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AN UNSTABLE CRYSTALLINE PHASE IN THE D-GLUCOSE–WATER SYSTEM*

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ABSTRACT

A crystalline phase of D-glucose was isolated from aqueous solution and found by optical-crystallographic study to be distinct from previously known forms of the sugar. Gas chromatography, polarimetric analysis, and hydrate composition indicated that the phase may be a hydrated form of β-D-glucose. In solution the phase is metastable at 38–50°, and is transformed into stable α-D-glucose monohydrate at 32–38°. In the dry state at room temperature, the crystals change to pseudomorphs of the α monohydrate form. The secondary nucleation or "false grain", that often occurs when D-glucose monohydrate crystallizes, is shown to be due largely to separation of this metastable phase, followed by transformation into small, thin crystals of the common form. Because of this behavior, the phase causes much difficulty in the commercial production of crystalline D-glucose.

INTRODUCTION

Previously known crystalline forms of D-glucose are the anhydrous α and β anomers and α-D-glucopyranose monohydrate. Solubilities and phase relationships of these forms have been published by Jackson and Silsbee[1], Newkirk[2], and Young[3]. Although modified crystal habits of α-D-glucose monohydrate have been reported[4], no distinct phases other than the aforesaid ones have been identified up to now.

In the commercial preparation of D-glucose monohydrate by gradual cooling of concentrated solutions, concurrent separation of the anhydrous α form sometimes occurs, which impairs the quality of the final product[5]. However, because of irregular growth, twinning, and modification of crystal habit by impurities, the anhydrous and monohydrate forms of D-glucose are not easily distinguished, under the microscope, by crystal form alone. Hence, during the course of some further work on crystallizing conditions, a study was made of the optical properties of crystals at various stages of

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growth. Under some conditions, composite particles were observed whose properties could not be reconciled with those of twin crystals of α-D-glucose in either the anhydrous or monohydrate form. The identity of these composite crystals, their properties, and the important role they play in the crystallizing operation are reported in this work.

RESULTS AND DISCUSSION

Fig. 1 is a photomicrograph of crystals, obtained by a small-scale laboratory method, after 16 h at 45°. The solution, from enzymic hydrolysis of grain sorghum starch, contained 74% of dry substance, 95% of which was D-glucose. The crystals

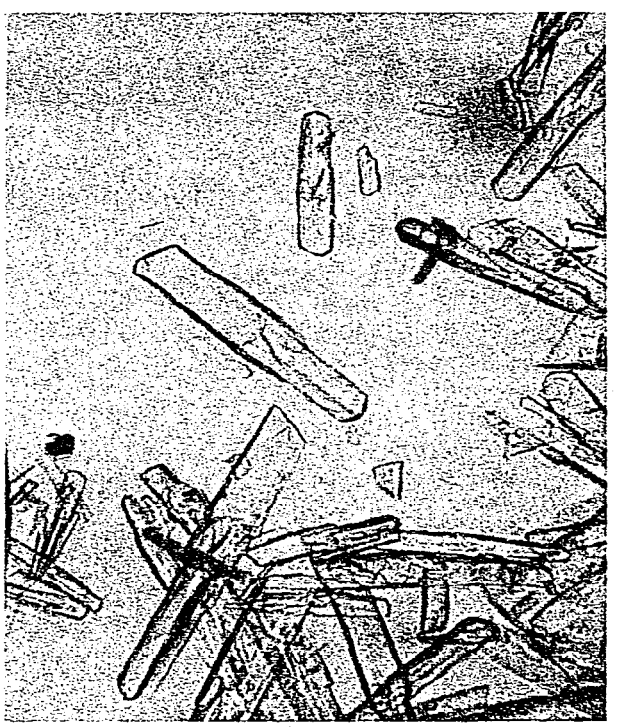

Fig. 1. Composite crystals of D-glucose monohydrate obtained at 45°. Magnification 70×.

appear to be composites of elongated plates and prisms, separated by a characteristic junction or intergrowth. In Fig. 2, a diagram of a typical particle, the plate and prism members are designated I and II for further reference. At first the particles were thought to be twin crystals of the monoclinic α-D-glucose monohydrate, despite an apparent difference in morphology of the two members. The latter could be due merely to hemihedral symmetry, with consequent different crystal habit of opposite faces.

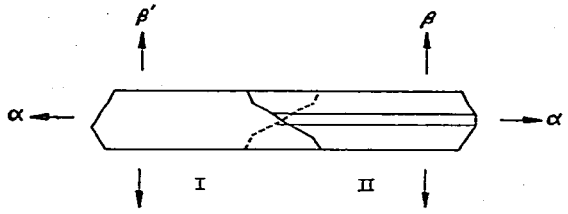

Fig. 2. Typical composite crystal of D-glucose monohydrate. Optical directions.

When the particles were examined in immersion media of various refractive indices, however, distinct differences were observed between the two members. Fig. 2 also shows optical directions for which refractive indices were determined. The index α was 1.520 for both members. Values of β' and β were 1.538 and 1.546, respectiveiy. Member I agrees in this respect with the known α-D-glucose monohydrate. Member II, however, is significantly different. Furthermore, in the immersion medium, form II appeared faintly blue, whereas I was invisible. Evidently the two crystal members differ in dispersion as well as in refractive index. These results indicate strongly that D-glucose monohydrate separates as more than one crystalline phase.

The crystallization experiments were repeated on a larger scale to obtain enough material for general analysis. Solutions from enzymic hydrolysis of corn starch, containing 74–75% of dry substance, 95% of which was D-glucose, were crystallized at 45°. Portions were removed from the crystallizer at intervals during the first 16 h, and separated by centrifuging, washing with methanol, and drying.

The dry crystals appeared under the microscope in the form of composites as previously described, and also as separate, small prisms. Enough of the latter were oriented suitably for determining the three principal refractive indices. Table I summarizes these data, together with corresponding values for the previously known crystalline forms of D-glucose.

TABLE I

OPTICAL-CRYSTALLOGRAPHIC PROPERTIES OF D-GLUCOSE

Compound	*Refractive index*[a]			*Optic axial angle*	*Elongation*
	α	β	γ		
Anhydrous α-D-glucose[b]	1.529	1.556	1.565	−60°	Negative
Anhydrous β-D-glucose[c]	1.544	1.554	1.554	−8°	Positive
α-D-Glucose monohydrate[b]	1.518	1.530	1.557	+68°	Negative
Phase II	1.520	1.546	1.550	−42° [d]	Negative

[a]25°; 5893 Å; all determinations ±0.002. [b]See ref. 6. [c]See ref. 7. [d]Calculated.

Anhydrous α and β-D-glucose both crystallize in the orthorhombic system. The α monohydrate is monoclinic, whereas phase II is probably orthorhombic, as inferred from symmetry of face development and optical properties.

Table I shows that phases I and II differ by 0.016 in the intermediate refractive index β. On the other hand, the crystals illustrated in Fig. 2 differed by only 0.008 in β and β'. This apparent discrepancy is due to the particular orientation of the crystals as they lie on the microscope slide. Crystals of the monoclinic α-D-glucose monohydrate commonly lie on their 001 faces; the optical directions of β and γ are inclined to these faces at angles[6] of about 45°. Consequently, a non-principal refractive index β', intermediate between β and γ, is observed. Phase II, on the other hand, exhibits the principal refractive index β. Because of these small differences in observed refractive indices, the presence of phase II may easily be overlooked.

Three possibilities were considered for the identity of phase II: (1), a hydrated form other than the common monohydrate, such as a hemi- or dihydrate; (2), a polymorphic form of α-D-glucose monohydrate; and (3), some other isomeric form of D-glucose.

Other hydrated forms were excluded by analysis for water of crystallization. *Anal.* Calc. H_2O for monohydrate: 9.09, dihydrate: 16.17, hemihydrate: 4.76%. Found: 9.1, 9.2.

From qualitative microscopic inspection, Sadovyi[8] inferred that α-D-glucose monohydrate exists in two polymorphic forms. As he apparently did not determine the true crystallography of the forms by morphology, optical properties, or X-ray diffraction, it is not clear whether he had observed more than simple differences in crystal habit. In the present work, X-ray diffraction analyses by the powder method were made on the composite particles. No phase of crystal structure different from that of the common monohydrate form was detected. The phase in question, however, was later found to be unstable, and to have reverted completely to the common form before analysis. Accordingly, at this point, the possibility of polymorphism was not ruled out.

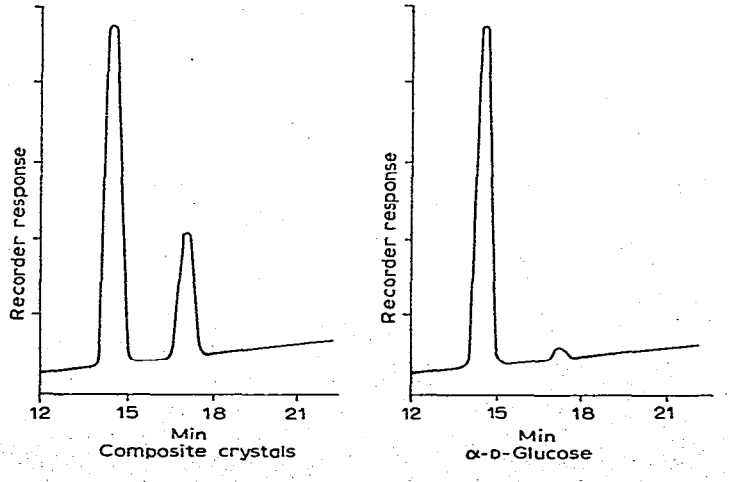

Fig. 3. Gas chromatograms from composite crystals of Fig. 1.

Finally, the possibility of an isomeric form was investigated by gas-chromatographic analysis of trimethylsilyl derivatives prepared from the crystals. This method was used because of the very small amounts of sample needed. Fig. 3 shows two chromatographic peaks for the composite crystals, one corresponding to α-D-glucose, and a second peak corresponding in retention volume to β-D-glucose. The latter amounted to 22%, as calculated from relative areas. The small peak in the curve for pure α-D-glucose is due to slight anomerization during the preparation of the derivatives. The previously known anhydrous β-D-glucose was excluded on grounds of optical-crystallographic properties and of hydrate composition. Since it was not found possible in this work to prepare phase II free of phase I, these and subsequent analytical results must be viewed only as indications of a possible identity of the unknown form. To establish firmly the existence of a hydrated form of β-D-glucose would require extensive analysis of the carefully purified compound.

Dry crystals, collected at various times during the larger-scale preparations, were analyzed polarimetrically to detect any β-D-glucose. During the first 2–3 h of crystallization at a constant temperature of 45°, a maximum of 14% was found. After a longer time the amount decreased, reaching 2–3% in 16 h.

A question may arise as to whether these indications of β-D-glucose could be due solely to small amounts of mother liquor remaining with the crystals upon isolation. Calculation shows, however, that as much as 27% of mother liquor would have to be present to account for the analyses. Since the crystals were free-flowing and very close to the composition of a monohydrate, the analyses should indicate the composition of the solid phase.

If phase II were a hydrated form of β-D-glucose it might be expected to be unstable even in the absence of free water because of the catalytic effect of the water of crystallization. The crystals, in common with those of the α monohydrate form would probably have an appreciable vapor pressure, and as a result a slow $\beta \rightarrow \alpha$ transformation would occur in the solid state, possibly starting at the surface of the crystal and progressing inward. Polarimetric analysis of the material after 24–48 h at room temperature indicated such a transformation proceeding at a rate of 2–3% of that in solution.

Further evidence of instability was found on microscopic examination, after three weeks, of the dry crystals shown in Fig. 1. Phase II had become completely opaque, whereas I was still transparent. The original characteristic junction was still visible, and sharply marked the boundary between opaque and transparent members. Apparently phase II first changes into a transparent pseudomorph and then undergoes gradual microcrystallization, analogous to the devitrification of a glass.

The crystals shown in Fig. 1 are unusually large and well formed. Far more often, the composite particles are very small and difficult to remove from the concentrated syrup in clean, dry form. They can be recognized in contact with the mother liquor, however, by their characteristic shape, as illustrated in Fig. 2. As crystallization continues, member II becomes progressively smaller and more rounded, and I becomes longer. Crystals of phase II appear to dissolve slowly and, at their expense,

small, thin crystals of I appear. Finally, after II has completely dissolved, a vestige of the original junction is apparent as an indentation at one end of I.

Separation of phase II is strongly influenced by temperature and concentration. Below 32° very little tendency to form this phase has been observed. Above 38° phase II forms readily up to 50°, the temperature at which the anhydrous α form begins to separate. Between 32 and 38° is a region of transformation. At concentrations close to saturation little tendency to form phase II is observed, even in the most favorable temperature-range. At higher concentration its occurrence is noticed increasingly.

The phase diagram of the D-glucose–water system[1] shows no inflection point in the monohydrate region except for the transition at 50° to the anhydrous form. The transformation of phase II at 32–38° must therefore be monotropic, that is, no true state of stable equilibrium occurs. Observations during the larger-scale, preparative work support this view. Polarimetric analysis for β-D-glucose indicated a progressive decline of the phase as crystallization continued, even though temperature and concentration were constant.

The effect of impurities usually found in starch hydrolyzates, on the crystal habit of the metastable phase II, is very pronounced. For example, in an experiment on a solution prepared by conversion of corn starch with hydrogen chloride, 90% of whose dry substance was D-glucose, phase II was in the form of long, slender needles. Under high magnification, the needle member showed terminal faces closely resembling those of II in Figs. 1 and 2.

In preparing α-D-glucose monohydrate by slowly cooling concentrated solutions, a serious problem of secondary nucleation often occurs at the expense of uniform growth of the seed crystals. This phenomenon, sometimes called "false grain", renders the product more difficult to separate from the mother liquor and impairs its quality. The cause of the difficulty has previously been thought to be the presence of foreign substances, especially of colloidal nature. Superior methods of purifying the solutions by ion-exchange and other treatment have brought much improvement[9,10]. The problem remains, however, even in the very pure solutions prepared by enzymic hydrolysis of starch[11], or those used for recrystallizing D-glucose monohydrate. To counteract the tendency, cooling is effected so gradually that the solution is always close to saturation[12].

In addition to the previously described laboratory experiments, some microscopic observations were made in the course of process studies on a scale of 200 l or more. Crystals of phase II were clearly identified during the early stage of cooling. On further cooling, excessive nucleation occurred. Conversely, when conditions of temperature and concentration were adjusted so that the metastable phase did not appear, the nucleation was much less. These observations, and the previous laboratory studies, point to a causal relationship between the occurrence of phase II and the incidence of excessive nucleation. The exact mechanism of the nucleation is not clear, but is probably related to the formation of a pseudomorph, as described earlier in this work. Upon subsequent dissolution, these pseudomorphic particles may release

large numbers of submicroscopic crystals of α-D-glucose monohydrate, which act as seeds to produce the small, thin crystals met in practice. The expedient of gradual cooling, to ensure a low degree of supersaturation, may well be to maintain the concentration below that at which the metastable phase II can separate.

If solutions are crystallized below 32°, separation of the metastable form can be avoided, even at moderate degrees of supersaturation. Practical means for maintaining the desired supersaturation, and at the same time keeping well below this limiting temperature, are disclosed in a recent patent[13]. Uniform growth of the stable α-D-glucose monohydrate is readily accomplished. Some tendency for secondary nucleation still occurs, but the small crystals are of the same form as the larger ones, with no evidence of the long, thin blades seen at higher temperature. Furthermore, because they are far less numerous, these small crystals can grow to normal size after slight adjustment of the conditions of temperature and concentration.

EXPERIMENTAL

Materials. — D-Glucose solutions were prepared by enzymic hydrolysis of grain sorghum or corn starch[13]. These were finally concentrated, under vacuum, in a circulating laboratory evaporator.

Apparatus. — A Submersion Rotator (Scientific Products, Evanston, Ill.) was fitted with 1.6 × 15-cm stoppered test tubes and rotated in a constant temperature water bath at 4 r.p.m. For larger-scale studies, a water-jacketed, stainless-steel crystallizer of 20 l capacity was used. Water from a constant-temperature bath was circulated through the jacket. A horizontal ribbon-agitator turned at 4 r.p.m.

Procedure. — Small-scale crystallization tests were made in test tubes after seeding with small amounts (0.1%) of wet crystals. The latter had been prepared beforchand by crystallizing at a lower temperature. Crystals were separated from the mother liquor by mixing with three volumes of 80% aqueous ethanol, filtering on sintered glass, washing with 95% ethanol, and drying. Larger-scale tests were made by successive crystallization of 20-l amounts of hydrolyzate, a 10% portion of each being used to seed the following one. Smaller amounts of seed gave crystals too fine to isolate from the syrup in clean, dry form. Crystals were separated by first diluting with a solution of D-glucose of lower concentration to decrease the viscosity of the mother liquor, filtering in a stainless-steel basket centrifuge, washing with anhydrous methanol, and drying. Methanol was used because of the definite small solubility of D-glucose in this solvent, with consequent lesser likelihood that residual mother liquor would separate as a heavy phase or amorphous precipitate.

General methods. — Starch hydrolyzates were analyzed for D-glucose by the D-glucose oxidase method[14]. Dry substance was determined by refractive index at 45° and reference to appropriate tables[15]. Gas-chromatographic analysis of trimethylsilyl derivatives was carried out by the method of Sweeley, *et al.*[16], modified according to Brobst and Lott[17]. A Hewlett–Packard model 810 gas chromatograph, equipped with a flame-ionization detector and provision for linear programming of temperature

was used. Columns, 6 mm × 30 cm were packed with 3% silicone rubber SE-58 on Chromosorb W. Temperature was programmed from 120 to 300° at 8° per min. Pure α-D-glucose always gave a small peak corresponding to β-D-glucose and amounting to 2–3% of the total. The polarimetric method of Hudson and Dale[18] was used for β-D-glucose. About 4 g of material, on anhydrous basis, was dissolved in water at room temperature and made up to 100 ml. Optical rotation was measured at various timed intervals and initial rotation obtained by extrapolation to the moment of dissolution. From accepted values of +18.7 and +112.2° for the β and α pyranose anomers, respectively, the composition was calculated. Water of crystallization was determined gravimetrically by heating for 16 h in a vacuum oven at 80°. Optical-crystallographic properties were determined with a Vickers polarizing microscope by common methods of chemical microscopy[19]. Immersion media were mixtures of dibutyl phthalate and α-bromonaphthalene, which had been checked at 25° with a precision Abbé refractometer.

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