for amorphous bulk polymers by Mandelkern, Quinn, and Flory (*10*). In their interesting paper, these authors explained the observed induction period and crystallization kinetics in terms of the concept of concurrent nucleation and crystal growth, just as has been done in the present work.

The present measurements were all made at one temperature. It would be of considerable interest to obtain data at several temperatures in order to determine the temperature coefficients of k and τ and to evaluate the effect of temperature on the crystallization kinetics.

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SUGAR CRYSTALLIZATION

X-Ray Diffractometer and Microscopic Investigation of Crystallization of Amorphous Sucrose

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An x-ray diffractometer method has been developed for determining the crystalline-to-amorphous ratio in a spray-dried sample of sucrose. The results obtained by application of this method to samples stored at 30.0 and 32.5% R.H. are discussed. The effect on the rate of crystallization of adding 5% finely ground crystalline sucrose to a sample of amorphous sucrose before storage at 30% R.H. has been measured. Observations made on these same samples with a polarizing microscope are used in conjunction with other evidence to postulate a mechanism for the crystallization process in spray-dried amorphous sucrose under the conditions of this experiment.

R ESULTS ON THE VARIATION OF MOISTURE CONTENT of amorphous sucrose with time when stored at relative humidities (R.H.) between 5.1 and 29.1% have been presented (*6*). This paper is concerned primarily with the development of an x-ray diffractometer procedure for measuring the extent of crystallization which occurs when amorphous sucrose is stored and determines whether or not the moisture content is a reliable index of crystallization. In addition, the x-ray procedure has been used to make a

quantitative study of the rate of crystallization of amorphous sucrose stored at relative humidities of 30 and 32.5% at 24° C. These particular conditions were chosen because the results of Makower and Dye (*6*) indicated that under these conditions crystallization occurs at a convenient rate.

There does not appear to be a previous investigation of the crystallization of amorphous sucrose. On the other hand a great deal of work has been done on the crystallization of amorphous lactose, because of the importance of the crystallization behavior of this sugar on the storage stability of many milk products. Supplee (*10*) observed that dried milk powder

absorbed water when exposed to a moist atmosphere, but upon standing the water content decreased. Troy and Sharp (*11*) suggested that Supplee's observation was due to absorption of water by the hydrophilic components of dried milk including amorphous lactose followed by the formation of crystalline α-lactose monohydrate. Because lactose monohydrate bound less water than amorphous lactose absorbed under the experimental conditions used, the dried milk powder lost weight when lactose crystallized. Herrington (*3*) has also made a rather thorough study of the transformation of lactose from the glassy to crystalline state, and in particular used the

polarizing microscope to follow the crystallization process. More recently, Henry, Kon, Lea, and White (2) also studied the crystallization of lactose in stored skim milk powder. Among other things they showed that the crystallization of lactose in skim milk powder is very sensitive to moisture content, the rate of crystallization changing many hundredfold for a change in moisture content from 5 to 7% at 37° C. In the case of sucrose, the critical moisture level at room temperature also appears to be in this range.

Experimental

The spray-dried sucrose used in this investigation was prepared by feeding a 43% aqueous solution of sucrose into a large pilot plant spray dryer. The particles varied between about 1 and 10 microns in diameter with an average diameter of about 5 microns. They were completely amorphous when viewed under a polarizing microscope.

A commercial x-ray diffractometer was used to determine the ratio of crystalline to amorphous sucrose in the samples under investigation. The method employed was similar to that used by Christ, Barnes, and Williams (1) for determining the relative amounts of the different types of crystalline penicillins in mixtures. The method consists in making a calibration curve by employing mixtures containing known amounts of the material under investigation and determining the ratio of intensities of a selected peak from the material of interest to a selected peak from a brass standard. Christ, Barnes, and Williams used the peak from a brass block at $2\theta = 49.13°$ for their standard. In this investigation the brass peak at $2\theta = 42.1°$ was used instead because the intensity of this peak was more nearly equal to the intensity of the selected sucrose peak at $2\theta = 24.8°$ when the sucrose sample was completely crystalline.

In all subsequent measurements the height of the brass peak was kept constant by slight adjustment of the wedge in front of the Geiger tube. This procedure compensated for any day-to-day variations in the x-ray tube and electronic circuits and also made it permissible to use the peak height as read off the recorder chart without correction for background or dead-time losses of the Geiger tube.

When this problem was first undertaken an attempt was made to follow the rate of crystallization by observing the change in peak height with time when a single sample was placed on the diffractometer and surrounded by an atmosphere of constant relative humidity. It soon became apparent, however, that this simple procedure was not satisfactory, because some of the crystallites which grew under these conditions were so large that erratic results, owing to preferred orientation, were obtained. A method had to be used, therefore, which included grinding the sample before measurement with the diffractometer. Since amorphous sucrose crystallizes rapidly when exposed to humid air, it was necessary to carry out the sample preparation procedures inside a conventional dry box. The difficulty of working in a dry box and the necessity of screening all of the sample in order to avoid fractionation, plus the fact that the samples were relatively unstable, made it impractical to use a screen smaller than 325 mesh. Particles of this size (~50 microns) are generally considered to be too large to give reliable relative intensities with an x-ray diffractometer (4). It was therefore necessary to determine how accurately the crystalline fraction in partially crystalline sucrose could be measured when 325-mesh powders were used. Extensive tests showed that essentially the same relative intensities were obtained from a 325-mesh sucrose sample as from a sucrose sample finely ground in a ball mill to particle size of about 10 microns. In addition, the same relative intensities and, to within about 5%, the same absolute intensity for a selected peak were obtained from different samples made from 325-mesh sucrose, provided care was used to follow the same procedure in packing the sample into the diffractometer sample holder. When measurements of peak height taken from the automatic recorder tracings for four separate samples were averaged together, the average deviation was reduced to 2.5%. About the same reproducibility was obtained when the area under the peak was used, but since the measurement of peak height was both faster and easier it was adopted in the final procedure.

In the course of these preliminary investigations it was noticed that whereas the relative intensities were the same for two sucrose samples, one of which was prepared from ordinary crystalline sucrose, and the other from spray-dried sucrose which had been allowed to crystallize by an extended exposure to a relative humidity of 30%, the height of the peak at $2\theta = 24.8°$ was about 10% less for the spray-dried sample. A possible reason for this difference was found when powder photographs were taken of these two samples in a conventional powder camera with the sample stationary. The graininess of the diffraction lines on these two photographs was different, and it was evident that the crystallite size was considerably smaller in the spray-dried sample than in the sample prepared from crystalline sucrose, even though both samples had been ground and sieved through a 325-mesh screen. Consequently, it was necessary to use spray-dried sucrose, which had been allowed to crystallize in an atmosphere with a relative humidity of 30%, for the crystalline phase when making known mixtures for the calibration curve.

Calibration Curve The calibration curve was made by plotting the ratio of the peak heights of the selected peaks for sucrose and the standard brass block against the concentration of crystalline sucrose. The data were obtained from mixtures prepared by thoroughly mixing weighed amounts of crystalline and amorphous sucrose in a conventional dry box. After mixing, the sample was placed in a diffractometer sample holder in the manner recommended by McCreery (5). It was immediately removed from the dry box and placed in position on the diffractometer.

A series of four runs was made quickly over the sucrose peak at $2\theta = 24.8°$ at a rate of 1° per minute. This was preceded and followed by two runs over the brass peak at $2\theta = 42.1°$. It was established that the sucrose peak did not begin to change in height because of crystallization of the amorphous phase during the time required to obtain the diffraction data. The average value obtained from four samples was used to determine each point of the calibration curve.

Sample Preparation Approximately 10 grams of amorphous sucrose, which had been stored in a vacuum desiccator over phosphorus pentoxide, was transferred to a large crystallizing dish contained in a vacuum desiccator, which in turn contained sulfuric acid of the appropriate concentration to give the desired relative humidity (9, 12). The transfer was performed in a dry box. The desiccator was then immediately evacuated and placed in a gloved box in which the relative humidity was maintained constant at about 10%. The box was kept in a constant temperature room (24° ± 1° C.). Each day the desiccator was opened and the sucrose stirred for about 10 minutes, after which samples were taken for the x-ray diffractometer, for moisture determination, and for observation under the polarizing microscope. The sucrose to be used for the diffractometer samples was ground in a mortar until it completely passed through a 325-mesh screen. It was then packed in the sample holder in the same way as the samples used for the calibration curve. Duplicate samples were run in every case. All of the above operations were performed inside the constant humidity box.

That portion of the sample to be viewed with the polarizing microscope was placed on a glass slide and covered with a drop of inert oil to slow down the absorption of moisture while under observation and approximately to match the refractive index of the sucrose particles. The moisture content was determined from the loss in weight of dupli-
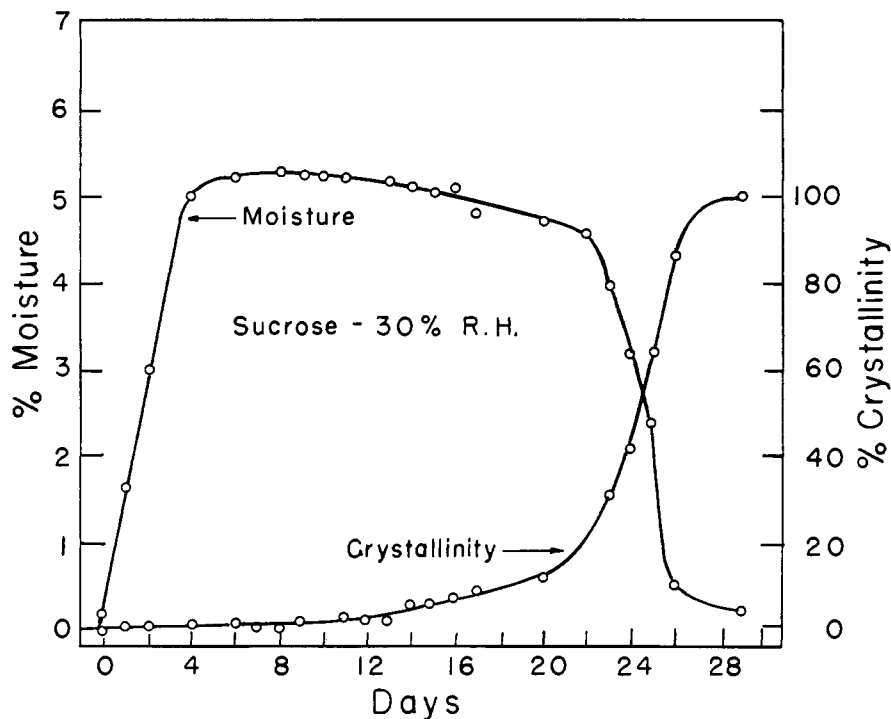
Figure 1. Effect of time of exposure on water content and crystallinity at relative humidity of 30.0% at 24° C.

cate samples placed in a vacuum oven at 105° C. for 16 hours.

Results and Discussion

The results obtained when amorphous sucrose was exposed to 30% R.H. at 24° C. are shown in Figure 1. Both water content and per cent crystallinity as determined by the x-ray diffractometer are plotted against time in days. It is evident from these data that the water content rises immediately and reaches its maximum value by the 6th day. The moisture content then remains nearly constant at 5.3% until about the 12th day, after which it decreases slowly to 4.6% on the 22nd day. A rapid decline in moisture then begins which corresponds to the rapid rise in crystallinity. The reaction is complete by the 28th day.

The decrease in time for complete crystallization with increase in relative humidity is very marked in the neighborhood of 30% R.H. This is illustrated by the results obtained at 32.5% R.H. which are shown in Figure 2. The moisture content of the samples rises rapidly, remains nearly constant for only 1 day, and then falls rapidly. The reaction is complete within 4 days.

A further illustration of the sensitivity of this reaction to relative humidity was obtained by exposing a sample of sucrose to a relative humidity of 33.5%; crystallization was complete in less than 2 days.

The samples of sucrose were observed each day with a polarizing microscope, and photomicrographs were taken for de-

tailed study. Observations made on the sample exposed to a relative humidity of 30% showed clearly that the initial step is the formation of a highly viscous solution. For the first several days the surface of the spray-dried particles were sticky, and when two particles touched they tended to fuse together. As a result the powder showed a decided tendency to cake when left undisturbed for 24 hours. At this stage crystallization had not progressed appreciably, and consequently caking appears to be due to the coalescing of the spray-dried

particles and not to crystallization.

After exposure to 30% R.H. for 7 days, it was observed that the surface of the sucrose particles were less tacky and that the particles acquired an electrostatic charge when the sample was stirred. This behavior is similar to that which would be expected if the surface of each particle were crystalline. If surface crystallization had occurred the crystalline layer would have to be submicroscopic in thickness because crystallization was not detected by observation with the polarizing microscope until the 11th day, which, as can be seen by reference to Figure 1, also coincides with the commencement of crystallization as determined by the x-ray diffractometer. Further evidence that the amount of material which crystallizes between the 7th and 11th day is very small is given by the fact that the moisture content remains essentially constant during this period. After 15 days at 30% R.H. photomicrographs show that crystallization had become general, but only on the surface of the particles. This fact confirms the assumption that crystallization commences on the surface of the particles. The first evidence of the formation of a crystalline crust was not obtained by microscopic observation or the x-ray diffractometer, but by the decrease in tackiness and the change in electrostatic behavior of the particles.

These observations, coupled with the final rapid rate of crystallization, suggest that under the experimental conditions used in this investigation, crystallization of the amorphous particles proceeds in two steps. The surface of the particles crystallizes first, followed by a rapid crystallization of the remaining amorphous sucrose inside each particle. It appears probable that the second step proceeds at a fast rate, because the crystalline crust

Figure 2. Effect of time of exposure on water content and crystallinity at relative humidity of 32.5% at 24° C.
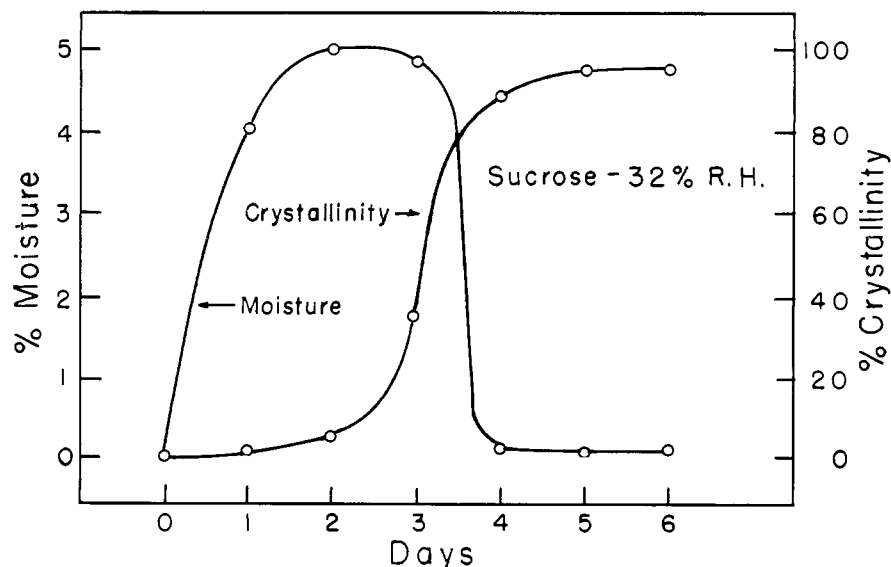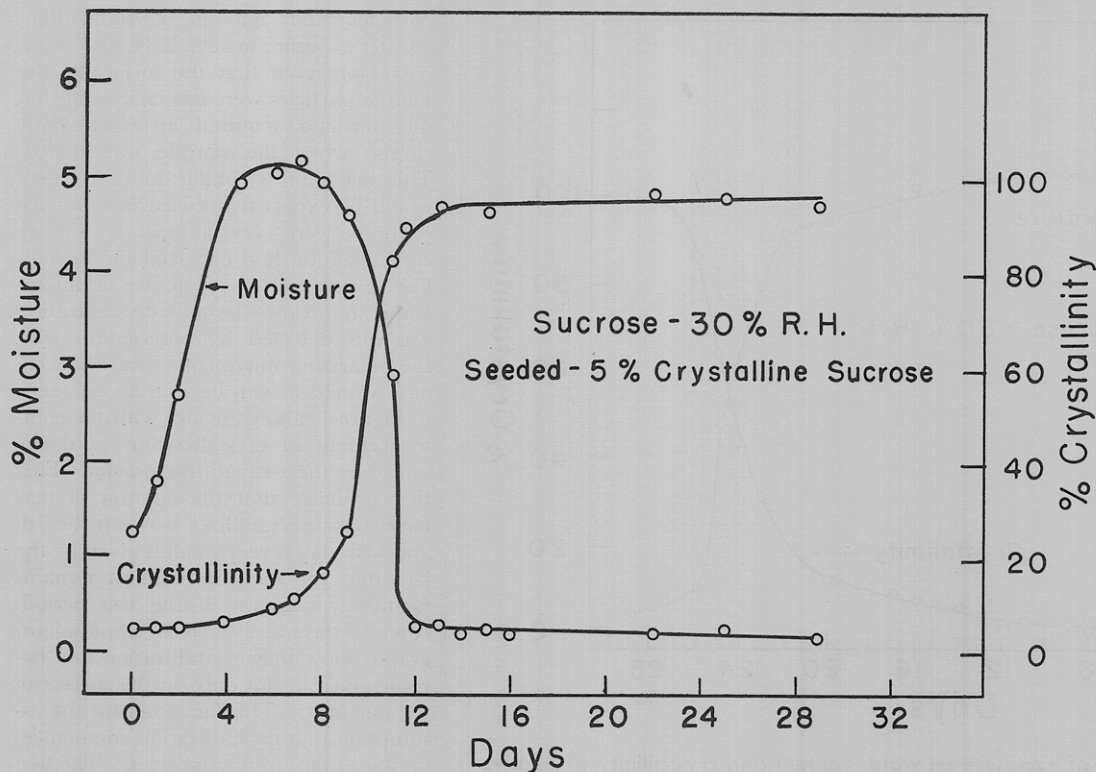
Figure 3. Effect of time of exposure on water content and crystallinity at relative humidity of 30.0% at 24° C.

Amorphous sucrose seeded with 5% by weight of finely ground crystalline sucrose

imposes a sufficient barrier to the diffusion of water released inside each particle owing to crystallization to raise the relative humidity within the particle by a few per cent. This would be sufficient to increase the rate of crystallization very markedly, and in this respect the reaction can be thought of as being autocatalytic.

The effect of mixing 5% by weight of finely ground crystalline sucrose with amorphous sucrose and exposing the mixture to a relative humidity of 30% is shown in Figure 3. The general shape of both the moisture content and crystallinity curve is seen to be similar to the unseeded sample exposed to the same relative humidity, except that crystallization commenced at a much earlier time. In the seeded sample amorphous sucrose began to crystallize rapidly on the 7th day rather than the 20th day as was observed for the unseeded sample and proceeded to completion within the next 5 days.

There appears to be little difference in the subsequent behavior of the 5% seeded sample and the unseeded sample after the latter has been exposed to a relative humidity of 30% for 14 days. This fact is in accord with the explanation suggested above that the initial step in the crystallization process is the formation of a crystalline crust on each particle. When finely powdered crystalline sucrose is added, it adheres to the sticky outer surface of the particles and supplies nuclei for the formation of the crystalline crust.
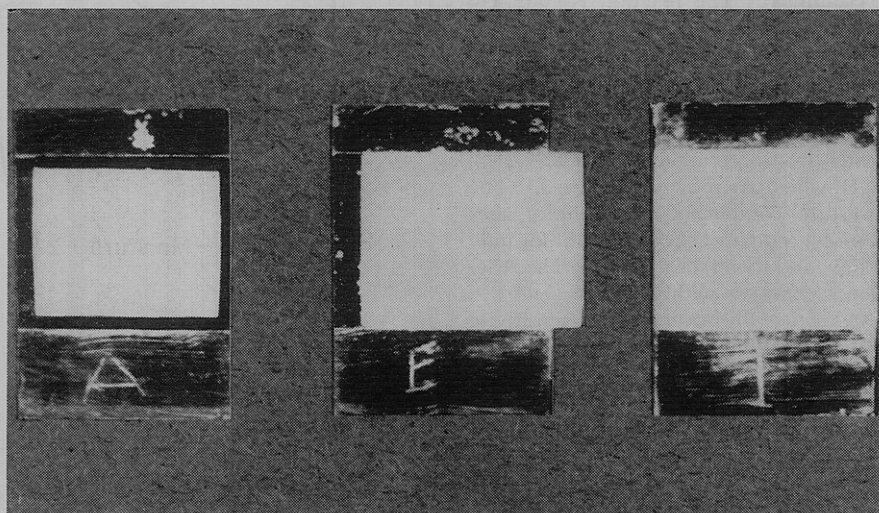
Additional evidence that a crystalline crust occurs on the sucrose particles was obtained by observing the caking properties of the different samples taken each day for x-ray examination. After the determination of crystallinity by the x-ray diffractometer, the samples used were left in their holders overnight exposed to room air, the relative humidity of which was about 50%. During the first several days of the experiment the unseeded samples all underwent marked shrinkage when exposed to room air for 24 hours and formed very hard brittle cakes. The seeded samples showed very little shrinkage under the same conditions. In all cases the samples had completely crystallized after exposure to room air for 24 hours. Figure 4 shows a photograph of three samples which had been exposed to a relative humidity of 30% for 2 days and then to room air for 24 hours. The samples from left to right are unseeded, seeded with 5% crystalline sucrose, and seeded with 15% crystalline sucrose, respectively. The middle sample was displaced to illustrate the fact that it was loosely held in the holder as a result of slight shrinkage. The 15% seeded sample still was held firmly by the brass sample holder and showed no indication of shrinkage.

A quantitative measure of this shrinkage for the unseeded sample was obtained by determining the bulk density of the sample made for the diffractometer and again after the same sample had been exposed to room air for 24 hours. The results are shown in Figure 5. The lower curve is a plot of bulk density of the sample when first removed from the desiccator, whereas the upper curve gives the bulk density of the same

Figure 4. Three samples prepared for x-ray diffractometer

sample after subsequent exposure to room air. The change in bulk density is appreciable in the early stages of the experiment, but as the exposure time to a relative humidity of 30% is increased the subsequent effect of room air on the diffractometer sample is decreased. On the 16th day the sample showed no shrinkage although it had been 90% amorphous on removal from the desiccator.

The crystallization of the surface of the particles evidently prevents them from flowing together when the sample is subsequently exposed to a high relative humidity. After the crust has formed, therefore, the sample does not shrink, and caking does not occur upon subsequent crystallization of the remaining amorphous sucrose.

The rate law derived by Makower and Dye (6) for unstirred samples is not applicable to the data obtained in this investigation because nucleation, in the present study, was induced by stirring. For example, there are no values of τ and k, which, when placed in their equations, can reproduce the long induction period and subsequent fast crystallization rate obtained for the sample held at 30.0% R.H.

The long induction period of the 30.0% R.H. sample is interesting, because it is reasonable to expect a stirred sample to nucleate, and consequently crystallize faster, than an unstirred one. The most likely reason for the longer induction period observed in this experiment as compared to that found by Makower and Dye (6) is the slightly lower (1° to 2°) temperature used in this experiment. It is well known that the viscosities of supercooled amorphous sugars change very rapidly with temperature (8). Since the rate of crystallization of sugars is dependent, among other things, on viscosity, lowering the temperature should increase the induction period.

It has also been shown (7) that the induction period, observed when amorphous bulk polymers crystallize, is very sensitive to temperature, actually varying inversely with the ninth power of ΔT, where ΔT is the difference between two temperatures at which the induction period was determined.

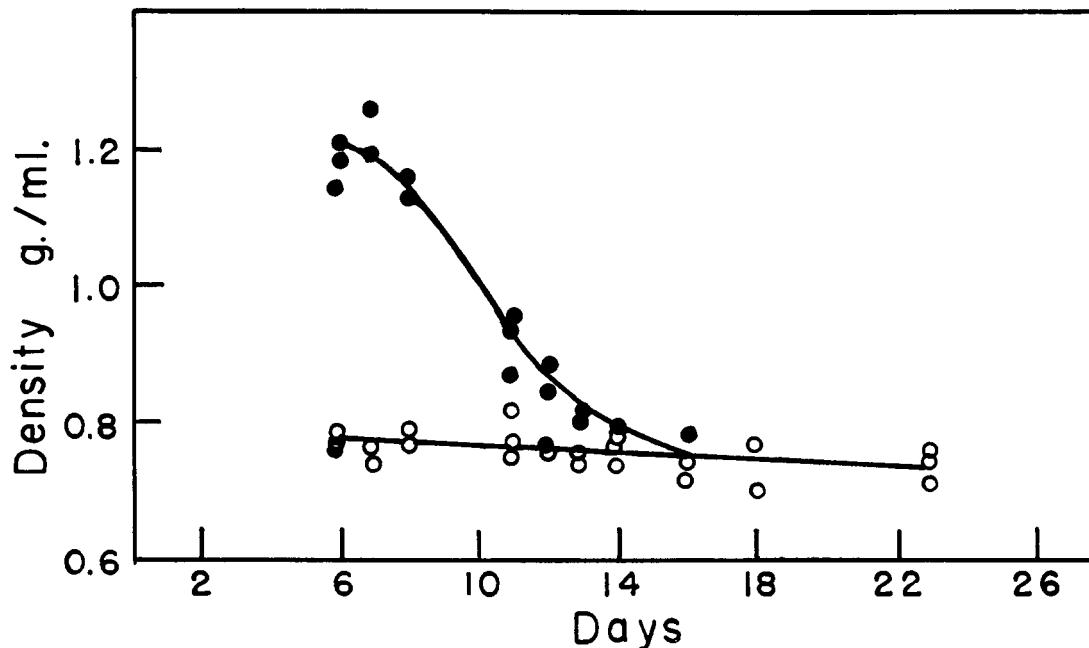The subsequent rapid crystallization rate following the induction period

Figure 5. Effect of time exposure on bulk density of samples

observed in the sucrose samples has been accounted for above by postulating that the water released during crystallization is trapped inside the crystalline-coated particles. Another alternative explanation is suggested by the similarity between the crystallization-time curves observed by the authors for sucrose and those obtained when bulk polymers crystallize (7). In the case of bulk polymers it has been assumed that the radius of the growing centers increase linearly with time. This postulate is not incompatible with those made by Makower and Dye, because the latter are not concerned with a single amorphous sample, but with hundreds of (assumed) independent particles. In this latter system, the crystals can only grow to a size limited by the size of the particles. Since the mass of each particle is small compared to total mass, an average rate of crystallization was used to calculate time required for a particle of average size to crystallize after the particle became nucleated.

The spray-dried sucrose samples used in this investigation actually fall between the two extremes of many small independent particles and one large sample. The particles in the authors' sample were initially larger than the particles in the samples employed by Makower and Dye and became still larger because of coalescing. They did not, however, form a single solid mass. The final rapid crystallization rate, therefore, may not be due entirely to the accumulation of water above the equilibrium value, but may also be due to the ability of the crystallites to grow to a comparatively large size by a mechanism similar to that proposed for bulk polymers.

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