

Dissolution of Starches in Dimethylsulfoxide and Variations in Starches of Several Species, Varieties, and Maturities¹

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ABSTRACT

Solubilization of starch in dimethylsulfoxide (Me_2SO)-water solutions was characteristic of the species, variety, and maturity of the plant of its origin. Starches from waxy corn (*Zea mays*), potato (*Solanum tuberosum*), rice (*Oryza sativa*), and several varieties each of navy bean (*Phaseolus vulgaris*) and lima bean (*P. lunatis*) were studied by microscopic, turbidimetric, and spectrophotometric-birefringence methods during dissolution in Me_2SO -water solutions. Differences in solubility properties include the length of the initiation period, during which no visible change occurs; the rate of swelling; the rate of loss of birefringence; the rate of dissolution; and the optimum Me_2SO -water concentration required to dissolve the starch. High concentrations of Me_2SO dissolve the starch granule from the outside whereas the mass of the granule was more soluble in lower concentrations of Me_2SO . Chemical analysis of the remnant from 85% Me_2SO indicates that it is highly branched amylopectin. The energy of activation for dissolution of swollen starch was approximately 24,000 calories.

Several publications have appeared on the solubility of starch in dimethylsulfoxide (Me_2SO) (1-3). They indicate a range of Me_2SO -water concentrations which will solubilize starch. Information on the difference in solubility properties of starches from various species was presented by Leach and Schoch (3), who also presented several photomicrographs of starch in Me_2SO .

Microscopic examination of bean starches in Me_2SO indicated differences in the behavior of starch from different bean varieties. Slight variations in Me_2SO -water concentrations greatly influenced the solubility. This paper describes the result of turbidimetric observations and the determination of other kinetic data for the dissolution of various starches in Me_2SO . Finally, several structural observations were made.

EXPERIMENTAL

Materials

The navy bean (*Phaseolus vulgaris*) and lima bean (*P. lunatis*) starches were prepared in this laboratory according to the method for corn starch of Watson (4) except that no 325-mesh screen was used. For comparative purposes three additional starches were selected: The White waxy corn (*Zea mays*) starch was produced by the Northern Regional Research Laboratory in 1950 using a sulfur dioxide steep. The potato (*Solanum tuberosum*) starch was purchased from Mallinckrodt and the rice (*Oryza sativa*) starch from Matheson, Coleman and Bell. The histories of the latter two starches are unknown. All of the starches were dried *in vacuo* over Drierite at room temperature.

¹Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

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A Walther-Slaubtechnik vortex classifier set at 30 m.³ per hr. was used to separate the fine starch grains from the coarse. Samples for the ground starch experiments were pulverized in a Crescent Dental Mfg. Co. Wig-L-Bug.

The Me₂SO, purchased from J. T. Baker Chemical, was labeled as containing 0.003% water. All Me₂SO water solutions are given as volume Me₂SO per volume of solution.

Since Me₂SO is hygroscopic, the rate of water uptake under the same atmospheric conditions used for our other experiments was determined with a Cary 90 infrared spectrophotometer at 3,500 cm.⁻¹ Over an 18-hr. period the average uptake was 0.02% water per hr. The standard was 95% Me₂SO. The Me₂SO, as received from the manufacturer, was stirred in a water bath at 39.7° C. during the test period.

Turbidimetric Measurements

All turbidimetric measurements were made on a Cary 14 spectrophotometer at 630 nm. A 1-cm. optical path flow cell was connected with Viton tubing to a reservoir in a thermostatic bath. The starch suspension was circulated at 125 ml. per min. with a Cole-Parmer Masterflex pump. Only 50 mg. each of the waxy corn, green Fordhook fines, and rice starches in 25 ml. of solvent were used. All other turbidimetric studies were done with 100 mg. of starch in 25 ml. of solvent. The observed optical density³ is a measure of the light scattered out of the optical path by a uniform suspension of starch grains, and obeys the Bouguere-Beer law for concentrations low enough that multiple scattering and shadowing are not important. Optimum solvent concentrations were determined at 47° C.

Birefringence Measurements

The birefringence measurements were made with the same circulation system, except that the optical cell was a vertical Pyrex tube of 3 mm. i.d. in the center of the compartment. The dimensions of the light beam at the tubular flow cell were reduced by inserting a plano-convex, fused silica lens of about 17 mm. clear aperture and 40 mm. focal length mounted in a 25.4 mm. diameter barrel in the V-block in the sample compartment with the plane lens surface about 25 mm. from the sample cell axis. After passing through the sample cell, the diverging beam and part of the scattered light were collected by a symmetrically placed similar silica lens and transferred to the detector through the standard optics.

Polaroid HN32 sheet, located in crossed orientation at each of the two fused silica lenses, gave an absorbance of 3.0 at 582 nm. when the cell was filled with 85% Me₂SO. For convenience, the instrument reading was reduced to 2.0 by means of a screen in the reference beam. When uniform birefringent grains are introduced between crossed polarizers, the light intensity passed by the second polarizer (analyzer) is proportional to the number of grains in dilute suspensions. Since our recordings were made on a logarithmic scale, the recording was replotted as percent transmission. A standard curve was drawn which showed a positive straight line relationship between percent transmission and concentrations from 5 to 200 mg. in 25 ml. of solvent.

³The term "optical density" is appropriate here rather than "absorbance", because the starch has almost no absorption at 630 nm.

Preparation of Starch Fractions

A 4% suspension of California small white bean starch in 85% Me₂SO was warmed to 50° C. with stirring and left overnight to facilitate solution. The next day it was centrifuged at 12,000 × g for 20 min. to precipitate the membranous remnants. Fresh 85% Me₂SO was added to the precipitate and it was warmed, stirred, and centrifuged. The washing with Me₂SO was repeated once more. The precipitate was then washed three times with absolute ethanol. Two volumes of absolute ethanol were added to the soluble matter from the first extraction with 85% Me₂SO-water. After standing a few minutes it was centrifuged and the precipitate washed twice with ethanol. These fractions were used for iodine staining and periodate oxidation.

Periodate Oxidation

The starch fractions were oxidized according to the method of Potter and Hassid (5).

Calculations

The graphic method was used to obtain the k value for dissolution and birefringence (Fig. 1) (6). Only the portion of the curve showing a decrease in optical density or birefringence was considered for these calculations. The dissolution values shown in Figs. 2 and 3 are half-lives (6). The "k" values for maximum optical density are the simple reciprocals of the time required to reach

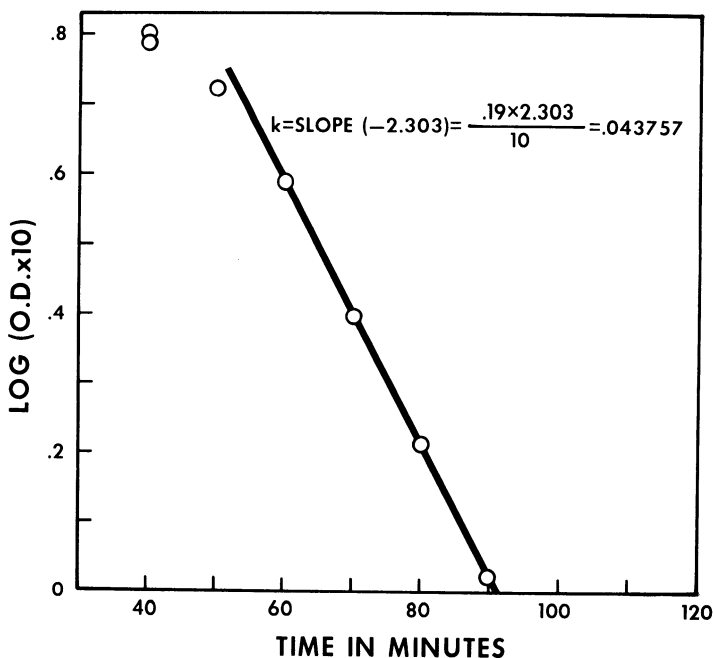


Fig. 1. Example of graphic determination of k. Graph shows dissolution of California small white bean starch in 85% Me₂SO at 39.7°C.

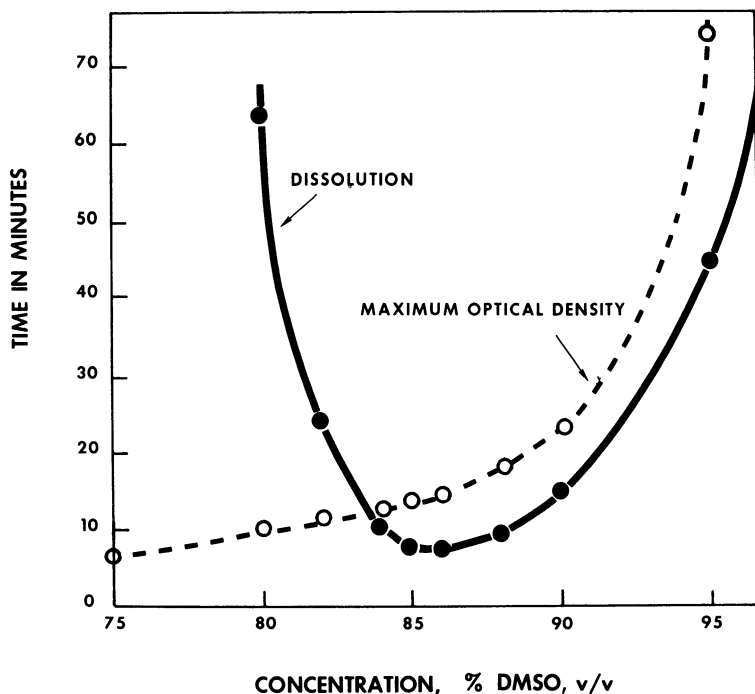


Fig. 2. Solvation of California small white bean starch in Me_2SO -water solvent at various concentrations and 47°C .; half-lives for dissolution and time to reach maximum optical density.

maximum optical density. In determining the birefringence k values, spectrophotometer readings were converted to concentrations.

Microscopic Examination

Starches were examined by phase, dark-field, bright-field, and polarized light microscopy. Only phase microscopy pictures are included in this paper, but the results of examination under the other conditions are discussed.

RESULTS AND DISCUSSION

In determining the optimum solvent concentration, both the time required to reach maximum optical density before dissolution reduced it and the rate of dissolution of half of the remaining starch were taken into account. The optimum concentration of 85% Me_2SO was chosen for the study of California small white bean starch (Fig. 2). All of the other starches were also studied in 85% Me_2SO so that the data for the several starches would be comparable. However, 85% Me_2SO is not the optimum concentration for all starches (Fig. 3).

Waxy corn, potato, and rice starch were included in our study so that the data could be compared with earlier reports on the solubility of starches in Me_2SO (1-3). Figure 4A shows how greatly the rate of solvation in Me_2SO differed among these starches. The initial optical density is inversely related to the size of

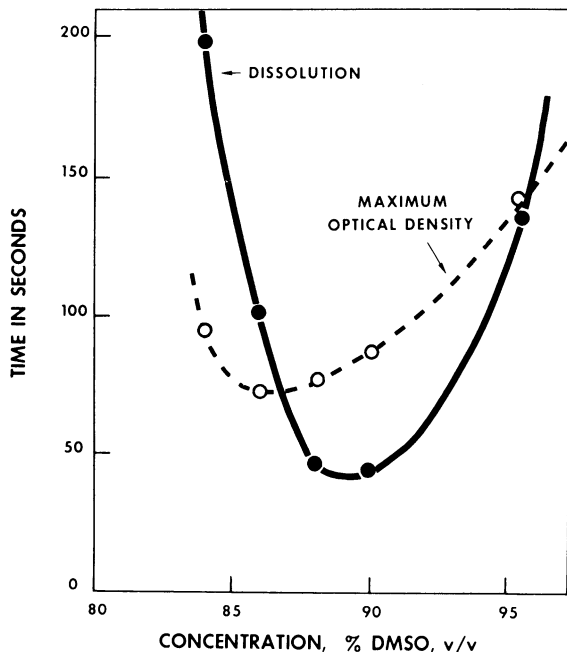


Fig. 3. Solvation of White waxy corn starch in Me_2SO -water solvent at various concentrations and 47°C .; half-lives for dissolution and time to reach maximum optical density.

the starch grains. The average size of rice starch granules is $5\ \mu$, waxy corn starch $12\ \mu$, bean starch $30\ \mu$, and potato starch $44\ \mu$.

In general, the solvation of starch in Me_2SO -water occurred in three steps which are observed both microscopically and by the spectrophotometer. First, initiation: During initiation no visible change occurred under the microscope (Fig. 5A); the granules remained uniformly refractile and birefringent and showed no change in shape or size. Neither was the spectrophotometer able to measure any change as illustrated during the first 15 min. for all three navy beans (Fig. 4B). Second, swelling: The spectrophotometer recorded swelling as an increase in optical density. Swelling is well illustrated for the waxy corn, potato, and rice starches during the first 5 min. (Fig. 4A); for the navy beans between 20 and 30 min. (Fig. 4B); and at various times during the first 30 min. for the lima beans (Fig. 4C). The bean starch granules showed a progressive swelling under the microscope, starting typically with a swelling at the apexes of the ellipsoid of revolution (poles) of the starch. These swellings started just below the surface and enlarged by both centripetal and circumferential involvement (Fig. 5B). The apparent birefringence and refractility of the swollen area decreased, but the birefringence of the unswollen portions remained strong. Third, dissolution: Dissolution was measured as a decrease in optical density by the spectrophotometer, illustrated after 40 min. for most of the bean starches and starting earlier for the other starches (Fig. 4). Microscopically the swellings burst

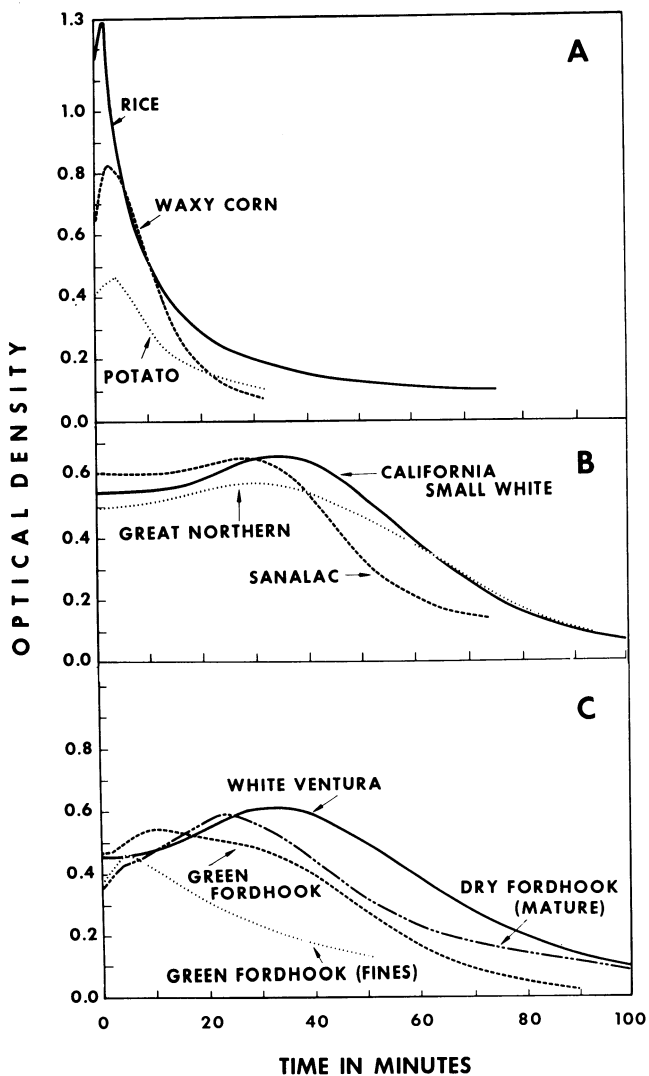


Fig. 4. Spectrophotometer turbidity recordings of solvation kinetics. A) Solvation of rice, corn, and potato starches at 39.7°C. in 85% Me₂SO-water; B) solvation of navy bean starches at 39.7°C. in 85% Me₂SO; C) solvation of lima bean starches at 39.7°C. in 85% Me₂SO.

much like balloons and left remnants, which at first included the center of the starch granules (Fig. 5C). The interior starch then dissolved leaving only the membranous remnants (Fig. 5D). It is of interest that each variety has its own characteristic solvation curve.

Solvation curves for lima bean starches show differences not only among bean varieties, but even between different maturities from the same variety of beans

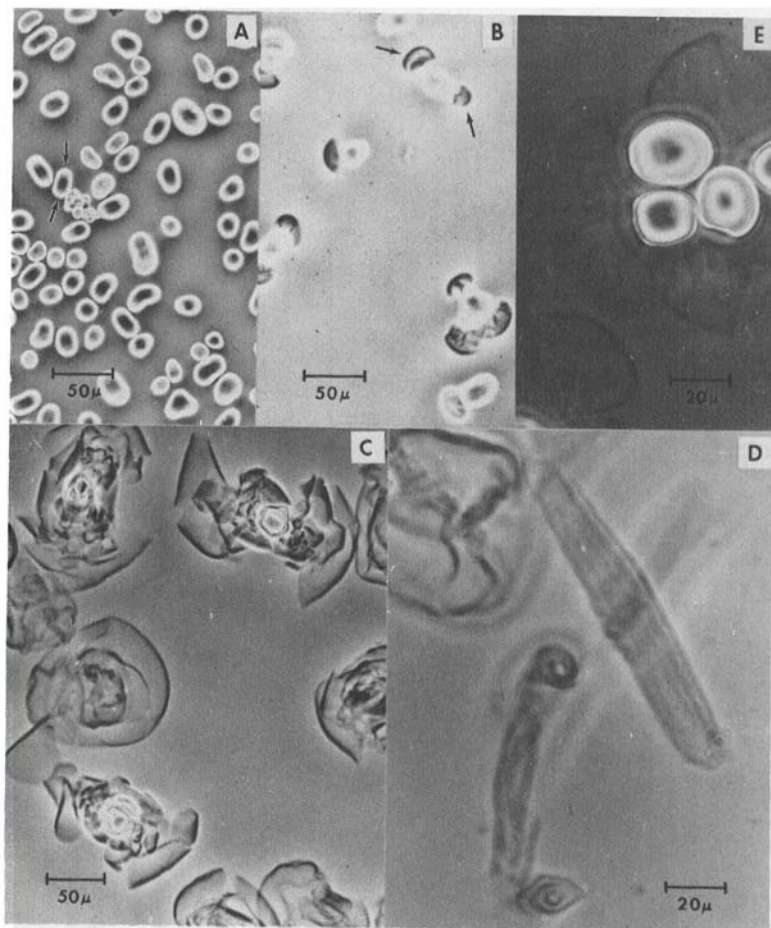


Fig. 5. Phase-contrast photomicrographs of starch grains in Me_2SO -water solvent. Unswollen remnants were birefringent when examined by polarized light microscopy. A) Unchanged California small white bean starch granules as they appear during induction. The arrows indicate the poles of a starch granule. B) California small white bean starch granules in 85% Me_2SO . Microscopic appearance corresponding to maximum optical density. Most of the original grains are still unswollen except for small areas at the poles indicated by the arrows. The unchanged starch together with the swollen material causes the increased scattering. C) California small white bean starch granules in 85% Me_2SO . Microscopic appearance during rapid dissolution. Few refractile unswollen remnants are still present. Note that the initially swollen poles (Fig. 5B) are now only torn fragments. D) Remnants of California small white bean starch granules in 85% Me_2SO . Microscopic appearance corresponding with minimum optical density. Some membranous remnants are curled like empty dried bean seed-coat or prune skins. Note the end view spiral. E) California small white bean starch granules in 95% Me_2SO . The outside has begun to dissolve but the mass of the granules is not yet affected. At this concentration swelling is slow and the swollen mass is very indistinct. It breaks up easily when stirred.

TABLE I. RATE CONSTANTS AND ACTIVATION ENERGIES FOR SOLVATION OF STARCHES IN 85% DIMETHYLSULFOXIDE-15% WATER

	Rate Constants, min. ⁻¹ ^a			Activation Energy Calories	
	k _{31.5}	k _{39.7}	k _{47.0}	31.5°-39.7° C.	39.7°-47° C.
California small white beans					
Dissolution	0.0150	0.0438	0.0898	24,700	19,500
Maximum o.d.	0.00909	0.0297	0.0769	27,300	25,800
Birefringence loss	...	0.0691	0.276	...	31,000
White waxy corn					
Dissolution	0.0415	0.100	0.277	20,300	27,700
Maximum o.d.	0.100	0.323	0.800	27,000	26,400
Birefringence loss	0.113 ^b	0.474	...	20,000	...

^aRate constants for dissolution and loss of birefringence were determined graphically. Maximum o.d. is simply the reciprocal of the full time.

^b26.0° C.

(Fig. 4C). The solvation curve was also determined separately for the fine grains. The first peak in the green Fordhook bean starch solvation curve corresponds to the fine granules and the later shoulder to the more coarse granules. At maturity there is a definite change in the nature of the granule distribution. The highest peak for the mature Fordhook bean starch seems to be between shoulders which correspond to the fine and coarse particles of the green Fordhook bean starch. Apparently the presence of fine granules obscures the initiation step for the starch from mature and reduces it for the starch from green Fordhook beans. A dimorphous starch population with the small granules having different properties is well known in some of the cereals (7).

The energy of activation for dissolution of California small white bean and White waxy corn starches was determined from 31.5° to 39.7° C. and from 39.7° to 47.0° C. (Table I). The energy of activation for reaching maximum optical density was also determined. The maximum optical density occurs at that point where the rates of dissolution and swelling are optically equivalent and represent no single reaction. Since it is a point, no true k can be calculated, but its reciprocal time of occurrence "k" behaves as true k , yielding an energy of activation very comparable to those calculated for dissolution (Table I). The differences in the activation energies for the two temperature ranges and for the two starches shown in Table I are consistent with the course of swelling of various starches in water at several temperatures (8).

Birefringence studies also showed unique differences among the three starches studied (Fig. 6). The greatest difference was due to particle size. Experimentally, because a dispersion of finer particles has a greater optical density due to light scattering, it will cause decreased response of the spectrophotometer set up with crossed polaroids to measure birefringence. Since birefringence of starch is due to its crystallinity, loss of birefringence ought to be a more strict measure of disorganization of the starch granules than swelling and dissolution.

California small white bean starch in 75% Me₂SO, as well as rice and White waxy corn starches in 85% Me₂SO, shows an initial increase in birefringence prior to the reduction of birefringence (Fig. 6). That it did not occur in the case of

the California small white bean starch in 85% Me₂SO suggests that the rise was a result of the less than optimal dissolution conditions. Grinding California small white bean starch caused the initiation stage to disappear, the initial transmission of light to be reduced, and the maximum slope of the curve to be greatly increased (Fig. 6).

The activation energies for the change of birefringence differ greatly and must reflect a difference in the structure of the starches (Table I). As expected the activation energies for dissolution and for the change of birefringence are very similar for White waxy corn starch at approximately the same temperature. The activation energy for the change of birefringence in the California small white bean starch is very large at 39.7° to 47° C.; however, over the same temperature range there is a small activation energy for dissolution. The rate of change of birefringence of the ground California small white starch decreased with time up to about 15 min. and then increased as indicated by the flat slope in Fig. 6.

The granules broken by the grinding procedure rapidly lost birefringence as indicated by the steep initial slope shown in Fig. 6. The unbroken granules began to lose birefringence later and hence the rate shows a perceptible increase at about 45 min. This indicates that the broken granules do not have the initiation stage which occurred in the other birefringence experiments in 85% Me₂SO. The initiation stage was not dependent upon granule size, from the comparison of rice and waxy corn starches with the California small white bean starch. Hence the experiment indicates that breaking the bean starch granules gave the solvent direct access to the starch, compared with impeded access in the intact granules.

Microscopic examination of California small white bean starch in 85% Me₂SO revealed that the granules began to swell usually at the poles. The area of swelling was well defined, with a sharp refractive index boundary between the swollen and unswollen portions of granules (Fig. 5B). As the swelling and the dissolution

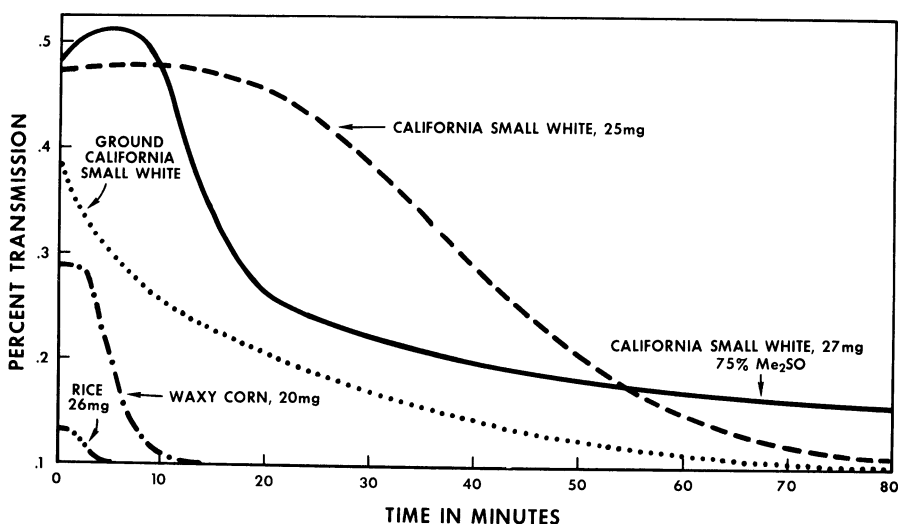


Fig. 6. Loss of birefringence by several starches at 39.7°C. in 85% Me₂SO-water except for plot labeled 75% Me₂SO.

progressed, eroded remnants of the original granules were surrounded by torn fragments of the amorphous swollen material (Fig. 5C). When dissolution had reached its maximum, filmy fragments (seen initially) remained in suspension (Fig. 5D).

Potato starch in 45% phosphoric acid or calcium nitrate has been reported to show very similar swelling behavior (9). Unfortunately, no photomicrographs were published. However, we have examined the swelling of both potato and California small white bean starches in 45% volume per volume phosphoric acid at room temperature. The swelling behavior of both starches was very much like the swelling of bean starch in 85% Me_2SO as shown in Fig. 5B. The most striking difference was the rate of swelling which was many times faster in the phosphoric acid. However, the bean starch swelled more than did the potato starch.

Dissolution of navy bean starches in 85% Me_2SO was characterized by a very long initiation period (Fig. 4B) usually followed by a localized swelling at the poles. The lima bean starches generally have shorter initiation periods and somewhat faster swelling (Fig. 4C). The faster rates suggest a somewhat less resistant barrier to solvent action.

Potato, waxy corn, and rice starches show no initiation period prior to dissolution in Me_2SO with turbidimetric measurements, and swelling occurs rapidly. Starches of these three species do show well-defined swelling, both turbidimetrically and microscopically; and waxy corn and rice starch lose birefringence more like normal California small white bean starch than like the ground bean starch.

Microscopic examination of California small white bean starch during solvation indicated that 95% Me_2SO dissolved the outside first (Fig. 5E). Apparently the action of 55% phosphoric acid on potato starch is also very similar to the action of 95% Me_2SO on bean starch (9).

After California small white bean starch was dissolved in 75 or 85% Me_2SO there were some remnants; these dissolved in 100% Me_2SO . Microscopic examination of washed preparations of the remnants of California small white bean starch which did not dissolve in 95% Me_2SO when stained with iodine reveals primarily blue particles showing some red edges. However, washed preparations of remnants from 85% Me_2SO were less bulky and stained red with iodine. To confirm the iodine staining and to quantitate the degree of branching, membranous remnants from 85% Me_2SO and alcohol-precipitated 85% Me_2SO -soluble material were oxidized by sodium periodate and titrated. The remnants had 14.3 anhydroglucose units per end group and the soluble materials 43. The whole starch had 35 anhydrous units per end group. Most amylopectins have been shown to contain 22 to 27 anhydroglucose units per end group while amyloses had 400 to 1,000 (5,10). The remnants are apparently highly branched amylopectin, whereas the soluble material is apparently a mixture of amylose and amylopectin.

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