Study of Cryostructuration of Polymer Systems. XVI. Freeze–Thaw-Induced Effects in the Low Concentration Systems Amylopectin–Water

VLADIMIR I. LOZINSKY,[1] LILIJA G. DAMSHKALN,[1] RUPERT BROWN,[2] IAN T. NORTON[2]

[1] A. N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, Vavilov st. 28, 117813 Moscow, Russia

[2] Unilever Colworth Research Laboratories, Colworth House, Sharnbrook, Bedford, MK44 1LQ, United Kingdom

ABSTRACT: Studies of the freeze–thaw behavior of low-concentrated (0.01–0.25 g/dL) water solutions and dilute pastes (0.5–1.0 g/dL) of maize starch amylopectin showed that cryogenic treatment of these systems resulted in the formation of precipitated matter, whose yield and thermal characteristics (melting temperature and enthalpy) depended on the initial polymer concentration and conditions of freezing, frozen storage, and thawing. Research of the kinetic features of these cryoprecipitation events revealed at least two stages for this process: (i) a rapid stage, when the precipitation of virtually all of the dissolved polysaccharide occurred while the system was freezing, and (ii) a slower stage, the rate of which was mainly dependent on the thawing regimes or duration of the sample storage frozen at subzero temperatures. Cryoprecipitation phenomena were observed to be most extensive at temperatures 1–2° below the melting point of the frozen system. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 75: 1740–1748, 2000

Key words: amylopectin; low-concentration water systems; freeze–thaw-induced precipitation; cryostructuration

INTRODUCTION

It has been known for many decades that aqueous pastes of various starches are not resistant to a cryogenic influence and undergo certain physical changes as a result of freezing, frozen storage and subsequent thawing.[1] For instance, such a low-temperature treatment of concentrated pastes gives rise to cryotropic gel formation, the final products of which, in the case of starches from common sources (e.g., potato, corn, wheat), are in the form of sponge-like texturates. Their morphology depends on the starch properties (amylopectin/amylose ratio), its concentration in the gelatinized system to be frozen, conditions of cryogenic processing, the presence of soluble admixtures, and so on. The physicochemical characteristics and structure of similar cryotexturates (cryostructurates, cryogels—the synonyms) have been studied by many researches,[2–8] and characteristics exclusive to the freeze–thaw-behavior of these systems have been recognized. At the same time, features inherent in the similar behavior of low-concentrated starch pastes or solutions, and especially those of the individual starch polysaccharides (i.e., amylose and amylopectin), have hardly been investigated at all, although the consequences of cryogenic treatment for these systems are of interest from both the academic and applied points of view, especially for water-soluble amylopectin (AP).

First of all, it should be pointed out that only dilute water solutions of poly(vinyl alcohol)

Correspondence to: V. I. Lozinsky.

Contract grant sponsor: Unilever Research; contract grant number: 161834/SS.

Journal of Applied Polymer Science, Vol. 75, 1740–1748 (2000)

(PVA), whose concentrated solutions are known to be capable of forming corresponding cryogels as a result of freeze–thaw treatment,[9] were begun to be studied in this respect about 30 years ago. At that time Labudzińska and Ziabicki,[10,11] and later Peppas,[12] used light-scattering technique to observe the promoting effect of the cryogenic treatment on the association of PVA macromolecules in such systems. Freeze–thaw-induced variations of the viscosity characteristics of similar solutions and the formation of cryoprecipitate fraction were described by Khukhrachik and Baramboim.[13] Only recently have the basic characteristics of this process been considered in more detail.[14] Therefore, the fundamental problem of similarity in the observed trends of cryogenic behavior exists: are they of universal character for all polymers possessing the ability to form similar physical cryogels, or are the data obtained specific only for PVA and differ considerably in the case of other macromolecular gelling agents?

On the other hand, AP, as the major component of various starches (especially those from waxy mutants), is used widely in the composition of numerous foodstuffs, including fluid ones, which are subjected to freezing in the course of storing or processing. In this regards, the freeze–thaw behavior of the AP-containing systems is of practical interest, as well. All these speculations have determined the goal of the present investigation: to study the basic characteristic features of the freeze–thaw behavior of low-concentration aqueous AP solutions and dilute pastes with the impact on the dynamics of cryogenically induced aggregation of the macromolecules of this biopolymer. The latter problem has been shown for the PVA-containing systems[14] to be of great importance for the understanding of trends in the behavior of freeze–thaw-induced association of similar macromolecules. Therefore, the investigation in this field may reveal similar thermal conditions, which are optimal for AP in these processes.

EXPERIMENTAL

Materials

The following substances were used in the work without additional purification.

Amylopectin from the maize starch, molecular weight (M_W) ~ 10^6–10^7 kDa and 1 terminal group per 25 glucose units (the manufacturer data), was purchased from Fluka (Switzerland; Catalogue no. 10120, Lot no. 321672/1293). Visible (VIS) spectral analysis[1] of the amylopectin–iodine complex showed amylose content less than 1%. SEC analysis on column (15 × 450 mm) filled with CL Sepharose 6B resin (Pharmacia Fine Chemicals, Uppsala, Sweden), eluent 0.05 N KOH, did not reveal the presence of the polymer fractions with M_W less than ~ 140 kDa.

Crystalline iodine, potassium iodide, and sodium acetate, all used in the preparation of reagent solution for the determination of PVA concentration, were purchased from Reakhim (Moscow, Russia) and were of chemically pure grade.

All the solutions were prepared with the deionized water.

Methods

Amylopectin solutions (0.01–0.25 g/dL) were prepared by the following procedures: weighed amounts of dry AP powder were dispersed in a known volume of distilled water and then heated with stirring on a boiling water bath for 20 min. The solutions thus obtained were filtered through sintered glass filter and were used in further experiments. More concentrated (0.5–1.0 g/dL) solutions of the same biopolymer were prepared in an analogous way, except the filtration step was modified. The solutions were filtered by passing the hot liquid through a polyamide (nylon) cloth with a mesh of 0.3 mm.

Quantitative determination of amylopectin concentrations in the solutions under study was done spectrophotometrically for the corresponding iodine–polysaccharide complexes essentially in accordance to the known standard procedure.[1] Calibration plots were first obtained; AP calibration solutions were prepared using distilled water as a solvent. In this analysis, 1 mL of the polysaccharide solution to be examined was mixed 1 mL of 0.2*M* Na-acetate buffer (pH 5.3), and then 2 mL of 0.002*M* I_2/KI solution was introduced. The VIS light spectra of the solutions thus prepared were recorded using the Model 557 ultraviolet (UV)-VIS-spectrophotometer (Hitachi, Tokyo, Japan).

Freezing and frozen storage of the samples was performed either in an MK-70 (MLW, former GDR) cryostat chamber (the accuracy of the temperature maintenance was ±2°C) or in the chamber of an F34MH (Julabo, Germany) programmable precision cryostat (the accuracy of the temperature maintained at ±0.01°C). In the former case, the defrosting of the frozen samples was carried

Table I Morphological Features of the Low-Concentrated AP Water Systems Stored Unfrozen and Subjected to a Cryogenic Treatment for the Same Time

Initial AP Concentration (g/dL)	Morphology of the Samples after Storage at Different Incubation Temperatures			
	+18°C	−6°C	−12°C	−24°C
0.01	Small amount of sedimented precipitate and transparent upper liquid	Cryoprecipitate and self-separated transparent supernatant	Cryoprecipitate and self-separated transparent supernatant	Cryoprecipitate and self-separated transparent supernatant
0.025	The same	The same	The same	The same
0.05	The same	The same	The same	The same
0.10	Sedimented precipitate and transparent upper liquid	The same	The same	The same
0.25	The same	The same	The same	The same
0.50	Jelly-like precipitate and transparent upper liquid layer	A very weak cryogel occupying a part of the sample's bulk, transparent upper liquid layer	A very weak cryogel occupying a part of the sample's bulk, transparent upper liquid layer	A very weak cryogel occupying a part of the sample's bulk, transparent upper liquid layer
1.0	Opaque very weak paste	The same	A weak cryogel	A weak cryogel

Duration of the incubation was 18 h, and defrosting of the frozen specimens was carried out in a cold room at 7 ± 2°C.

out in a cold room at +7 ± 2°C, and in latter case this was done with controlled heating at a preassigned rate using the microprocessor-controlled facilities of the cryostat.

Calorimetric studies were carried out with a Model DASM-4 adiabatic differential scanning calorimeter (DSC) (SKBBP, Russia) in the temperature range of 10–100°C, using a positive going scan rate of 2° min^{-1}.

RESULTS AND DISCUSSION

Phenomenology of Amylopectin Cryoprecipitation

It is well known[15–17] that amylopectin-containing aqueous solutions and pastes are nonequilibrium systems. They are capable of phase-separating in the course of storing, and the rate of such a liquid–solid segregation process depends on the initial AP concentration, molecular weight characteristics of the polysaccharide, its degree of crystallinity, as well as on the storage temperature (the lower the temperature, the higher the rate of the retrogradation phenomena). Even very dilute AP water solutions are not storage-resistant. This statement, even at the higher extent as it will be demonstrated below, turned out to be valid for the freeze–thaw behavior of such solutions. In this work we studied the low-concentration AP water systems with a rather broad range of polymer content, which overlapped by two orders of magnitude, namely 0.01–1.0 g/dL. This allowed one to trace the AP precipitation processes of interest commencing from the practically transparent dilute molecular solutions up to the semi-dilute systems, which were translucent low-concentrated pastes, that is, they were virtually colloid-type dispersions. Table I describes qualitatively the appearance of all these samples after either being exposed unfrozen at +18°C for 18 h or subjected to a cryogenic treatment at various freezing temperatures, and Table II presents the quantitative data on the efficiency of AP precipitation and cryoprecipitation.

Although the insoluble matter was separated from the AP solutions stored both at room and negative temperatures, the morphology of the precipitates and cryoprecipitates, as well as of the viscous jelly-like species and cryogels, differed significantly (Table I). Thus, light microscopy investigation of the specimens formed from the 0.1-g/dL initial solutions under the conditions indi-

Table II Yields of the Precipitates and Cryoprecipitates from the Low-Concentrated AP Water Systems Stored Unfrozen and Subjected to a Cryogenic Treatment for the Same Time

Initial AP Concentration (g/dL)	Yields of the Precipitates and Cryoprecipitates Formed as a Result of the Samples Stored at Different Incubation Temperatures[1]			
	+18°C	−6°C	−12°C	−24°C
0.01	—[2]	—	—	—
0.025	—	—	—	—
0.05	84.0 ± 0.6	92.5 ± 0.5	94.4 ± 1.6	90.0 ± 5.0
0.10	80.0 ± 0.5	96.0 ± 0.7	96.0 ± 1.8	90.8 ± 4.3
0.25	88.0 ± 3.2	96.0 ± 2.0	96.0 ± 2.2	92.0 ± 2.0
0.50	91.0 ± 2.0	92.3 ± 1.2	90.8 ± 1.6	86.4 ± 1.9
1.0	83.0 ± 3.0	93.6 ± 1.4	89.2 ± 1.7	85.5 ± 2.7

[1] Insoluble matter was separated by the centrifugation at 4500 rpm for 30 min; yield values were computed as $(1 - C_i/C_m) \times 100\%$, where C_i was the initial AP concentration and C_m was the measured polysaccharide concentration in a supernatant after the centrifugation.

[2] —, The case, when C_m value was lower than the sensitivity limit of the analytical method used (see text).

cated has revealed that cryoprecipitates possessed smaller sized particles and a more compact structure compared to the larger particles of the AP precipitate, which possessed a "looser" structure. In the range of AP concentrations studied, practically all the solutions were affected by freeze–thaw influence, i.e., the cryogenic treatment caused the formation of either cryoprecipitates (≤0.25 g/dL) or weak cryogels, which occupied a portion of the sample's bulk (0.5 g/dL), and, with the increase in polymer concentration up to 1.0 g/dL, occupied the whole volume of the sample. Both of these latter kinds of cryogels could be compacted by a mild centrifugation (4500 rpm for 30 min), and the "residual" AP concentration in the supernatant liquid could be measured easily with the iodine-staining procedure, thus allowing one to estimate the yield of such a cryostructuration (Table II).

It was shown that for the most dilute (0.01–0.025 g/dL) aqueous solutions of AP, practically all solutes precipitated, both at positive (+18°C) and negative temperatures. This was supported by the fact that despite the high sensitivity of the spectrophotometric procedure used (<0.002 g of AP dL^{-1}), there was insufficient signal to detect the presence of soluble polysaccharide in the supernatant.

In the concentration range of 0.05–0.25 g/dL, the following main tendencies were found. The efficacy (yield) of the phase segregation processes was also high. An increase in the AP concentration resulted in some growth in the yield of an insoluble fraction, and decrease in the freezing temperature from −6 to −24°C led to only a slight diminishment of the yield values under the thawing conditions used (7°C). In all cases, the yield of cryoprecipitates was higher (this is more important) than the yield of precipitates formed from the equi-concentrated solutions at positive temperatures. Such results obviously meant that cryogenic treatment induced stronger association of AP macromolecules, giving rise to a more pronounced formation of insoluble aggregates than in the unfrozen liquid systems. This fact has demonstrated one of the principal effects inherent in the processes of cryotropic gel formation in general, namely, the promotional influence of the freeze–thaw treatment on the efficiency of gelation (since every AP cryoprecipitate particle could evidently be considered as a small-size cryogel, the effect under discussion is valid for the precipitation processes, as well). The major reason for such an effect has been recognized[18,19] to be the cryoconcentrating phenomena, that is, the increase in solute concentration in unfrozen regions of the system's bulk. These unfrozen inclusions are known as "unfrozen liquid microphase."[19,20] As a result of similar cryoconcentrating effects, the entanglement and associative interactions of AP chains were intensified, thus favoring intermolecular H-bonding and the formation of the microcrystallinity zones, thereby, enhancing the AP retrogradation.

Table III Influence of the Defrosting Rate on the Yield of the Matter Cryogenically Precipitated from the AP Water Solutions

Conditions of the Cryogenic Treatment				
Initial AP Concentration (g/dL)	Freezing Temperature (°C)	Frozen Storage Duration (h)	Thawing Rate (°C/min)	Yield of the Cryoprecipitated Matter (%)
0.5	−12	18	~ 3	73.3 ± 3.1
			0.30	78.5 ± 1.8
			0.03	93.0 ± 1.3

Upon increasing the initial AP concentration from 0.5 to 1.0 g/dL, the yields of both precipitates and cryoprecipitates decreased somewhat, which could be assigned to a certain growth in viscosity of the polymer solutions, thus influencing the diffusion rates of the interacting polysaccharide chains. For the subsequent experiments we chose the initial AP concentration of 0.5 g/dL because in the case of similar solutions, the most well-reproducible results were obtained and the cryostructurates formed (weak cryogels occupying a part of the sample's bulk; see Table I) turned out to be the most convenient ones for getting reliable phase separation by centrifugation followed by quantification of AP in the supernatants.

Influence of the Cryogenic Treatment Parameters on the Cryoprecipitation of Amylopectin

It has been found that cryogenic treatments in the freezing temperature range from −6 to −24°C had an insignificant effect on the yield values (Table II), and the preliminary tests showed that the length of the frozen storage time ceased to have any marked effect on the pattern observed after the 8- to 10-h exposition; because of this, we further concentrated the studies on the influence of thawing conditions.

Usually, the cryostructuration processes resulting in the formation of thermoreversible (physical) cryogels are rather sensitive to the thawing regimes, in this case the fundamental characteristic feature is as follows: the slower the thawing rate, the higher the efficiency of the gel formation. For instance, it was shown for the water–PVA systems that the properties of final specimens strongly depended on the defrosting conditions. Thus, the gel strength of slowly thawed samples was several times higher than the strength of samples of equal initial polymer concentration thawed at moderate rates, and even no cryogels were formed when the thawing rate was higher than about 10°C/min.[21] Therefore, the influence of this principal parameter (thawing rate) on the efficiency of the cryostructuration of AP low-concentrated systems was examined. The data obtained are listed in Table III.

These results evidently showed that a decrease in the thawing rate gave rise to an increase in the efficiency of AP cryoprecipitation similar to the aforementioned reinforcement of PVA cryogels[21] formed from the concentrated polymer solutions or analogously to enhancement of the PVA cryoprecipitation from the less concentrated systems.[14] Such a similarity in the freeze–thaw behavior of the synthetic polymer and natural polysaccharide testified to the universal character of the mechanisms governing the noncovalent cryostructuration of the polyol-type macromolecular gelling agents. One may also conclude that when the frozen samples described in Tables I and II were thawed-out by means of standing in a cold room at 7°C, the heating rate was closer to 0.03 rather than to 0.3°C/min, because the value of the cryoprecipitation yield of ~ 91% (the example 0.5 g/dL, −12°C in Table II) was nearest to that of 93% (the example 0.03°C/min in Table III).

The differences between the samples thawed with various rates were reflected not only on the values of cryoprecipitation yield, but also on the characteristics of the insoluble matter formed. These features were characterized using a highly sensitive, differential adiabatic scanning calorimetry. For these studies, AP cryoprecipitates were harvested by centrifugation at 4500 rpm for 30 min in order to prepare thicker dispersions. This was necessary because of the sensitivity requirements (0.001 J) of the calorimeter used. Then the sediment was resuspended in a known volume of pure water at 18°C until AP concentration was about 1 g/dL. An aliquot of this suspension was

Table IV Influence of the Regimes of a Cryogenic Treatment on the Thermal Characteristics of AP Cryoprecipitates

	Conditions of the Cryogenic Treatment			Thermal Properties of the Cryoprecipitates	
Initial AP Concentration (g/dL)	Freezing Temperature (°C)	Frozen Storage Duration (h)	Thawing Rate (°C/min)	Melting Temperature (°C)	Melting Enthalpy (J/g)
0.5	−6	18	~ 3	ND	< 0.01
			0.30	44 ± 1	~ 0.1
			0.03	46 ± 1	1.2 ± 0.2
	−12		~ 3	ND	0.01–0.05
			0.30	44 ± 1	0.05–0.10
			0.03	47.5 ± 0.5	0.43 ± 0.04

ND, not determined (no possibility for the correct determination).

injected into the probe head of the calorimeter (see Experimental). The exact concentration of the polysaccharide in the suspensions was determined with the iodine-staining reaction after heating the samples on a boiling water bath to dissolve the cryoprecipitated material. It should be also pointed out that for the AP precipitate, no melting enthalpy could be detected with the DSC instrument used, whereas for the AP cryoprecipitates a melting event was clearly recorded. These results showed that precipitation of AP from the 0.5 g/dL water solution resulted in formation of only a very small amount of ordered zones (microcrystallites), whereas during cryoprecipitation of the same initial system the retrogradation processes were reinforced significantly, resulting in much more extensive AP crystallization, and the melting of these cryogenically induced microcrystalites was registered by the calorimeter. The results of calorimetric experiments are summarized in Table IV.

A decrease in the thawing rate, giving rise to the growth of yield values (Table III), also facilitated an increase in the absolute values of thermal effects assigned to the melting of respective cryoprecipitates. The data of Table IV demonstrate that the slower the thawing rate, the higher the melting temperature and specific melting enthalpy. In the case of the samples defrosted rapidly (~ 3°C/min), the DSC curves were practically indistinguishable from the baseline. A least-squares fit to the endothermic peaks for the samples thawed at the rate of 0.3°C/min could be determined only with a computer-assisted procedure. However, at the same conditions, melting peaks for the slowly thawed cryoprecipitates were clearly recorded. This meant that the amount and length of intermolecular noncovalent links (ordered contacts, microcrystallites), manifesting a cooperative thermal effect under the melting of AP cryoprecipitates, increased considerably, when controlled slow heating was used for thawing the frozen samples. Also a larger melting enthalpy was inherent in the cryoprecipitates formed at −6 than at −12°C, but similar differences were registered when the specimens were thawed at a rate of 0.03 C/min; however, at higher thawing rates these differences were insignificant. All these data clearly pointed to the important role performed by the thawing stage in the cryostructuration of AP. The key role of this stage also was confirmed by subsequent studies of the dynamics of cryoprecipitation of the given polysaccharide.

Dynamics of the Formation of Cryogenically Induced Amylopectin Cryoprecipitates

The observed influence of thawing rate on the intensity of AP cryoprecipitation undoubtedly meant that this was associated with the residence time of the samples at definite negative temperatures during thawing, since the slower the defrostation process, the longer was the time at which a system resided at the temperature range where more possibilities for the polymer–polymer interactions were realized. Therefore, we asked the question: what is "the most effective" temperature interval for these interactions? In order to illuminate this problem, the experiments were conducted in accordance with the following scheme.

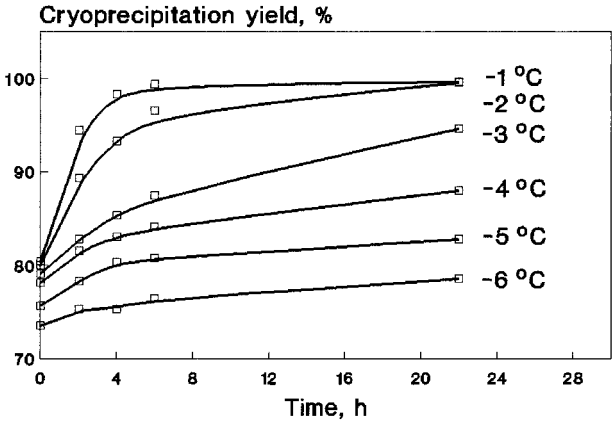

Figure 1 Variation of the cryoprecipitation yield in the course of the incubation of frozen samples (initial AP concentration: 0.5 g/dL) at different subzero temperatures; the yield values are the averaged ones of the three runs in separate experiments.

Portions (5 mL) of freshly prepared 0.5-g/dL water solutions of AP were poured into glass vials, stoppered, and placed into the liquid coolant (ethanol) in the cryostat chamber having a temperature of −24°C. After storage for 1 h (under which conditions all the samples froze in a short time), the temperature in the cryostat was increased (the rate of the heating was 1.64°C/min) up to several predetermined levels (−6, −5, −4, −3, −2, or −1°C) and was maintained constant at these temperatures for 22 h. During this incubation period several samples were removed from the cryostat after definite time intervals and heated to room temperature rapidly (~ 3°C/min) so as to avoid the influence of thawing dynamics on the cryoprecipitation yield. Insoluble matter formed in the samples was separated by centrifugation, and the polysaccharide concentration in the supernatants was determined with the iodine reaction. The use of similar temperature profiles allowed us to follow the dynamics of cryoprecipitate formation at each of the above subzero temperatures. The kinetic regularities established are depicted in Figure 1 as the time-dependent variation of cryoprecipitation yield. The values of yield at zero time correspond to the moments when the temperature in the cryostat chamber reached the incubation temperature. The experimental error upon the determination of the yields was the same as indicated for similar values in Tables II and III, i.e., not exceeding ±5%.

These studies have revealed several rather interesting trends in the freeze behavior of similar AP-containing systems. First, the process of cryoprecipitation itself was found to consist of at least two stages, namely, an initial fast event and a much slower secondary stage. A considerable portion of dissolved polymer transferred into insoluble matter simply as a result of freezing the system. In other words, ice crystallization, which was accompanied by a rapid increase in AP concentration in the unfrozen parts of the system, exerted a powerful action on the interchain interaction of the polysaccharide to an extent that the efficacy of association of AP macromolecules rose significantly. It was enough only to freeze the initial polymer solution and thaw it rapidly to reach a > 70% yield of a cryoprecipitate. Upon increasing the temperature to one of the subzero points in the range − 6 to −1°C, a slower growth in yield values took place. The rate of this "secondary" increase in yield depended on the particular temperature selected. It is seen from the graph that at −6 and −5°C, the formation of new fractions of cryoprecipitate proceeded with low efficiency. The temperature region from −4 to −3°C was a transition zone, whereas at −2 and, especially at −1°C, the rate of the second stage of the cryoprecipitation process increased considerably. At higher negative temperatures (i.e., at −0.5°C) similar experiments turned out to be difficult to carry out, because some of the samples began to thaw and, therefore, the reproducibility of the results was very poor. In general, for the range from −6 to −1°C, one may conclude that the efficiency (rate) of the second stage of AP cryoprecipitation increased with the increase in incubation temperature.

It was of interest that in the case of polymers possessing lower capability of associating in water media under the same freeze–thaw conditions (e.g., PVA), a somewhat different pattern was observed. The temperature dependence of the cryoprecipitation yield for the low-concentration PVA water solutions was of extreme character, and with the highest intensity, cryostructuration occurred in the vicinity of −2°C,[14] whereas below and above this negative temperature the yield values were lower. Undoubtedly, these distinctions in the freeze–thaw behavior of AP and PVA low-concentrated solutions could be associated with the different primary structure of these two polymers (branched AP in contrast to linear PVA) and also to a higher degree of crystallinity of maize amylopectin (≥35%) compared to atactic PVA (≤20%), thus determining the higher potential of the macromolecules of the polysaccharide to experience polymer–polymer interactions.

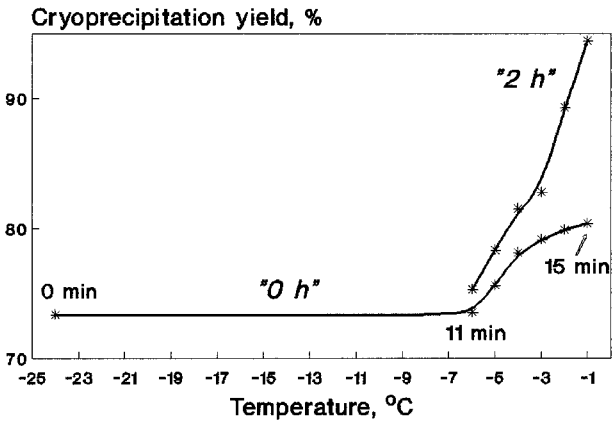

Figure 2 Variation of the cryoprecipitation yield in the course of the temperature increasing from −24 to −1°C at the rate of 1.64°C/min.

In the course of a fast thawing (~ 3°C/min), the frozen system "passed" through the temperature interval −3–0°C in 1 min; in the case of moderate thawing rate (0.3°C/min), this residence time was lengthened to 10 min; and at a slow thawing rate (0.03°C/min), the system resided at these "favorable" temperatures for 100 min. Only this latter period was obviously long enough for a marked increase in cryoprecipitation yield to be achieved, as it is seen from Figure 1 (curves −3, −2, and −1°C). The intensity of the AP aggregation processes during the time when the frozen samples "passed" through this temperature range in the course of their heating can be inferred from the data of Figure 2. The curve "0 h" shows the yield of the cryoprecipitates at the points when the temperature in the cryostat chamber reached the incubation temperature, and the small digits near these points indicate how many minutes elapsed from the beginning of heating of the respective frozen samples from −24°C (freezing temperature) to the particular incubation thermal conditions. For comparison purposes the curve "2 h" gives the yield values after the 2-h exposure at the corresponding subzero temperatures.

It is clearly seen that in the course of heating the samples from −24 to −6°C with the above-noted rate of 1.64°C/min (time required, about 11 min) practically no variation of the yield values occurred, but then, upon the transition through the "boundary" temperatures of −6 to −5°C, for only a further 4 min, the cryoprecipitation phenomenon markedly accelerated, and its yield increased by an additional ~ 10%. A subsequent 2-h incubation of the frozen specimens at the most favored temperature range of −2 to −1°C gave rise to an even greater growth in the yield values. These data evidently demonstrate the major importance of the subzero temperature range for the efficiency of cryostructuration processes. Just at these temperatures, as it was proved elsewhere by the nuclear magnetic resonance and ESR investigations of water–PVA systems,[22,23] does a certain amount of a liquid solvent arise in the still macroscopically frozen sample, in addition to its unfrozen liquid microphase. This "new" liquid facilitates the increase in mobility of polymer chains in the cryogenically concentrated medium, thus promoting intermolecular association. In the case of frozen AP solutions, Figures 1 and 2 suggest that the same situation obviously also existed for this polymer, since the acceleration of the second stage of cryoprecipitation was observed. In addition, the data of Table IV, which pointed to the significant increase in the amount of ordered regions in the slowly thawed cryoprecipitated pellets compared to the samples thawed more rapidly, testified that such an ordering took place at the subzero temperatures, and the insoluble phase formed at the first stage of the cryoprecipitation process was mainly amorphous. It is thought that this point is the principal feature for the cryostructuration of AP-containing systems.

CONCLUSIONS

The formation of AP cryoprecipitates as a result of a cryogenic influence on low-concentration water solutions of this biopolymer is an interesting example of the noncovalent cryostructuration of gelling polymers at all. While the freeze–thaw treatment of the concentrated amylopectin or starch gelatinized pastes was known to give rise to the fabrication of respective cryogels in the whole sample's bulk, the same influence on the low-concentration systems was shown here to result in the cryotropic gel formation within the small pellets of a cryoprecipitate phase. Such a freeze-induced "micro-structuration" may apparently be considered as a preliminary stage of the gelation processes of the more concentrated systems, resulting in the cryogels occupying the total volume of the corresponding samples. At the early stages of the formation of similar cryogels the association of AP macromolecules into primary clusters, their aggregation into the microgel grains and subsequent building of larger particles should have an effect on the properties of any

resultant macrogel. In this case, as it was also pointed out earlier for the water–PVA systems,[14] the small structured pellets of the cryoprecipitate phase function as the "primary bricks" during formation of a gel in the total bulk of the system. Therefore, the regularities inherent in the freeze–thaw behavior of the low-concentration AP solutions are of importance for a better understanding of the fine mechanisms of cryotropic gelation of more concentrated systems based on this natural polymer.

The authors thank Unilever Research for the permission to publish this work and for financial support and E. N. Kalistratova for the DSC measurements.

REFERENCES

1. Richter, M.; Augustat, S.; Schierbaum, F. Ausgewòhlte methoden der Störkechemie. VEB Fachbuchverlag: Leipzig, 1969.
2. Schulze, W. E.; Yu, D. T.; MacMasters, M. M. Störke 1964, 16, 41.
3. Schierbaum, F.; Richter, M. Die Nahrung 1964, 8, 487.
4. Chan, W. S.; Toledo, T. R. J Food Sci 1976, 4, 301.
5. Von Eberstein, K.; Hopcke, R.; Konieczny-Janda, G.; Stute, R. Störke 1980, 32, 397.
6. Eliasson, A. C.; Kim, H. R. J Texture Stud 1992, 23, 279.
7. Ferrero, C.; Martino, M. N.; Zaritzky, N. E. Int J Food Sci Technol 1993, 17, 191.
8. Navarro, A. S.; Martino, M. N.; Zaritzky, N. E. J Food Eng 1997, 34, 411.
9. Lozinsky, V. I. Russian Chemical Reviews, English Edn 1998, 67, 573.
10. Labudzińska, A. Polimery 1967, 12, 411.
11. Labudzińska, A.; Ziabicki, A. Kolloid Z und Z Polymere 1971, 243, 21.
12. Peppas, N. A. Makromol Chem 1975, 176, 3433.
13. Kukharchik, M. M.; Baramboim, N. K. Vysokomolekul soed 1972, 14B, 843 (in Russian).
14. Damshkaln, L. G.; Simenel, I. A.; Lozinsky, V. I. J Appl Polym Sci 1999, 74, 1978.
15. I'Anson, K.; Morris, V. J.; Ring, S. G. In: New Approaches to Research on Cereal Carbohydrates; Hill, R. D.; Munck, L., eds.; Elsevier Science Publishers BV: Amsterdam, 1985, p 115.
16. Biliaderis, C. G. In: Developments in Carbohydrate Chemistry, Alexander, J.; Zobel, H. F., eds.; American Association of Cereal Chemists: St. Paul, MN, 1993; p 87.
17. German, M. L.; Blumenfeld, A. L.; Guenin, Ya. V.; Yuryev, V. P.; Tolstogozov, V. B. Carbohydr Polym 1992, 18, 27.
18. Lozinsky, V. I. Proc 216-th Ann ACS Meeting, Div of Polymeric Materials: Science and Engineering, ACS, Boston, 1998, 79, p 238.
19. Sergeev, G. B.; Batyuk, V. A.; Stepanov, M. B.; Sergeev, B. M. Doklady Akademii nauk SSSR 1973, 213, 891 (in Russian).
20. Sergeev, G. B.; Batyuk, V. A. Uspekhi khimii 1976, 45, 793 (in Russian).
21. Domotenko, L. V.; Lozinsky, V. I.; Vainerman, E. S.; Rogozhin, S. V. Vysokomolekul soed 1988, 30A, 1661 (in Russian).
22. Gusev, D. G.; Lozinsky, V. I.; Vainerman, E. S.; Bakhmutov, V. I. Magn Reson in Chem 1990, 8, 651.
23. Mikhalev, O. I.; Sepinski, M.; Lozinsky, V. I.; Kapanin, P. V.; Chkheidze, I. I.; Alfimov, M. V. Cryo-Letters 1991, 12, 197.