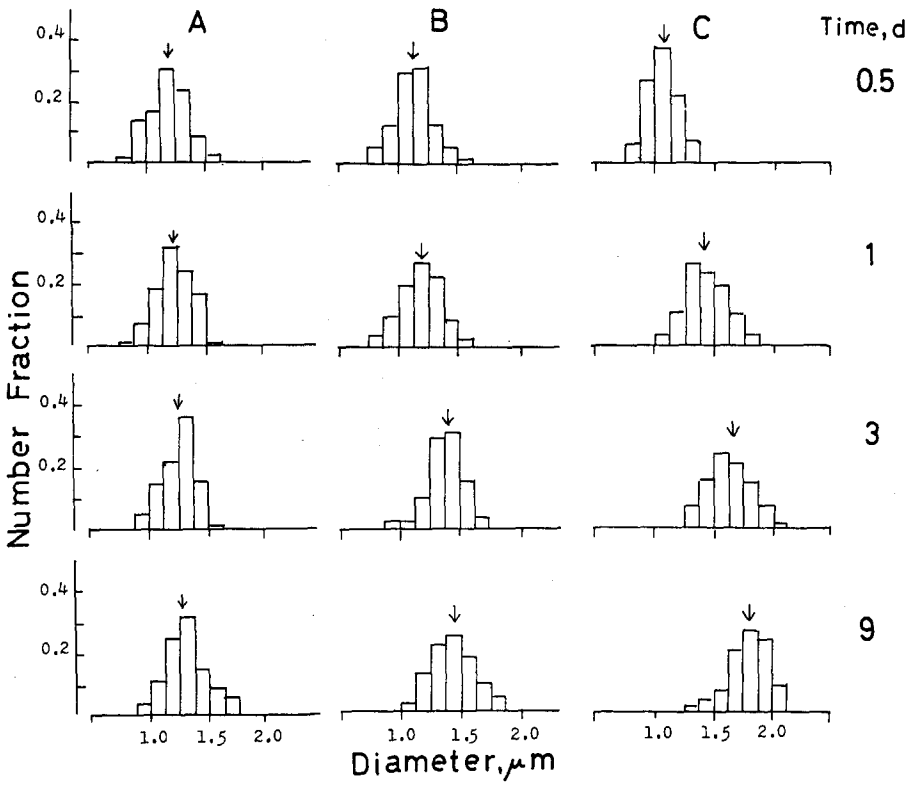

Fig. 4. Particle-size distributions of amylose particles retrograded from 0·1 M (A), 0·3 M (B) and 0·5 M (C) KCl aqueous solutions.

retrogradation. Figure 5 also shows that the particle volume is dependent on the KCl concentration. Comparison of each $\bar{V}_n$ at the same percentage of retrogradation indicates that the particle volume increases with increasing the salt concentration. This result is also shown photographically in Figs 2 and 3. These photomicrographs represent the particles obtained at the stage of retrogradation of about 80% from 0·1 and 0·5 M KCl aqueous solutions, respectively.

DISCUSSION

Babor and Kaláč (1969) considered that the retrogradation of amylose proceeds through a two step process; the first step is the formation of

TABLE 1
The Data for the Particle Size of Retrograded Amylose Samples

Samples				
KCl conc. (M)	*Time (days)*	*Retrogradation (%)*	$\bar{D}_n$ *(μm)*	$\bar{V}_n$ *(μm)*^{3}
0·1	0·5	75·0	1·19	1·77
	1·0	80·7	1·22	1·93
	3·0	92·6	1·25	2·07
	9·0	93·6	1·26	2·15
0·3	0·5	50·0	1·14	1·55
	1·0	62·0	1·19	1·79
	3·0	83·2	1·40	2·71
	9·0	87·6	1·45	3·15
0·5	0·5	28·0	1·10	1·40
	1·0	39·5	1·44	3·09
	3·0	64·4	1·64	4·56
	9·0	78·0	1·80	5·96

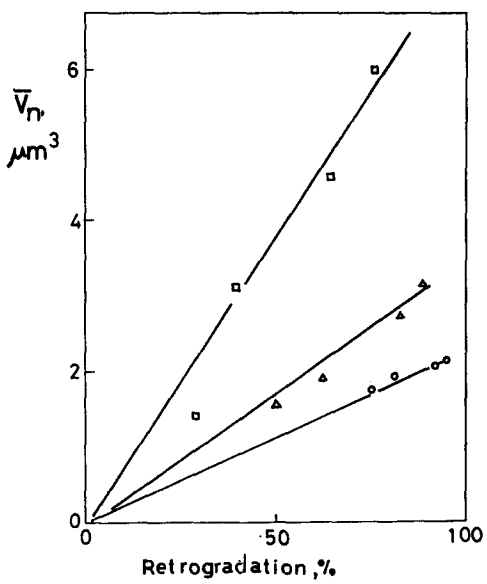

Fig. 5. Plots of $\bar{V}_n$ versus retrogradation percentage for the amyloses retrograded from 0·1 M (○), 0·3 M (△) and 0·5 M (□) KCl aqueous solutions.

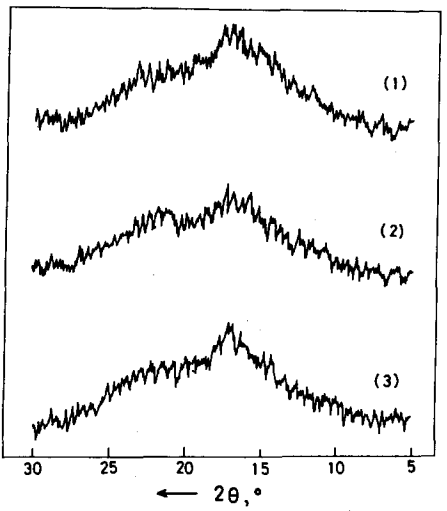

Fig. 6. X-ray diffraction patterns of retrograded amyloses. (1) 0·1 M KCl, 9 days retrogradation; (2) 0·3 M KCl, 9 days retrogradation; (3) 0·5 M KCl, 9 days retrogradation.

stable nuclei in the solution, and the second is the further growth of the nuclei by the addition of amylose molecules to their surfaces. The results presented in this paper confirm their hypotheses. The results shown in Fig. 5 suggest that nucleation and growth do not take place simultaneously, and the nucleation step ceases within a short period after the onset of retrogradation. The surprising uniformity in size of retrograded amylose particles also supports their proposal. This means that the retrogradation of amylose resembles polymer crystallisation in terms of its nucleation and growth mechanism. However, the X-ray diffraction patterns for these amyloses showed only an amorphous halo with very weak B-crystalline peaks (Fig. 6), which suggest that the major portion of these precipitates are amorphous in character.

Recently Davies *et al.* (1980) have reported that sphaerocrystalline particles were formed during the high temperature retrogradation of maize starch paste. The mechanism of the retrogradation reported here differs from that of the high temperature process particularly in relation to the nucleation mechanism. The high temperature retrogradation was reported to be initiated by the inclusion of fatty acids

with amylose, but the particles obtained in this study were formed without such an inclusion process. The sphaerocrystalline particles are seen to be similar in surface texture and shape but not in overall dimensions to those of the particles described here.

Figure 5 also shows that increasing the concentration of KCl causes an increase in the final size of particle produced. This fact suggests that KCl reduces the number of nuclei formed in the initial stage, and this effect results in a decrease in the rate of retrogradation. It was found that the aqueous amylose solution (0·4%) containing 2·5 M KCl was stable over two months apparently through the KCl suppressing the nucleation process in an otherwise unstable amylose solution. It is possible, therefore, that the effects of various ions in enhancing or suppressing starch retrogradation, which is known to follow the Hofmeister series (Samec, 1936; Morsi & Sterling, 1963), may be explained largely through their different effects on the nucleation process.

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