alone approximately equalling the entire computed result listed in Table II. In view of the sensitivity of the calculations to the type of repulsion potential chosen it would seem that an adequate accounting for the observed results cannot be made until a more detailed picture of the repulsive forces is provided.

Summary

The heats of formation at 25° of sodium chloride–sodium bromide solid solutions have been measured at intervals of 10 mole per cent. A cubic equation has been fitted to these data by the method of least squares. Equations are given from which the partial molal heat contents relative to the pure solids can be calculated.

By assuming the entropy of formation is that of random mixing, the free energies of formation were obtained.

The limits of solubility have been determined graphically by the method of common tangents from 0 to 350°K. The critical solution temperature and concentration have also been calculated. With these data the solid solubility diagram has been constructed.

A comparison of the heats of solution as obtained in this research with those calculated by Tobolsky has been made. The differences noted have been discussed in terms of possible inadequacies in Tobolsky's development.

PITTSBURGH, PENNSYLVANIA RECEIVED JUNE 1, 1948

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF IOWA STATE COLLEGE]

An Investigation of the Streaming Birefringence of Amylose Solutions[1]

BY JOSEPH F. FOSTER AND IRWIN H. LEPOW[2]

Starch is now considered to consist of two components, the amylose fraction being essentially a linear polymer while the amylopectin fraction is highly ramified. The problem of distinguishing between such polymeric types by the usual physical-chemical studies of their solutions is difficult since both have high frictional coefficients. On the other hand it seems possible that the two types of polymers might behave quite differently with regard to their orientability in a streaming gradient since this property depends primarily on length rather than just asymmetry or frictional coefficient. For this reason an extensive study of the streaming birefringence of these starch fractions has been undertaken.

Peterlin and Samec[3] attempted to orient both amylose and amylopectin from several types of starch without success and concluded both fractions to be very nearly spherical. The present paper shows on the contrary that the amylose component is readily orientable.

Experimental

Apparatus.—The concentric cylinder apparatus was patterned after that described in detail by Edsall, *et al.*[4] It consists of two concentric stainless steel cylinders of mean radius 3.22 cm. separated by a gap of 1.0 mm. The inner cylinder is driven through a flexible shaft by a 0.22-h.p. 15-volt direct current motor. The armature and field circuits of this motor are separately excited by two direct current generators. This enables control of the motor by means of the standard Ward–Leonard circuit and enables the attainment of constant speeds with adequate torque over a wide range, from approximately 100 to 3000 r.p.m. The outer cylinder is jacketed for circulation of water.

The filter-isolated green line of a type AH-4 General Electric mercury arc is used as the light source. This is condensed on a slit placed at the focal point of a collimating lens. The essentially parallel light then passes in succession the polarizing Nicol prism, the lower window of the apparatus, the gap between the cylinders, upper window, analyzing Nicol, 90° prism and observing telescope. For making measurements of magnitude of double refraction a $\lambda/4$ plate (and sometimes a quartz half-shadow wedge) are placed in the path just ahead of the analyzer.

It will be noted that the gap width in this apparatus is four times as great as that in the apparatus of Edsall.[4] This reduces the maximum gradient attainable, but greatly simplifies the optical problem.

Solvent.—One of the best molecular solvents for starch and its fractions is ethylenediamine. Glycerol, while not in itself a good solvent for amylose, can be added in almost unlimited quantity to solutions of amylose in ethylenediamine without impairing the clarity or reducing the stability. In this way solutions of high viscosity can be prepared, greatly enhancing the ease of orientability in the streaming gradient. All runs reported in this paper have been made in such mixed solvents. Furthermore, no effort was made to dehydrate the reagents,[5] since it would be almost impossible to keep the solutions anhydrous. The exact composition of the solvents is not important so long as the viscosity is known. Viscosities were measured (in Ostwald type viscometers) on several mixtures of ethylenediamine and glycerol at 19, 25 and 40°. Plots of log η *vs.* temperature for the various compositions were very nearly linear and parallel. Viscosities at other temperatures and compositions were obtained by interpolation from this graph.

Materials Studied.—All amylose samples studied were prepared by fractionation after the method of Schoch[6] which involves crystallization of the complex formed with one of the higher (4–6 carbon) alcohols. All were recrystallized from one to five times.

Methods.—The solutions were made up entirely by weight. The amylose was first dissolved in the ethylenediamine, contained in a glass-stoppered flask, with the aid of mechanical agitation. The glycerol was then added,

(1) Journal Paper No. J-1552 of the Iowa Agricultural Experiment Station, Ames, Iowa; Project No. 817. Supported in part by a grant from the Corn Industries Research Foundation.

(2) Present address, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York.

(3) Peterlin and Samec, *Kolloid Z.*, **109**, 96 (1944).

(4) J. T. Edsall, C. G. Gordon, J. W. Mehl, H. Scheinberg and D. W. Mann, *Rev. Sci. Instruments*, **15**, 243 (1944).

(5) Eastman Kodak Co. 95–100% ethylenediamine and General Chemical Co. C. P. glycerol were used without further purification.

(6) T. J. Schoch, THIS JOURNAL, **64**, 2957 (1942).

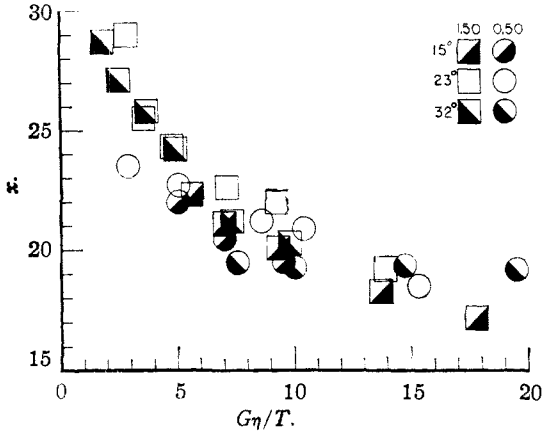

Fig. 1.—Relation between extinction angle and gradient for corn amylose at two concentrations, 0.50 and 1.50% by weight, and at three temperatures. The solvent is 49.5% ethylenediamine, 50.5% glycerol. The viscosity of the solvent is, respectively, 2.3, 1.4 and 0.75 poise at the three temperatures.

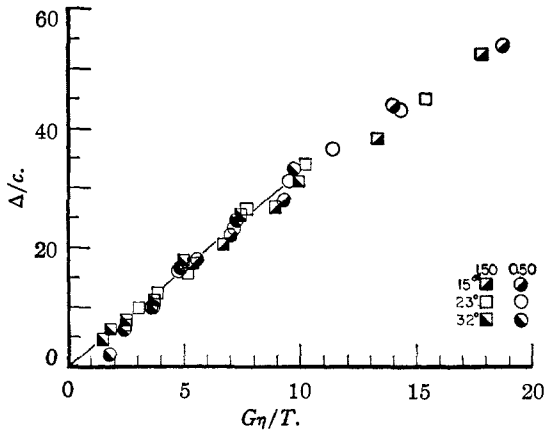

Fig. 2.—Relation between the magnitude of birefringence and gradient for corn amylose at two concentrations, 0.50 and 1.50% by weight, and at three temperatures. The solvent is 49.5% ethylenediamine, 50.5% glycerol.

thoroughly mixed and poured into a 50-ml. glass-stoppered dropping funnel which served as the filling tube for the cylinders. Finally, just before running the solution into the apparatus, it was placed under vacuum (water aspirator) for fifteen to thirty minutes. This serves to remove dissolved gases and thus eliminates to a large extent formation of foam or bubbles in the cylinders.

Readings were first made of the extinction position by crossing the Nicols and rotating them in the crossed position in the usual manner. Instead of making repeated readings of a single extinction position the practice was to make single readings of each of the four equivalent extinction positions at a given speed. The sense of rotation was then reversed, the four readings made, and χ calculated for each of the four positions by halving the difference in reading in the two senses and subtracting from 45°. The χ values reported thus represent an average of four readings at four positions approximately 90° apart.

Measurements were usually made at two or three different temperatures by varying the temperature of the reservoir from which water was circulated through the stator. Measurements of the phase difference were ordinarily made at each temperature also, using the Senarmont compensator in the usual manner.

Results

Measurements of the extinction angle, χ, are summarized in Figs. 1, 3 and 4, measurements of magnitude of birefringence [expressed as Δ/C, the rotation of the Senarmont compensator (in degrees) divided by the concentration of solute (in per cent. by weight)] in Figs. 2 and 5. In all cases the independent variable is the quantity $G\eta/T$, *i. e.*, the velocity gradient (in sec.$^{-1}$) times the viscosity (in poise) divided by the absolute temperature. Use of this parameter enables direct comparison of extinction angles obtained in different solvents and at different temperatures. This is not quite true of Δ/C measurements since these depend in addition on the index of refraction of the solvent.[7] In order to standardize the method for use in comparing amyloses from various sources, it was deemed important to study the effect of certain variables in the results. For this purpose one sample of corn amylose, three times recrystallized, was studied under a wide variety of conditions, and the results of these studies are summarized in Figs. 1, 2 and 3.

Effect of Solute Concentration.—Although it is not expedient to extrapolate flow birefringence results to infinite dilution it is important to make measurements at several solute concentrations to determine possible effects of solute–solute interactions. Figures 1 and 2 present results obtained on 0.5 and 1.5% corn amylose in a solvent consisting of 49.5% ethylenediamine and 50.5% glycerol. Interactions would be expected to manifest themselves in a decrease in χ values and in an increase in Δ/C values. No such trends are evident and it seems safe to conclude that molecular interactions are unimportant even at 1.5% concentration. Data on 1.0% corn amylose under the same conditions are presented in Fig. 3 and agree well with the other data. To eliminate excessive congestion of points only the two extremes of concentration are given in Figs. 1 and 2.

These results were somewhat surprising since considerable interaction was observed in the case of fibrinogen even at 0.5% concentration.[8] However, this difference is probably to be expected from the fact that amylose carries little or no ionic charge.

Effect of Solvent Composition.—Figure 3 summarizes results of extinction angle measurements on solutions of 1.0% corn amylose in five different solvent compositions. The excellent agreement is apparent and indicates the shape of the dissolved molecules to be substantially independent of solvent composition over this range. Unfortunately Δ/C values cannot be compared directly in the various solvents since

(7) For a comprehensive review of the theory of flow birefringence the reader is referred to a chapter by J. T. Edsall, in "Advances in Colloid Science," Interscience Publishers, New York, N. Y., Vol. I, 1942, p. 269. A brief outline of the theory is given by J. F. Foster and J. T. Edsall, THIS JOURNAL, **67**, 617 (1945).

(8) J. T. Edsall, J. F. Foster and H. Scheinberg, *ibid.*, **69**, 2731 (1947).

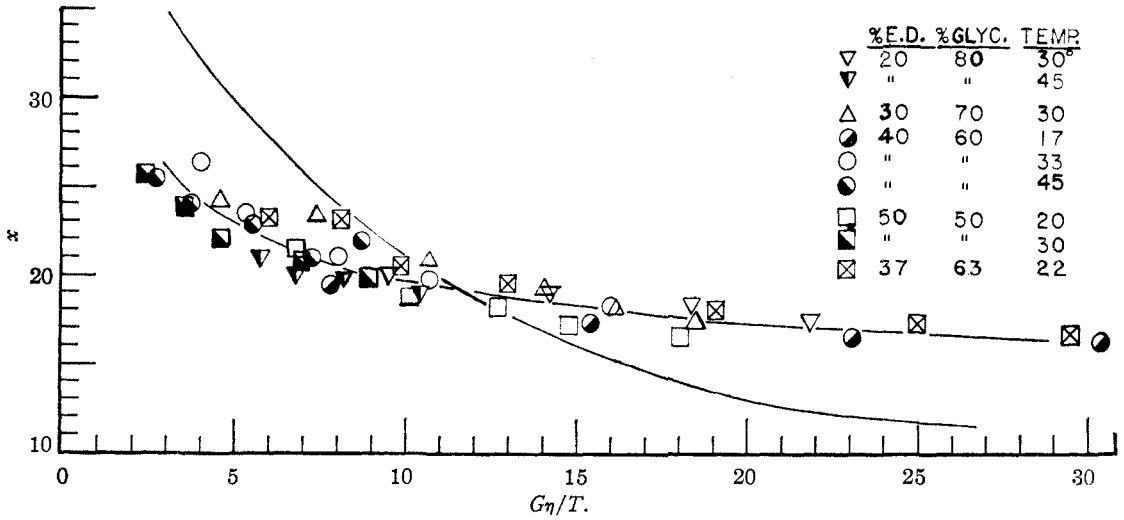

Fig. 3.—Relation between extinction angle and gradient for corn amylose, concentration 1.0% by weight, in various mixtures of ethylenediamine and glycerol, and at various temperatures. The solid curve cutting across the experimental one is calculated for simple orientation of rigid rods of length 800Å. The solvent viscosities range from 0.85 poise for 50% glycerol at 30° to 3.2 poise for 80% glycerol at the same temperature.

they depend also on the refractive index of the medium.

Successful runs have also been made in ethylenediamine–water with results in satisfactory agreement with those reported here.

Effect of Temperature.—Figures 1 to 5 present data obtained at several temperatures ranging from about 15° to 45°. Agreement between runs at different temperatures on the same solution is very satisfactory.

Comparison of Amyloses from Various Sources.—In Figs. 4 and 5 are plotted results obtained on amylose preparations from tapioca, potato and lily bulb starch. For comparison the mean curves for corn amylose, taken from Figs. 1 and 2, are included. Tapioca, potato and lily bulb preparations appear to be practically indistinguishable and all much more orientable than corn, presumably indicating a higher degree of polymerization. Foster and Hixon[9] found significant differences in intrinsic viscosity between amyloses from these starches, corn having the lowest value. Since the two investigations were made on different preparations direct comparison cannot be made. To be meaningful such comparison should be made on fractionated preparations. Studies of this nature are in progress.

Comparison with Theory.—For comparison with the experimental data theoretical curves are included in Figs. 3 and 4 calculated on the basis of simple orientation of rigid particles.

(9) J. F. Foster and R. M. Hixon, *ibid.*, **65**, 618 (1943).

The models used, selected to give optimal fit of the experimental data, were highly elongated ellipsoids (rods) of length 800 Å. in the case of corn and 1000 Å. in the case of lily bulb and tapioca amylose.[10] Since most of the experimental data yield values of χ lower than those for which the

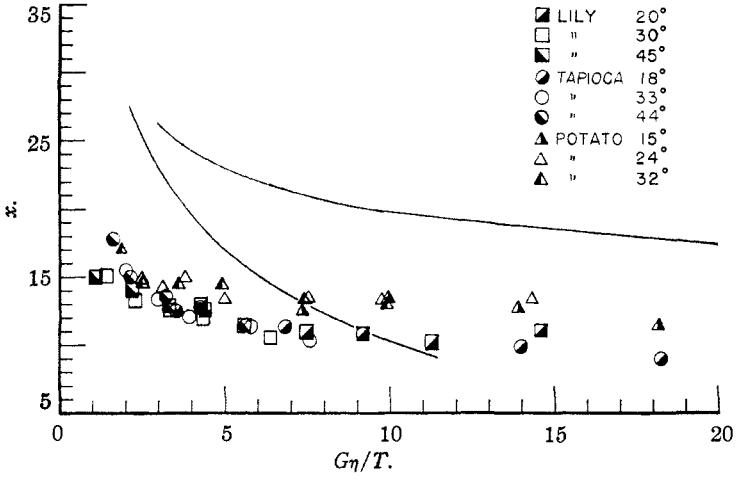

Fig. 4.—Relation between extinction angle and gradient for lily bulb, tapioca and potato amyloses, at various temperatures. The solvent is 49.5% ethylenediamine, 50.5% glycerol. Upper solid curve is for corn amylose, taken from Fig. 1. Lower solid curve is theoretical for case of 1000 Å. rods.

limiting law of the three-dimensional orientation theory can be applied, we have based our calculations on the empirical plot for the relation between χ and α ($=G/\theta$) and between Δ and α which have

(10) Interestingly, B. A. Dombrow and C. O. Beckmann, *J. Phys. and Colloid Chem.*, **51**, 107 (1947), have reported values of about 600 and 900 Å. respectively for the acetates of these two amyloses, based on results of sedimentation, diffusion and viscosity. Undoubtedly this agreement is entirely fortuitous for the reasons discussed here.

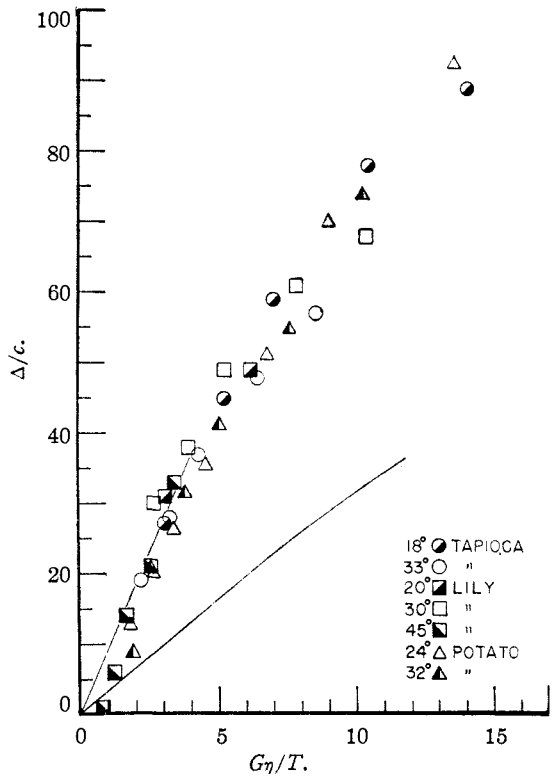

Fig. 5.—Relation between the magnitude of birefringence and gradient for lily bulb, tapioca and potato amyloses at various temperatures. The solvent is 49.5% ethylenediamine, 50.5% glycerol. The curve for corn amylose, taken from Fig. 2, is given for comparison.

been found to agree well with the experimental data on fibrinogen.[8]

The experimentally-determined extinction angle curves are seen to be much flatter than predicted by theory. In fact, in the case of Fig. 4 the curve is effectively horizontal at $G\eta/T$ values above about 5. Certain published results on other systems exhibit this apparent tendency of χ to level off at a value greater than 0°, for example in the case of myosin,[11] polystyrene,[12] nitrocellulose[13] and polyisobutylene.[14] Polydispersity is to be expected in all of these cases and might account for the observed results. Equations developed by Sadron[15] enable one to predict the behavior of such systems. Using these equations it has been found possible to fit the experimental curve for corn amylose quite closely by assuming a mixture consisting of 80% of molecules 500 Å. in length and 20% 1200 Å. The longer and flatter curve exhibited by the other amyloses presumably could be fitted in a similar manner although a more complex system would need to be assumed.

An alternative explanation of these results lies in the probability that the molecules are not correctly pictured as rigid rods but are flexible. In such cases the observed effect would be predominately one of distortion of the molecules, the so-called photoelastic effect. That this effect is in reality an important one is indicated by a consideration of a second type of deviation from the simple orientation theory, namely, the fact that Δ/C continues to increase almost linearly with gradient (Figs. 2 and 5) even though χ is far below 45°. On the basis of the orientation theory the birefringence should be approaching saturation under such conditions. Again this behavior has been observed in the cases mentioned above. Von Muralt and Edsall[11] suggested this as due to photoelasticity even before the development of an adequate orientation theory. On the other hand, Sadron[15] concluded that such behavior could be accounted for on the basis of polydispersity. However, Δ/C curves calculated for the hypothetical system which fit the extinction angle curve for corn did not fit the experimental birefringence curve at all well, but showed the usual tendency toward saturation.

W. Kuhn and H. Kuhn have dealt at length with this very complex problem of the behavior of non-rigid molecules in a streaming gradient.[16] A thorough experimental test of their theory must await the preparation of polymers of a high degree of homogeneity. Their theory predicts a birefringence curve with positive curvature for non-rigid solutes Presumably in the case of an intermediate degree of rigidity one might expect a linear increase in the briefringence as observed in the above mentioned systems.

Zvetkov and Frisman[14] have pointed out that by elimination of $\alpha(= G/\theta)$ between the expressions for χ and Δ it is possible to calculate a quantity which should be proportional to the intrinsic birefringence of the solute molecules, $n_1 - n_2$. For the case of simple orientation in a homogeneous system $k(n_1 - n_2)$ should be constant, independent of gradient. On an intuitive basis it would seem that in a polymer-homologous system of rigid molecules, $k(n_1 - n_2)$ should, if anything, decrease with increasing gradient, since the shorter molecules, having a lower intrinsic birefringence, would weight the results more at higher gradient. On the contrary $k(n_1 - n_2)$ would be expected to increase with increasing gradient if the molecules are being significantly elongated by the shearing force. Thus, while the method is still only empirical, it does appear to have possibilities for distinguishing between the two effects. Zvetkov and Frisman found an almost linear increase in $n_1 - n_2$ with increasing gradient in the case of polyisobutylene, indicating photoelasticity to play an important part in this system.

The amylose data have been evaluated in this manner, again resorting to the empirical long-

(11) A. Von Muralt and J. T. Edsall, *J. Biol. Chem.*, **89**, 351 (1930).
(12) R. Signer, *Trans. Faraday Soc.*, **32**, 296 (1936).
(13) R. Signer and H. Gross, *Z. physik. Chem.*, **A165**, 161 (1933).
(14) W. Zvetkov and E. Frisman, *Acta Physicochim. U. R. S. S.*, **20**, 61 (1945).
(15) C. Sadron, *J. phys. radium*, [7] **9**, 381 (1938).
(16) W. Kuhn and H. Kuhn, *Helv. Chim. Acta*, **26**, 1394 (1943); **28**, 1533 (1945); **29**, 71 (1946).

range $\chi - \alpha$ and $\Delta - \alpha$ plots previously mentioned. The resulting $k(n_1 - n_2)$ (in arbitrary units) is shown in Fig. 6, as a function of $G\eta/T$. For comparison the calculations have been made in the same way for the published data on zein[17] and fibrinogen.[8] In the case of the proteins $k(n_1 - n_2)$ is seen to be very nearly constant whereas in the case of the amyloses the function increases almost linearly with gradient. The difference in behavior between the proteins and the amyloses is, indeed, striking, and would seem to indicate a much lower degree of rigidity in the latter case. The fact that the plots extrapolate close to the origin might be taken as indicating the molecules in the resting solution to be nearly isotropic and hence, presumably, spherical. Such conclusions, based on an empirical application of simple orientation theory to a situation where it evidently does not apply, should be accepted only with reservations.

The configuration of amylose in solution is of considerable interest in view of its known ability to assume a helical configuration in complexes with iodine and the higher alcohols. It seems unlikely that such helices pre-exist in solution since amylose in the helical (V) modification tends to go over spontaneously, upon exposure to water vapor, to the crystalline modifications characterized by more elongated chains. Furthermore the helical configuration would probably lead to a fairly rigid structure.[18] It seems more probable that the situation, particularly in a strong solvent such as used here, is a random coiling such as is assumed by Kuhn.[16]

Acknowledgments.—The authors are indebted to Dr. T. J. Schoch of the Corn Products Refining Co. who supplied some of the amylose samples studied; to Prof. B. S. Willis of the Department of Electrical Engineering, Iowa State College, for aid in the design of the motor control circuit used; and to Dr. J. T. Edsall, Harvard Medical School, for his interest and helpful suggestions.

(17) J. F. Foster and J. T. Edsall, *ibid.*, **67**, 617 (1945).

(18) R. E. Rundle and R. R. Baldwin, THIS JOURNAL, **65**, 554 (1943), have reported observations of flow dichroism on amylose–iodine complex in aqueous solutions. Unfortunately these observations were only qualitative, but doubtless in this case the complex molecule would be quite rigid.

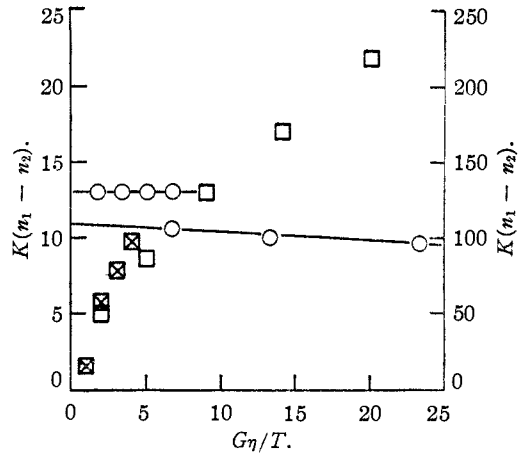

Fig. 6.—The function $K(n_1 - n_2)$(in arbitrary units) as a function of gradient for corn amylose (□) and tapioca and lily bulb amylose (⊠). For comparison there are included values calculated from the published data on fibrinogen (upper curve) and zein (lower). The right-hand ordinate refers to proteins.

Summary

The amylose component of starch is readily orientable in a streaming gradient. A study has been made of the effect of temperature, solute concentration and solvent composition using as solvent mixtures of glycerol and ethylenediamine. Comparison of amyloses from corn, tapioca, potato and lily bulb starches give results in qualitative agreement with their relative intrinsic viscosities. The results are discussed on the basis of the orientation theory with the conclusion that the effect in the case of the amyloses is predominantly due to elongation of coiled molecules rather than to simple orientation.

AMES, IOWA RECEIVED MAY 17, 1948