Effects of Salts on Crystallization Kinetics and Rheological Behavior of Concentrated α,α-Trehalose Solutions

M. CERDEIRA, M.C. PUPPO, S. MARTINI, AND M.L. HERRERA

ABSTRACT: The effect of addition of $CaCl_2 \cdot 2H_2O$ and $MgCl_2 \cdot 6H_2O$ on crystallization kinetics and the rheological behavior of a 70% trehalose solution was studied by polarized light microscopy and dynamic oscillatory rheometry. Salts caused a delay on nucleation with induction times for crystallization longer for Mg^{2+} than for Ca^{2+}. Divalent cations were incorporated into crystals, changing the growth of certain faces preferentially, which resulted in changes in morphology. Addition of $CaCl_2 \cdot 2H_2O$ in a high salt/trehalose molar ratio dramatically increased complex modulus (G*), indicating a stronger viscoelastic behavior. A gel-like structure was formed when Ca^{2+} was added to trehalose solution. The behavior of Mg^{2+}/trehalose solutions cannot be considered viscoelastic in nature but as a fluid-like system.

Keywords: trehalose, polarized light microscopy, rheology, crystallization kinetics, divalent cations

Introduction

Amorphous and crystalline states of certain sugars are relevant in biological, pharmaceutical, and food sciences. Undesired or uncontrolled crystallization processes have become a main issue in the food industry because crystallization of the solid phase may significantly affect the shelf life of a product. For example, storage stability and quality of milk powders are significantly affected by the physical state of lactose, one of the main components in regular milk powders (Jouppila and Roos 1994). Crystallization of lactose in ice cream, condensed milk, and milk powder is considered undesirable, whereas in products such as milk chocolate, lactose crystallization is desirable. Likewise, sucrose crystallization evidenced by graining in boiled sweets is considered to be a defect, whereas the fine crystals present in fondant icing are desirable because they help icing retain its shape in confectionery products (Hartel and Shastry 1991).

Among saccharides, trehalose is particularly effective in terms of its ability to preserve and maintain activity of biomolecules. This sugar has no toxicity and is currently being used in several industries, such as food, medical, cosmetic, and other bio-industries, to preserve biomaterials under dry conditions. Despite its importance in cryopreservation and desiccation protection, the properties that make trehalose an effective protective agent are still poorly understood. There are several hypotheses that attempt to explain why trehalose is particularly effective, none of which completely accounts for experimental observations. Green and Angell (1989) have proposed that the higher glass transition temperature (T_g) of the trehalose/water system (compared with that of other glass-forming monosaccharides and disaccharides) could be responsible for its superior protective properties. Furthermore, they have shown that there is a strong correlation between the protective ability of protective agents and their T_g's. On the other hand, Crowe and others (1994) have noted that vitrification is not sufficient for preservation. Vitrification alone does not explain, for example, why another carbohydrate, dextran, which has a significantly higher T_g than trehalose, is a much less effective cryoprotectant than trehalose. According to the water replacement hypothesis, first proposed by Crowe and others (1994, 1998), as the systems are dried or frozen, the bonding of the trehalose hydrogens with the polar head groups of the lipids that constitute biomembranes replace those of hydration water at the membrane-fluid interface. In such a way, in the opinions of these authors, it prevents the phase transition and the accompanying leakage upon rehydration.

The study of delay/inhibition of sugar crystallization in supercooled liquids may increase the range of applications of sugar for specific purposes. The quality characteristics of the product, such as effectiveness of encapsulation and process parameters, are strongly influenced by the crystallization behavior of the matrix. Although previous studies indicated that the presence of salts may affect important properties of aqueous sugar systems, related to their protective role (Miller and others 1997, 1998; Mazzobre and Buera 1999; Mazzobre and others 2001a, 2001b; Gallo and others 2002; Longinotti and others 2002), there is a lack of information on the effect of salts on microstructural and rheological properties of aqueous sugar-salt systems. The objective of this study was to analyze the effect of divalent cations on crystallization kinetics and the rheological behavior of concentrated aqueous trehalose solutions. Morphology of crystals was described by polarized light microscopy (PLM), and growth was quantified in terms of normalized crystal area with time. Knowledge of crystallization kinetics and rheological behavior could give some insight into the role of trehalose-salts interactions on the mechanism of protection.

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Materials and Methods

Raw materials

α,α-Trehalose dihydrate (α-D-glucopyranosyl-(1-1)-α-D-glucopyranoside) from *Saccharomyces cerevisiae,* 99%, obtained from

Sigma (Sigma-Aldrich, St. Louis, Mo., U.S.A.) was used without any further purification. High-performance liquid chromatography (HPLC) water was used for all experimental work. $CaCl_2 \cdot 2H_2O$ and $MgCl_2 \cdot 6H_2O$ were analytical-grade. As in trehalose solutions, crystallization rate was determined by the counteracting effects of supersaturation and viscosity (Gallo and others 2002); the 70% trehalose solution, which showed the highest crystallization rate and the maximum solid content for the same crystallization temperature, was selected for this study. The solution was prepared by carefully weighing cold water and trehalose. $CaCl_2 \cdot 2H_2O$ was added to this solution in 0.4:1, 0.6:1, 0.7:1, and 1:1 molar ratios and $MgCl_2 \cdot 6H_2O$ in 0.4:1 and 0.7:1 molar ratios of salt/trehalose; there was no crystallization for at least 1 wk for the $MgCl_2 \cdot 6H_2O$ 1:1 molar ratio.

Isothermal crystallization

Trehalose solution was heated to 70 °C with stirring for 1 h, after which the solution was placed in an 80-mL water-jacketed glass cell at crystallization temperature. The solution in the glass cell, mechanically stirred by a magnetic stirrer at a fixed speed (60 rpm), was cooled to crystallization temperature and kept isothermally until crystallization started. The cooling rate was calculated from the initial slope of the temperature record of the sample, as measured by a copper-Constantine thermocouple. The results of several runs were averaged to obtain an average cooling rate of 10.0 ± 0.5 °C/min. Temperature was controlled by using a programmable LAUDA ethylene glycol:water (3:1) bath model RK 8 KP (Werklauda, Königshofen, Germany). Selected crystallization temperatures were 15 °, 20 °, and 25 °C. When a few crystals of 0.2 μm were detected on the microscope, a slurry of trehalose solution was placed on a microscope slide, and growth was studied in a quiescent way. The induction time for crystallization, that is, the time interval between trehalose solutions reaching crystallization temperature and the detection of the first crystals, was considered as zero time. It is very difficult to quantify crystallization kinetics with microscopy when both nucleation and growth take place quiescently (Gallo and others 2002). To obtain results with statistical significance, nucleation was performed in a dynamic way. Since trehalose is currently of much interest in biology and food science for its very efficient preservation properties against water stresses induced by dehydration or freezing, growth was performed quiescently. The crystallization process, in a sense, simulated an industrial process or an encapsulated material in which sugars crystallized forming many initial crystals, and after which they grew quiescently while the product was packaged and stored.

Optical microscopy

A Leitz microscope model Ortholux II (Ernst Leitz Co., Wetzlat, Germany) with a controlled-temperature platform was used to follow crystallization over time. The platform temperature was controlled by a Lauda TUK cryostat (Werklauda). Image acquisition started when the first crystals appeared (denoted as time zero). At that time, a slurry of the solution was collected with a pipette from the glass cell and was placed on the slide at crystallization temperature, and photographs were taken for 3 h with a Leitz-Vario-Othomat (Ernst Leitz Co., Wetzlat, Germany) camera under polarized light. Magnification of 100× was used for all photographs.

Image analysis

The growth of the overall crystal surface area was calculated by summing the areas occupied by all of the crystals within the region of interest (ROI). The ROI was a rectangle with the same dimensions as the original micrograph (0.46 mm^2) and remained constant for each image that was recorded. The crystal surface area for each image was normalized by dividing the surface area occupied by the crystals by the area of ROI. A plot of normalized area against time provides an easy way to determine how quickly a "surface" is being covered (Howell and Hartel 2001). Results are the average of 3 runs. Standard deviations are shown in graphics.

X-ray diffraction (XRD)

Samples were analyzed for their crystallinity by using a Philips 1730 X-ray spectrometer fitted with a system for temperature control (Philips Argentina S.A., Capital Federal, Argentina). The temperature of the sample holder placed within the refraction chamber was controlled through a programmable Lauda UK 30 cryostat. Ethylene glycol in water (3:1, vol/vol) was used as coolant. $K_{\alpha 1 \alpha 2}$ radiation from copper was used at 40 kV, 20 mA, and a scanning velocity of 1°/min from 5 to 30°. Positions of diffraction lines varying more than 0.01 can be considered different.

Rheological measurements

Tests were carried out in a Haake (Karlsruhe, Germany) CV20 rheometer using a 1-mm gap parallel-plate sensor. The equipment was driven through the Haake software osc. 2.0. The linear viscoelastic range of the solutions was determined by measuring the complex modulus (G*) as a function of deformation (frequency = 1 Hz). From these results, experiments were conducted at the same deformation (d = 3%), within the linear range to cause no structural damage to the sample. At a time equal to induction time (denoted as time zero for growth studies), solutions were placed on the lower plate, which were maintained at crystallization temperature (15 °, 20 °, or 25 °C). Calcium and magnesium salts were added at the same molar ratio as used in the crystallization studies. The dynamic behavior of solutions was studied through the storage shear modulus (G′) and the loss shear modulus (G″) frequency sweeps. The general viscoelastic behavior of the solutions was studied through G* and the viscous-to-elastic moduli ratio (tan δ). tan δ was calculated using Eq. 1:

$$tan\ \delta = \frac{G''}{G'} \quad (1)$$

Results are the averages of 3 replicates.

FTIR experiments

Samples were analyzed using an FTIR Bruker IFS-66 spectrometer (Bruker, Rheinstetten, Germany) fitted with a system for temperature control, using the KBr pellet and Nujol techniques. FTIR spectra were obtained at crystallization temperature scanning between 4000/cm and 400/cm.

Results and Discussion

Induction times of crystallization

Table 1 shows the induction times obtained when samples were crystallized dynamically at 15 °, 20 °, and 25 °C. No induction time for crystallization was found when trehalose solution was crystallized at 15 ° and 20 °C, that is, crystallization started at the moment when the solution reached crystallization temperature. At 25 °C there was a short induction time, in agreement with the lower supersaturation at that temperature (Gallo and others 2002). Addition of $MgCl_2 \cdot 6H_2O$ in a ratio of 0.4:1 salt/trehalose slightly delayed the start of crystallization at all temperatures ($P < 0.05$). In a 0.7:1 molar ratio, there was a very significant difference ($P < 0.001$) in induction times for trehalose solution with and without the addition of $MgCl_2 \cdot 6H_2O$, with higher crystallization temperatures giving longer induction times. No crystallization was found for addition of $MgCl_2 \cdot$

$6H_2O$ in a higher ratio for at least 1 wk. Addition of $CaCl_2 \cdot 2H_2O$ also prolonged induction times for crystallization at all temperatures selected in this study with higher molar ratio (from 0.4 to 0.7) giving longer induction times. For a molar ratio of 1:1, induction times were similar to the ones found for a molar ratio of 0.6. Salts caused a delay of nucleation, which may mean that they are able to be incorporated in trehalose crystals. In addition to this mechanism, $CaCl_2 \cdot 2H_2O$ at a molar ratio of 1:1 may also act as heteronuclei, accelerating nucleation through the catalytic actions of such impurities. In all the experiments performed in this study, trehalose was in solution state, which means that the only polymorphic form possible for trehalose crystals was the dihydrate (Sussich and others 2001).

In a previous study (Gallo and others 2002) we found that in trehalose solutions crystallized without agitation crystallization rate was determined by the counteracting effects of supersaturation and viscosity. In that study, crystallization rate was faster for solutions crystallized at 20 °C. In the present study, however, induction times were shorter for 15 °C, which is somewhat surprising. Agitation favored diffusion and, as at 15 °C, the thermodynamic driving force for crystallization (the supersaturation) is higher; nucleation occurred at shorter times. The different behavior found for the system when nucleation occurred with or without agitation shows the great importance of processing conditions in the crystallization process of diffusion control systems.

Effect of crystallization temperature

Figure 1 shows the effect of crystallization temperature on crystal size for a 70% trehalose solution crystallized at 10 °C/min with an agitation rate of 60 rpm. Photographs were taken 2 min after the start of crystallization. Crystal morphology was very similar for the 3 selected crystallization temperatures, but the size increased with temperature. This effect was also found for the trehalose solution with addition of salts. Although nucleation is a precursor to crystal growth, the latter dominates at higher temperatures. Crystal growth dominates at lower degrees of supersaturation and, therefore, bigger and fewer crystals per field were found for 25 °C.

Effect of salts on crystalline microstructure

Figure 2 shows representative PLM images of the trehalose solutions cooled to 20 °C (60 rpm) both (a) without and with addition of 0.4:1 molar ratio of (b) $MgCl_2 \cdot 6H_2O$ and (c) $CaCl_2 \cdot 2H_2O$. After a time equal to induction time, samples were placed on microscope slides (zero time for growth kinetics) to complete crystallization. The images shown were at time intervals from 0 to 3 h and portrayed dramatic differences in kinetics and crystal properties as a result of addition of salts. Salts diminished crystal size in agreement with the effect in delaying crystallization especially for the addition of $CaCl_2 \cdot 2H_2O$. However, the main effect was on crystal morphology, indicating that growth mechanism was also modified. After 7 min of the start of crystallization, it is very clear that crystals formed in trehalose solutions with salts grew in a 2-dimensional fashion, showing even a needle shape for $CaCl_2 \cdot 2H_2O$ in molar ratios higher than 0.4:1 (data not shown). On the other hand, the trehalose solution without the addition of salts grew 3-dimensionally, showing well-defined prismatic crystals with sharp edges. When impurities are incorporated into a crystal, they usually change the growth of certain faces preferentially, which results in changes in morphology.

Effect of salts on crystal growth

The normalized crystal area (NCA) with time of crystallization for the 70% trehalose solution and with addition of 0.4:1 mol ratio of $CaCl_2 \cdot 2H_2O$ or 0.4:1 and 0.7:1 mol ratio of $MgCl_2 \cdot 6H_2O$ is reported

Table 1—Induction times of crystallization (τ) for all samples at the 3 selected crystallization temperatures (15 °, 20 °, and 25 °C)

	τ (min)		
Sample	15 °C	20 °C	25 °C
Trehalose (T)	0	0	2.5 ± 1.4
$MgCl_2$/T 0.4:1	2.0 ± 1.2	6 ± 1.7	5 ± 2.2
$MgCl_2$/T 0.7:1	60 ± 2.9	120 ± 3.5	130 ± 4.1
$CaCl_2$/T 0.4:1	5 ± 2.1	8 ± 2.3	6 ± 2.3
$CaCl_2$/T 0.6:1	22 ± 1.8	25 ± 1.8	35 ± 2.5
$CaCl_2$/T 0.7:1	35 ± 2.6	40 ± 2.3	55 ± 2.8
$CaCl_2$/T 1:1	23 ± 2.0	25 ± 2.0	38 ± 2.7

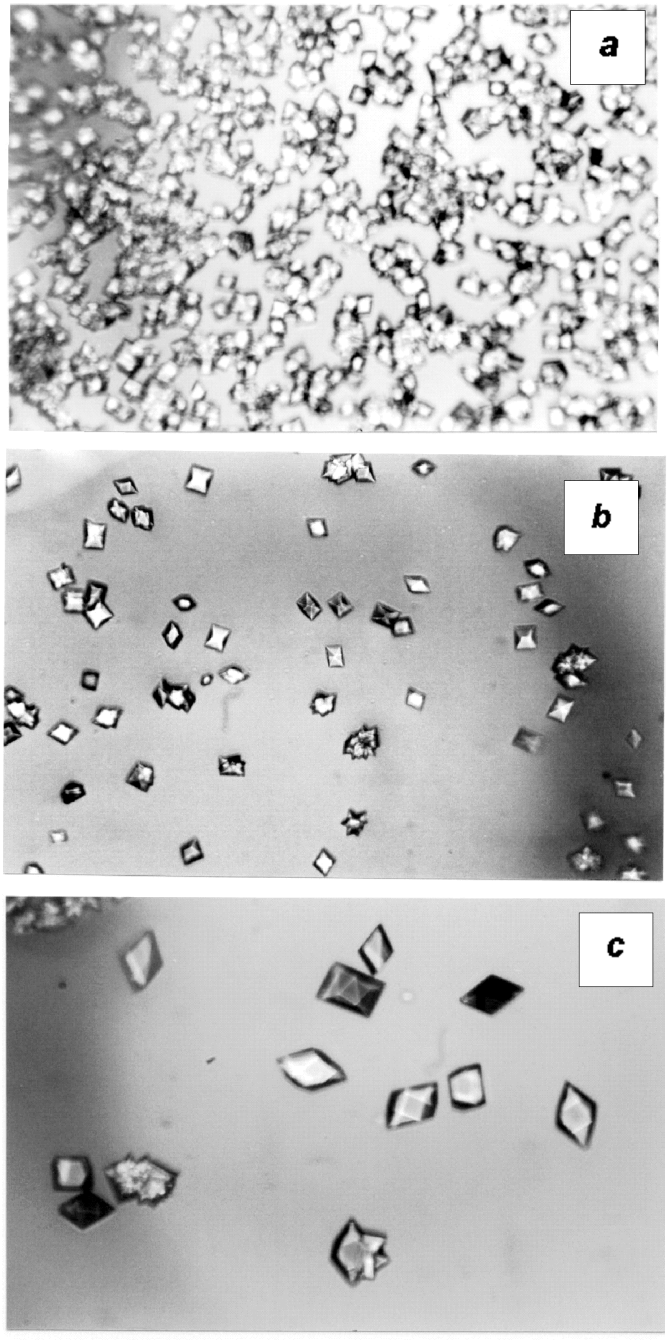

Figure 1—Effect of crystallization temperature on crystal size for a 70% trehalose solution 2 min after the start of crystallization at (a) 15 °C, (b) 20 °C, and (c) 25 °C

in Figure 3. Addition of $CaCl_2 \cdot 2H_2O$ in ratios of 0.6:1, 0.7:1, and 1:1 were not evaluated because at these concentrations, crystals were needle-shaped and accumulated in a way that made it difficult to evaluate accurately the covered area. Reported values correspond to crystal growth. Zero time is the start of crystallization, that is, induction times were subtracted from the total time at crystallization temperature. At a crystallization temperature of 15 °C, crystal growth was faster for the 70% trehalose solution without addition of salts. In the beginning, the NCA was high (0.61). After 180 min, the NCA was significantly lower for the addition of Ca^{2+} at 0.4:1 and Mg^{2+} at a 0.7:1 molar ratio ($P < 0.001$). Addition of Mg^{2+} at a 0.4:1 molar ratio also diminished the NCA significantly ($P < 0.05$). At 20 °C, the growth rate was also higher for the 70% trehalose solution without salts up to an NCA of 0.62. For a higher NCA, growth was higher with addition of Ca^{2+}, being the final NCA value being significantly higher ($P < 0.05$). Solutions with Mg^{2+} showed the same behavior as at 15 °C. At a crystallization temperature of 25 °C, growth rate was faster for the 70% trehalose solution without salts up to a NCA of 0.2. For higher NCA, addition of Ca^{2+} and Mg^{2+} in a 0.4:1 ratio accelerated growth with a significantly higher NCA after 180 min ($P < 0.05$). Addition of Mg^{2+} in a 0.7:1 molar ratio always delayed growth, with a 180-min NCA value significantly lower ($P < 0.001$). The effect of salts on crystal growth rate was strongly dependent on crystallization temperature. The lower the temperature, the greater the delay in growth. At 15 °C, molecular diffusion is slower and was the determining factor. Growth behavior of the solution with Ca^{2+} was more affected by viscosity than solutions with Mg^{2+} as shown in Figure 3. In a previous study, we found that Ca^{2+} was very efficient in delaying trehalose nucleation. For crystallization studies without agitation, no crystals were found after 400 min

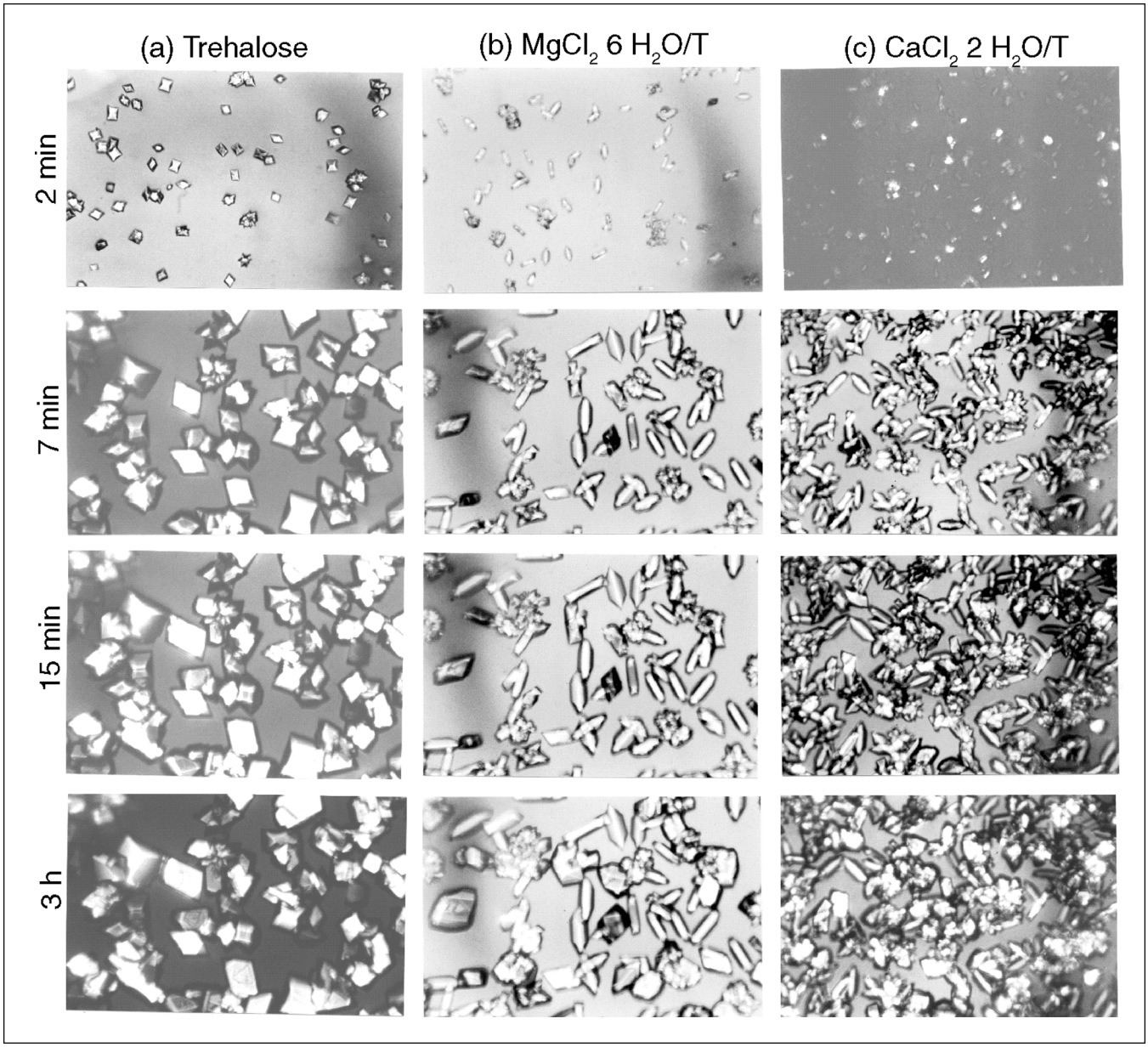

Figure 2—Growth kinetics followed by PLM of the 70% trehalose solution (T) without salts (a) and with the addition of $MgCl_2 \cdot 6H_2O$ (b) and $CaCl_2 \cdot 2H_2O$ (c) at 0.4:1 salt-trehose ratio.

at crystallization temperature (Gallo and others 2002). This effect was so dramatic that it strongly supported the hypothesis of specific interactions between Ca^{2+} and trehalose in addition to an incorporation of Ca^{2+} into trehalose crystals. When nucleation occurred with agitation, however, induction times were in agreement with the polarizing character of cations, that is, Mg^{2+} delayed nucleation more efficiently than Ca^{2+}. On the contrary, Ca^{2+} showed a greater effect on crystal growth at low temperature. As reported in the literature, ionic polysaccharides had the ability to form gels in the presence of divalent cations such as Ca^{2+} (Braccini and others 1999). Dronnet and others (1998) pointed out the importance of gelation and the structural conformational changes that take place when pectins interact with Ca^{2+}. Although the presence of COOH groups is very important to achieve gelation, in the case of trehalose, Ca^{2+} could delay crystallization because of a gel-like effect due to interactions with Ohio groups. Chlorides, however, did not show an effect on crystallization kinetics. Chlorides of monovalent cations such as NaCl or KCl did not modify induction times of crystallization or growth kinetics. To deeply investigate this hypothesis, oscillatory dynamic rheometry was performed within the linear viscoelastic range to study tehalose solution viscoelasticity, and then the solutions were filtered after 3 h at crystallization temperature, and crystals were analyzed by XRD and FTIR spectroscopy.

Rheology of trehalose-salt solutions

At a time equal to induction time, samples were placed on the rheometer plate for rheological measurements. Storage modulus (G′) and loss modulus (G″) were frequency-dependent as evidenced by the ascendant slope of the modulus compared with frequency curves (data not shown). As a representative example, Figure 4 shows the variation of G′ and G″ with salt molar ratio for both salts at 20 °C and a frequency of 1 Hz. For other frequencies, the same behavior was found in all cases. G′ of a system is essentially a measure of its elasticity, or solid-like character. G′ for trehalose solution was very low (Figure 4) and remained low with addition of

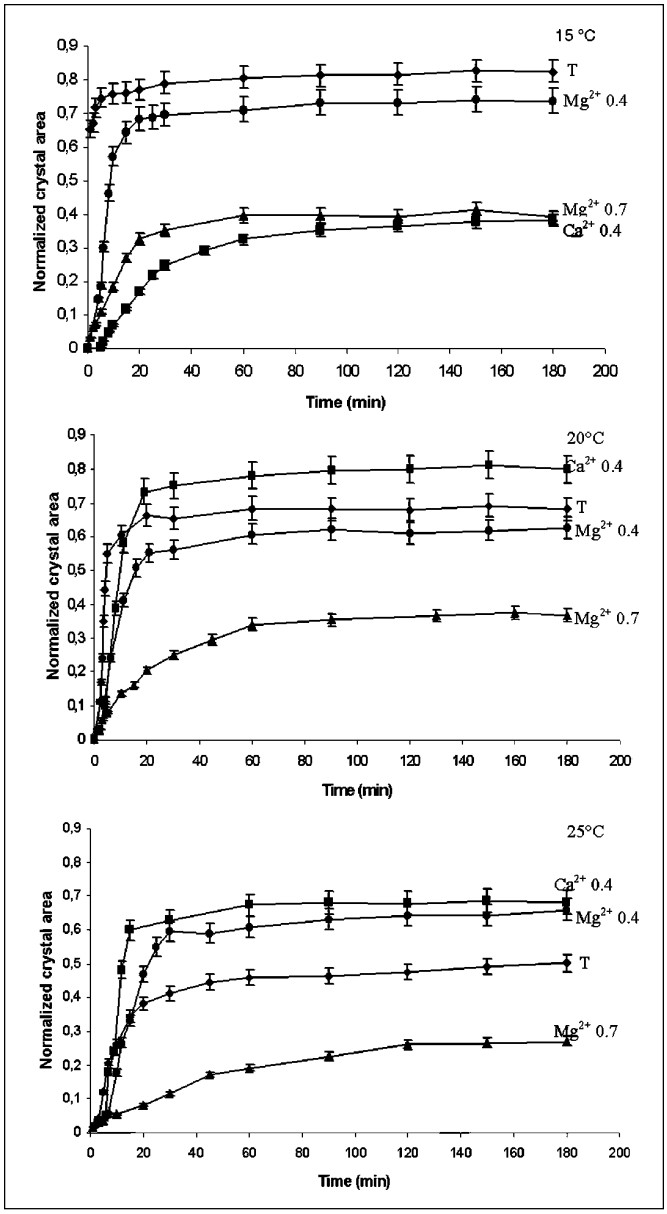

Figure 3—Normalized area plotted against time at different crystallization temperatures (15 °, 20 °, and 25 °C) for 70% trehose solution and with the addition of $CaCl_2 \cdot 2H_2O$ at 0.4:1 molar ratio and $MgCl_2 \cdot 6H_2O$ at 0.4:1 and 0.7:1 molar ratio

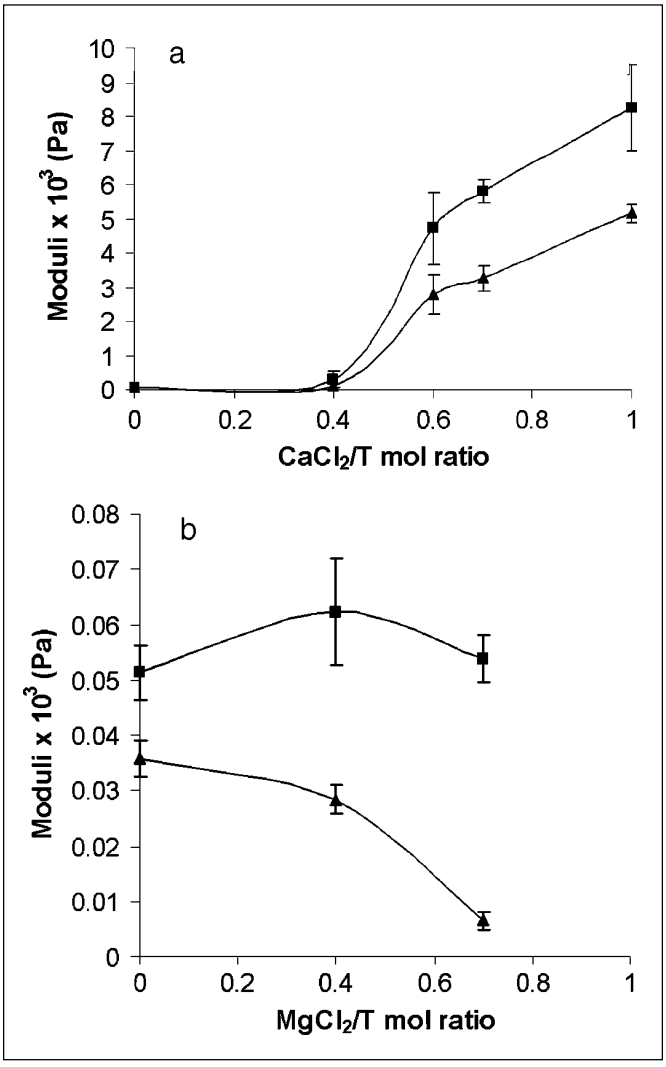

Figure 4—Variation of storage modulus (G′) and loss modulus (G″) with salt molar ratio at 20 °C and a frequency of 1 Hz (a) $CaCl_2 \cdot 2H_2O$ and (b) $MgCl_2 \cdot 6H_2O$

Table 2—Complex modulus (G*) and tan (δ) for all samples crystallized at 20 °C.

	Sample						
Parameter	Trehalose 70%	$CaCl_2$/T 0.4:1	$CaCl_2$/T 0.6:1	$CaCl_2$/T 0.7:1	$CaCl_2$/T 1:1	$MgCl_2$/T 0.4:1	$MgCl_2$/T 0.7:1
G* (Pa)	62 ± 6	325 ± 29	5477 ± 92	5812 ± 95	9731 ± 92	68 ± 9	54 ± 4
Tan (δ)	1.4 ± 0.4	1.9 ± 0.4	1.7 ± 0.1	1.6 ± 0.4	1.6 ± 0.2	2.2 ± 0.1	8.4 ± 0.5

$CaCl_2 \cdot 2H_2O$ at molar ratios lower than 0.4:1 (Figure 4a). For higher molar ratios, G′ increased in a very significant way ($P < 0.05$), indicating that a stronger structure was formed. G″ describes the viscous behavior and is a measure of the liquid-like behavior of a system. For the addition of $CaCl_2 \cdot 2H_2O$, G″ showed the same behavior as G′, but its values were significantly higher than G′ values ($P < 0.05$), which is indicative of the predominant viscous behavior of the solutions. The general viscoelastic behavior of the materials is described by G*. The phase shift, tan (δ), is the ratio of G″/G′ and therefore describes the viscous/elastic behavior of the system. As the value of δ approaches 0°, the G″ value approaches zero; therefore, the sample behaves like an ideal solid and is called perfectly elastic. As δ approaches 90° (stress is 100% out of phase), the G′ value approaches zero and all of the energy will be dissipated as heat and the sample will behave liquid-like. At intermittent values, the samples are considered to be viscoelastic in nature. Therefore, tan (δ) is an excellent indicator of the structural integrity of the sample, indicating the proportion of the material structure attributable to the 3-dimensional network and the samples liquid phase. G* and tan (δ) values for trehalose solutions crystallized at 20 °C with and without the addition of salts are summarized in Table 2. Values correspond to a frequency of 1 Hz. Addition of $CaCl_2 \cdot 2H_2O$ did not modify the values of tan (δ) obtained for trehalose solutions without salts ($P < 0.05$). Thus, the proportion of the material that behaves as a solid was not modified. G*, however, significantly increased with molar ratio salt-trehalose, indicating a stronger viscoelastic behavior. That is, a short-range structure was formed when Ca^{2+} was added to trehalose solution. Trehalose solutions with addition of $MgCl_2 \cdot 6H_2O$ showed a different behavior. G′ values significantly diminished with molar ratio salt-trehalose, whereas values of G″ show no significant differences from 0 to 0.7:1 molar ratio ($P < 0.05$). G″ values were always higher than G′ values, which means that for these solutions, viscous behavior prevails over elastic behavior. Tan (δ) values increased with addition of $MgCl_2 \cdot 6H_2O$. The high value found for the 0.7:1 molar ratio solution indicates that the trehalose-Mg solutions behavior cannot be considered viscoelastic in nature but a fluid-like system (Table 2). G* values were very low, indicating that no short-range structure was formed because of addition of Mg^{2+} as happened with Ca^{2+} addition. Although Mg^{2+} delayed nucleation more efficiently than Ca^{2+}, a gel-like formation occurred only with addition of Ca^{2+} and, therefore, growth was more strongly modified at low temperatures (15 °C) in the presence of Ca^{2+} (Figure 3).

X-ray diffraction analysis

Figure 5 shows X-ray diffraction patterns of trehalose solutions crystallized at 20 °C (a) without salts and with addition of salts in 0.7:1 (salt/trehalose) molar ratio (b) $MgCl_2 \cdot 6H_2O$ and (c) $CaCl_2 \cdot 2H_2O$. Although the patterns were similar for the 3 samples analyzed, significant differences were found between them. The trehalose pattern showed 3 strong signals at 13.63 °, 17.47 °, and 23.80 °. Addition of salts caused an increase in the trehalose 13.63 ° signal intensity, especially with $CaCl_2 \cdot 2H_2O$. Signal positions were significantly modified, being 2θ values 13.77 °, 17.63 °, and 23.90 °, and 13.93 °, 17.77 °, and 24.03 ° for $MgCl_2 \cdot 6H_2O$ and $CaCl_2 \cdot 2H_2O$, respectively. The increase of 2θ values indicates a decrease of diffraction distances, in agreement with the different morphologies found for trehalose solutions with and without salts. According to these results, divalent cations seem to be incorporated in trehalose

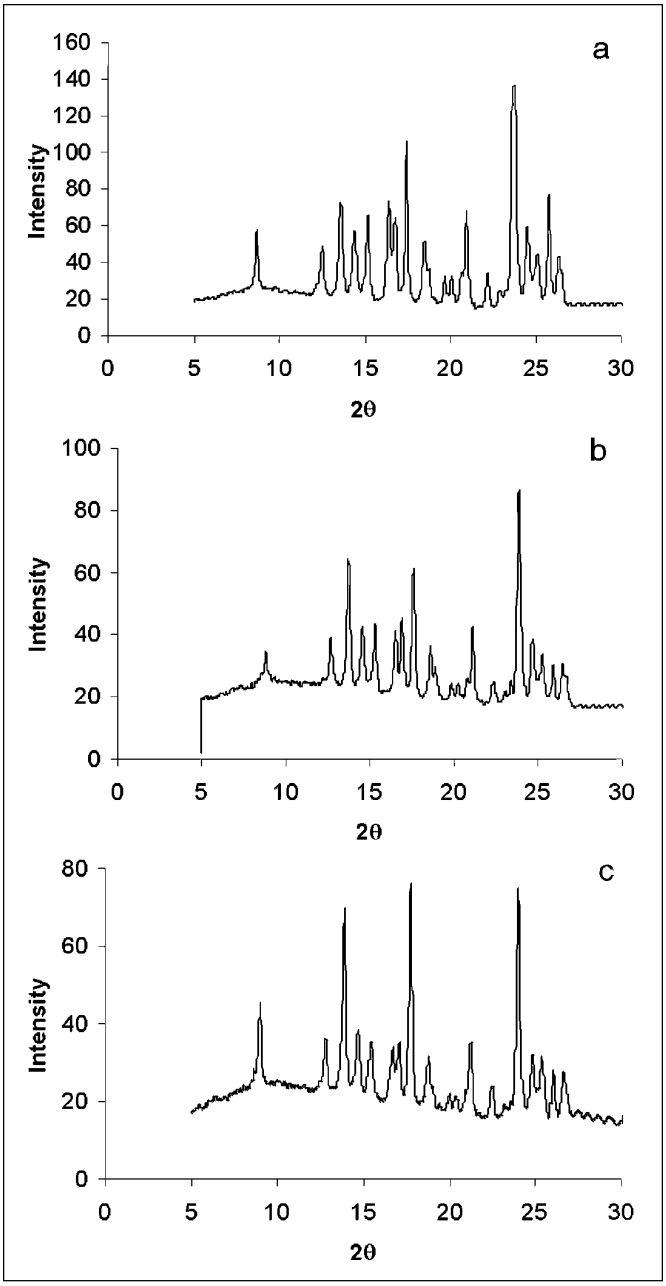

Figure 5—X-ray diffraction patterns of trehalose solutions crystallized at 20 °C (a) without salts, (b) with $MgCl_2 \cdot 6H_2O$ 0.7:1 salt/T ratio, and (c) with $CaCl_2 \cdot 2H_20$ 0.7:1 salt/T ratio

crystals. Although signal positions were significantly modified, the patterns still correspond to trehalose pattern, indicating that no adduct was formed. Patterns also showed that salts did not crystallize in the conditions selected in this study.

FTIR spectroscopy

Figure 6 reports FTIR spectra of trehalose solutions crystallized at 20 °C (a) without salts and with addition of salts in 0.7:1 (salt-trehalose) molar ratio (b) $MgCl_2 \cdot 6H_2O$, and (c) $CaCl_2 \cdot 2H_2O$. In the trehalose spectrum, a region between 3600/cm and 3300/cm corresponds to the O-H stretching modes, whereas a region close to 1600/cm is only attributable to H-O-H bending modes of water. A region between 3000/cm and 2900/cm corresponds to C-H stretching mode, whereas a region between 1200/cm and 1000/cm corresponds to C-O stretching. Positions and intensities of peaks were significantly modified by the addition of both salts, indicating strong interactions with OH and CO. However, the changes found in FTIR spectra did not support the formation of an adduct between trehalose and Mg^{2+} or Ca^{2+}. This conclusion is in agreement with the results found by XRD.

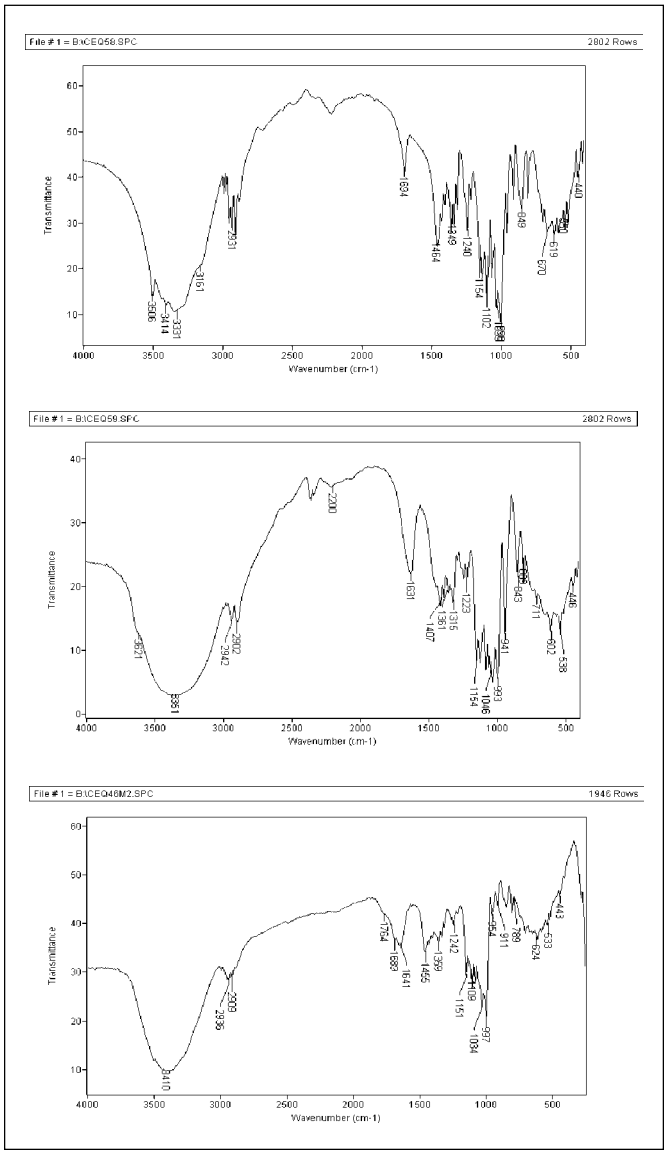

Figure 6—FTIR spectra of (a) trehalose crystals, (b) Ca^{2+}/trehalose 0.7:1 crystals, and (c)Mg^{2+}-trehalose 0.7:1 crystals

Conclusions

The divalent cations Mg^{2+} and Ca^{2+} were able to modify crystallization kinetics of trehalose solutions, delaying nucleation and modifying crystal growth. The effect on nucleation was in agreement with their polarizing character. The effect on growth, however, was strongly dependent on temperature. Both divalent cations, Ca^{2+} and Mg^{2+}, modified crystal morphology because they were incorporated into crystals. Viscoelastic behavior of trehalose solution was dramatically modified by addition of Ca^{2+} because of its ability to form a gel-like structure. The different behavior found when nucleation occurred with and without agitation showed the great importance of processing conditions in the crystallization process of diffusion control systems.

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