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[CONTRIBUTION OF THE NORTHERN REGIONAL RESEARCH LABORATORY[1]]

Starch Granule Swelling in Water Vapor Sorption

BY N. N. HELLMAN, T. F. BOESCH AND E. H. MELVIN

Microscopic measurements of the swelling of individual starch granules occurring with the sorption of water vapor at various relative pressures are reported for corn, potato, tapioca and waxy corn starch. The linear granule swelling in a water-saturated atmosphere over the vacuum-dry dimension is as follows: corn, 9.1%; potato, 12.7%; tapioca, 28.4%; and waxy corn, 22.7%. For all except tapioca starch, there is practically no hysteresis in the function of swelling *vs.* relative humidity of the atmosphere with which the starch is equilibrated. For all starches the function of swelling *vs.* moisture content shows an absorption–desorption loop with the desorption leg giving smaller granule dimensions for equal water content.

As part of an investigation of the water-sorptive properties of starch, it became of interest to determine the volume changes accompanying the sorption of water vapor. After preliminary studies, density methods were abandoned owing to ambiguity in interpreting the results when water was used as an immersion medium and difficulties of wetting, granule penetration, and degassing for non-aqueous immersion media. To avoid such uncertainties it was decided to follow the dimensional changes of individual granules directly. The starch granules were mounted in a conditioning chamber and microscopic measurements were made as they absorbed or desorbed water vapor to come into equilibrium with salt solutions.

Experimental

Materials.—Corn, potato, tapioca and waxy corn starches were chosen for investigation. The corn, potato and waxy corn starches were extracted at this Laboratory. The starch was separated from the ground-up source material by use of distilled water. Drying temperature of the extracted products in processing did not exceed 45°. The tapioca starch was a first-grade commercial starch.

Apparatus and Procedure.—The following techniques, though somewhat elaborate, were found to be necessary to obtain meaningful experiments. It was found early in this work that the increase in linear swelling which occurred when starch sorbed water vapor between two suitable low humidities may be only 1–2% of the vacuum-dry dimension. For the same humidity interval at high humidities, however, the linear swelling may be 5–10% of the vacuum-dry dimension. To demonstrate the approach and attainment of equilibrium for the small swellings at low humidities, it was therefore necessary to measure the granule dimensions with a very high precision, whereas for the large swellings at high humidities it was also necessary to control precisely the vapor pressure and temperature of the water vapor. A large number of simpler experimental arrangements were tried before success was achieved with the following apparatus and procedures.

A conditioning chamber, the thickness and length of a microscope slide, was constructed of brass. One-eighth-inch brass tubing rolled flat to $^1/_{16}$ inch (the thickness of the slide) was soldered flush into the slide and led to a $^1/_2$-inch diameter central hole. The central hole was covered top and bottom by $^3/_4$-inch circular cover slips which fit into recesses in the slide surface and were cemented to the slide by Plicene. A metal stop, covering two-thirds of the center hole in the slide and the same thickness as the metal slide in the region undercut to accept the cover slips, was inserted to prevent collapse of the cover slips under vacuum.

Before being cemented to the brass slide, the under side of the top cover slip was brushed with a very thin coat of a dilute mucilage solution. When the mucilage was nearly dry, the starch sample was dusted lightly onto the cover slip. The point of attachment of the starch granules to the mucilage layer could be seen with the microscope to be very small, yet this mounting procedure prevented the granules from moving in the subsequent evacuations and humidifications and presenting different profiles.

After assembly, the conditioning chamber was attached snugly to the microscope stage by short brass lugs screwed into the stage. A temperature-controlling coil was taped over the slide. The coil consisted of $^1/_8$-inch copper tubing bent into flat loops through which water could be circulated from a thermostat. The coil was shaped to allow free movement of the microscope objective in the region of observation.

The remainder of the equilibration apparatus was built around a T-bore, three-way stopcock. One arm was connected to a rotary oil vacuum pump, the second arm to a standard taper joint which connected to a water-jacketed small container for salt solution, and the third arm to the conditioning chamber on the microscope stage. A mercury manometer was connected to the arm going to the slide to permit measurement of the water vapor pressure at equilibrium. To permit flexibility of movement of the microscope, the entire apparatus was carried on a rod bolted to the microscope stage. The two portions of the equilibration apparatus were attached to each other by a very short section of rubber tubing wired at each end to assure a tight connection.

For the microscopic measurements, an oil immersion 97 × objective and a sensitive movement 15 × ocular micrometer with traveling spider silk bifilar cross hair were used. Because of the spherical shape of the starch granules, the apparent granule diameter changed with position of focus. It thus became necessary to make all measurements at a fixed depth of focus. To achieve this, the sample was illuminated with monochromatic light from a sodium vapor lamp that intensified the normally objectionable diffraction halos around each granule. It was then relatively simple to focus to a constant diffraction pattern about the granule, measure at the apparent granule boundary, and in this manner to achieve unusually high precision in the dimensional measurements. Ten readings of each dimension were customarily taken. The standard deviation of the measurements varied from 0.006 to 0.01 unit of the micrometer drum, and the standard deviation of the mean of the 10 readings was 0.002 to 0.003 unit. Since the average granule diameter measured was about 5 drum units, the precision of the dimensional determinations was about 0.1%. The calibration of the microscope equipment used was 1 drum turn = 0.002314 mm.

Equilibrations were accomplished by evacuating the salt solutions and slide simultaneously until the vapor pressure of the salt solution was reached. The stopcock of the equilibration system was then turned to connect the salt solution to the slide with the maintenance of the vacuum. Water from a thermostat at 25.10 ± 0.05° was circulated continuously through the salt solution jacket and the temperature controlling stage coil. Approximately five to eight hours were required for equilbration although generally 24 hours were allowed. Each granule measured was gener-

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

ally cycled at least twice through sorption and desorption unless the experiment was interrupted by mishap. The dimension of a single granule was independent of the number of cycles, reproducibility being as good as that obtained in repeated readings of a granule at one stage of a cycle. The variability of the individual granules was determined in 9 different granules of corn starch and 5 different granules of waxy corn starch. At any given humidity, maximum variability in swelling found among the granules of each starch was 2% of the vacuum dry dimension. Since the granules were measured in random orientation, these data also serve to show that swelling is approximately isotropic. The authors extend this conclusion to tapioca and potato starches as well, since starch granules are spherocrystals which would ideally show isotropic swelling. Nevertheless, it is recognized that potato starch granules, which are egg-shaped, may show some anisotropy. Corn starch granules from the floury and horny endosperm did not differ in swelling.

The water sorption isotherms of the starches were determined gravimetrically to correlate granule dimension with water content. The equilibration procedures have been previously reported.[1] Moisture contents are reported to 0.01% to indicate the precision (maximum range of duplicates = 0.05%) which was found in the determination of water contents. However, it is recognized that the inability to control relative humidity and sample history will limit the accuracy of these data to approximately 0.1% of water.

Results

The swelling of corn, potato, tapioca and waxy corn starch occurring when water vapor is sorbed from atmospheres of different moisture content is shown in Table I. The results reported are for

TABLE I

SWELLING OF STARCHES UPON SORPTION OF WATER VAPOR

Relative humidity, %	Increase over vacuum-dry diameter, %							
	Corn		Potato		Tapioca		Waxy corn	
	Absorption	Desorption	Absorption	Desorption	Absorption	Desorption	Absorption	Desorption
8		1.5	1.9	2.2	3.6	1.1	1.5	1.8
20	1.9	1.9	4.0	4.1	4.6	2.4	5.2	4.5
31	2.6	2.5	5.4	5.8	5.5	3.9	7.0	6.4
43	3.3	3.3	8.0	8.0	7.6	5.2	9.1	8.9
58	4.1	4.1	9.4	9.4	9.6	7.3	10.7	10.6
75	5.4	5.5	10.3	10.4	12.5	11.4	13.5	13.3
85		6.6		11.0	16.8	16.1	15.9	15.3
93	7.3		11.6	11.7	22.8	21.9	18.5	18.8
100	9.1		12.7		28.4		22.7	

single granules each of corn, potato and tapioca starches whose vacuum-dry granule diameters were 16.96, 38.23 and 20.16 μ, respectively. The waxy corn starch data are the average of three granules whose vacuum-dry granule diameters were 12.22, 9.66 and 9.89 μ. In other experiments in which mishaps prevented obtaining the complete absorption–desorption cycles, eight other corn starch granules, two other waxy corn starch granules, and one other granule each of potato and tapioca starch were measured. The swelling in the portions of the cycles obtained agree with the reported data within 2% of the vacuum-dry diameter. Swelling data in the range of 0–85% R.H. for six granules of another sample of corn starch, which had been prepared from corn of the following year's crop, showed linear swelling above 43% R.H. from 1–4% greater than the reported values.

The amount of water which these starches sorb under similar conditions has been previously reported for corn, potato and tapioca starch.[2] The water sorption isotherm for waxy corn starch is shown in Table II. The water sorption from saturated vapor is difficult to determine. However, the most careful equilibrations yield the following water contents: corn, 39.9%; potato 50.9%; tapioca 42.9%; and waxy corn, 51.4%. By combining the swelling and water sorption data at a given relative humidity, the relation between swelling and moisture content for the starches can be obtained, and is shown in Fig. 1.

TABLE II

SORPTION OF WATER BY WAXY CORN STARCH AT 25°

Relative humidity, %	—H₂O, (g./100 g. dry starch)— Absorption	Desorption
8	4.53	7.19
20	8.67	10.49
31	10.44	12.34
43	12.37	14.44
58	14.78	16.90
75	18.55	19.68
85	21.71	22.70
93	27.71	29.66

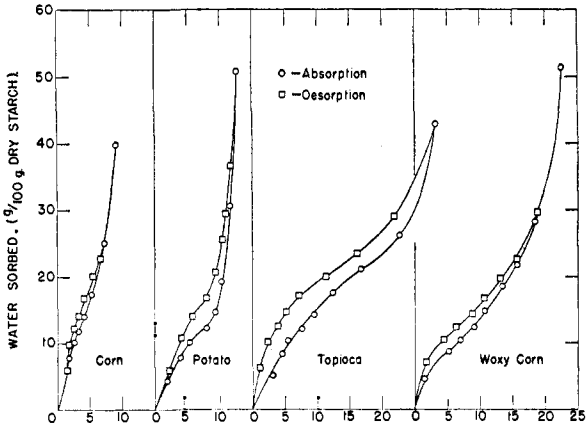


Fig. 1.—Swelling of starches as a function of water content.

Discussion

The painstaking and tedious nature of these measurements limited the number of granules which were measured, and only one source was used for each type of starch. Since the variability between the different granules of the same starch is small in comparison to the observed swelling, it is felt that the conclusions which follow can be safely drawn; nevertheless, one should consider them of an exploratory nature.

A marked difference was found in the swelling exhibited by the four starches at 100% R.H. The relatively small amount of linear swelling of corn starch, 9% in a water-saturated atmosphere over vacuum-dry diameter as compared to 28% observed for tapioca starch, indicates considerable difference in the physical structures of the starch granules and the structural disposition of the sorbed water. Conversely, similar swelling properties of tapioca starch and waxy corn starch may indicate similar physical structure on a sub-micro but supermolecular scale, despite their known chemical difference. Waxy corn starch is composed almost

(2) N. N. Hellman and E. H. Melvin, THIS JOURNAL, 72, 5186 (1950).

completely of the branched polymer, amylopectin; whereas tapioca contains 17–19% of the linear polymer, amylose.

The relative lack of dimensional hysteresis as a humidity (Table I), particularly in the case of corn and potato starch, is unusual, especially in view of the known hysteresis of the water sorption as a function of relative vapor pressure. In tapioca starch where a sorption–desorption loop is present, the desorption results in smaller swelling than does absorption.

In the curves of swelling as a function of water content (Fig. 1), an absorption–desorption loop is prominent in the data for all starches, the desorption values lying anomalously below the absorption curve. Similar anomalous "hysteresis" has been reported for the length swelling of cotton[3] and for the length swelling of human hair[4] as a function of humidity, yet the mechanism for such "shrinking" behavior seems obscure.

Assuming the specific volume of starch to be 0.67, as indicated by pycnometric densities in water, and that volumes are additive in the sorption process, the linear expansion for isotropic swelling accompanying a 1% addition of water would be 0.5%. The initial swelling of dry potato and tapioca starch occurs at approximately this rate. The swelling at high moisture contents may also occur at this rate, but the effect of intergranular porosities on increasing the water sorption of bulk starch at high humidities prevents our obtaining the relation between swelling and granule water content in the highest moisture range. Since calculations indicate that significant amounts of water can be held in intergranular porosities only above 99% R.H., this ambiguity exists for only the last 3–5% of the water sorbed.

The greater portion of the swelling curves of the starches reported exhibits smaller or larger rates of swelling than above calculated. A smaller rate of swelling could result from sorption occurring in voids. To account for the small swelling of corn and waxy corn starch at low humidities, however, the voids must extend to molecular dimensions. A rate of swelling larger than above calculated could result from hydration occurring at junction points of a three-dimensional molecular network causing an opening up of the structure beyond the volume of the water introduced. Such an assumed three-dimensional network structure would also explain the oberved anomalous "hysteresis" in the function of swelling *versus* water content in a manner similar to the "ink bottle"[5] explanation of hysteresis in the function of vapor pressure *vs.* water content. Extent of hydration of the junctions of the starch polymer chains would determine the extent of swelling. Then, for the same granule size on absorption, only the junctions would be hydrated, whereas on desorption the junction and its associated void would be filled with water.

Acknowledgment.—The authors are grateful to Dr. Majel M. MacMasters for providing the starch samples for this work.

(3) G. E. Collins, *Textile Inst. J.*, **21**, T311 (1930).

(4) H. T. White and P. B. Stam, *Textile Res. J.*, **19**, 136 (1949).

(5) S. Brunauer, "The Adsorption of Gases and Vapors," Princeton University Press, Princeton, N. J., 1945, p. 398.

PEORIA, ILL. RECEIVED AUGUST 30, 1950

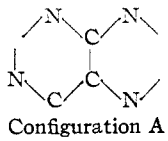
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING OF THE UNIVERSITY OF PENNSYLVANIA]

Studies in Imidazoles. II.[1] Imidazo[b]pyrazines[2,3]

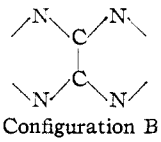
BY EDGAR SCHIPPER[4] AND ALLAN R. DAY

Imidazo[b]pyrazines, members of a previously unknown ring system, were prepared and several derivatives were tested as potential antimetabolites. The synthesis of the imidazo[b]pyrazines was accomplished by reactions of the corresponding diaminopyrazine with ethyl orthoformate, acyl halides and urea, respectively.

In the first paper of this series[1] the synthesis and chemical reactions of imidazo[b]quinoxalines were described. It was pointed out that these compounds possessed the oxamidine moiety (Configuration B) which bears close structural analogy to Configuration A, a grouping present in the ring systems of a variety of essential metabolites.

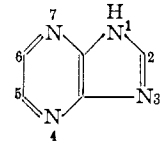


Configuration A



Configuration B

Another type of molecule containing Configuration B is the hitherto unknown ring system of imidazo[b]pyrazine



This molecule, as can be seen, is a "cross" between purines and pteridines, retaining the imidazole moiety of the former and the pyrazine ring of the latter. Furthermore, imidazo[b]pyrazines possess an apparent structural relationship to benzimidazoles, one member of which, 5,6-dimethylbenzimidazole, recently has been shown to constitute an

(1) For the first paper in this series see E. Schipper and A. R. Day, THIS JOURNAL, **73**, 5672 (1951).

(2) From a thesis submitted February, 1951, by E. Schipper to the Department of Chemistry and Chemical Engineering of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(3) Presented in part at the 118th Meeting of the American Chemical Society in Chicago, Ill., September 8, 1950.

(4) National Institutes of Health Predoctoral Research Fellow 1949–1950.