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Streaming orientation of amylose and amylose complexes

Donald Zucker
Iowa State College

14

STREAMING ORIENTATION OF AMYLOSE AND AMYLOSE COMPLEXES

by

Donald Zucker

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Head of Major Department

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TABLE OF CONTENTS

INTRODUCTION

All starches, regardless of origin, are alike in that they are polymers of glucose having 1,4 glucosidic linkages of the α-configuration. In nearly all starches there are two kinds of such polymers in varying proportions. One kind appears to have no linkages except the 1,4, being thus a straight chain compound. The other variety is branched, having side chains attached by 1,6 linkages. The first of these is called amylose; the second, amylopectin. In this thesis amylose is of primary importance, amylopectin being of interest only in so far as it interferes with the work on amylose.

From the preceding, it is obvious that all amyloses are chemically identical except in the degree of polymerization (commonly abbreviated d.p.), that is, in the number of glucose units per molecule. Considerable work has been done to determine the d.p.'s, or molecular weights, of various amyloses. Both chemical and physical methods have been applied. Chemical methods generally are not subject to error due to aggregation, but can be seriously affected by incomplete reaction and side reactions, degradation of the sample, and the presence of amylopectin.

Physical methods include the application of the ultracentrifuge, determination of osmotic pressure and

intrinsic viscosity, and streaming orientation. All of these methods are subject to error due to aggregation, and the interference of amylopectin. In addition, molecular weights cannot usually be determined from streaming orientation studies of amylose because of lack of rigidity of the individual molecules, and because the spatial arrangement of the molecule is not known.

The object of this study was to overcome as many as possible of the sources of error applicable to the methods of streaming orientation, to determine by this technique the length and molecular weight distributions of several amylose samples, and to obtain all data possible by this method concerning the purity of the samples and rigidity of the molecules.

It seemed probable that the lack of rigidity and uncertainty of structure could be overcome by using the amylose-iodine complex. It was also likely that the interference of amylopectin could be eliminated, since it forms a less stable and differently colored complex with iodine. To prevent precipitation of the complex, and to permit enough light to pass through the sample, very dilute solutions would have to be used. As a result, the birefringence would be too low to measure, and another means would have to be used to determine the orientation angle. Fortunately, such a means was available in the dichroism exhibited by this complex.

Study of the flow dichroism of the amylose-iodine complex was a major part of this investigation. It was necessary to devise and build special apparatus for this work. Efforts were made to find a solvent and conditions of solution such that aggregation and degradation were overcome, as it appeared that if this could be done, and if the above-mentioned advantages could be realized, the method would be one of the best available for amylose molecular weight determinations.

Birefringence studies were undertaken on the uncomplexed amyloses. It was believed that use of the proper solvent could overcome aggregation and degradation, but the lack of rigidity, the question of spatial arrangement, and the effect of amylopectin would not be eliminated. A comparison of results obtained by dichroism of the complex and by birefringence of the uncomplexed amylose was considered advisable.

REVIEW OF LITERATURE

Preparation and Properties of Amylose

Separation from amylopectin

Early methods. It has long been recognized that there are at least two components in starch. Early methods for separating these components were mostly based on solubility. Usually investigators simply leached whole starch with hot water, but there were variations*. For example, Ling and Nanji (46) first heated a starch paste to 130°, froze it, allowed it to thaw, then extracted with water at 60°. Other methods include the electrophoretic separation of Sameo (70,71) and preferential adsorption on cellulose fibers such as cotton (56,83). The different methods gave different results, and although many workers called their products "amylose" and "amylopectin", the materials referred to by these names were not always the same for all procedures. Other terms were also used (2,56) such as "α-amylose" and "β-amylose".

There were two major difficulties: one, the actual separation, and the other, lack of criteria for the extent

*For a brief review of methods used prior to 1930, as well as a detailed discussion of his own method, see M. E. Baldwin, J. Am. Chem. Soc., 52, 2907 (1930).

of separation. These problems have been fairly well solved, as will be discussed in other sections to follow.

Precipitation with alcohols. T. J. Schoch largely eliminated the difficulty in separating amylose from amylopectin by his work with butanol precipitation (33,74,85). He found that when starch was autoclaved with water and the resulting dispersion saturated with n-butanol, a crystalline precipitate formed on cooling. This precipitate was predominately amylose in the form of a loose butanol complex. Recrystallization by the same method raised the purity still further. Later work (75) showed that many alcohols of four or more carbon atoms could be used, but that n-pentanol and Pentasol (a commercial mixture of 5-carbon alcohols) were best. Schoch also prepared amylose without autoclaving, by refluxing starch, Pentasol, water, and buffer for several hours with continuous stirring, then cooling this mixture (45).

Subfractionation of amylose

No procedure so far developed even approximates a complete separation of amylose into fractions which are homogeneous with respect to chain length, except perhaps by a prohibitive number of repetitions of the process. However, some fractionation was accomplished by Schoch by a recently developed partial precipitation method based on the

previously described alcohol precipitation method used for separation of amylopectin and amylose (45). The two processes were identical except that instead of saturating the hot starch suspension with butanol or Pentasol, only a small quantity of the alcohol was added, the mixture then being cooled. The resulting precipitate was removed, the supernatant liquid was heated, more of the alcohol was added, and the process repeated. This was continued until all the amylose was removed. Beginning with several different natural starches, Schoch and co-workers prepared many amylose fractions of different average chain lengths. Some of these samples were among those investigated in the work reported later in this thesis.

Structure

Chemical. Chemical evidence for the nature of amylose can be found in any good carbohydrate text (61, p. 569). It is based on hydrolysis of amylose and its methylated derivatives, with identification of the products. An amylose molecule is shown in Fig. 1.

Solid state. The physical structure of solid amylose depends on the method of preparation. Granular starches have extended chains in one of two modifications which can be distinguished from each other by their x-ray diffraction

patterns. Katz (30,32) showed that, in general, the cereal starches show one pattern, called the "A" pattern, and the tuber starches show another, called the "B" pattern. He also showed that starches retrograded at temperatures above

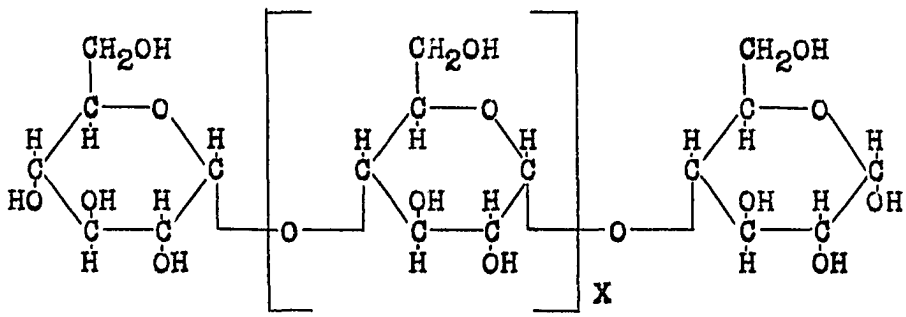

Fig. 1. Structure of an amylose molecule.

50° produced "A" patterns, while those retrograded at low temperatures gave "B" patterns. Bear and French (4) were able to show continuous variation from one pattern to the other by varying the retrogradation temperatures. They also showed that the "B" modification had a larger unit cell, agreeing with the observation that this form was more highly hydrated.

Amylose freshly precipitated by alcohols has another form called by Katz the "V" modification (29,31,69). This has been fairly well established as having a helical structure. Hixon and Rundle have recently reviewed the evidence for this structure (24, p. 675).

Solution. In molecular dispersions of amylose the molecules could conceivably have a randomly coiled, a helical, or a linear and fully-extended structure; or they could have some other structure not yet postulated. Foster and Lepow (15) concluded from streaming orientation studies that the molecules are stretched somewhat by flow gradients, which supported the random coil theory*. Kuhn (39,40,41) developed the theory of polymer molecules at rest and in flowing liquids. He concluded that these polymers would be alternately compressed and stretched, the effects being greatest at 45° to the direction of flow. While Schmidt and Marlies (73, p. 250) state that amylose is one of the more rigid of polymers, they indicate that it is not entirely rigid. We may consequently expect that amylose is at least somewhat coiled and that some length variations will occur, with attendent change in properties based on length.

If amylose is among the more rigid, we may expect a relatively high shape resistance, that is, resistance to change of the distance between the ends of the molecule. Kuhn (41) states that molecules with high shape resistance undergo practically no change in length with a single rotation, but do suffer a slight extension with many rotations, since the average extending force is greater than the average contracting force.

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*Similar structures have been found for other polymers (16,23,79).

Iodine complex. Hanes (19) noted that six-membered cycloamyloses are formed by certain enzyme reactions with amylose, and therefore postulated a helical structure for amylose, with six glucose residues per turn. Freudenberg (17), by building models of such chains, showed that in this case the hydroxyl groups should be directed outward, leaving the inside of the helix essentially hydrocarbon in character. He therefore considered the iodine in the amylose-iodine complex to be in the center of the helix because a blue color is characteristic of iodine in hydrocarbon solutions, rather than in polar solvents.

Neither Hanes nor Freudenberg offered further evidence for their theories, but it has since been shown that the main points of Freudenberg's proposal were correct. Bear (3) pointed out that the x-ray pattern for the amylose-iodine complex was similar to that of the previously mentioned "V" form of crystalline amylose. Subsequently both the "V" forms and the iodine complex were prepared in better forms. Rundle (65) showed clearly by x-ray analysis that they were helical and identical, and in the case of the iodine complex, this element occupies the center of the helix. The helices are 13 Å in diameter, 8 Å in period along the helix axis (77), and have six glucose residues per turn as Hanes suggested.

Thus we have in the amylose-iodine complex an

amylose complex of known spatial arrangement. Furthermore, it is probably quite rigid.

Molecular weight

Osmotic pressure (61, p. 522). According to the van't Hoff equation

$$M = RT/(\pi/c) \qquad (1)$$

where M is the molecular weight, c is the concentration of solute, and π is the osmotic pressure. This is strictly valid only at infinite dilution. Meyer (49) stated that the simplest way to arrive at the value of π/c at infinite dilution is to determine π at various concentrations, graph the results according to the equation

$$\pi/c = a + bc \qquad (2)$$

and extrapolate to zero concentration.

This method, when properly applied to well prepared samples, gives a number average molecular weight. For amylose values of 10,000 to 286,000 have been reported. It is highly probable that the higher values represent aggregated samples. In fact, Meyer (49) stated that osmotic pressure measurements with amylose are worthless unless it is highly degraded first, which, of course, does not permit determination of the original molecular weights.

Viscosity. The same difficulty, aggregation, that is encountered in osmotic pressure work also renders doubtful many of the results obtained by viscosity measurements. In addition, the Staudinger equation used,

$$\lim_{c \to o} \left[\frac{\eta_{sp}}{c}\right] = K_m M \qquad (3)$$

has been found by Staudinger (82), Meyer (50), and Schulz (76) to be invalid in many cases. Furthermore, the constant is found by determining the viscosities of a suitable sample whose molecular weight has been determined osmometrically, thus introducing the errors of this method.

Thus it is apparent that d.p.'s determined by viscosity may well be much too high. This is especially of interest here because the d.p.'s reported later in this thesis are much lower than the values calculated from Schoch's and Hassid's viscosity data for the same samples.

From viscosity data by Schoch and from their own osmotic pressure measurements (62), Potter and Hassid found for amylose in 1 N KOH the relationship

$$[\eta] = .00166 \cdot \text{d.p.} \qquad (4)$$

or, in the form of the Staudinger equation,

$$[\eta] = 1.02 \cdot 10^{-5} M \qquad (5)$$

where M is the molecular weight. However, Foster and Hixon (13,14) found that Kuhn's equation (38)

$$[\eta] = KM^{\alpha} \qquad (6)$$

was more applicable for amylose dissolved in ethylenediamine. They found for such solutions that

$$[\eta] = 3.5 \cdot 10^{-4} M^{1.5} \qquad (7)$$

Ultracentrifuge. Molecular weights may be found by particle distribution in a centrifuge which in some cases may be of relatively low speed, or by sedimentation rate in one of higher speed. These methods have an advantage over viscosity and osmotic pressure methods in that an indication of homogeneity is obtained. Meyer (49) claimed that aggregation rendered questionable most of the results obtained by this method. Molecular weights of 300,000 to 1,800,000 have been reported (44). Beckmann and Landis (5) reported values from 17,000 to 225,000 for a corn amylose separated by electrophoretic means. Nearly 50% of the sample fell in the range 31,000 to 61,000.

Mercaptalation. Wolfrom (87) found a d.p. of 150 for a methylated potato starch which had a d.p. of 7000 by viscosity. No explanation for the discrepancy was given. The value by mercaptalation is in better agreement with the

number average obtained by streaming orientation of the iodine complex than is the viscosity result. It may be significant that with a methylated cellulose, where aggregation may be less serious and viscosity measurements therefore more reliable, Wolfrom's method gave results in good agreement with results obtained by viscosity measurements.

End group assay. Hydrolysis of a molecule of methylated amylose gives one tetramethyl glucose from the end of the chain, the remainder of the molecule forming 2,3,6-trimethyl glucose (61, p. 572). If the sample is free of amylopectin and if the sample is reasonably completely methylated, the number of tetramethyl glucose molecules is equal to the number of amylose molecules originally present. Thus a number average molecular weight is obtained. Since amylopectin has many short branches, a small percentage of this as a contaminant will greatly increase the number of end-groups, giving low results for the molecular weight. Conversely, incomplete methylation could give too low a value for the number of end groups, consequently too high a molecular weight.

Another method based on end-group determination is oxidation with HIO_4. This reaction was first carried out with simple sugars by Malaprade (47), and with corn starch by Jackson and Hudson (26). Potter and Hassid (62) used

periodate oxidation in an attempt to ascertain the degree of branching of starches. They determined the molecular weights of their samples by osmotic pressure, and also by oxidation, thereby finding the number of non-reducing groups per molecule. They obtained values for amylose from 0.8 to 3.3, nearly all being above 1.0. These values, according to Potter and Hassid, may indicate a slight branching of the amylose, or they could also result from a trace of amylopectin.

Newton and Peckham (53, Chap. XI) have given a rather good review of starch oxidation, with an excellent section on the periodate method.

Orientation Theory

Rotation of molecules in flow fields

Production of a flow field. The simplest way to produce a flow gradient is to pass the fluid under examination through a tube. However, although this method has been used in some cases, it is practically useless where an accurate knowledge of the gradient is required, being useful only when relative behavior under different conditions is to be investigated.

The apparatus usually used consists of two concentric cylinders, the one being fixed and the other rotating. One

such apparatus* is described in detail by Edsall (11,51). The rotating cylinder is driven at known speeds. The radii R_1 and R_2 of the inner and outer cylinders respectively, and the gap width d between them are known, and since R_1 is nearly equal to R_2 in most machines, the flow gradient G is given approximately by

$$G \cong \frac{R_1 \Omega}{d} \cong \frac{R_2 \Omega}{d} \qquad (8)$$

where Ω is the angular velocity, 2π times the revolutions per second. The dimensions of G are t^{-1}.

When the angular velocity of the rotating cylinder exceeds a certain critical value (Ω_c), turbulence develops in the liquid, and the results are at present useless theoretically. For the case of inner cylinder rotation, Taylor (84) developed the equation

$$\Omega_c^2 = \left(\frac{\eta}{\rho}\right)^2 \frac{\pi^4}{2P} \frac{R_1 + R_2}{d^3 R_1^2} \qquad (9)$$

in which η is the viscosity of the liquid, ρ is its density, and P is a numerical factor given by the equation:

$$P = 0.0571\left(1-.0652 \frac{d}{R_1}\right) + \frac{0.00056}{1-.0652\ d/R_1} \qquad (10)$$

*Similar machines were used by Kundt (42) in 1881, and by Maxwell (48) in 1874. These earliest machines were used to investigate flow theories, rather than behavior of sols as such.

Irregularities of any kind might, of course, lower the value of the critical velocity.

The critical velocity is much higher when the inner cylinder is fixed and the outer rotates, but even with this advantage, rotation of the outer cylinder is not much used because of the mechanical difficulty in preparing such a machine.

Inglis (25) considered the effect produced when the cylinders are not quite concentric. He concluded that the average flow is less near the stationary cylinder, more near the moving cylinder.

Rotary diffusion constant. Before considering the various orientation theories, it would be well to consider the rotary diffusion constant. For any particle, there are really three constants, corresponding to rotation about each of its three axes. However, we are concerned only with long, thin ellipsoids of revolution. With such a particle the two short axes are the same, and consequently the constants are identical. Rotation about the long axis is so free and so unaffected by ordinary flow gradients that it contributes practically nothing to the phenomena of orientation with which we are concerned, so this constant may be ignored.

We therefore consider one rotary diffusion constant

and rotation in a 2-dimensional system, following Boeder's treatment (7). If Δn is the number of particles per unit volume, whose axes lie between the angles ϕ and $\phi + \Delta\phi$, we may define a distribution function $\rho(\phi)$ by the equation:

$$\rho(\phi) = \lim_{\Delta\phi \to 0} \frac{\Delta n}{\Delta \phi} \tag{11}$$

Now assume that an external force, such as a flow gradient, has established a preferred direction of orientation, so that ρ is not constant. Then Brownian motion will tend to cause molecules to shift from the preferred position to the less preferred; that is, to make the distribution more constant. The net number of molecules, dn, whose orientation shifts across the angle ϕ from lower to higher values in the time dt due to Brownian movement is

$$\left(\frac{dn}{dt}\right)_{\phi} = -\Theta \frac{\partial \rho}{\partial \phi} \tag{12}$$

This can be considered the definition of Θ, the rotary diffusion constant.

Perrin (58) derived an expression for the relaxation time τ of an elongated ellipsoid of revolution in terms of the time τ_o of a sphere of the same volume, and the dimensions of the ellipsoid. Edsall (11), using the relationship

$$\frac{\tau}{\tau_o} = \frac{\Theta_o}{\Theta} \tag{13}$$

wrote Perrin's equation in the form

$$\theta = \frac{3KT}{16\pi\eta a^3}\left(-1 + 2\log_e \frac{2a}{b}\right) \qquad (14)$$

which is applicable for molecules in which $a > 5b$. In this equation η is the viscosity of the solvent, K is the Boltzmann constant, T is the absolute temperature, a is the semimajor axis of the ellipsoid, and b is the semiminor axis. When this equation is written in the form

$$a = \left[\frac{3KT}{16\pi\eta\theta}\left(-1 + 2\log_e \frac{2a}{b}\right)\right]^{\frac{1}{3}} \qquad (15)$$

it can be seen that changing the ratio a/b will make only a small change in a. Consequently if θ can be found and if even a rough guess can be made as to the value of b, the length 2a can easily be calculated.

The diameter of the amylose-iodine complex in the crystalline state is known (13 Å) and can be assumed to be about the same in solution, so we can easily use this equation for finding lengths of these molecules once their rotary diffusion constants are known.

Early approximations in rotation theory. Jeffery (27) made one of the first successful attacks on the problem of the behavior of particles subjected to a flow gradient. He assumed an ellipsoid of revolution, so large that Brownian

motion was negligible. His results showed that the particle rotates most rapidly when the long axis is perpendicular to the flow lines, slowest when this axis is parallel to them.

Since many, if not most, macromolecules are small enough to be affected by Brownian motion, another treatment was necessary. Boeder (7) derived an expression

$$\frac{\partial \rho}{\partial \phi} + \alpha \rho \sin^2 \phi = C \qquad (16)$$

based on the assumption that the particle is confined to a plane including the direction of flow and the direction of the flow gradient. While this assumption was not entirely valid, the particles do spend much of their time in or near this plane. In this equation, ρ is the orientation distribution function as a function of ϕ, and $\alpha = G/\theta$.

Boeder's solution to his equation gives results very similar to results obtained later from more rigorous derivations, and to most experimental results. In this 2-dimensional case, it is found that for small values of α, ρ is a maximum at 45° to the flow lines; the position of the maximum shifts to lower angles for higher values of α. In the latter case it is also found that more complete orientation occurs; that is, the particles spend more of their time in the preferred position.

The 3-dimensional case was also considered by Boeder.

For low values of α, he found that the angle between the preferred position and the flow lines (called the extinction angle) is given by

$$\chi = \tfrac{1}{2} \arctan \frac{6}{\alpha} \qquad (17)$$

This can easily be changed to the form*

$$\chi = \frac{\pi}{4} - \frac{\alpha}{12}\left(1 - \frac{\alpha^2}{108} + \cdots\right) \qquad (18)$$

The equations of Peterlin and Stuart. The most satisfactory expressions for χ and for the magnitude of the flow birefringence obtained to the present time are those of Peterlin and Stuart (59). They obtained χ as a function of α, a, and b, but until recently it was prohibitively difficult to use their expression except in the modified form applicable only at low values of α:

$$\chi = \frac{\pi}{4} - \frac{\alpha}{12}\left[1 - \frac{\alpha^2}{108}\left(1 + \frac{24}{35}\cdot\frac{a^2-b^2}{a^2+b^2} + \cdots\right)\right] \qquad (19)$$

Their expression for the magnitude of flow double refraction is

$$\frac{\Delta n}{n} = \frac{2\pi}{n^2} c(g_1-g_2)\cdot f(\alpha, \tfrac{a}{b}) \qquad (20)$$

*The expansion required, taken from the Handbook of Chemistry and Physics, 29th edition, Chemical Rubber Publishing Co., p. 244, is arctan $X = X - (1/3)X^3 + (1/5)X^5 - (1/7)X^7 + \ldots$. Boeder's expression is first converted to the form $\chi = \frac{1}{2}(\pi/2 - \arctan \alpha/6)$.

Here n is the refractive index of the solution at rest. Δn is the difference between the refractive indices of the flowing liquid for the extraordinary and ordinary rays, c is the volume fraction of the colloid, (g_1-g_2) is an optical factor dependent on the refractive indices of the colloid and solvent and on the particle dimensions, and $f(\alpha,\frac{a}{b})$ is a distribution function. The refractive indices of the particles and the effects of their dimensions are practically constant for very long particles such as amylose, a change of length producing only negligible changes in them. Thus, for a homologous series varying only in chain length, but all particles being long, Δn is essentially proportional to concentration and the distribution function*. However, the same difficulty was formerly encountered here as in the case of the Peterlin and Stuart expression for χ; the distribution function was too complex to handle except at low α's**.

In 1949, Scheraga, Edsall, and Gadd (72) applied the Mark I calculator at Harvard University to the problem, and prepared tables of χ and f, the distribution

*This, of course, depends on the assumption that the solution is dilute enough to prevent particle interaction.

**It should also be pointed out that all this work is valid, according to Peterlin and Stuart, only if all dimensions of the particles lie between 10 and 1000 Å.

function*, as functions of α for values of α from 0 to 200 for a monodisperse system. These are the best data available at the present time, and were used in the work to be presented later in this thesis. Plots of the data for an axial ratio of 50 are shown in Fig. 2.

Polydisperse systems

Many experimental curves for χ vs. α and for Δ** vs. α do not fit the theoretical curves obtained from the equations of Peterlin and Stuart. For example, Signer and Gross (78) found that the birefringence of a solution of nitrocellulose increased approximately linearly with α, although at the gradients attained, it should have deviated considerably. Also, the χ values for a preparation of rabbit myosin appeared to approach 12° instead of 0° as a limit at higher gradients (51).

In an attempt to account for these discrepancies, Sadron (68) developed equations showing the effect of polydispersity in colloidal systems. The effects of the various

*The symbol f may also be used to represent the relative birefringence of a sample, normalized to a value of 1 for a completely oriented sample, as calculated from α by means of the theoretical work of Peterlin and Stuart. α in turn is obtained either from experimentally determined values of χ, again using the Peterlin and Stuart equation, or from θ for a hypothetical sample, using Perrin's equation for θ.

**Δ is the directly observed birefringence of a solution. For a monodisperse system of rigid particles, Δ should be proportional to f as calculated from the corresponding χ values.

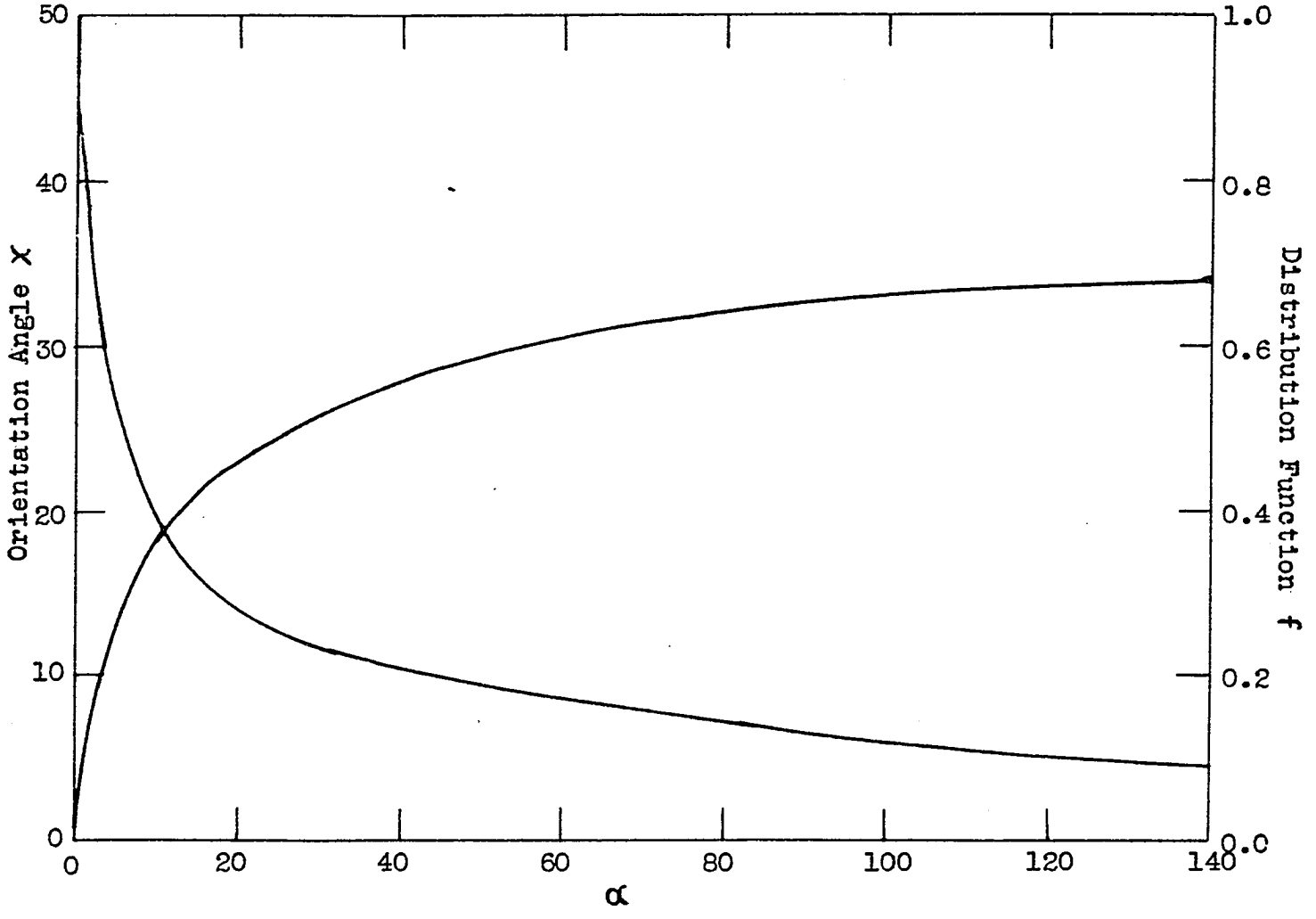


Fig. 2. χ and f as functions of α for a monodisperse system.

components are summed up in the following ways:

$$\tan 2\chi = \frac{\sum_{1}^{i} w_i f_i \sin 2\chi_i}{\sum_{1}^{i} w_i f_i \cos 2\chi_i} \qquad (21)$$

$$f^2 = \left[\sum_{1}^{i} w_i f_i \sin 2\chi_i\right]^2 + \left[\sum_{1}^{i} w_i f_i \cos 2\chi_i\right]^2 \qquad (22)$$

χ_i and f_i are the extinction angle and birefringence respectively which one would have if the ith component alone were present, and w_i is the weight fraction of the ith component. In these equations it is assumed that the concentration of each component is low, and that they act independently of each other.

Birefringence

Theory of double refraction

Optical anisotropy. An excellent discussion of optically anisotropic materials may be found in the text by Jenkins and White (28, Chap. XV), or in any good physical optics textbook, and consequently need not be undertaken here. Rundle and French (67) have investigated the optical properties of amylose, and its iodine complex in crystalline form, and have found them anisotropic. Form birefringence

requires that the particles be small compared to the wavelength of light, and as these crystals were from 5 to 20 microns in size, their birefringence could not be due to the shape of the crystals. Hence the birefringence of amylose is at least partially inherent in its internal structure. According to the work of Rundle and French and that of Silberstein (80), it appears that when amylose is in the extended form, such as in the "A" or "B" modifications, light with the electric vector parallel to the long axis is retarded most. When amylose is in the helical form, light with the electric vector normal to the helix axis is retarded most.

Form birefringence. In 1912 Wiener (86) showed that if a system were composed of a large number of ellipsoids, all oriented in the same direction and small compared to the wavelength of light, the system would be anisotropic even if the particles themselves were optically isotropic, provided their refractive indices were different from that of the immersion liquid. This is sometimes called form birefringence. The slower component vibration is parallel to the greater dimensions of the particle, regardless of the refractive index (8, p. 295); that is, the particle is optically positive.

Since molecularly dispersed amylose particles are considerably smaller, even in their longest dimension, than

the wavelength of the visible light used to observe the system, there will obviously be form birefringence in an amylose solution, as well as the inherent birefringence already mentioned.

Double refraction of partially oriented amylose. As shown above, amylose molecules individually show both form and inherent birefringence. An extended chain type of molecule should show birefringence in a solvent of any refractive index, for the inherent birefringence is always positive, and the form birefringence is zero or positive, never negative. However, greatest retardation of the velocity of light probably occurs for the component whose electric vector is normal to the long axis for a helical molecule (67); hence, such a molecule should have negative inherent birefringence. One would then expect it to be possible to choose a solvent of such refractive index that the negative inherent and the positive form birefringence exactly cancel each other and leave no birefringence at all. This would probably require nearly monochromatic light.

The above paragraph referred to an individual molecule only. A sufficiently large number of such molecules oriented entirely randomly would show no birefringence at all, regardless of the properties of the individual molecules. In a flow field, however, there is a preferred orientation, and the individual molecules spend more of

their time in this position than in any other. The solution then becomes anisotropic if the individual molecules exhibit double refraction. With increasing flow gradients, the molecules become more and more aligned, and the solution anisotropy increases, reaching a maximum when all molecules are completely oriented in the preferred direction. This, of course, requires an infinite flow gradient, and can only be approached, not attained.

Since we must therefore deal with systems of partially oriented molecules, knowledge of the function relating gradient to relative birefringence of the solution is necessary if one wishes to obtain all the information possible from flow birefringence.

Boeder (7) derived the distribution function for the 2-dimensional case (equation 16). While this function was useful, it was not accurate enough for precise work. Peterlin and Stuart (59) developed a more rigorous equation for the distribution function, but its complexity prevented its use except at low α until the recent work of Scheraga, et al. with the Harvard Mark I calculator.

As stated on p. 21, for the systems dealt with in this thesis Δn is proportional to the distribution function. Thus the birefringence measured for a solution should be proportional to the value of f as calculated for a monodisperse system from the experimentally determined χ values; if it is

not, the molecules are non-rigid, polydisperse, or both.

Measurement of birefringence

Extinction position. When two Nicol prisms or Polaroid discs are aligned with the principal sections oriented at 90° to each other, practically no light gets through both of them. If a birefringent material is placed between them with its optic axis parallel to the principal section of either prism or disc, there is still no light transmitted, for the added material does not alter the light from the polarizer in any way. However, it can easily be shown (28, Chap. XVI) that with the added material in any other position, transmission practically always occurs. Hence, if the sample is rotated until extinction occurs, its optic axis is known to be parallel to the axis of either polarizer or analyzer. Instead of rotating the sample, it is an obviously equivalent process to rotate the polarizer and analyzer, always keeping them crossed. This is the procedure in flow birefringence, since it is not practical to set up the cylinder system to rotate about the light beam.

Most workers have used only one extinction position of the four available for determining the extinction angle. However, Foster and Lepow (15) improved upon this technique by measuring all four extinction angles, then reversing the

cylinder rotation and again measuring all four. One set of readings was subtracted from the other, and the differences averaged. The average was halved and subtracted from 45° to give χ.

Magnitude of birefringence. By a simple process, one can show (28, Chap. XVI) that the phase difference between two rays of light, initially in phase, one parallel to the optic axis of a material through which it passes, and the other perpendicular, is proportional to the birefringence of the material; that is, to the difference of its refractive indices parallel to and perpendicular to the optic axis.

There are various ways to measure this phase difference, using such devices as the quartz wedge (8, p. 276), Babinet (28, p. 360), Soleil (28, p. 361), and Senarmont compensators. The techniques for the use of the Babinet and Soleil compensators are quite similar to that of the quartz wedge. The Senarmont compensator (quarter-wave plate) is the most suitable of the above compensators for measurement of phase shifts of the magnitude encountered in flow birefringence work.

To use the Senarmont compensator, one adjusts the polarizer or the material to be examined so that their optic axes are at 45° to each other, the polarizer and analyzer being crossed. If the sample orientation is

predetermined by the experimental set-up, as it is for a given sample at a specified gradient in a given flow birefringence apparatus, then the necessary adjustment must be made by rotating the polarizer and analyzer. Next the sample is removed or made isotropic (in a flow birefringence apparatus, flow is stopped) and a quarter-wave plate is inserted between the analyzer and the sample (or the place where the sample is to be, if it has been removed). This plate is rotated until extinction is attained. Then the sample is replaced or rendered anisotropic by flow, and the analyzer alone rotated to the new extinction position. If Δ is the angle through which the analyzer is rotated and δ is the phase difference to be determined, it can be shown (12) that

$$\Delta = \frac{\delta}{2} \tag{23}$$

Thus the analyzer rotation obtained by this method is proportional to the birefringence of the sample.

Apparatus. Details of the apparatus have been varied with different workers, but the same principles apply in all cases. The apparatus of Edsall (11,51) is typical. There is a light source directed downward, and beneath this is a polarizer followed by concentric cylinders which are set up so that the inner cylinder can be rotated

at various speeds. The polarized light from above passes through the gap between the cylinders, the light path being parallel to the axis of the cylinders. Below this there is a mount for the quarter wave plate, an analyzer so mounted that it can be rotated with the polarizer, or independently of it, and finally, a prism and telescope at the bottom for observation.

Dichroism

Definition

Dichroism is the property of exhibiting one color when viewed by transmitted light whose electric vector is vibrating parallel to the optic axis, and another color for light whose electric vector is vibrating in the perpendicular direction*.

*According to Webster's New International Dictionary, 2nd ed. (1946), dichroism is defined as

1. Cryst. The property of presenting different colors by transmitted light, when viewed in two different directions, the colors being unlike in the direction of unlike or unequal axes.
2. Physics. The property of some bodies of differing in color with the thickness of the transmitting layer, or, in liquids, with the degree of concentration of the solution.
3. Dichromatism.

While optics texts generally use the term "dichroism" in either the first or second sense, a few make a distinction by using "dichroism" for the first meaning, and "dichromatism" for the second.

Theory of dichroism

Inherent dichroism. The absorption of light by colored anisotropic crystals usually varies with the angle of the plane of polarization of the light (8, p. 279; 35, p. 140). Since the absorption band for light whose electric vector is vibrating parallel to the optic axis occurs at a different wavelength than the absorption band for light with its electric vector perpendicular to the first, the light transmitted will have a different wavelength distribution, hence, (usually) a different color.

According to Raman and Bhagavantam (64) who worked with colored organic materials, the absorption frequency of such materials along the axis is decreased by interaction between the atomic dipoles induced by the light wave, and the absorption frequency normal to the axis is increased. Krishnan and Dasgupta (36) reported similar behavior of alkali nitrates in the ultraviolet region.

It must be understood that the term "color" must not be limited to the visible spectrum, but should be used to include also light in the infrared and ultraviolet spectrum. For example, an absorption band for light vibrating in one plane might be in the red region; for light vibrating at 90° to the first plane it might be in the ultraviolet or infrared. If there were no appreciable absorption of

any other wavelengths, the material in the first case would be blue or green, whereas in the other, it would be colorless to the eye. If we consider only visible color, the example given would not fit our definition of dichroism, nor would the term "dichroism" be applicable etymologically since there would be only one color involved. Yet the principle would be identical to that involved in a case where, for example, the two absorption bands were in the red and green regions, giving green and red colors respectively. Therefore it is not reasonable to limit the term "dichroism" only to cases wherein there are actually two different colors visible to the eye when the plane of polarization of the transmitted light is rotated.

The term "dichroism" (or the more general term "pleochroism") may even be used in cases where there is no visible color at all. Krishnan (37) wrote of the pleochroism of several materials, referring entirely to absorption in the ultraviolet, the materials being transparent to visible light.

Dichroism due to birefringence. Procopiu (63) developed an equation for dichroism resulting from the fact that reflection of light from an interface depends in part on the refractive indices of the materials on either side of the interface. Since the refractive index of many

materials, such as amylose, is different in different directions, reflection of light striking an amylose-solvent interface would be expected to be different for light of different planes of vibration. There would then be a difference in transmission corresponding to the difference in light removed by reflection, producing the same result as true dichroism.

The equation Procopiu derived is

$$\sin 2\delta = \frac{(n_1-n_2)(n_1 n_2-n^2)}{4n^3} kc \qquad (24)$$

Here n_1 and n_2 are the refractive indices of the solid particle, n is the refractive index of the solvent, c is the concentration, δ is an experimental measure of the dichroism of the solution (see the next section, "Experimental determinations"), and k is a constant. The equation is derived on the assumption that n, n_1, and n_2 are not too greatly different.

Experimental determinations

Qualitative. Detection of dichroism in crystals involves nothing more than passing white light through the sample and through a Nicol prism*, rotating one of them

*Nearly always Polaroid is quite satisfactory, but if accurate observation of color were needed, the slight color of this material might be objectionable.

and observing the color and intensity of the transmitted light (8, p. 279; 35, p. 140). It makes no difference whether the light is passed first through the sample or the polarizer. When the sample in question consists of many submicroscopic particles such as macromolecules suspended in a fluid, it is necessary to orient the particles at least partially, then to proceed as above.

Various techniques have been used for orientation of such particles. Magnetic and electric fields have been used (10,22,43,60), also flow through a tube (18,60,89) and between concentric cylinders (66,89), and in some cases samples were prepared by dying an already oriented material such as cellulose or gelatin (1,52).

Rundle and Baldwin (66) using the concentric cylinder method for producing a flow gradient and so orienting the sample, were able to detect flow dichroism of the amylose-iodine complex. They were not able to measure the orientation angle or the extent of dichroism with their apparatus.

Quantitative. Most workers have attempted, not only to detect dichroism where it existed or could be produced, but also to make some quantitative measurements with it. For example, Heller (22) used magnetic dichroism of magnetite sols to study aggregation. Freundlich, et al. (18),

used flow dichroism as developed in a tube to study aging and temperature effects on V_2O_5 sols. They measured the extent of the dichroism when flow was great enough to produce essentially complete orientation, and also noted that the flow rate required to achieve this state depended partly on the age of the sol.

The determination of the magnitude of dichroism can be carried out by using two Nicols or Polaroids, after the angle of the absorption axis of the sample has been found. A polarizer is placed between the light source and the sample, with the optic axis of the polarizer and the absorption axis of the sample at an angle of 45° to each other. The analyzer is placed between the sample and the eye. Its optic axis is placed perpendicular to that of the polarizer, and then it is rotated until a minimum is reached. The angle through which it is rotated is δ.

Zocher (89) studied dichroism in flow in tubes and also between concentric cylinders. He showed that the magnitude of dichroism of a sol containing completely oriented particles is given by

$$k_1 - k_2 = \frac{2 \log_e \tan (45 + \delta)}{d} \qquad (25)$$

In this equation, k_1 and k_2 are the absorption coefficients of the solvent for light vibrating parallel to and

perpendicular to the optic axis, and d is the depth of solution examined.

Nikitine (54,55) carried out experiments with the dichroism of flow of fluorescein solutions. His interest was mainly in photodichroism, the Weigert effect.

APPARATUS

A simplified diagram showing the main parts of the apparatus as set up for visual observation of birefringence is shown in Fig. 3. The various components will be taken up individually.

The optical and mechanical parts of the machine were essentially those built by Foster and Lepow (15), only a few very minor changes being made. The photoelectric apparatus was designed and built for these studies.

Mechanical

Cylinders

A cross-sectional view of the cylinders and accessories is shown in Fig. 4. The inner cylinder and the inner portion of the outer cylinder are made of stainless steel. Water at a constant temperature is circulated through the space between the walls of the outer cylinder. The sample is admitted at the bottom, any excess being discharged at the overflow.

Cylinder drive

The inner cylinder is driven by a shunt wound D.C. motor, the two being connected by a long flexible shaft.

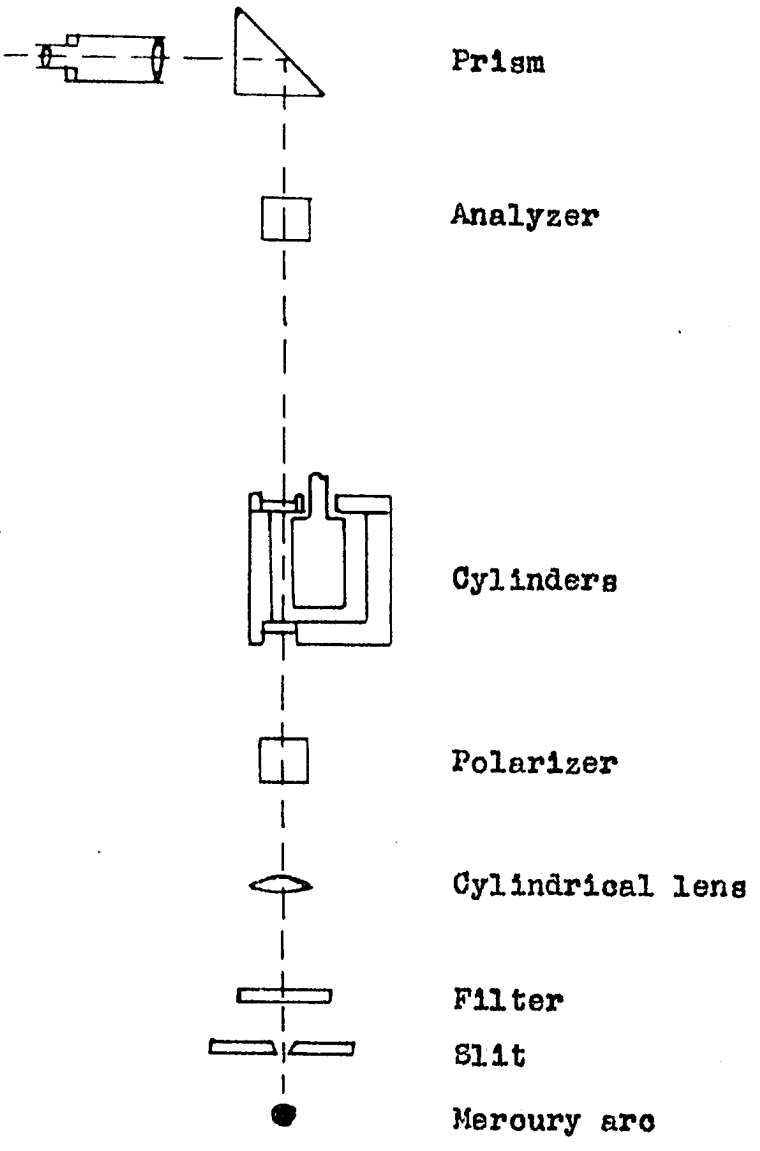


Fig. 3. Apparatus for measuring birefringence of flow.

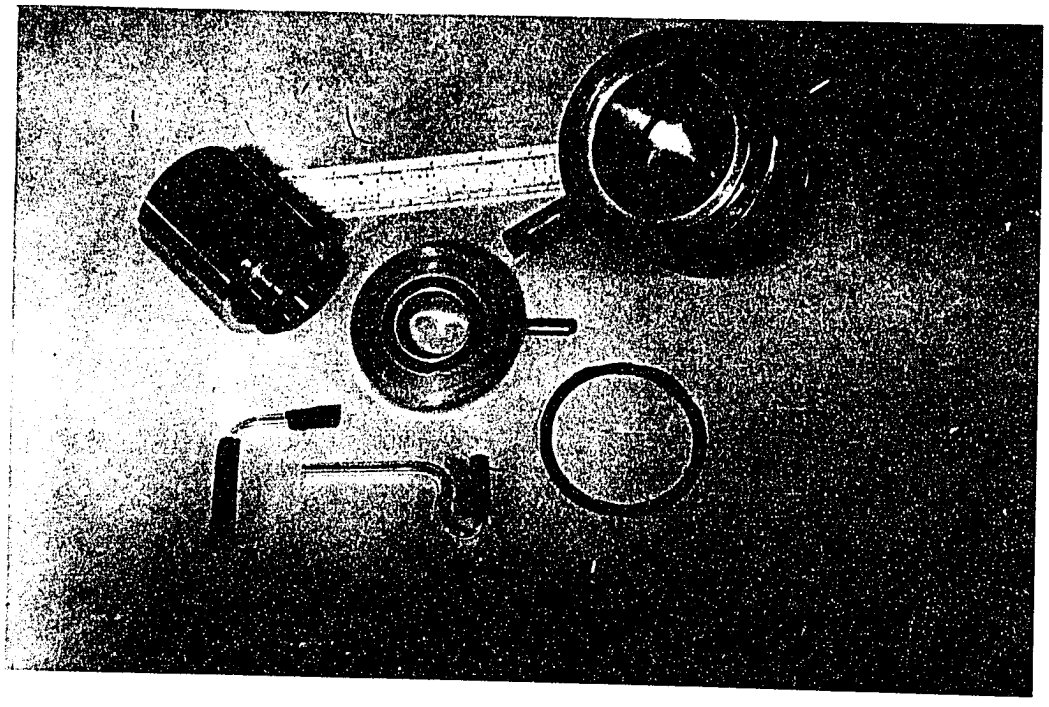


Fig. 4. Cylinders for production of flow gradients.

The power for the field and armature of this motor is supplied from separate D.C. generators. Independent control of the field and armature currents is thus possible, and facilitates speed control. Fairly steady speeds can be maintained from about 50 RPM to a maximum of 350 to 2400 RPM, depending on the viscosity of the solution between the cylinders. By reversing the direction of the current flow in the motor field, the cylinder can be rotated in the opposite direction.

The speed of rotation is ascertained by means of a stroboscope operating at a frequency of 3600 on-off cycles per minute. This light illuminates a disc mounted on the drive shaft. The disc is marked with four radial lines at 90° intervals, three being of equal lengths, and the fourth longer.

Polarizer and analyzer coupling

The polarizer and analyzer are operated by worm gears which engage large gears on their mounts. The worm gear shafts are coupled by right angle gears and a long vertical shaft. Turning the latter shaft, or either worm gear shaft (the upper is used in practice) causes both the polarizer and the analyzer to rotate at the same rate and in the same direction. This apparatus is shown in Fig. 5 and 6.

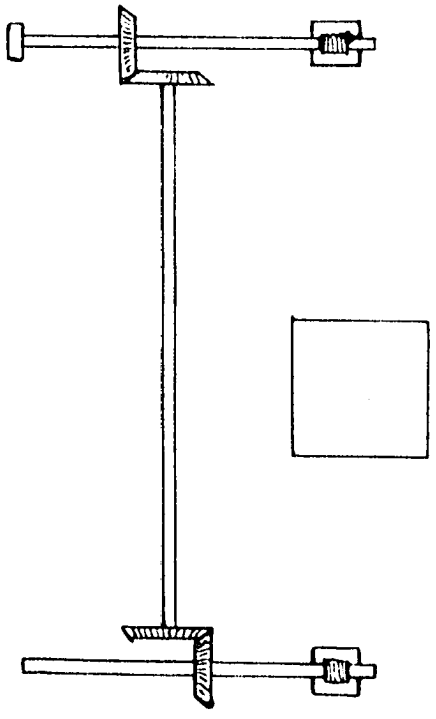


Fig. 5. Polarizer-analyzer coupling

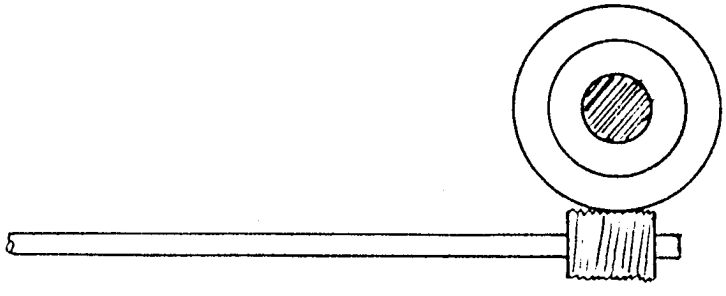


Fig. 6. Top view of analyzer drive

If either is to be rotated independently, one of the right angle gears coupling the vertical shaft with the analyzer worm gear drive is uncoupled. This is a simple operation, requiring only loosening of a set screw.

Mounting

The cylinders and light source are mounted on a rigid bench of heavy angle iron. An iron shaft rises vertically from this bench and is fastened securely to the wall at the top. This shaft provides a solid support for the optical and electrical apparatus used for observation and measurement. Other shafts carry the analyzer mount and drive. These shafts are fastened to the above vertical shaft.

The D.C. motor is mounted on the wall. The D.C. generators and the A.C. motor used to drive them are on the floor.

Optical

Light source

A General Electric type AH-4 mercury vapor bulb is operated from the 110 volt A.C. line with a suitable transformer. Filters are used to isolate the desired lines of the mercury spectrum.

A cylindrical lens and slit provide collimation of the light. The slit is parallel to the long axis of the bulb and to the tangent to the cylinders at the point where the light passes through them. The cylindrical axis of the lens is parallel to the slit.

Polarizer and analyzer

At first Nicol prisms were used for both the polarizer and the analyzer. Polaroid discs, mounted between round plates of glass, were later substituted. These had a larger aperture, did not deviate the light rays, and appeared to give better extinction.

Apparatus for visual observation

The analyzer is placed at a height from the floor of about 5 feet, just below eye level. Above this there is a prism to reflect the ray horizontally at eye level. A telescope focussed on the gap between the cylinders is mounted in front of the prism. To help keep out stray light, a black, curved shield, fitting the face of the observer closely, is attached to the telescope.

Photoelectric Measuring Circuit

Purpose

The almost complete extinction found when crossed Nicols or Polaroids are parallel to or perpendicular to the

optic axis of a birefringent sample is not usually observed in the case of flow dichroism. Generally the light intensity at the minimum is great enough that minima can be determined visually only with a low degree of precision if at all. Readings of the position of maximum intensity, necessary for determinations in adjacent quadrants in dichroism work (see p. 89), are virtually impossible by visual methods.

With photoelectric methods various means are available for opposing most of the signal so that a high sensitivity can be used to measure the small unopposed signal, thus permitting accurate detection of small variations in the light transmitted by the dichroic sample.

The light intensity of the transmitted beam may be moderately high, but is more often low because of the considerable absorption by the amylose-iodine complex. In addition, the cross-sectional area of the beam is very small (.06 cm^2), so that the total flux reaching the phototube is not sufficient for an ordinary phototube. A 931-A multiplier phototube is therefore used.

General description of apparatus

An outline of the photoelectric apparatus is shown in Fig. 7. Light from a source modulated at 120 cycles, after passing through the mechanical and optical apparatus previously described (with the prism and telescope removed, however) strikes the cathode of the multiplier phototube.

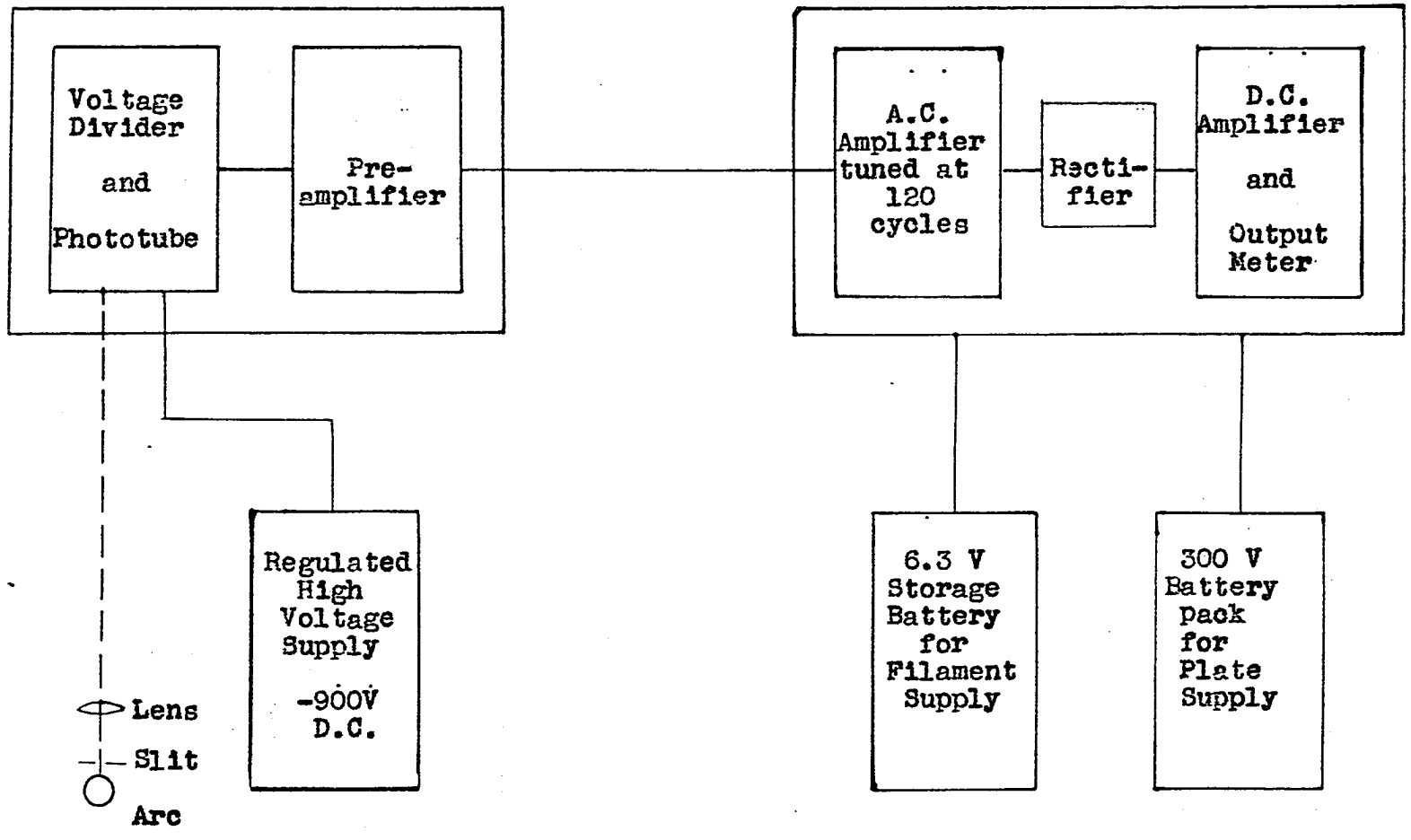


Fig. 7. Outline of electronic apparatus

The phototube output is amplified with little frequency discrimination, and is then amplified further by an A.C. amplifier tuned to 120 cycles. The desired signal is increased by the tuned amplifier, but the D.C. and random frequency A.C. output resulting from stray light, phototube dark current, etc. are not. The output from the tuned amplifier is rectified, partially opposed when necessary by a stepwise variable voltage obtained from a battery of small dry cells, then is amplified again and measured by a microammeter provided with suitable shunts.

All components are shielded, the shielding being grounded. Coaxial cable is used to make all external connections.

Light source

Modulation. The same light source is used as for visual observation. This source, like other A.C. arcs, is modulated at twice the line frequency. Such modulation is very convenient, for it permits use of a tuned A.C. amplifier in the electronic circuit used with the phototube, with the advantages listed in the preceding section.

Any change in frequency of the light causes a change in the output of the tuned amplifier. An A.C. power supply with a stabilized frequency is therefore to be desired. It would be better if the frequency were not an even multiple

of line frequency. If the light source power supply and the tuned amplifier were designed to operate at 100 cycles, for example, the probability of detecting stray fields from unshielded A.C. lines would be much reduced.

Voltage stability. Variation in line voltage varies the intensity of the mercury arc light, therefore also the output of the photoelectric circuit. A voltage regulation device which does not alter the wave form of the current would be advantageous. However, a Sola constant voltage transformer did not help at all. A simple, but usually effective way to overcome both voltage and frequency problems is to work late at night, shutting off all nearby ovens and refrigerators. This method was resorted to several times.

High voltage supply

The high voltage supply for the phototube is shown in Fig. 8. Taps are provided at -600, -750, and -900 volts. A potential of -900 volts appears to work best. Switch S_3 and resistance R_1, mounted on the front panel, together with resistance R_3 inside the housing permit adjustment of the V.R. tube current to any desired value. Switch S_2 is a microswitch arranged to open the primary circuit when the lid to the housing is raised.

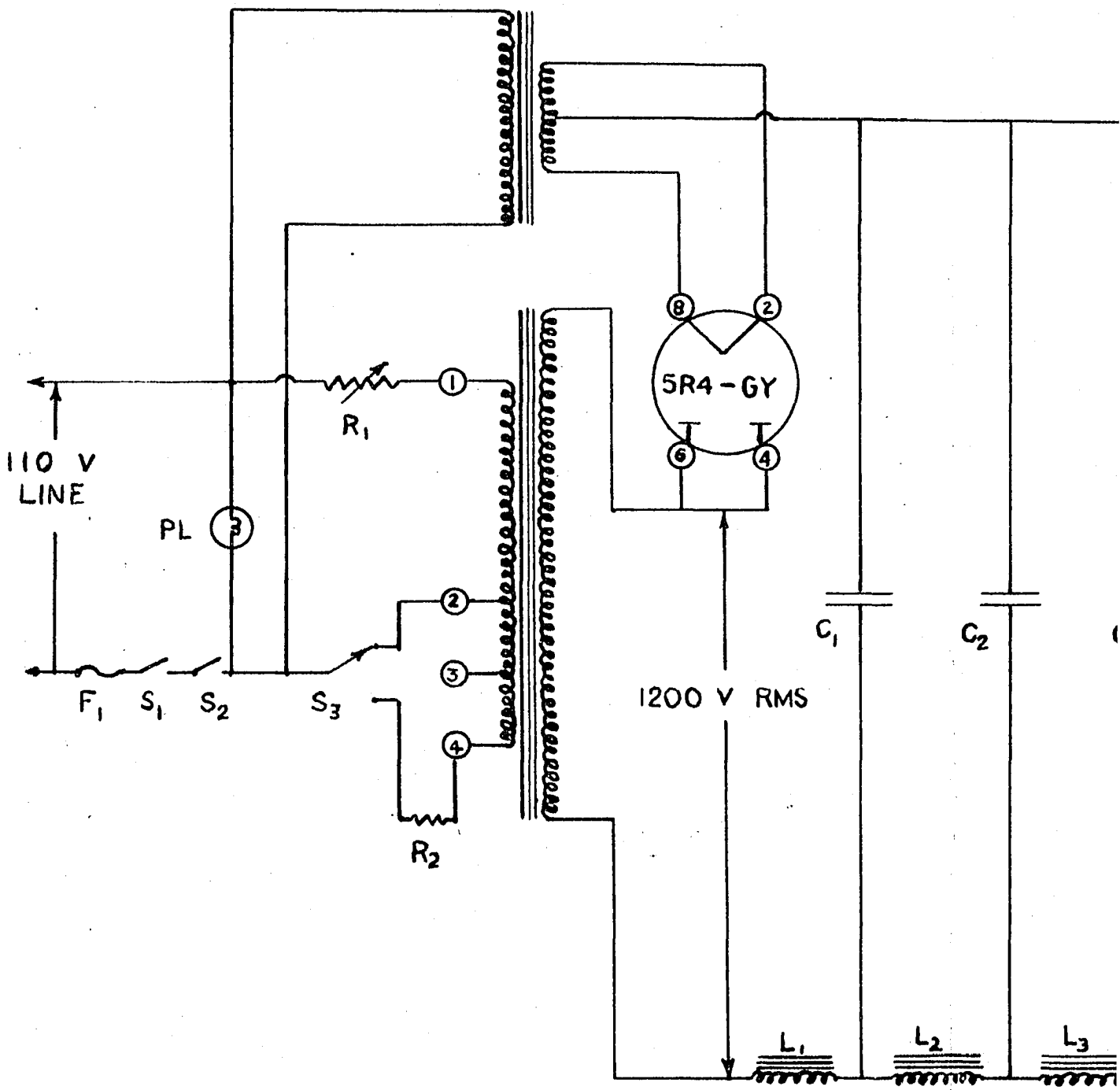
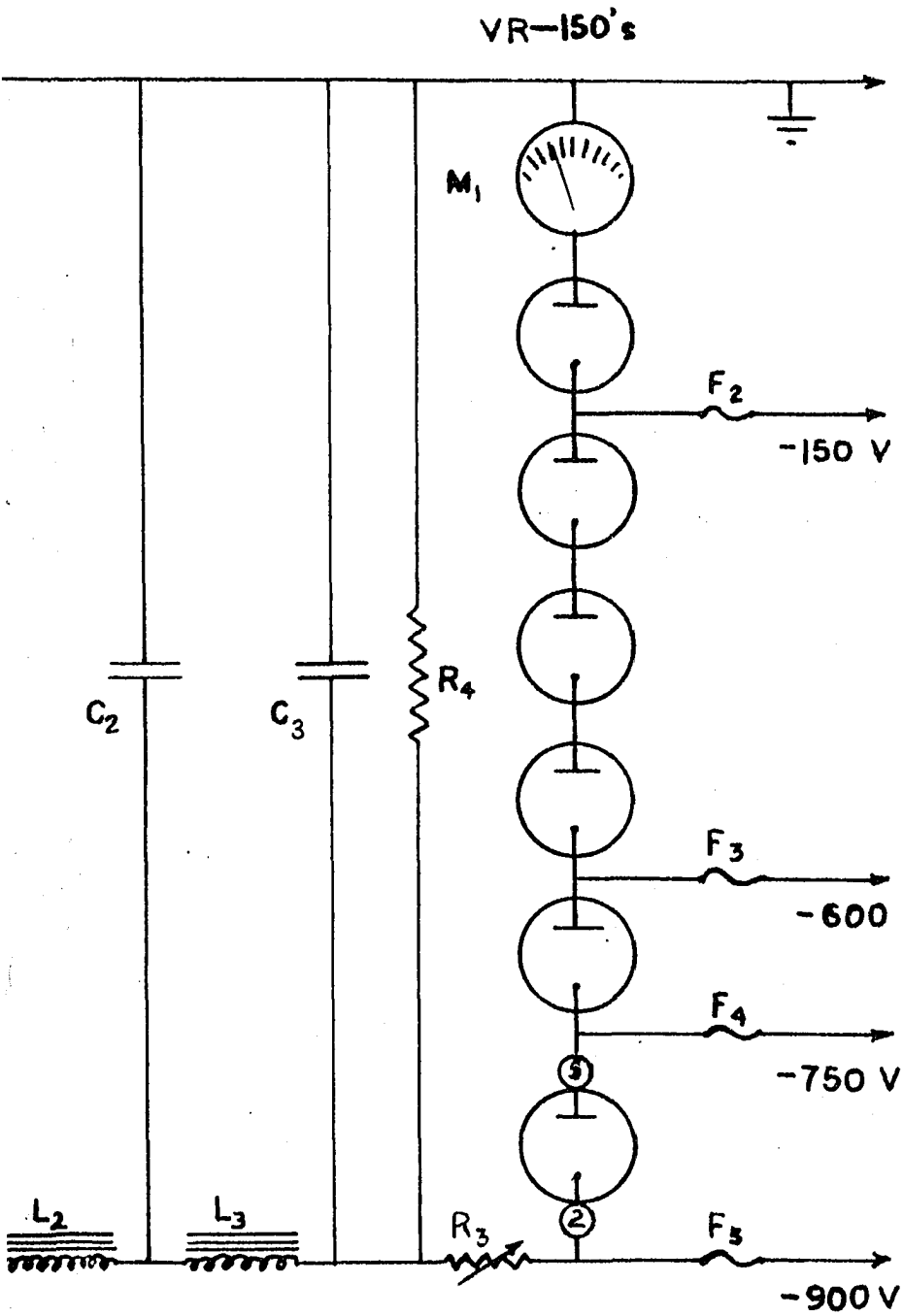


Fig. 8. High voltag



S_1	Lid safety switch
S_2	Power switch
R_1	25 ohms, 25 W
R_2	10 ohms, 50 W
R_3	25,000 ohms, 50 W
R_4	1 megohm, 2 W
C_1	2 mfd., 2500 V
C_2	4 mfd., 1500 V
C_3	9 mfd., 1200 V
L_{1-3}	16 henries
M_1	50 ma. meter, approx. 0.035 volts drop at full scale
Pl	Pilot light
F_1	2 ampere fuse
F_2	5 ma. fuse
F_{3-5}	1/32 ampere fuses

8. High voltage supply.

Phototube and preamplifier

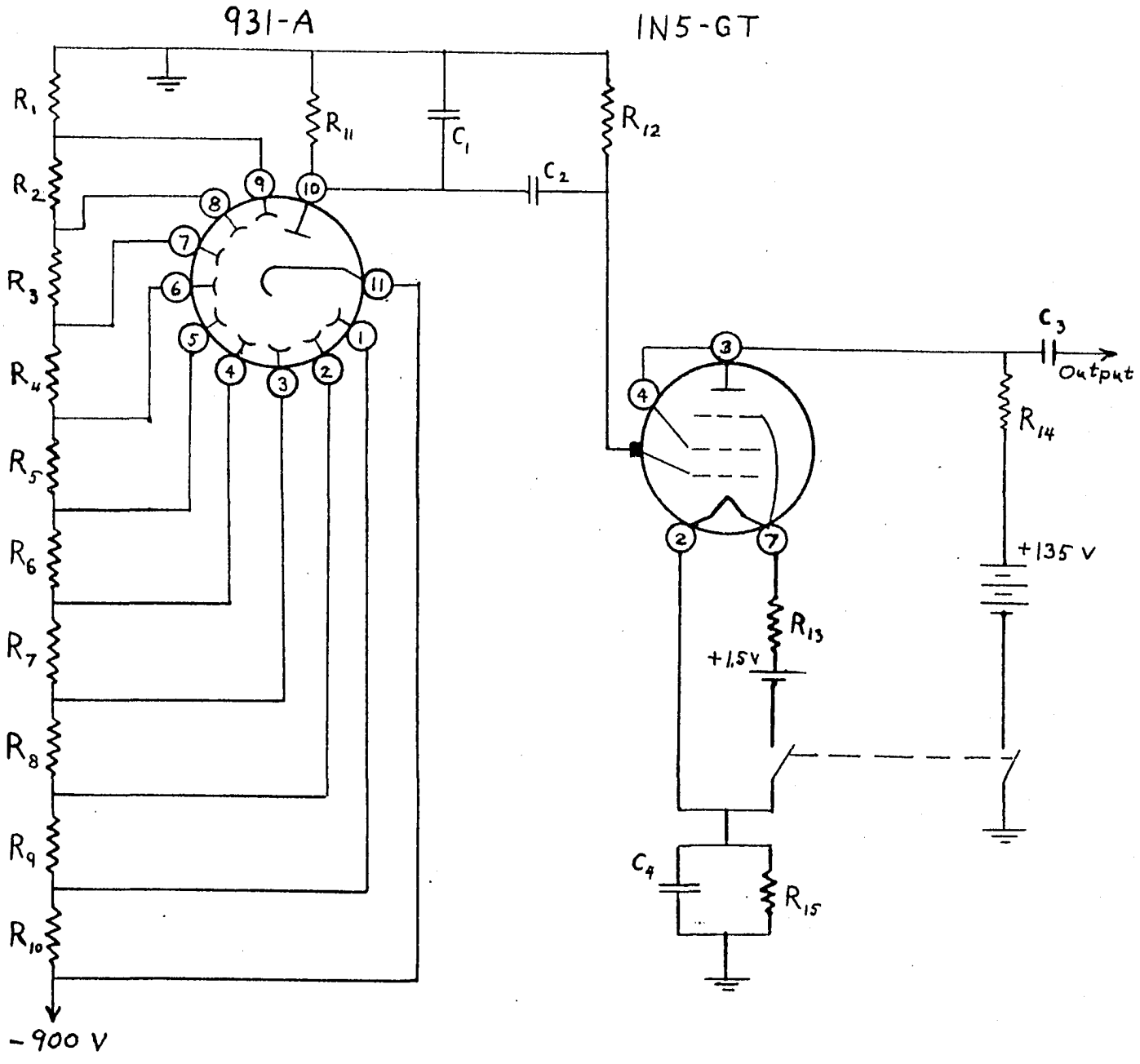
The 931-A multiplier phototube, preamplifier and preamplifier batteries are housed together in a fairly air-tight metal box. Sacks of magnesium perchlorate are placed inside as a drying agent, and a drying tube of the same material is attached to a small vent in the case. The on-off switch handle is covered with a thin rubber tube cemented to the case. These precautions keep the air inside dry for the life of the batteries.

The circuit for this unit is shown in Fig. 9. Steady signals as from daylight leaks are not transmitted past condenser C_2, and high frequencies are mostly by-passed by C_1. Thus this unit has a slight frequency discrimination.

To help produce steadier response, all resistors in this unit are wire. Use of a 1P21 tube instead of the 931-A was considered, but was not found necessary.

A piece of exposed and processed film is placed below the phototube, so arranged that it can be placed in or out of the light path by an external control. This film reduces the light transmitted to about 0.12%, and is used to protect the phototube when bright light is encountered.

With the phototube inoperative (high voltage off) a series of small known 60 cycle voltages was applied to its anode, and the output of the preamplifier was measured with



R_1	15K ohms	R_{13}	2.6 ohms	C_1	0.001 mfd.
R_{2-10}	20K ohms	R_{14}	295K ohms	$C_{2,3}$	0.01 mfd.
$R_{11,12}$	800K ohms	R_{15}	1000 ohms	C_4	10 mfd.

Fig. 9. Phototube and preamplifier.

an A.C. vacuum tube voltmeter. The voltage gain was found to be about 17, being approximately linear up to an output of at least 9 volts RMS which is considerably more than necessary. Testing with an audio oscillator of approximately constant output showed that the gain was essentially the same at 60 and at 120 cycles.

Tuned amplifier and output

The final stages of the apparatus are shown in Fig. 10. The power supply, not shown in the figure, consists of seven Burgess #5308 45 volt B-batteries in series in a grounded metal box, connected to the amplifier with a shielded cable. A 6.3 volt storage battery provides filament power. A well-regulated, line operated power supply mounted in a separate chassis could perhaps be used, but when such a supply was mounted in the same chassis, considerable difficulty was experienced with unsteady output and pickup of stray 120 cycle signals even though the tuned stage was isolated fairly well from the power supply. This power supply consisted of a 5Y3 used as a full wave rectifier together with a filtering and V.R. tube regulating circuit, and was probably the source of the undesired 120 cycle signal. Battery operation completely eliminated these difficulties. It would be desirable when using battery operation to redesign the output circuit for lower current requirements to lengthen battery life.

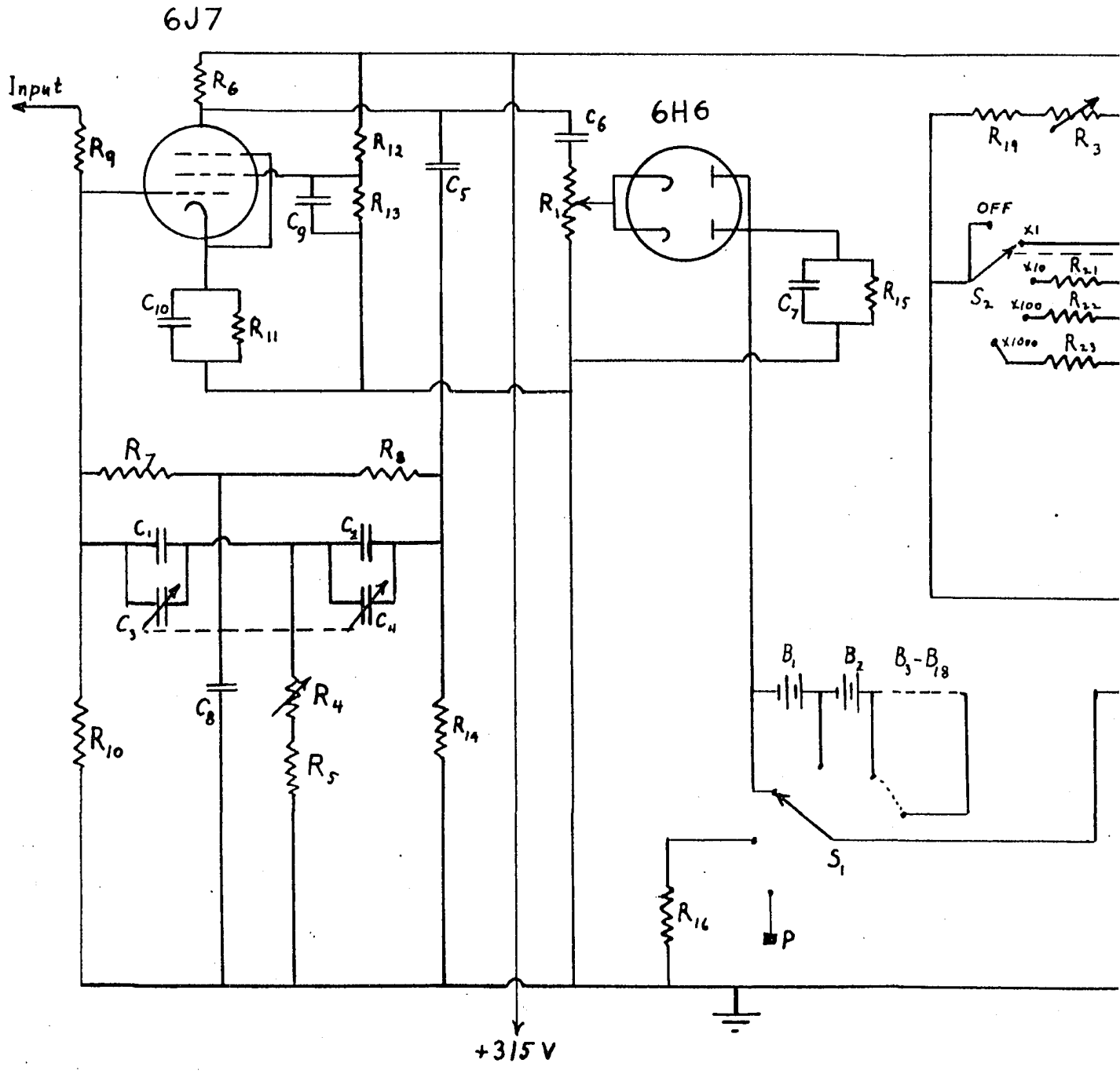
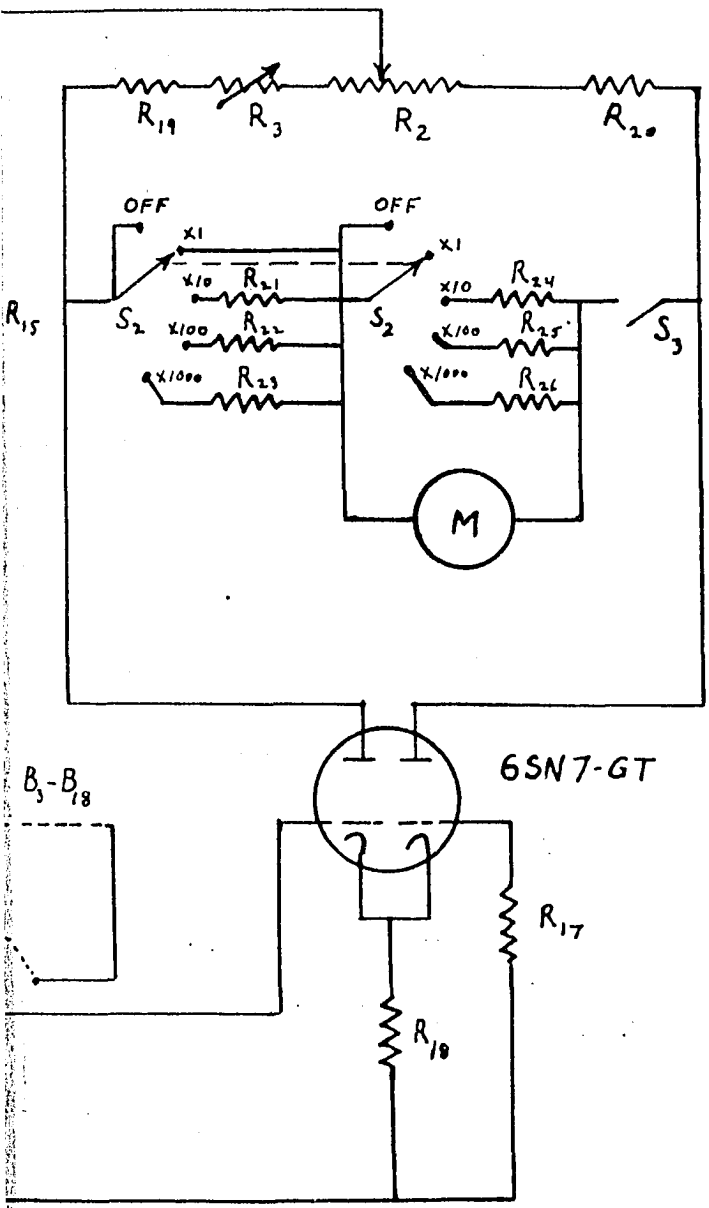


Fig. 10. Tuned amplifie



R_1	250K ohms
R_2	10K ohms
R_3	1000 ohms
R_4	100K ohms
$R_{5,6}$	245K ohms, wire
$R_{7,8}$	490K ohms, wire, ±1%
R_9	399K ohms, wire
R_{10}	1 megohm, wire
R_{11}	2500 ohms, wire
R_{12}	750K ohms
R_{13}	1 megohm
R_{14}	889K ohms, wire
R_{15}	5.6 megohms
$R_{16,17}$	300K ohms
R_{18}	470 ohms
$R_{19,20}$	7500 ohms
R_{21}	1588 ohms
R_{22}	1747 ohms
R_{23}	1763 ohms
R_{24}	196 ohms
R_{25}	18.0 ohms
R_{26}	1.80 ohms
$C_{1,2}$	2400 uuf., matched
$C_{3,4}$	325 uuf., matched
C_{5-7}	0.01 mfd.
C_8	7600 uuf.
C_9	0. 05 mfd.
C_{10}	10 mfd.
M	50 uA meter
P	External terminal post
B_{1-18}	3-volt batteries
S_1	Grid bias selector
S_2	Output factor
S_3	Meter cutout

Tuned amplifier and output.

The tuned circuit was taken from the circuit of Zimm's light scattering apparatus (21,88) without appreciable change except that a two-gang variable condenser is used for tuning. Once the apparatus is tuned, retuning is apparently unnecessary until the power supply battery voltage begins to drop with age and use. In one case the first evidence that the batteries were low was a lowered maximum output of the 6SN7-GT together with inability to tune the amplifier. The power supply voltage was then found to have dropped from 315 to 52 volts.

The output from the tuned stage is rectified in a conventional manner, the variable resistor R_1 serving as a gain control. The negative voltage developed by the rectifier circuit is opposed when desired by a positive voltage of 3 to 54 volts obtained from dry cells. As will be shown later the output of the 6J7 is no longer linear with the more extreme signals, but they are nevertheless useful at times. In most measurements, 10-40 volts were needed at this point to keep within the limits of the output tube.

Resistors R_2 and R_3 permit meter zero adjustment, and together with the opposing voltages selected by S_1 enable the operator to keep the meter on scale at the highest sensitivity practical (the limit is established by the unsteadiness of the output), even with considerable signal strength. Shunt resistors used with the output meter were

selected to give tenfold steps in sensitivity. The two-gang switch used to select the shunt resistance also adds the proper series resistance to maintain the total meter circuit resistance at a constant value to help preserve linearity of this stage.

In the normal operating range the preamplifier was shown to be linear, and the output tube can be kept fairly well in its linear range by selection of the proper grid bias as controlled by S_1. No attempt was made to preserve linearity of the tuned stage, and it is almost certainly the cause of practically all the non-linearity discussed in the next section.

Characteristics

A known voltage* was applied to the input of the tuned amplifier and the output was measured. Since the voltage gain of the preamplifier was already known, and since the phototube current required to produce a given voltage was easily calculated, the current gain of the entire apparatus was readily found. At an equivalent output**

*The known voltage at the proper frequency was obtained by using the phototube and preamplifier with the mercury arc light source, measuring the preamplifier output with a vacuum tube voltmeter. Then the voltmeter was removed, the tuned amplifier connected, and the output read.

**Equivalent output is the actual output given by the output meter plus the product of the grid bias voltage as selected by switch S_1 and the output change produced by a one volt change in this voltage, minus the output with no light to the phototube.

of 45 milliamperes the gain was about 200,000. From the response data presented below, it was found that the gain in the linear range was about 700,000. With the present apparatus, the usual practical limit of detection of output current change is 10-50 microamperes, although a few times with very low light levels somewhat smaller changes have been detected. Thus phototube current changes of about 10^{-5} to 10^{-4} microamperes can be detected, very much less than the dark current value given by the RCA Tube Handbook for this tube.

Since the putput meter could be read to 0.1 microampere, and since such sensitivity would be useful if attainable, it was desirable to locate the source of the instability that limited the useful range. Various tests were made for this purpose. With the high voltage supply off, therefore with no phototube current, the output drifted only very slightly with time (several microamperes per hour) and there was no detectable short time instability at all. Amplifier instability was thus ruled out. Then the high voltage was turned on but the light path was blocked. There were then fluctuations of 0.2-0.3 microamperes, with occasional jumps of 0.5-0.6 microampere. These fluctuations are of no great consequence, so apparently the instability in use is due either to the light source or to the random emission of the phototube. If the light source were the major cause of trouble, the fluctuations should

increase in proportion to the signal strength. They do increase somewhat, but only by a small fraction of what one would predict, so apparently phototube random emission is primarily responsible. Admission of a very small amount of light from a flashlight when no other light was present increased the output variations to about 20 microamperes from the previous 0.2 microampere, showing that random A.C. phototube current is increased with greater signal strength, even though the signal itself is not random*. It appears that improvement could be obtained by selection of a tube of lower noise level.

By admitting varying known amounts of light from the mercury arc to the phototube and measuring the equivalent output, the linearity curve of Fig. 11 was obtained. The output is practically linear for the first ten milliamperes. At 40 milliamperes sensitivity to change has dropped to about 1/9 the value at low signal strengths.

To control the amount of light admitted to the phototube, a birefringent crystal (the quarter-wave plate was convenient) was used between crossed Polaroids. There was virtual extinction when the axis of the plate was parallel to the axis of either Polaroid. The intensity of

*Bennett and Free (6) state that with this type of tube the noise level is proportional to its D.C. output.

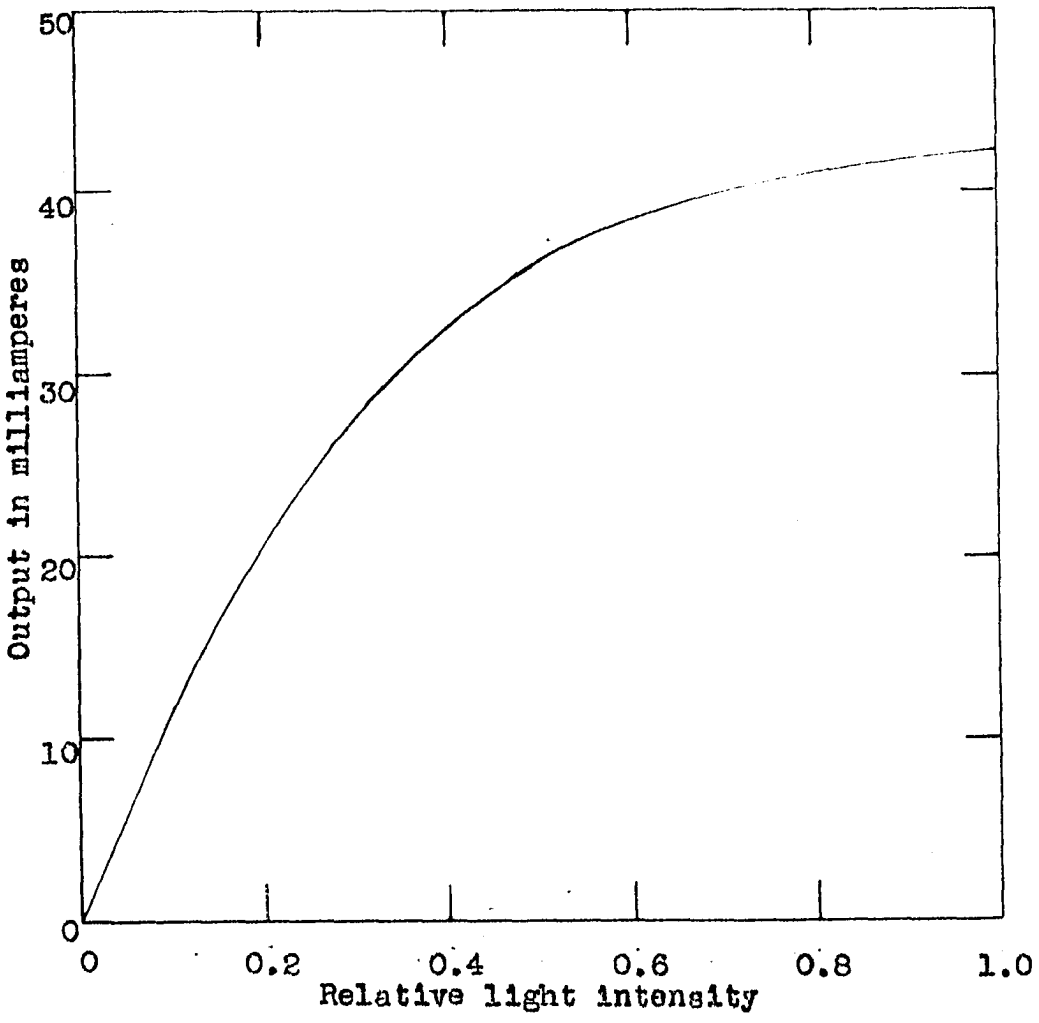


Fig. 11. Output vs. light intensity

the light transmitted when the quarter wave plate was rotated through an angle β is given by

$$I = \tfrac{1}{2} I_o \sin^2 2\beta \qquad (26)$$

where I_o was the initial intensity of the beam.

Applications

Curves showing output as a function of analyzer position, with the cylinder at rest and rotating at 450 RPM in each direction, are shown in Fig. 12. The sample used was the iodine complex of Schoch's tapioca amylose, T-7/9-A(13b).

It can be seen from these graphs that discrepancies between readings taken from adjacent quadrants occur in this case as in birefringence. An attempt was made to eliminate the difference between readings taken from maxima and those taken from minima by correcting for the variation in output at zero gradient. Results were no better than without correction.

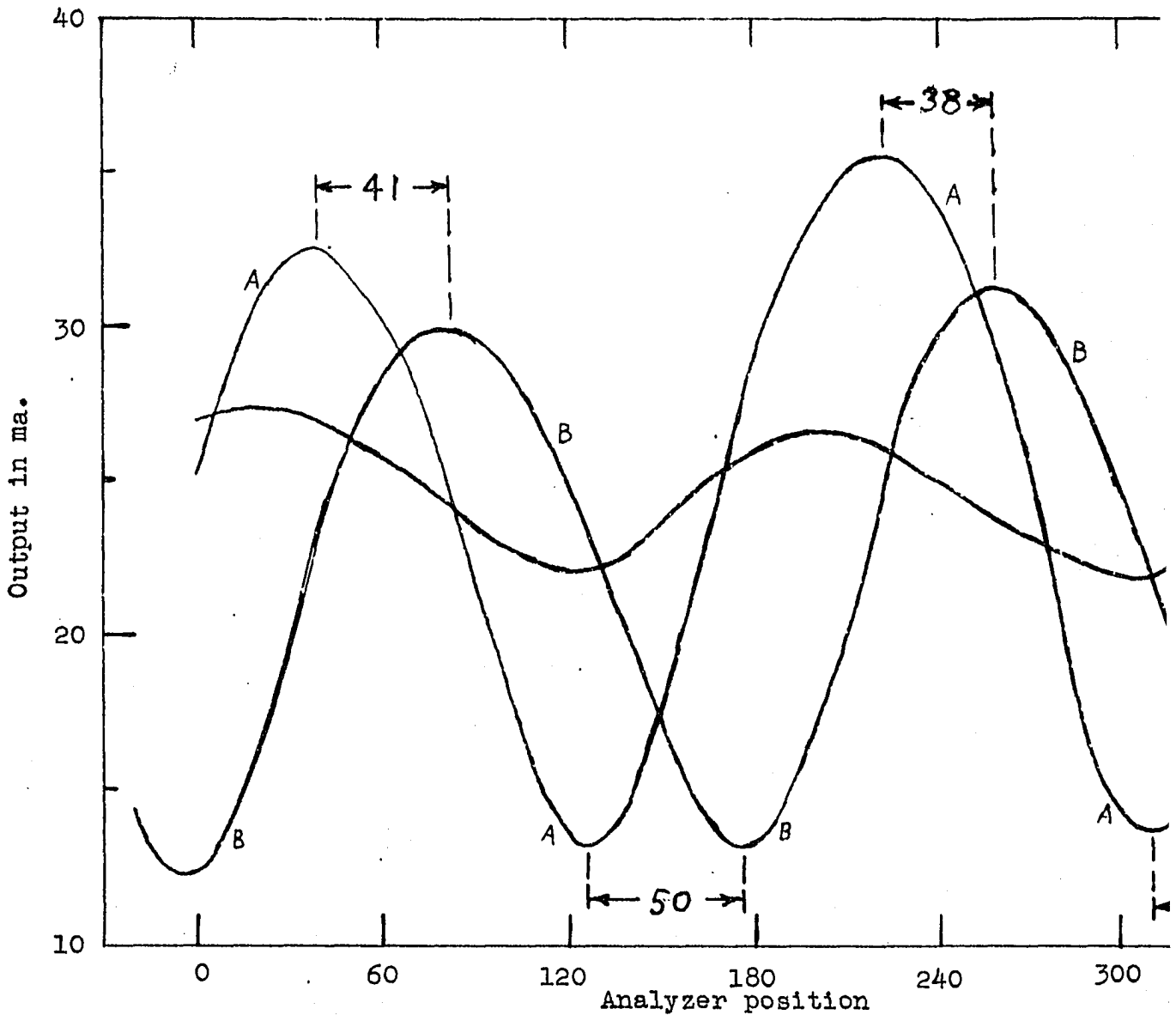


Fig. 12. Output vs. analyzer position for an amylose-iodine complex.

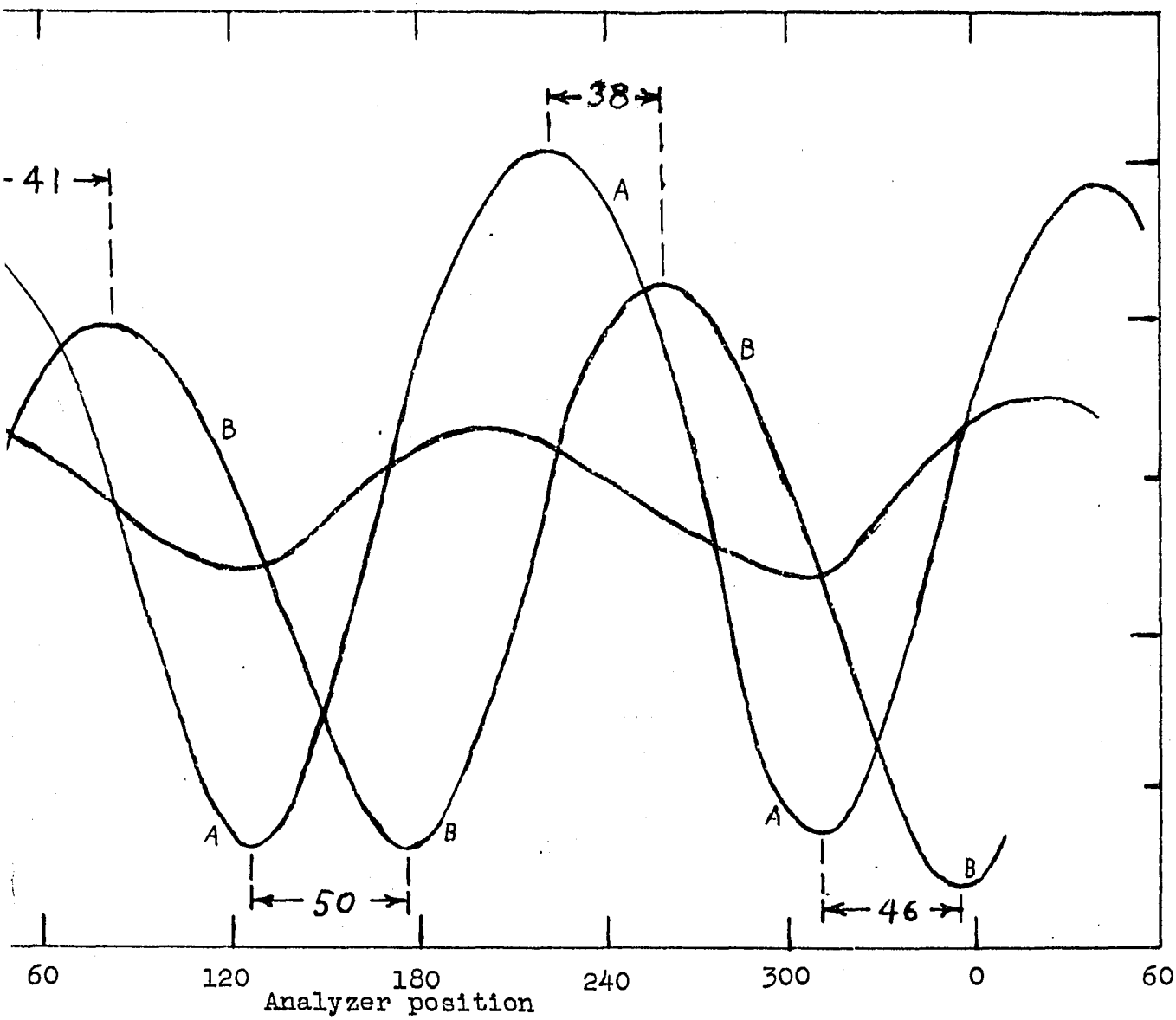


Fig. 12. Output vs. analyzer position for an amylose-iodine complex.

Table 1

B-A readings as obtained from a graph of output vs. analyzer position, and readings obtained from this graph after correction for zero gradient output.

	B-A readings	Determined from	Averages	
Original curves	38, 41	maxima	40	44
	46, 50	minima	48	
Corrected curves	37, 49	maxima	43	45.5
	46, 50	minima	48	

B-A (that is, 2 χ) values obtained at maximum and minimum points of the output curves for both the original and corrected curves are shown in Table 1. Since somewhat more than a complete 360° cycle was investigated, minima and maxima for each sense occurred twice, giving two values for B-A from maxima, and two for minima. These are listed separately in the table, and averages are given. Until an explanation for the peculiar behavior is found, it appears best to use an average of readings obtained at maxima and at minima.

MATERIALS

Carbohydrate Samples

A number of amylose samples of varying purity and length distributions were obtained through the courtesy of Thomas J. Schoch, W. Z. Hassid, and Richard L. Smith. Schoch (45) prepared samples of amylose from corn, potato, and tapioca starches. He first separated the amylose from the amylopectin by a Pentasol precipitation, then recrystalized it using n-butanol or Pentasol. The various preparations were fractionated by partial precipitation with cyclohexanol or n-octanol, the latter being preferred. Many of these samples were made available for our studies. Their code numbers and intrinsic viscosities*, as determined by Schoch, are listed in Table 2.

Others of Schoch's samples were sent to Hassid, who did considerably more work on them. He further purified them by butanol precipitation and showed by periodate oxidation that he had practically eliminated the amylopectin, some of which remained in Schoch's samples (20). One of

*Intrinsic viscosity is defined as the limit of the ratio of specific viscosity to sample concentration as the latter approaches zero. The specific viscosity in this case is

$$\eta_{sp} = \frac{\eta_{solution}}{\eta_{solvent}} - 1 = \eta_{relative} - 1$$

Table 2

Intrinsic viscosities and birefringence results for Schoch's series of amylose preparations.

Code Number	$[\eta]$	d.p	%	$G\eta/T = 5$			$G\eta/T = 10$			$G\eta/T = 20$		
				χ	$\frac{\Delta}{c}$	k(Δn)	χ	$\frac{\Delta}{c}$	k(Δn)	χ	$\frac{\Delta}{c}$	k(Δn)
C-146-A(11c)	0.80	480	1.00	26.5	14	9	26.0	23	16	25.0	39	24
C-146-A(1b)	0.94	570	1.00	25.2	16	10	23.8	28	16	21.9	47	23
C-148/150-A(13b)	1.12	680	1.00	20.1	20	9	20.5	32	15	20.1	51	23
P-5/6-A(12d)	1.34	810	1.40	21.1	22	10	18.7	42	16	17.5	-	-
P-5/6-A(12c)	1.37	830	0.81	22.5	23	12	19.9	41	18	18.0	72	27
P-5/6-A(8b)	1.37	830	1.33	20.8	25	12	18.6	47	18	17.5	79	30
C-148/150-A(13a)	1.65	990	1.00	19.2	30	12	18.1	48	18	17.0	-	-
P-5/6-A(7b)	1.78	1070	0.80	20.9	-	-	18.0	-	-	-	-	-
P-5/6-A(2a)	1.78	1070	1.34	17.4	36	13	15.8	62	20	15.5	-	-
P-5/6-A(4b)	1.86	1120	1.62	16.7	43	16	15.0	-	-	-	-	-
T-7/9-A(15c)	2.31	1390	1.00	13.3	52	15	13.3	83	22	13.9	124	36
T-7/9-A(15b)	2.94	1770	0.93	12.3	65	18	13.1	91	25	-	-	-

these pectin-free samples, a potato subfraction numbered P-7/9-A(17e)$_9$ (the subscript "9" is Hassid's), was contributed by Hassid for use in this investigation. Its d.p. was 890 by osmotic pressure, and 900 by periodate oxidation. Before Hassid's work, Schoch found this sample to have an intrinsic viscosity of 1.45, corresponding to a d.p. of 875, according to the equation of Potter and Hassid (equation 4).

R. L. Smith provided a defatted potato starch containing about 25% amylose, and two amylose fractions precipitated from it with n-butanol (81). The first of these fractions (Batch V_{A_1}) precipitated immediately on cooling the butanol-saturated amylose solution. After removal of this fraction, the supernatant liquid was allowed to stand at room temperature for 27 days, during which time the second fraction gradually precipitated (Batch V_{A_2}). Mr. Smith also provided a sample of potato amylopectin (Batch V_{B_7}) used in some of the experiments.

Reagents

Commercially available materials

Glycerol. Reagent Grade Baker and Adamson 95.0% glycerol, code number 1782 was used. Several samples of

this were assayed by specific gravity determination and found to be within .05% of the stated concentration. This material was used without change for birefringence work. For use with iodine complexes in the dichroism experiments, it was first treated with iodine to remove any traces of easily oxidizable matter which might be present. To each 50.0 grams of glycerol, 0.1 ml. of 0.09 N iodine solution was added. This was warmed or allowed to stand overnight, most or all of the yellow color disappearing.

Ethylenediamine. This was 95-100% material from Eastman Kodak Co., code number 1915.

Pyridine. Paragon No. 5029 was used.

Laboratory preparations

Iodine solution. This was made up in KI solution by R. O. McIntire according to the procedure given by Kolthoff and Sandell (34, p. 592). The exact normality was 0.0900.

Glycerol-ethylenediamine mixtures. Since the amyloses on which flow birefringence were to be run were dissolved in ethylenediamine and then added to glycerol, it was necessary to know the viscosities of glycerol-ethylenediamine mixtures in which the two components had

the same ratio as in the total samples, and at the same temperatures. Therefore, a number of such mixtures were prepared and the viscosities determined at temperatures from -15° to +70°. These were graphed so that the viscosity of any such mixture containing from 6% to 60% ethylenediamine could easily be found to within 2-4% for any temperature within the range given.

Pyridine-glycerol-water-iodine mixtures. As will be discussed in the next section, the samples for dichroism were dissolved in 15% (by volume) pyridine. Five ml. of such a solution was treated with 0.30 ml. iodine solution and added to 50.1 grams of glycerol*. Therefore, a solution was made up in this manner but without the amylose, and its viscosity was determined to be 109.9 cp. at 25.00°, this being the temperature at which all dichroism runs were made.

*Fifty grams of glycerol treated with 0.1 ml. of iodine solution as above.

EXPERIMENTAL METHODS AND RESULTS

Birefringence

General technique

Orientation angle. Samples of amylose were dissolved in mixtures of ethylenediamine and glycerol, except for three dissolved in aqueous KOH and glycerol. In general, each solution was filtered or centrifuged, evacuated, and then placed in the annular space between the cylinders of the machine. The Nicols were crossed, the cylinder rotated slowly, and extinction angles read for both senses of rotation. From these readings, χ was found for this speed of rotation as described on p. 28. Successive determinations were made at higher speeds, until the sample clouded up so that insufficient light was transmitted, or until the highest speed of the machine was reached.

When birefringence was low, for example at low values of $G\eta/T$, readings in adjacent quadrants did not agree well; that is, they were usually 80-85°, or 95-100° apart instead of 90° as they should have been. These discrepancies became less at higher birefringence. Readings in opposite quadrants generally differed by 180°, deviating from this only by amounts easily ascribed to reading errors.

Magnitude of double refraction. The quantity Δ, proportional to the birefringence of the sample (see p. 30), was measured for most of the samples described so far. Curves for Δ vs. $G\eta/T$ are all similar, so only one is shown in this section (Fig. 13).

For Δ determinations, the polarizer should have been set at 45° to the average A reading* for the particular speed at which Δ was to be found. Actually, the error caused by a polarizer setting as much as 5° off from its proper position was negligible, hence in most cases the average A reading for all speeds was taken, and the polarizer was set at 45° to this average**. In the few cases where the A reading range was more than 10° from the lowest to the highest gradient, the polarizer was set at 45° to the average of the A readings for the lower gradients, and Δ was then found for these gradients. Then the polarizer was set at 45° to the average of the A readings for the higher gradients, and Δ was found for these gradients. In

*For settings in different quadrants, one need only add 90°, 180°, or 270°; that is, the settings may be at 45°, 135°, 225°, or 315°.

**Since the only scale available was mounted on the analyzer, and since a change of 90° did not change the theoretical behavior, the polarizer was set by crossing the two with no cylinder rotation, then setting the analyzer at the desired reading.

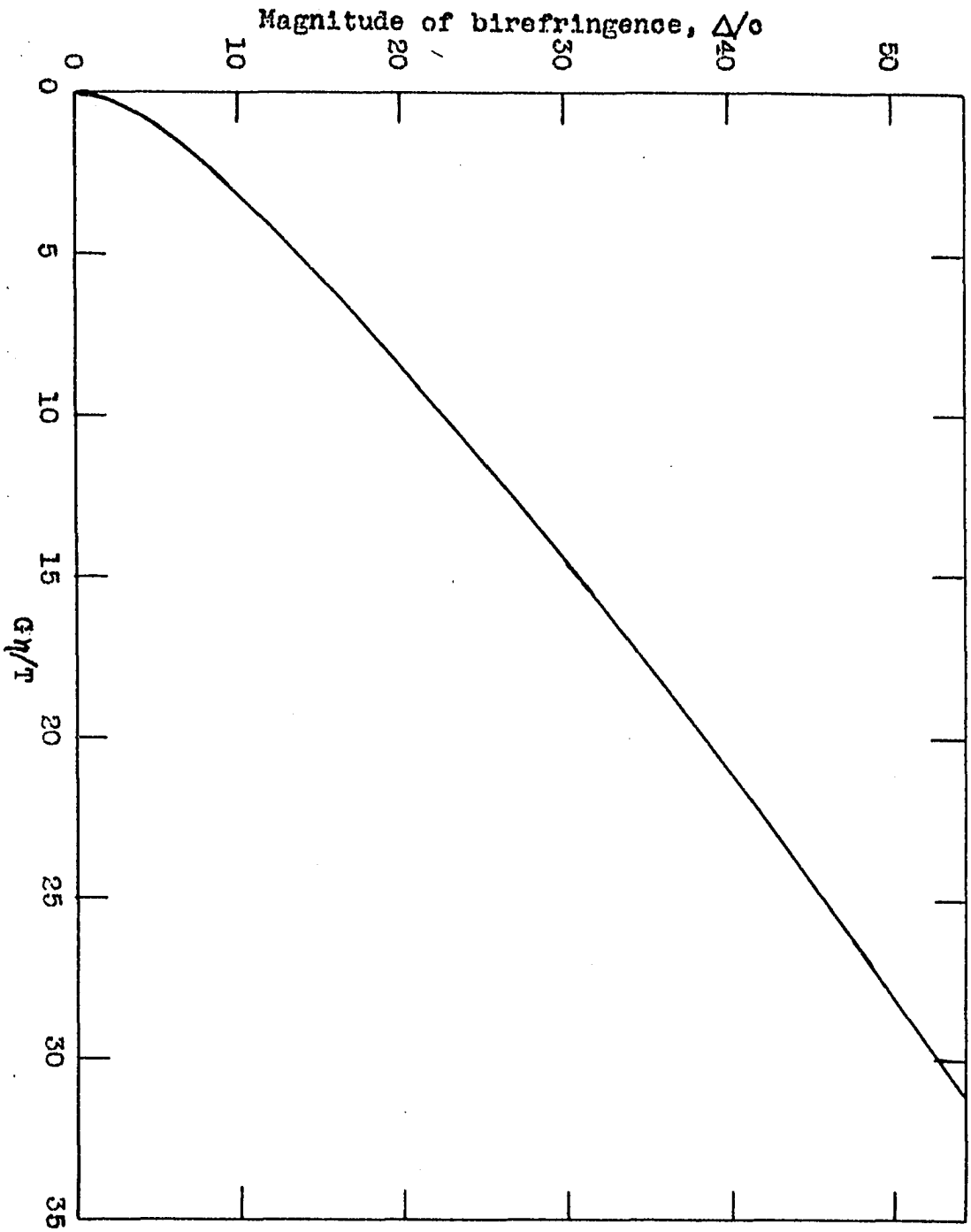


Fig. 13. Δ/c vs. Gη/T for corn amylose C-146-A(11c).

no case was it necessary to divide the range into three groups of readings.

Having set the polarizer in its proper position at 45° to the average of the A sense readings as just described, and with the analyzer at 90° to the polarizer, a quarter-wave plate was inserted between the analyzer and sample. With no cylinder rotation, the plate was rotated until the extinction position was attained. The analyzer was then uncoupled, and the positions of the polarizer and quarter-wave plate were not changed again during the determination. The reading of the analyzer scale was noted, and was checked by rotating the analyzer several times and returning to the extinction position. The average of several such extinction position readings was taken as the zero reading.

The cylinder was then rotated in the A direction at a known speed, and analyzer scale readings at the new extinction position taken several times. The difference between the average of these readings and the zero reading was Δ.

Temperature. At room temperature the viscosity of the solvent was high enough that sufficiently low values of $G\eta/T$ could be attained only by cylinder speeds so low that operation was unsteady. At the other extreme, it was in most cases impossible to obtain values as high as desired

because the viscosity of the solvent at room temperature was not high enough.

These troubles were overcome by using several temperatures for most samples. Usually a sample was run first at a higher temperature, in most cases 38°, at which the viscosity of the solvent was small enough to permit measurements at low $G\eta/T$. Then the sample was run at about 20°, and finally at about 0.5-2.0°. The solvent viscosity at the low temperature was great enough to give a higher $G\eta/T$ than was obtainable at room temperature, even though the cylinder could not be rotated as rapidly.

Variations. The preceding technique was varied somewhat at times. The variations will be discussed later in connection with the individual samples for which they were made.

Preparation of solutions

Amylose samples. The origin of the amylose samples was discussed on p. 62, under "Materials". These samples contained some moisture, generally 5-10%. There were several ways in which this moisture was undesirable; however, since it was considered best to keep operations of any kind on these samples at a minimum, and since none of the effects of water in such small quantities was believed serious, the samples were generally not dried.

The water present would be expected to change the viscosity of the solvent, but the solutions usually contained about 1% amylose, and if 10% of this were water, it would only mean adding 0.1% water to the final solution. Presence of moisture not allowed for in weighing the sample would also cause an error in the calculated concentration, but this would have no effect on the values of χ, the most important part of the results. The value of Δ/c would be in error, of course, but this error would be the same at any gradient, and would consequently not alter the shape of the curve for Δ/c vs. gradient. This, as will be seen later, is more important than the actual value. Only in a comparison of several samples would an error in concentration matter, and even here we might expect the error introduced to be less than the other experimental errors of the method.

Solution of the sample. After the sample was weighed, it was added to the proper quantity of ethylenediamine, and the bottle was immediately stoppered and shaken very vigorously. Best results were obtained by placing the sample in the lid of a weighing bottle, and the ethylenediamine in the bottom of the bottle. In this way stoppering of the bottle was done simultaneously with adding the sample to the ethylenediamine. Even this much delay

allowed some lumping of the sample, but it was much less serious than when other methods were used.

When solution was reasonably complete (there were practically always a few undissolved lumps, however, even if the sample was rolled or shaken for several days or a week), glycerol was added, and the solution was then centrifuged or filtered. After this, it was evacuated for 15-30 minutes to remove dissolved air. The solution was then ready to use.

Concentration used. The glycerol was added solely to increase the viscosity of the solvent*, hence its concentration was varied somewhat. It was finally decided that the calculations would be simplified if one glycerol concentration was adhered to wherever practical, and a solvent of 30% ethylenediamine-70% glycerol was selected. The materials were weighed out to within about 0.1% of their proper values; that is, the ethylenediamine was kept between 29.9 and 30.1%. Solvents with other proportions were prepared with about the same precision.

*When referring to a solution prepared (actually or hypothetically) for streaming orientation, the term "solvent" is used throughout this thesis to denote the liquid in which the sample is suspended or dissolved when the sample is ready for the determination, not the liquid in which the sample was first dissolved. The term "initial solvent" will be used for this latter material.

Amylose concentrations varied considerably, the highest being 3.0% and the lowest, 0.2%, but most samples were made up to contain about 1.0%.

Effect of amylose concentration

The first few samples run were prepared with amylose concentrations of 1 to 2%. It was soon decided that less total work would be involved if all concentrations were standardized at 1.00%. Eventually, however, there arose the question of whether or not this concentration was low enough to give valid results, since all of the existing theories assumed low concentrations. Also, the quantity of sample provided by Hassid (p. 62) was extremely limited, and it was essential to use as little of it as possible. Hence, it was desirable to learn from a more abundant sample just how low a concentration could be used with satisfactory results.

Concentration effects were checked with two samples of the group provided by Schoch. The first was a potato amylose; the second, a corn amylose.

Potato amylose, P-5/6-A(7b). This sample was studied in concentrations of 3.0, 1.5, 0.8, and 0.2%. In the higher concentrations a definite precipitate developed when the sample was run. The lower concentrations showed only a cloudiness, but the change from the rather considerable

precipitate in the 3% solution to the faint haze of the 0.2% solution was regular and gradual, and it appeared that the quantity of insoluble material was roughly proportional to the total quantity of sample present. This indicated that a more or less definite fraction of the sample was made insoluble in the machine.

The samples of highest and lowest concentrations were rerun; that is, after reaching the highest gradient in the determination of χ (Δ values were not obtained, having been found earlier for these samples), the process was repeated beginning from the lowest gradient. In both cases it will be seen that the lengths (Fig. 14) at low gradients were less for the reruns than for the first runs, but that at the highest gradients attained, the lengths from the first runs and from the reruns became identical.

The lengths determined at various concentrations were shorter at lower concentrations, and this effect appeared even at the lowest concentration studied. However, the lengths at a concentration of 1.5% were only about 12% longer than at a concentration of 0.8% at low $G\eta/T$, and only about 5% longer at high gradients. Length differences at concentrations of 0.8% and 0.2% were even less, becoming negligible at higher gradients. It appeared then that while the earlier results obtained with concentrations of 1% were

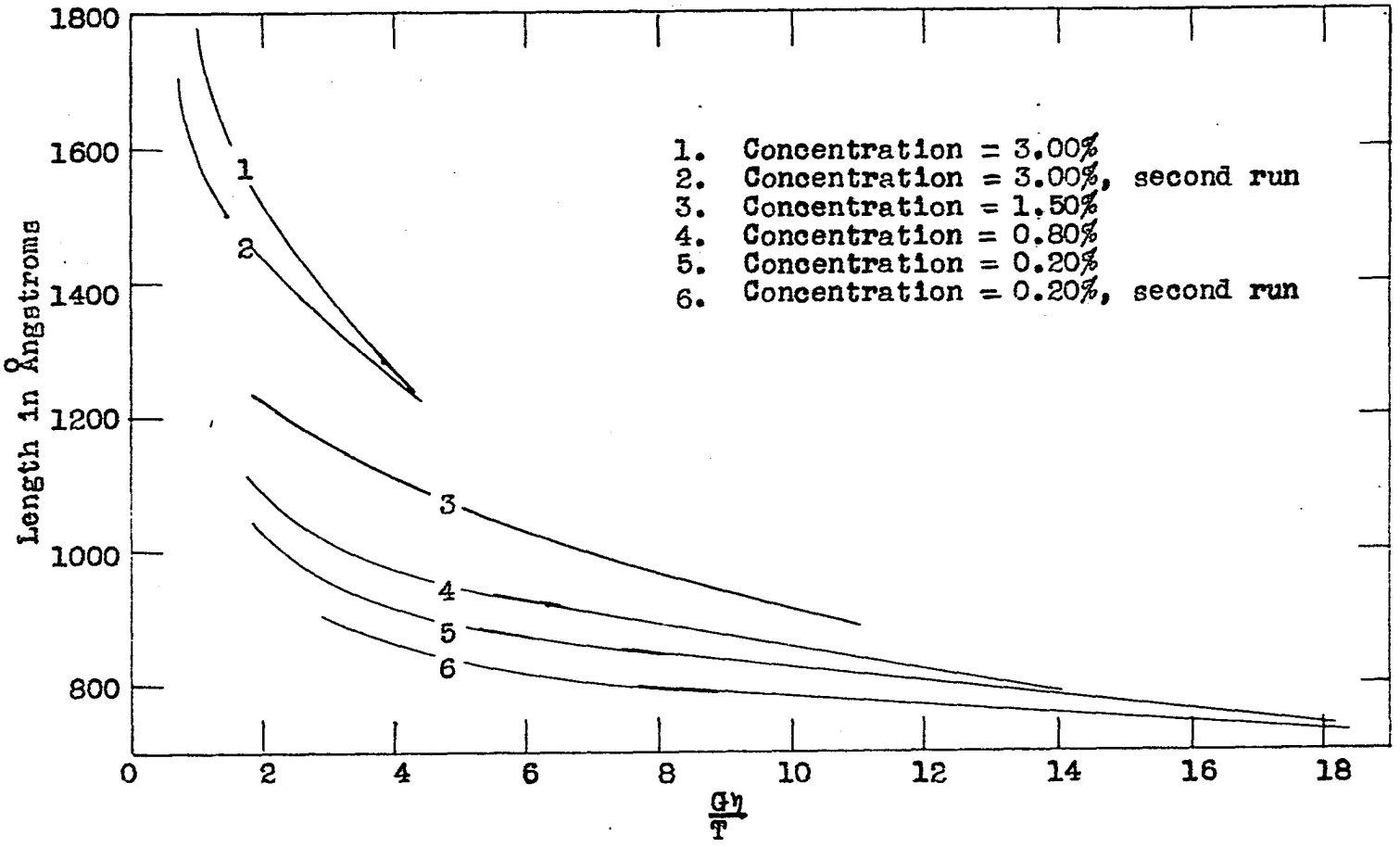


Fig. 14. Length vs. $G\eta/T$ for potato amylose P-5/6-A(7b) at various concentrations.

probably somewhat in error, due to molecular interaction of some sort, such deviation was very likely less than the errors due to amylopectin, reflection, and other experimental sources of inaccuracy. It did not seem worth while to repeat these experiments.

When the concentration of amylose was 0.2%, some difficulty was experienced in determining χ at low gradients. It was apparent that lower concentrations were impractical unless only the behavior at high gradients was of interest.

Corn amylose, C-148/150-A(13b). This sample was run at concentrations of 0.8, 0.4, and 0.2%. All three gave practically identical results. As with the corresponding concentrations of the potato amylose of the preceding paragraph, a cloudiness developed during the runs. The turbidity increased with increasing concentration.

It may or may not be significant that when the sample of lowest amylose concentration was rerun, shorter lengths were obtained at all gradients instead of only at the lower gradients as in the case of the potato amylose above.

Results of these runs are shown in Fig. 15.

Potassium hydroxide as initial solvent

The potato and corn amylose discussed above were also prepared with 1.00 N aqueous KOH as the initial solvent, the

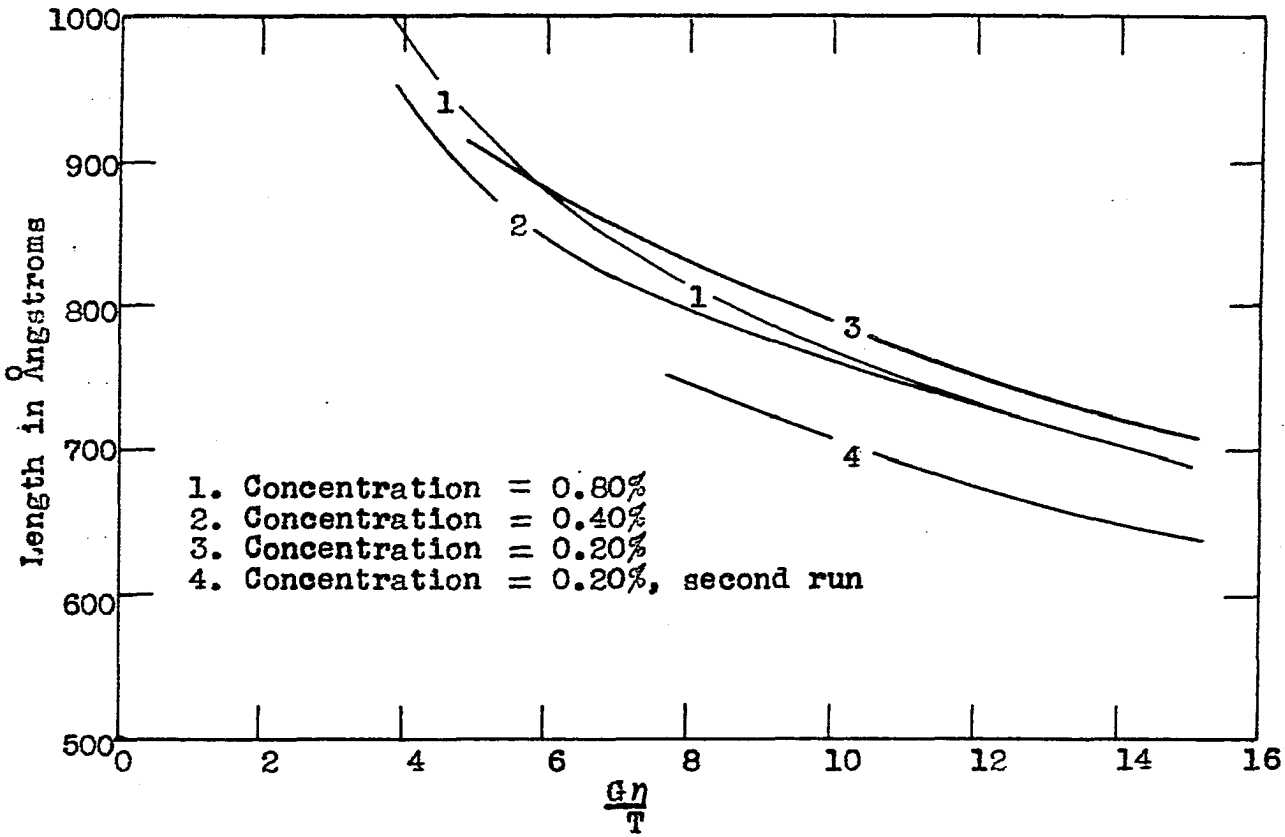


Fig. 15. Length vs. $G\eta/T$ for corn amylose C-148/150-A(136) at various concentrations.

final solvent being 17.4% 1.00 N KOH and 82.6% glycerol with a viscosity of 48.6 centipoises at 25°C. The potato amylose concentration was 0.2%. Two solutions of the corn amylose were prepared, one having 0.2% amylose and the other 0.8%. The lengths in these cases were about the same as when ethylenediamine was the initial solvent.

After the KOH-glycerol solution of the potato sample was run, it was neutralized with HCl solution and the pH adjusted to about 5. The viscosity of the solution was determined to be 12.0 centipoises at 25°. The solvent viscosity was assumed to be about the same, since its amylose concentration was only 0.16% after neutralization. The sample apparently became highly aggregated, for the birefringence became quite low, and the lengths, though difficult to determine, were obviously much greater than prior to neutralization, when compared at similar values of $G\eta/T$.

Effect of contamination with amylopectin

It seemed likely that the precipitates developed in the amylose solutions were caused by the presence of amylopectin. The initial rise in χ with increasing gradient could also have been due to this impurity. To investigate these possibilities, the purest amylose sample available, that provided by Hassid, was prepared with varying quantities of added amylopectin.

This sample, when run and rerun at 25°, behaved

somewhat like the potato amylose, P-5/6-A(7b), in that the lengths at lower gradients were somewhat shorter during the second run (Fig. 16). The difference, however, was not great. Values obtained at 15° and again at 25° agreed with the second run rather than the first.

The sample originally was made up to 0.2%. An amylopectin sample was made up in the same manner and concentration. Thus, when the latter solution was added to the former, the solvent composition and the total carbohydrate concentration remained constant. However, amylopectin was added and the amylose concentration was decreased. In this manner, the amylopectin content of the total carbohydrate present was increased from zero (neglecting any amylopectin initially present in the amylose) to 38%. As can be seen from the curves of Fig. 17, the chief effect of amylopectin is to cause a low χ value at low gradients, the value rising for a while with increasing gradient. The effect was not serious until the amylopectin content was increased to about 17%. However, a precipitate developed after each run, and it is quite likely that this included most of the amylopectin added to that time. Therefore, the actual concentrations of dissolved amylopectin might have been considerably smaller than the values given in Fig. 17. For example, the highest concentration may have been 21% instead of 38%, and the next highest may have been 12% instead of 17%. At the highest amylopectin concentration, the lengths at higher gradients appeared to be

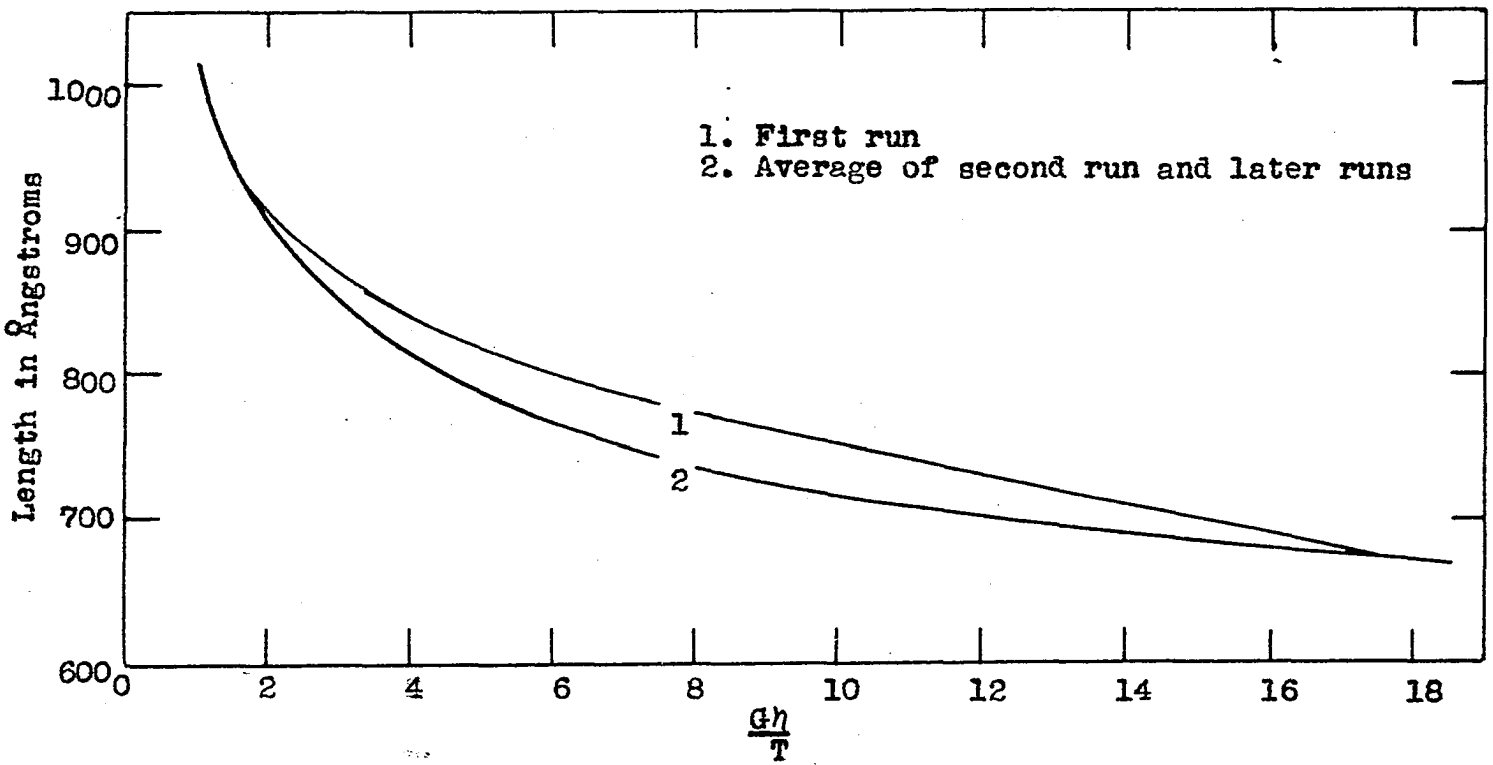


Fig. 16. Length vs. $G\eta/T$ for Hassid's amylose sample.

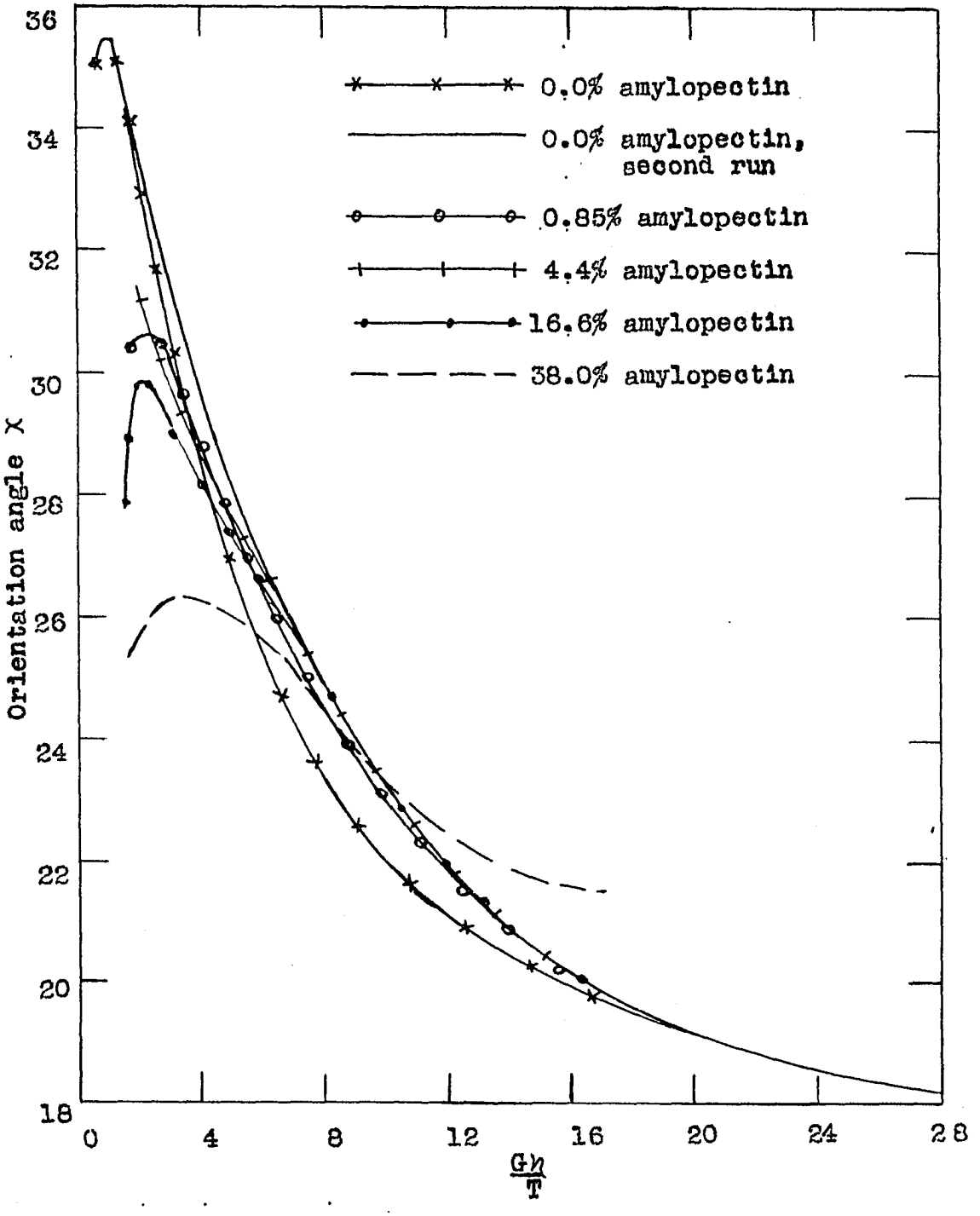


Fig. 17. χ vs. Gη/T for Hassid's amylose plus varying quantities of amylopectin.

shorter than when there was less amylopectin.

χ values for three of these amylose-amylopectin mixtures were rechecked at several points after completion of the first run in each case. The results checked well with the χ vs. gradient curves first found for the mixtures, which is rather surprising unless precipitation was already essentially complete at a low gradient. This was more likely to be so with these samples than with any others run, because the precipitate from the preceding run was present to provide nuclei for the amylopectin added.

Nature of the precipitate developed by flow

Precipitation and separation. A solution of corn amylose, C-148/150-A(13a), was prepared with an amylose concentration of 1.00% in a solvent consisting of 30% ethylenediamine and 70% glycerol. Part of this was set aside, and the rest was run slowly through the machine at 0.5-2.0°, while the inner cylinder was rotated at the highest speed possible. The cloudy solution was then centrifuged until clear, and the supernatant liquid was decanted. This decantate and the original sample which had been set aside were analyzed for carbohydrate content by optical rotation, and the decantate was found to have a carbohydrate content of 0.76%. Both the original solution and the decantate were run in the usual way, and the results are shown in Figs. 18 and 19.

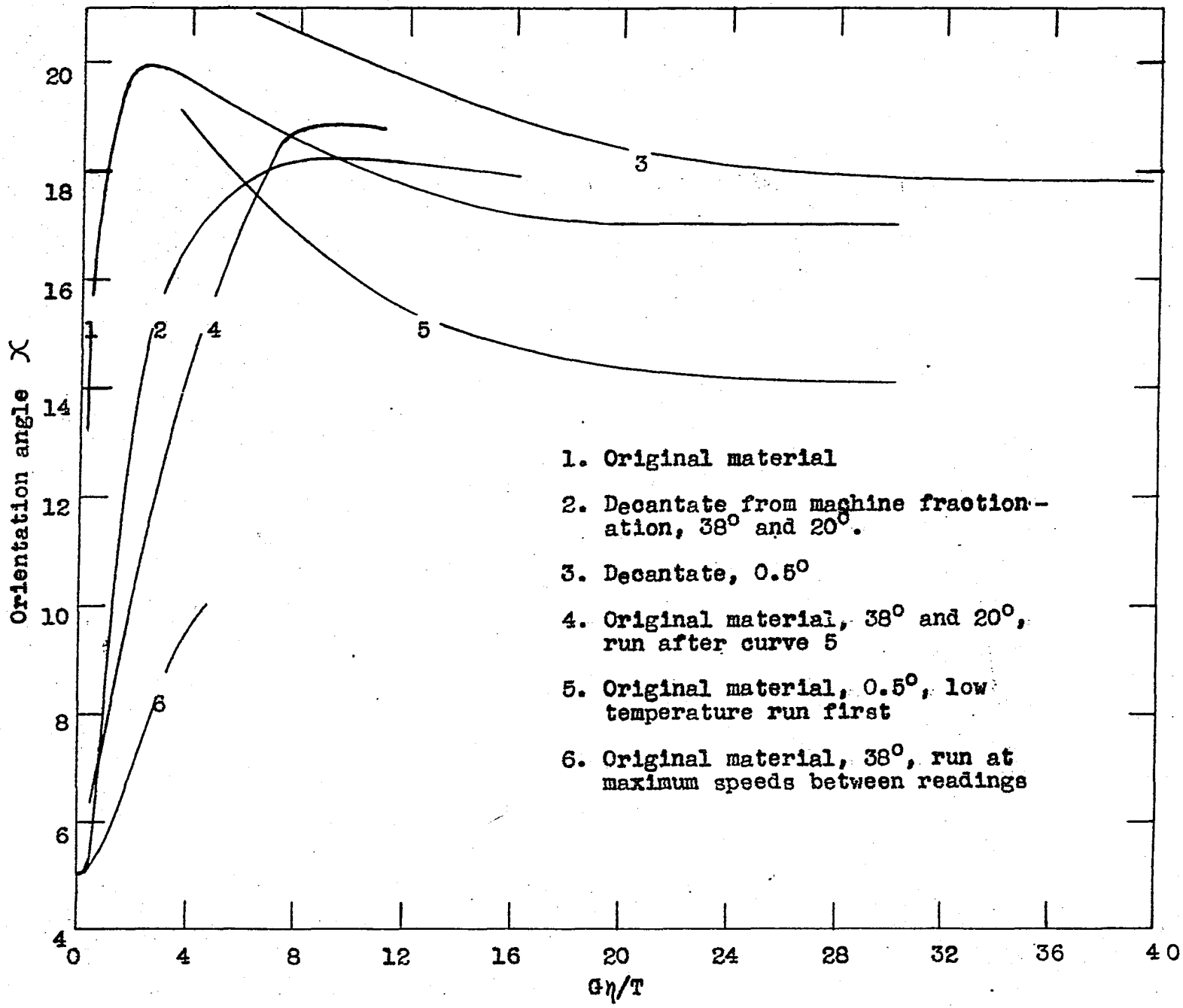


Fig. 18. χ vs. Gη/T for corn amylose C-148/150-A(13a).

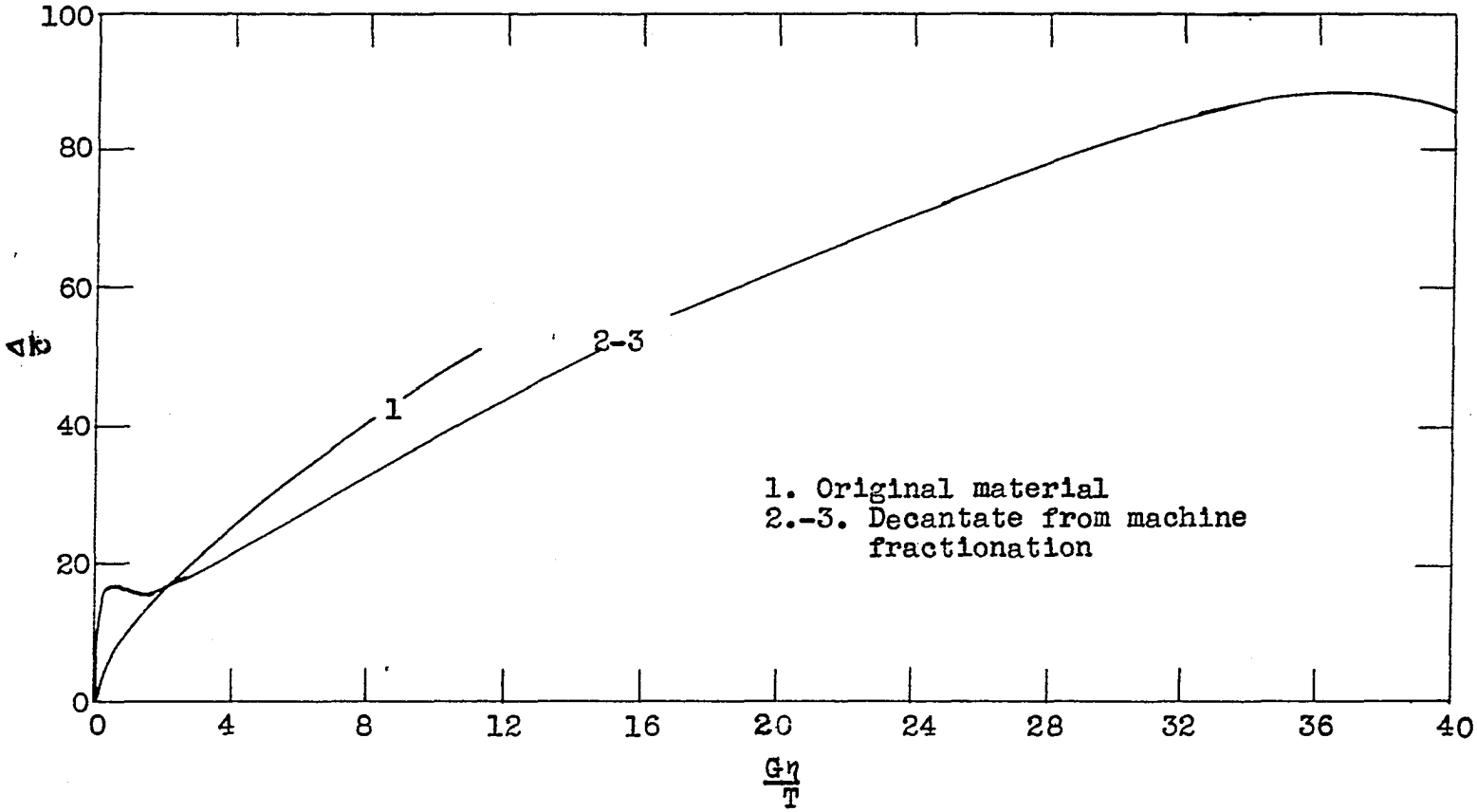


Fig. 19. Δ/c vs. $G\eta/T$ for corn amylose C-148/150-A(13a)

For comparison, several other runs made with solutions of the original sample are also shown in Fig. 18. In one case the usual temperature order was reversed, a run being made first at 0.5°, then at 24.0°, then at 38.0°. In another case a run was made at 38° only, but with the cylinder being driven at top speed at frequent intervals during the run.

During the decantate run, a gradient was attained which was somewhat higher than that at which the precipitate was first formed. It may have been for this reason that a slight additional precipitate was formed during the run. This precipitate was removed by centrifugation, and the carbohydrate concentration in the solution was checked by optical rotation. It was found to contain 0.68% carbohydrate.

The major object of the preceding work was the obtaining of the precipitate developed by running the solution through the cold machine at the highest cylinder speed possible. This precipitate, unfortunately, was lost because of failure of a centrifuge cup. Therefore, another solution of the same composition as the first was run through the machine at the same temperature, but this time a somewhat higher gradient was attained through improved motor operation. The resulting precipitate was removed by centrifugation as

before, and the supernatant liquid was discarded. The latter was not analyzed, but from the gradient used in the precipitation process, and from the results obtained with the first sample so treated, it was estimated that the carbohydrate concentration in the liquid was about 0.70%; that is, about 30% of the total starch was precipitated.

The precipitate from the above separation was washed twice with solvent, allowing it to stand several hours each time before centrifuging. Assuming the dissolved amylose to be more or less uniformly distributed throughout the sample and wash liquid each time, it was estimated that the precipitate after the second washing and decantation was contaminated with about 7-14% of its weight in soluble material that had not been washed out.

The precipitate was then washed with anhydrous ethanol until it was estimated that the remaining liquid was about 2% original solvent, 98% ethanol. It was next washed once with 80% methanol, then extracted with more of the same material in a Bailey-Walker apparatus for 6 hours, and overnight with anhydrous methanol. Subsequently it was dried at room temperature (about 25°) for 20 hours at a pressure of about 60-70 mm. After this it was allowed to stand over magnesium perchlorate.

An attempt was made to dissolve the precipitate in 1 N KOH. This was done, using about half of the precipitate,

two days after the vacuum drying. The sample was apparently dissolved after about 9 days, and the solution was neutralized. An iodine titration indicated that about 75% of the sample was amylose. The remainder may have been amylopectin, but it might also have been undissolved amylose, or degradation products resulting from the long standing in KOH. Oxygen had not been excluded.

Correlation of streaming orientation and intrinsic viscosity

A number of Schoch's samples other than those so far discussed were also run. A complete list is given in Table 2, together with their intrinsic viscosities in 1 N KOH and the d.p.'s calculated from them by equation 4. This table also shows some values of χ and Δ/c as determined by birefringence, and the absolute birefringence $k(n_1-n_2)$. The latter were based on the use of the distribution function for monodisperse systems (see p.120).

Dichroism

General technique

Orientation angle. Iodine complexes of amylose samples were prepared in various ways to be described in the section, "Preparation of the Complex". The complexes were

then poured into glycerol, thoroughly mixed, and transferred to the machine. The extinction positions were determined as in the case of birefringence studies, except that the photoelectric apparatus was used instead of the visual method, and the analyzer alone was rotated, the polarizer being removed. In addition, the positions of maximum intensity were located. The latter gave the positions of the normals to the preferred orientation positions.

Since pairs of readings taken at about 180° intervals agreed well, it was considered sufficient to locate only one minimum and one maximum position at each gradient and in each sense of rotation. A similar procedure could have been followed in birefringence work, only two positions of minimum intensity being located instead of four, provided the two were in adjacent quadrants.

Magnitude of dichroism. This was measured essentially as described on p. 36. However, an approximation was made similar to that used in the determination of the magnitude of birefringence, p. 68. If the readings at all gradients in one sense of rotation did not cover a range of more than about 10°, the middle of the range was taken, the polarizer was set at 45° to this angle*, and all readings for the

*The polarizer and analyzer were crossed, and the analyzer was set at 45° to the prescribed angle. This fixed the polarizer at the desired angle.

sample were made with this setting.

Permissibility of this approximation was established by finding δ values at various gradients using polarizer settings at 45° to each orientation angle instead of the average, then repeating these determinations with the polarizer in each case 5° or 10° from its proper position. In nearly all cases an error of 10° in the polarizer setting caused only a negligible change in δ. An error of 5° in polarizer setting caused no detectable error in any case.

Preparation of the complex

Concentration of the amylose complex. It was found that in all cases the concentration of the amylose complex, calculated as percent amylose in the final solution, should be between 0.001% and 0.003%, with best results obtained at about 0.0015% to 0.002%. This was obtained by weighing 1 mg. of amylose on a microbalance*, dissolving it in 5.0 ml. of the initial solvent, complexing with 0.3 ml. of iodine, and finally adding the complex to 50.1 g. of iodine-treated glycerol. When the concentration was too low, the difference between the maximum and minimum light intensity was too

*Alternatively, larger quantities were weighed and dissolved in correspondingly larger volumes of the initial solvent, then a 5.0 ml. aliquot part was taken.

small for accurate work. When it was too high, the light transmitted was insufficient.

KOH as initial solvent. At first amylose samples were dissolved in 1 N KOH solution, which was then neutralized with HCl and diluted (no significant difference was found when the KOH solution was diluted before neutralization). The amylose solution, now containing KCl, was treated with iodine solution and added immediately to the glycerol. When not added at once to the glycerol, evidence of aggregation appeared within 5 seconds after adding the iodine, and individual particles or clumps of particles could be seen with the naked eye in about 15 seconds. Since it required at least 2 or 3 seconds for getting the complex mixed with the glycerol (10 to 15 seconds for thorough mixing), it was evident that serious aggregation must have occurred, and the lengths determined were probably in error.

Aggregation continued, though at a greatly reduced rate, even after addition to glycerol. This was shown by the continued rapid dropping of X with time; that is, the particles were growing longer. In 3 or 4 hours the dichroism vanished, even though the solution appeared unchanged to the eye. It is interesting to note that a similarly colored, apparently finely dispersed, but non-dichroic

solution was formed when an aggregated complex was added to glycerol 15 seconds after addition of iodine.

As a result of the increase in length occurring during a run, and apparently taking place much more rapidly during preparation of the sample, average lengths determined with 1 N KOH as the initial solvent are useful only to establish an upper limit. Values obtained for 5 representative samples are shown in Fig. 20.

Attempts were made to reduce or eliminate aggregation occurring before the addition of glycerol by combining the glycerol and amylose solution first, then adding iodine, and also by adding the iodine to the glycerol, then adding the amylose solution. In the first case, no amylose-iodine complex was formed at all for several hours, a slight green color* developing eventually. In the second case a slight green color appeared at once, but did not become more intense on standing, even after addition of more iodine. Neither sample showed sufficient dichroism for use.

Water as initial solvent. It seemed probable that the KCl and KI (from the I_2 solution) present in the above solution were at least partially responsible for the aggregation observed. Therefore an attempt was made to prepare an

*The green was a result of the blue of the complex, combined with the yellow of the excess iodine.

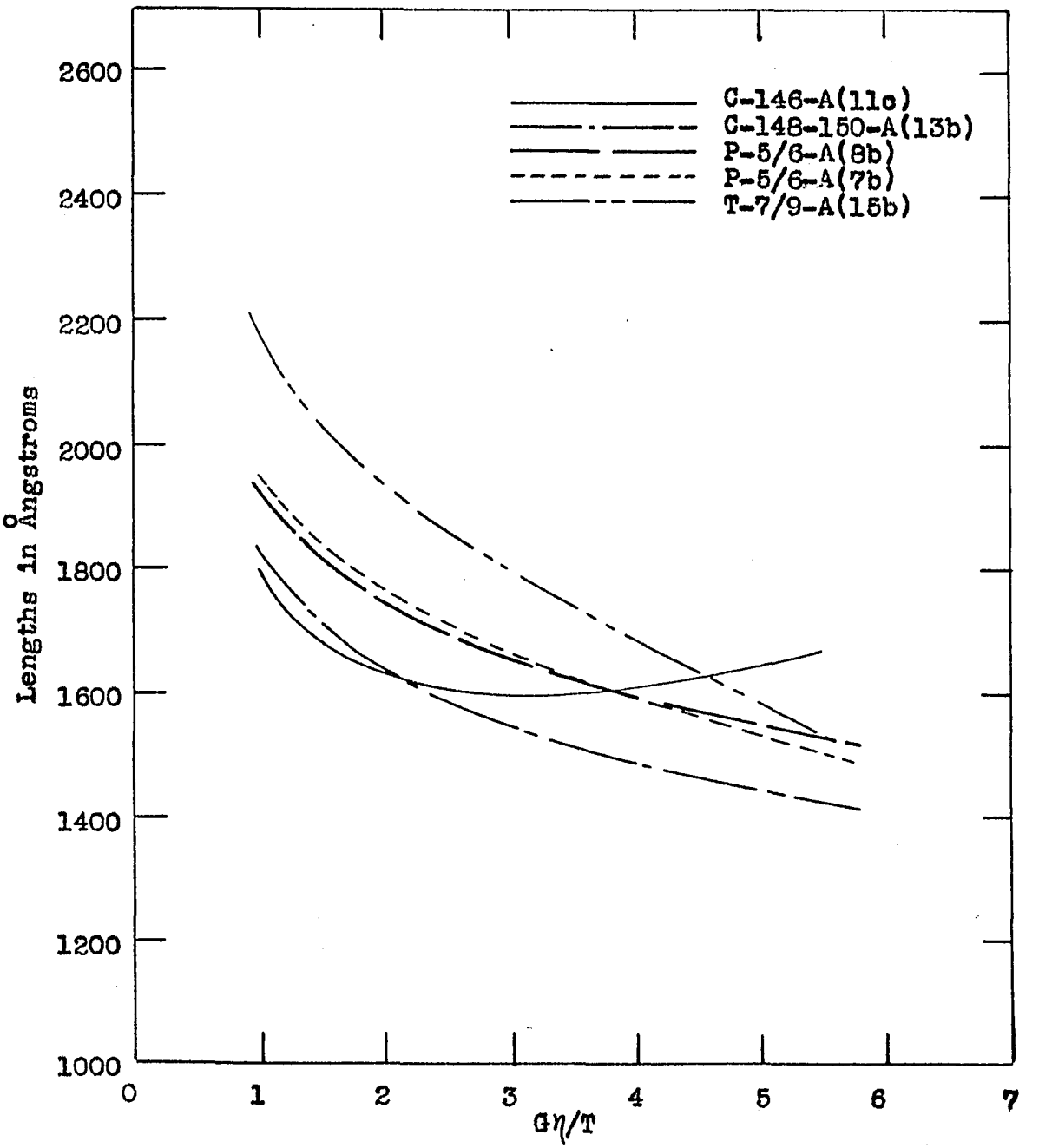


Fig. 20. Lengths vs. $G\eta/T$ for amylose-iodine complexes in KOH as initial solvent.

aqueous amylose solution. The best sample available, that provided by Hassid, was used in these experiments.

This sample would not dissolve appreciably in hot water. More drastic measures were then tried, the sample being autoclaved with water for 1½ hours at 15 pounds gage pressure (120° C.) Enough dissolved to give a blue color when aqueous iodine (not in KI) was added. When this solution was added to glycerol containing a little excess iodine, the blue color disappeared at once, leaving only the yellow of iodine. Addition of KI did not restore the blue color.

Two attempts with the autoclave failed to accomplish more than a very slight extent of solution. Some other technique was obviously needed, since longer autoclaving might have resulted in appreciable degradation. No further attempt was made to investigate the effect of KI under these conditions.

Aqueous pyridine as initial solvent. According to Pascu and Mullen (57), aqueous pyridine in suitable concentrations can cause gelatinization of starch. Since pyridine can be removed from aqueous solutions by distillation (the volatile component is a constant boiling mixture of 57% pyridine, boiling at 92.6° C.), it seemed advisable to try such a solution. If the starch should dissolve, an

excess of water could be added and the pyridine removed by evaporation.

Even after standing overnight in 40% (by volume) pyridine, the amylose sample (Hassid's) showed no signs of solution. It dissolved readily, however, when warmed to 80°-90°. The pyridine was removed by evaporation, water being added from time to time. When the absence of odor indicated essentially complete removal of the pyridine, the amylose was complexed with I_2 in KI and left 2½ minutes. When the solution was still not visibly aggregated after this time, it was apparent that some improvement had been made, so the sample was added to glycerol and run. There appeared to be some aggregation during the run, as shown by a slight drop in χ, but it was much less serious than with previous samples.

The lengths so determined were about the same as those obtained when 1 N KOH was used as the initial solvent (Fig. 21). This was probably due to the relatively long time between iodine and glycerol addition, 2½ minutes as compared to 2 or 3 seconds when KOH was used. One could expect better results than ever before obtained, by using this method and adding glycerol immediately after complexing. However, since another procedure, described in the next paragraphs, ultimately eliminated practically all apparent

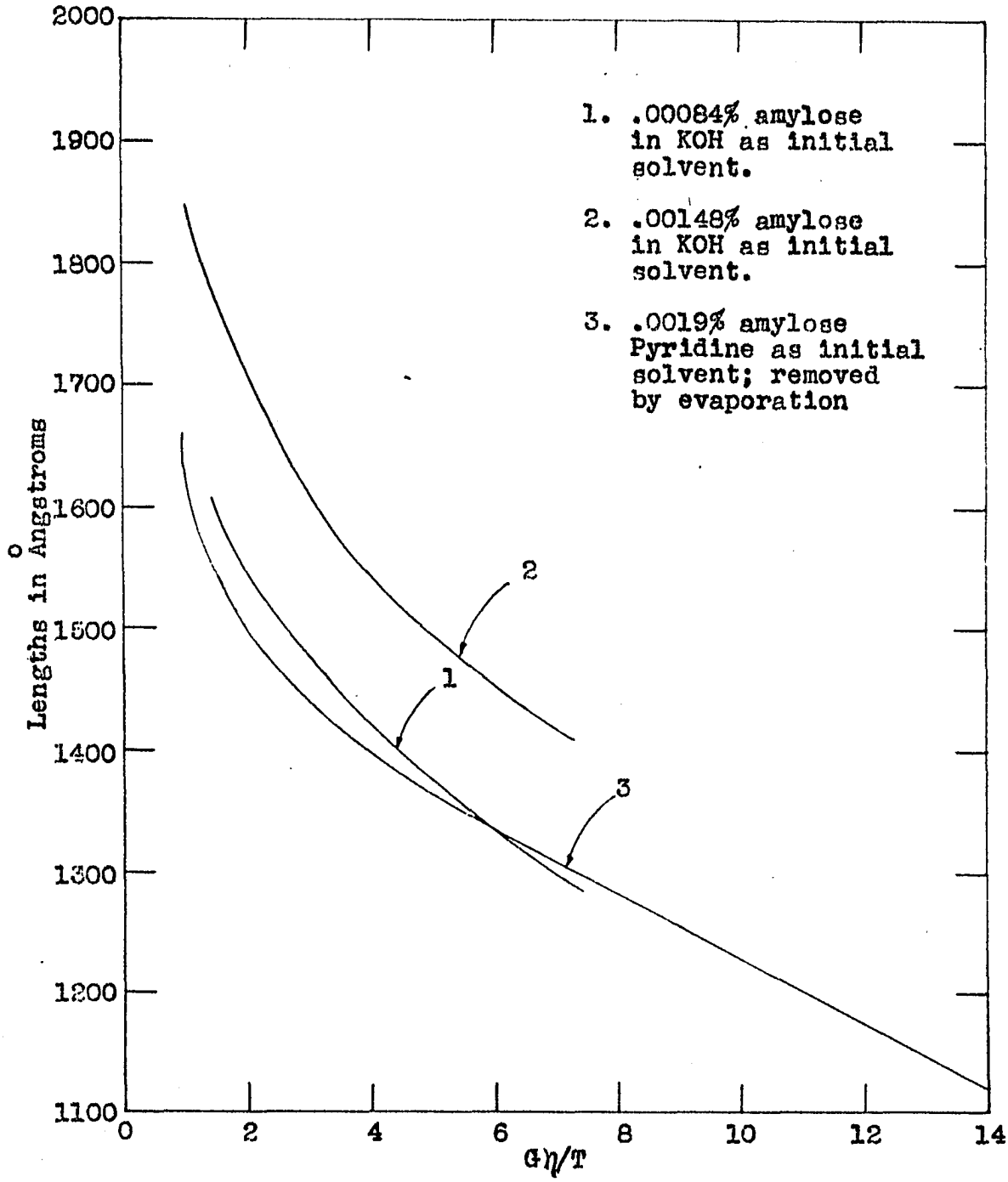


Fig. 21. Lengths vs. $G\eta/T$ for the iodine complex of Hassid's amylose.

aggregation, there was no need to investigate this method further.

Preliminary tests showed that the amylose-iodine complex could be formed in the presence of pyridine if the latter were not too concentrated. The first rough checks showed the limit in pyridine concentration to be around 20%. However, when Hassid's sample was dissolved in hot 40% pyridine, then cooled and diluted to 20% pyridine, it formed only a very feeble, muddy green color with I_2 in KI. This sample was added to glycerol, 5 seconds after iodine addition, but had too little dichroism to measure. The process was repeated, allowing the amylose solution to stand for 10 minutes with iodine before adding glycerol, but the results were no better.

More of the amylose was dissolved in 37% pyridine. This time it was diluted to give a pyridine concentration of 10% by volume. Iodine solution was added, and 5 seconds later it was poured into glycerol and run. This time the dichroism was quite satisfactory; there was no evidence at all that aggregation was occurring during the run, and the lengths obtained were shorter than any previously found. Apparently this process was the best developed up to that time, but there was still the possibility that some aggregation was occurring between the addition of iodine and the

time when aggregation, if present, was apparently stopped (or more probably, tremendously delayed) by addition of glycerol.

To check this possibility, two more samples were prepared and run. In one of these, 2½ minutes were allowed to pass between the addition of iodine and glycerol; in the other, 8 minutes elapsed. The average length increased somewhat, indicating aggregation. It would no doubt be possible to run a series of samples with varying complexing times, and extrapolate back to zero time (the curve for the above work at one gradient is shown in Fig. 22), but it appeared better to try to eliminate this aggregation altogether, or at least reduce it.

The next samples were tried with 15% pyridine* as the initial solvent. When this was found to give samples with satisfactory dichroism, a series of 5 samples was prepared in which the complexing time varied from a few seconds to 8½ minutes. These samples all agreed very well with each other, indicating that aggregation had been practically stopped at all stages. A curve of length vs. $G\eta/T$ for

*At first the amylose samples were dissolved in hot 30-40% pyridine, and diluted, but it was soon found that a pyridine concentration of 15% by volume was quite satisfactory, although perhaps a trifle slower. When a hot solution was cooled, a precipitate developed in a few hours. Consequently when a larger volume of solution was prepared, and aliquot parts taken for the individual samples, the solution was reheated and cooled each time a sample was taken from it.

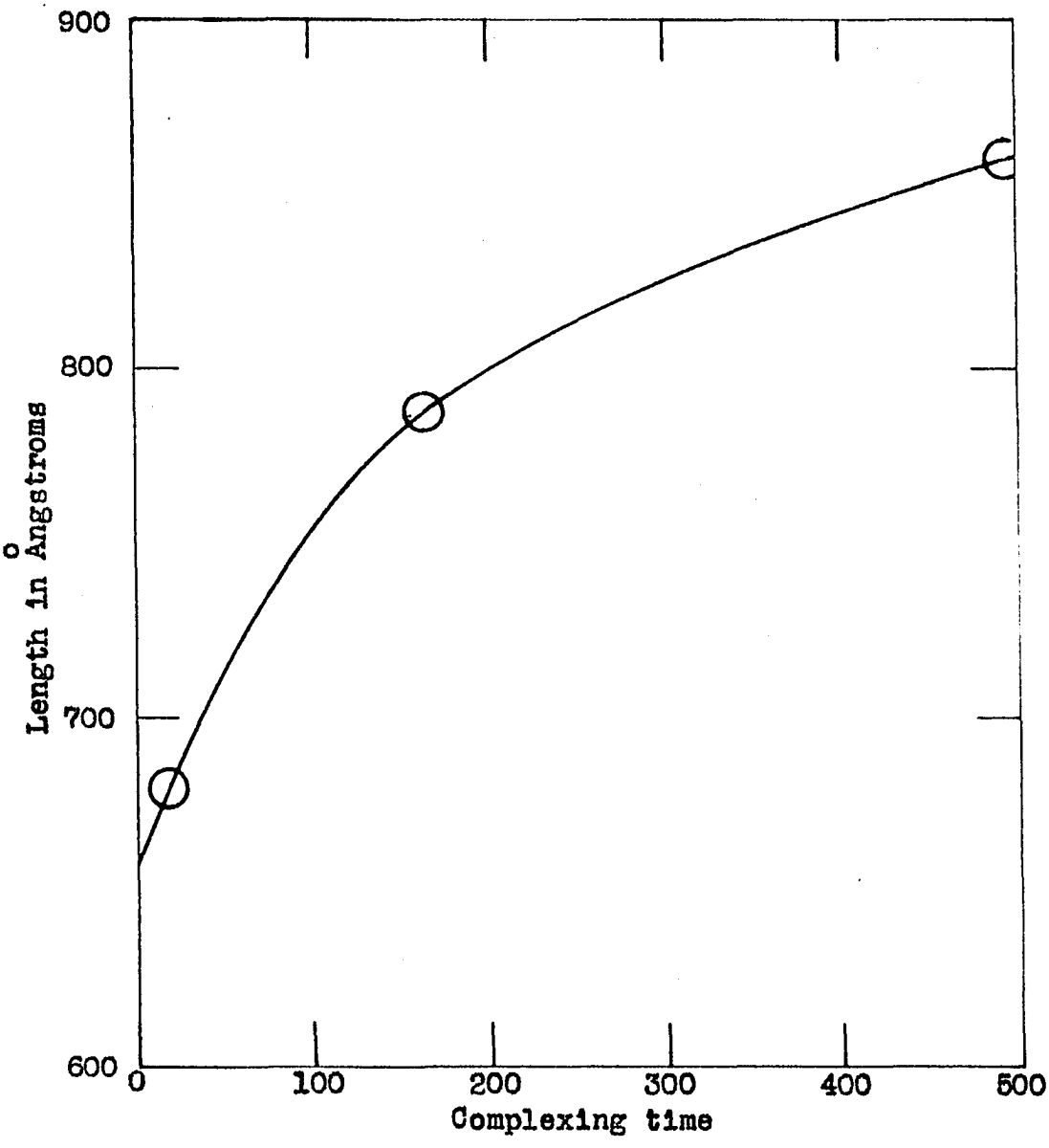


Fig. 22. Average lengths of the iodine complex of Hassid's amylose sample at $G\eta/T = 18.8$. Lengths determined with samples containing 10% pyridine at the time of complexing.

these samples is shown in Fig. 23. The only questions now were whether or not the particles were molecularly dispersed in the first place, and whether similar results could be obtained with less pure amyloses such as were provided by Schoch.

Probability of molecular dispersion. It has already been shown that when the pyridine concentration in the initial solvent is just short of the point where no complex can be formed, there is no measurable tendency to aggregate. In addition to the samples prepared as above, others were run under different conditions. All of the samples, including those described above, were either dissolved in hot 37% pyridine and diluted to 15%, or dissolved initially in 15% pyridine. Most were heated until dissolved, then cooled quickly and complexed; but several were cooled slowly, and one was cooled quickly, then allowed to stand 11 minutes before complexing. In several cases the iodine was added while the amylose solutions were still hot, the solutions then being cooled quickly. All of these techniques gave practically the same results*. When the iodine was added to the hot

*Later, Schoch's amylose P-5/6-A(8b) was run with complexing times of 10 seconds and 5 minutes, and once also with a delay of 11 minutes between cooling and iodine addition. All three runs gave practically identical results. The initial solvent was 15% pyridine.

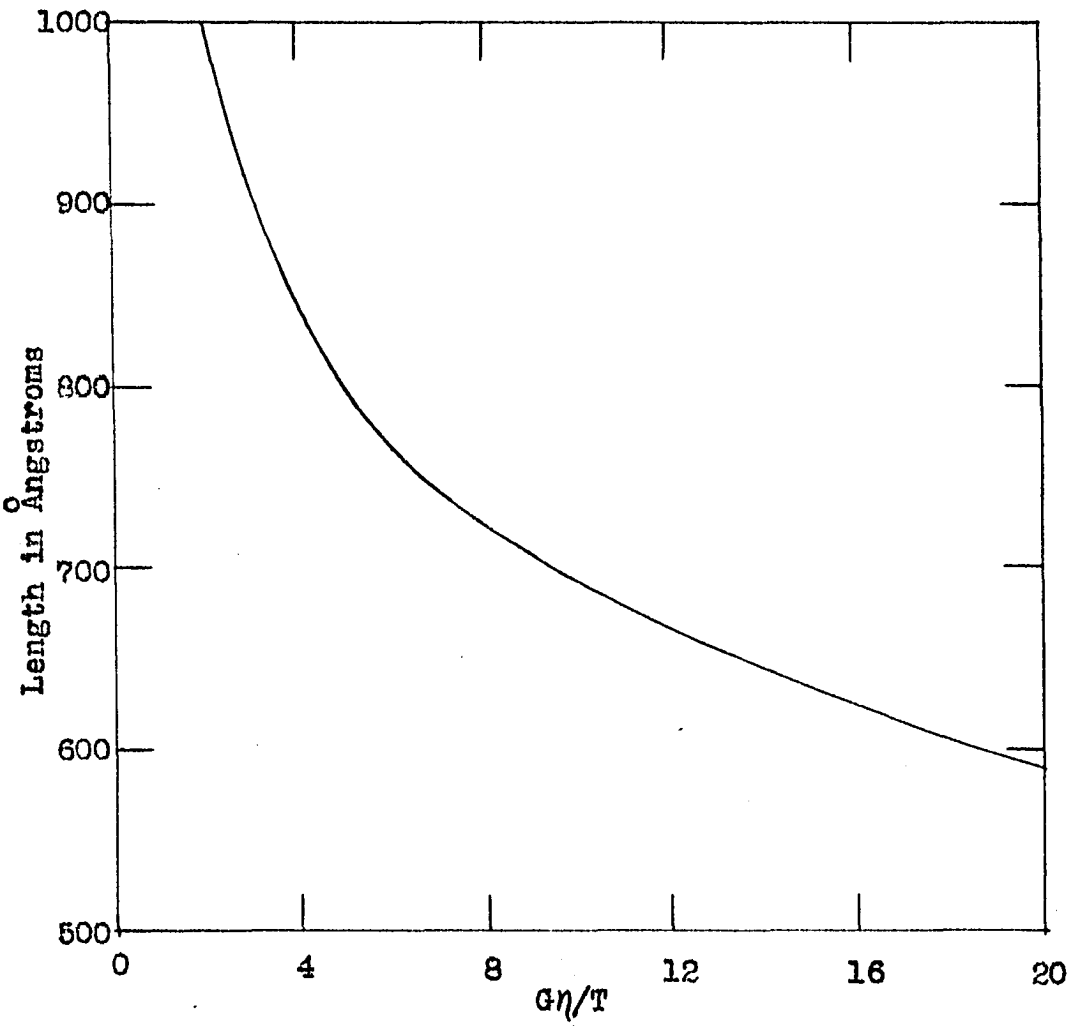


Fig. 23. Length vs. $G\eta/T$ for the iodine complex of Hassid's amylose. 15% pyridine as initial solvent.

solution, which was then cooled slowly, the particles were a little longer, indicating some aggregation during the cooling process.

Two samples gave shorter lengths. In one case amylopectin had been added until the original amylose comprised only 5% of the total starch present. This sample had iodine added while it was hot, and it was found later that this technique was not satisfactory for samples containing amylopectin. The sample passed little light and had only a little dichroism. In the other case the pyridine solution of the amylose had stood three days, and was then heated to dissolve the sample. Subsequently experiments with samples left for varying times showed that the longer a sample stood in 15% pyridine at room temperature, the harder it was to dissolve by warming. For example, it took several hours of heating to dissolve even a very small fraction of a sample that had stood for a month or more. It is therefore possible that the sample was not completely dissolved, the shorter chains being more completely dissolved than the longer. Oxidation degradation might also have occurred.

It should be added that even these latter two samples gave lengths only 6-8% lower than the average of the others, which is not really significant.

Effect of amylopectin. A considerable quantity of amylopectin was dissolved in 15% pyridine, giving a solution of perhaps 10-100 times the carbohydrate concentration used with amylose. When iodine in KI was added, there was no color at all except the yellow-brown obtained with pyridine-iodine solutions alone. Apparently the amylopectin-iodine complex is not stable enough to form under such conditions.

A sample containing 40.5% of Hassid's amylose and 59.5% of amylopectin was prepared in two ways. First, one part was dissolved in hot 15% pyridine, complexed, and cooled quickly. It had a poor, muddy greenish-blue color, and when added to glycerol and run, there was not enough dichroism for even rough measurements.

Another part of this sample was dissolved hot, then cooled quickly and complexed. A good color was obtained, and the complex was highly dichroic when run in the presence of glycerol. Furthermore, the results agreed with those previously found.

A similar sample containing 5% of Hassid's amylose and 95% of amylopectin also gave a good color and dichroism, and agreed with previous average lengths, when the amylose solution was cooled quickly before adding iodine.

Five of Schoch's amylose preparations, which had not been completely freed of amylopectin, were tried (see the next section). When iodine was added to the hot solutions,

poor results were obtained; in fact, the two corn amylose samples and the shorter of the two potato amyloses gave so little dichroism when complexed that satisfactory measurements could not even be made. When the iodine was added to the cold solutions, however, satisfactory results were obtained in all five cases.

Results on various amylose preparations

By the time the proper method for preparing the sample had been established, Hassid's amylose had already been rather thoroughly investigated. No more work was done on this sample.

As mentioned in the section just preceding this, five of Schoch's amylose samples were investigated. While the first attempts to run these, preparing the complex by adding iodine to the hot solutions, did not work out satisfactorily, interesting information of a positive nature was nevertheless obtained. As with all samples, these showed no blue color at about 80°, but developed the complex color on cooling. It may be important to note that the shorter the chain length, the colder the solution had to be to obtain maximum color intensity.

Whole potato starch and two amylose fractions prepared from it were run, the results being shown in Fig. 24. The whole starch contained about 25% amylose, which was

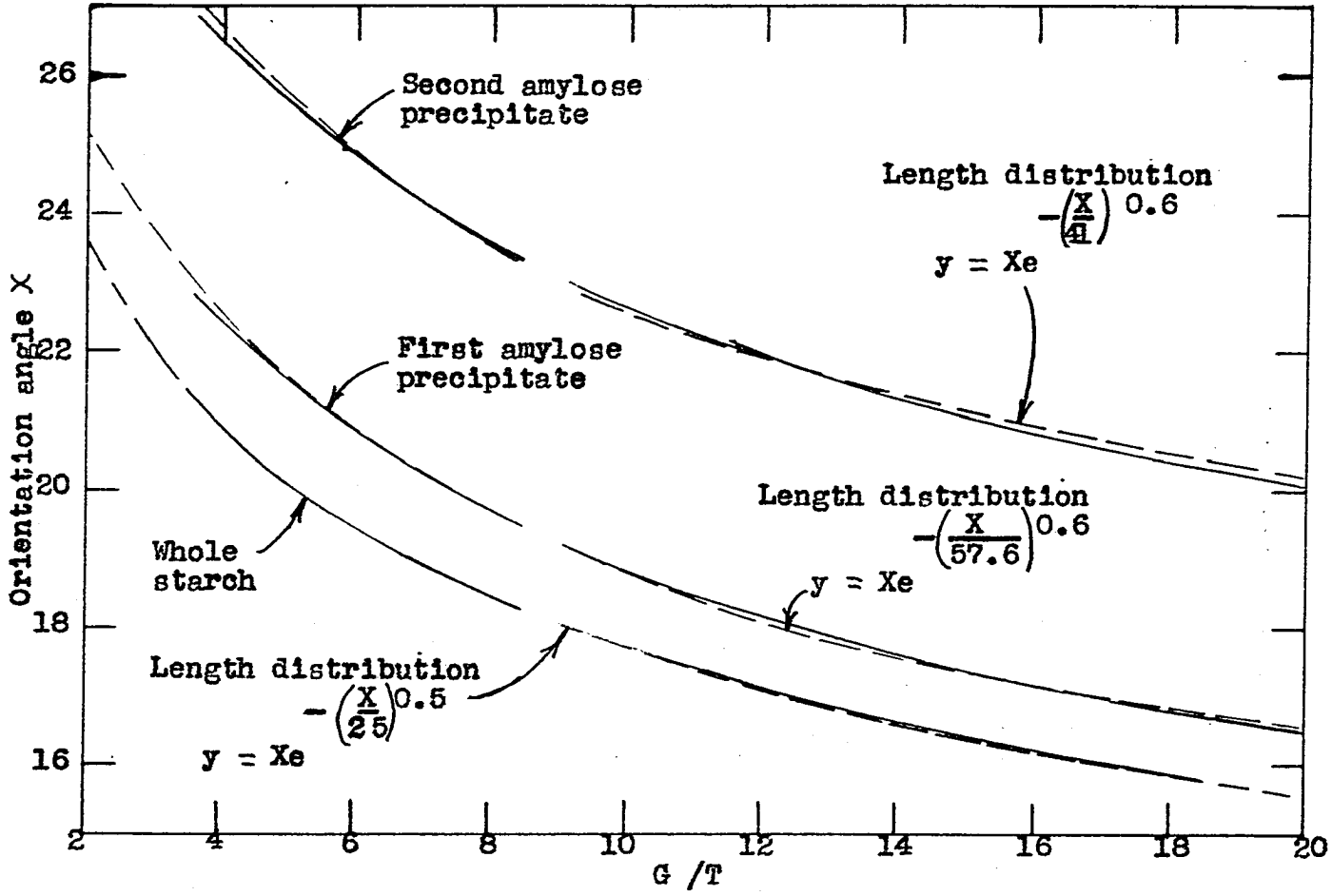


Fig. 24. X vs. $G\eta/T$ for the iodine complexes of the amyloses of R. L. Smith. The solid lines are experimental curves; the dotted are theoretical.

five times as much as in the most unfavorable sample tried (5% amylose, 95% amylopectin), and gave quite satisfactory results.

When the whole starch was fractionated (81), most of the amylose present was removed in the first precipitate. One would therefore expect the average lengths of the amylose in this precipitate to be about the same as in the whole starch, or perhaps a little shorter because of degradation occurring during solution of the sample in the autoclave. The latter appears to be the case.

The second fraction precipitated only on long standing, and one would therefore expect this to consist of shorter molecules. The experimental results confirmed this prediction.

Curves of χ vs. $G\eta/T$ for the sample discussed in this section are shown in Figs. 24 to 29. Some of these graphs also show theoretical curves fitted to the experimental, as described under "Discussion".

The curve for Δ vs. $G\eta/T$ for the iodine complex of Hassid's sample is shown in Fig. 30. The values of Δ were also found for the other samples investigated. The curves of Δ vs. $G\eta/T$ for these had about the same shape as the curve for Hassid's sample, and contributed no additional information.

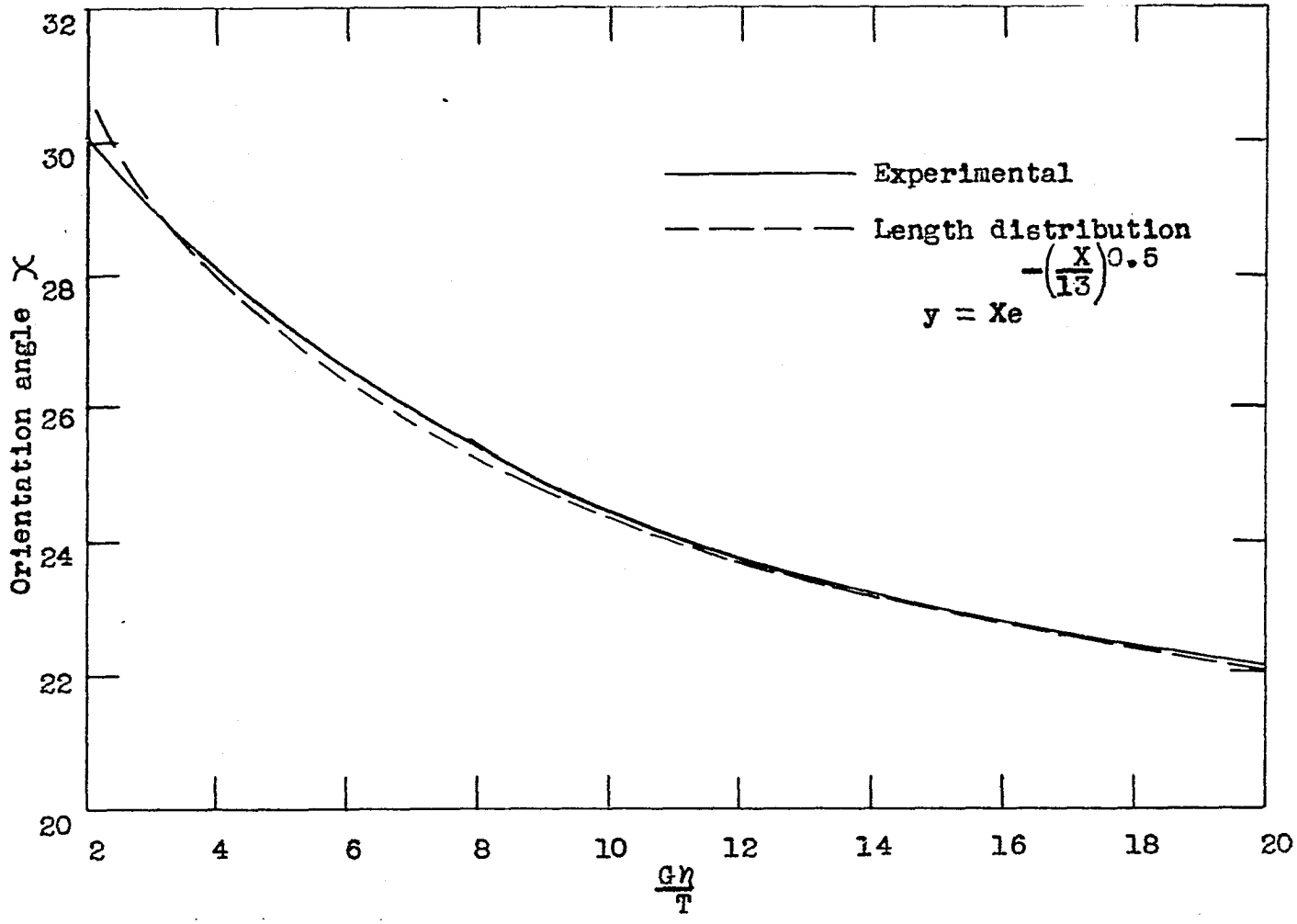


Fig. 25. χ vs. $G\eta/T$ for the iodine complex of Hassid's amylose.

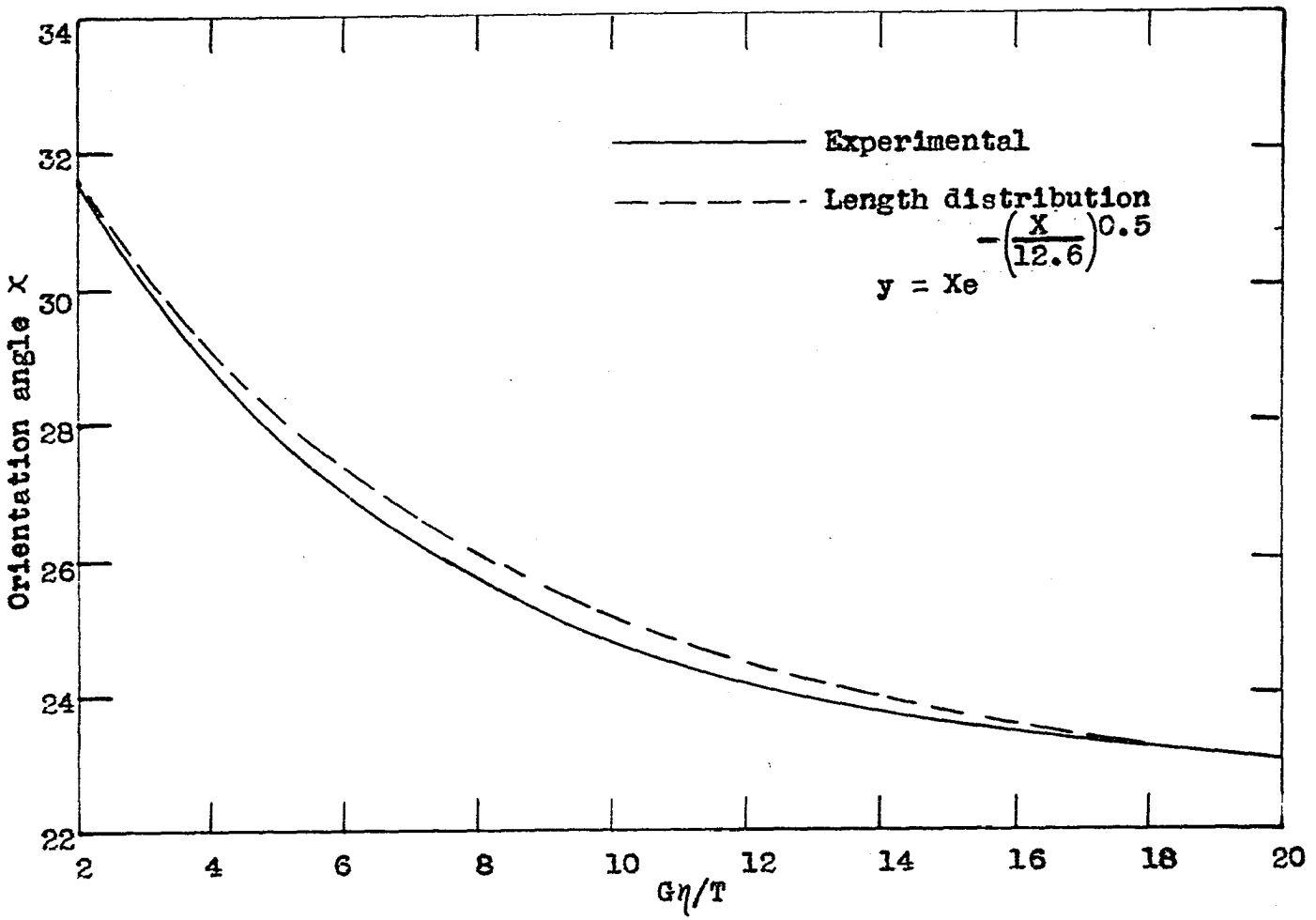


Fig. 26. χ vs. $G\eta/T$ for the iodine complex of potato amylose P-5/6-A(8b).

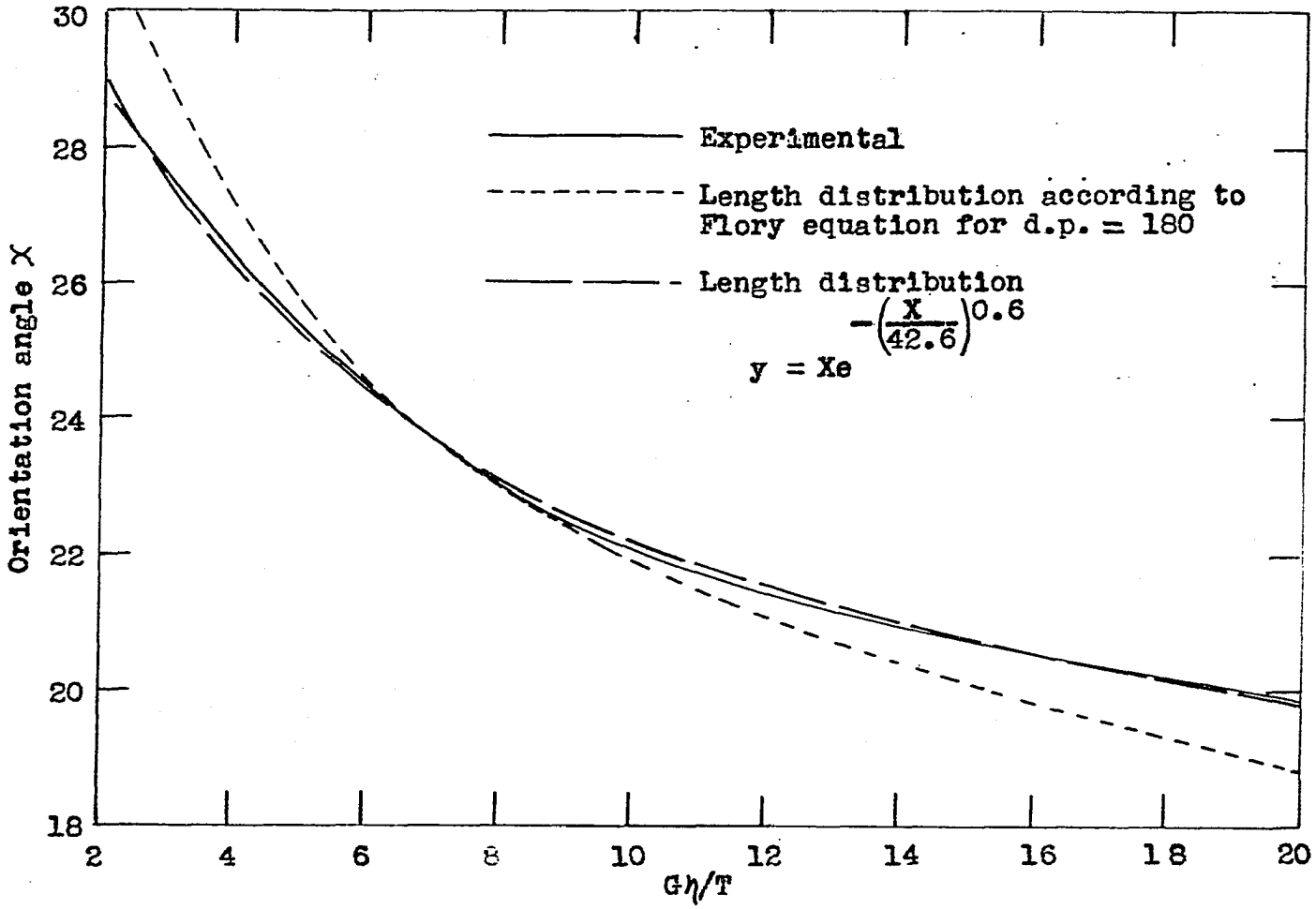


Fig. 27. χ vs. Gη/T for the iodine complex of potato amylose P-5/6-A(7b).

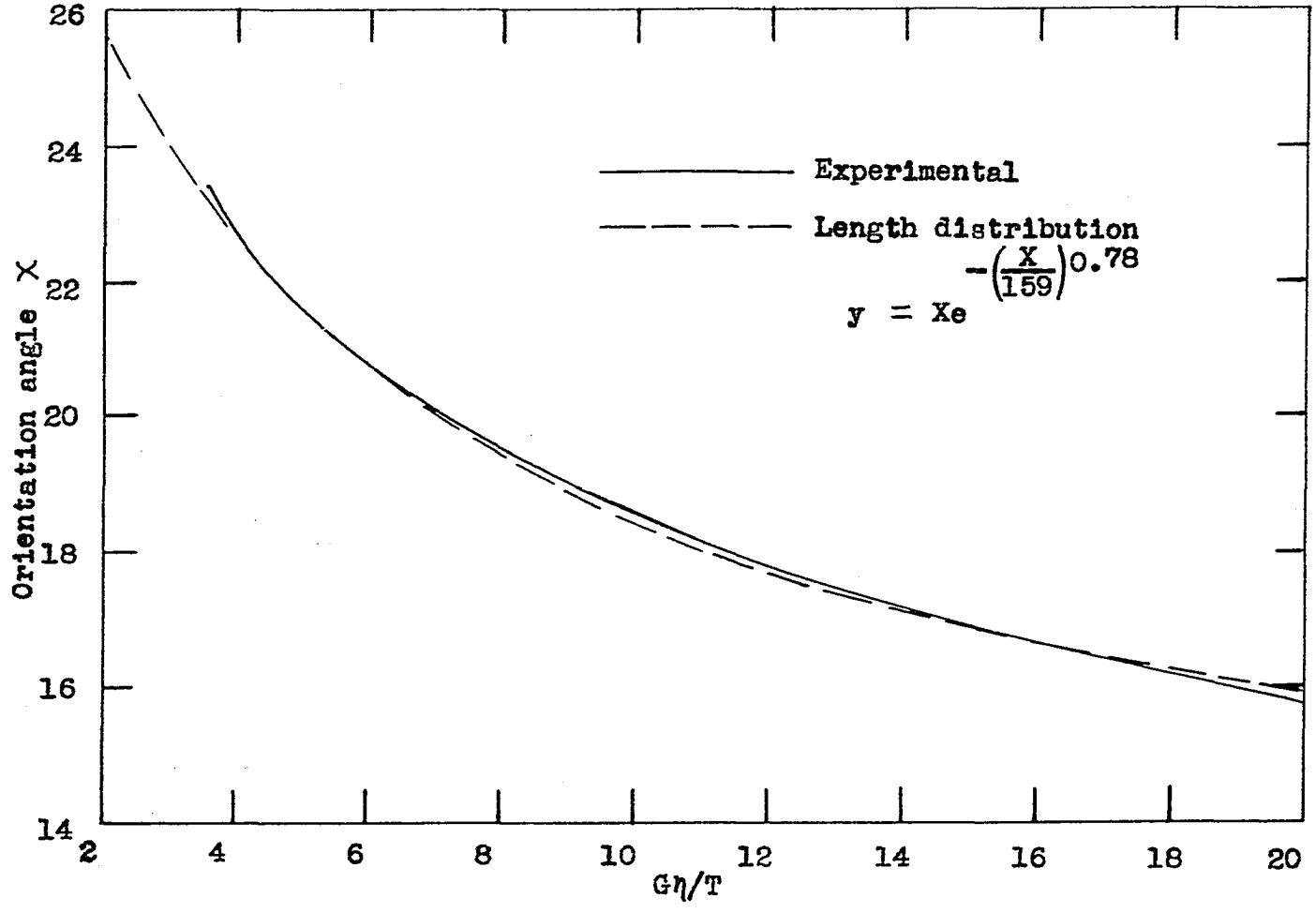


Fig. 28. X vs. $G\eta/T$ for the iodine complex of tapioca amylose T-7/9-A(15b).

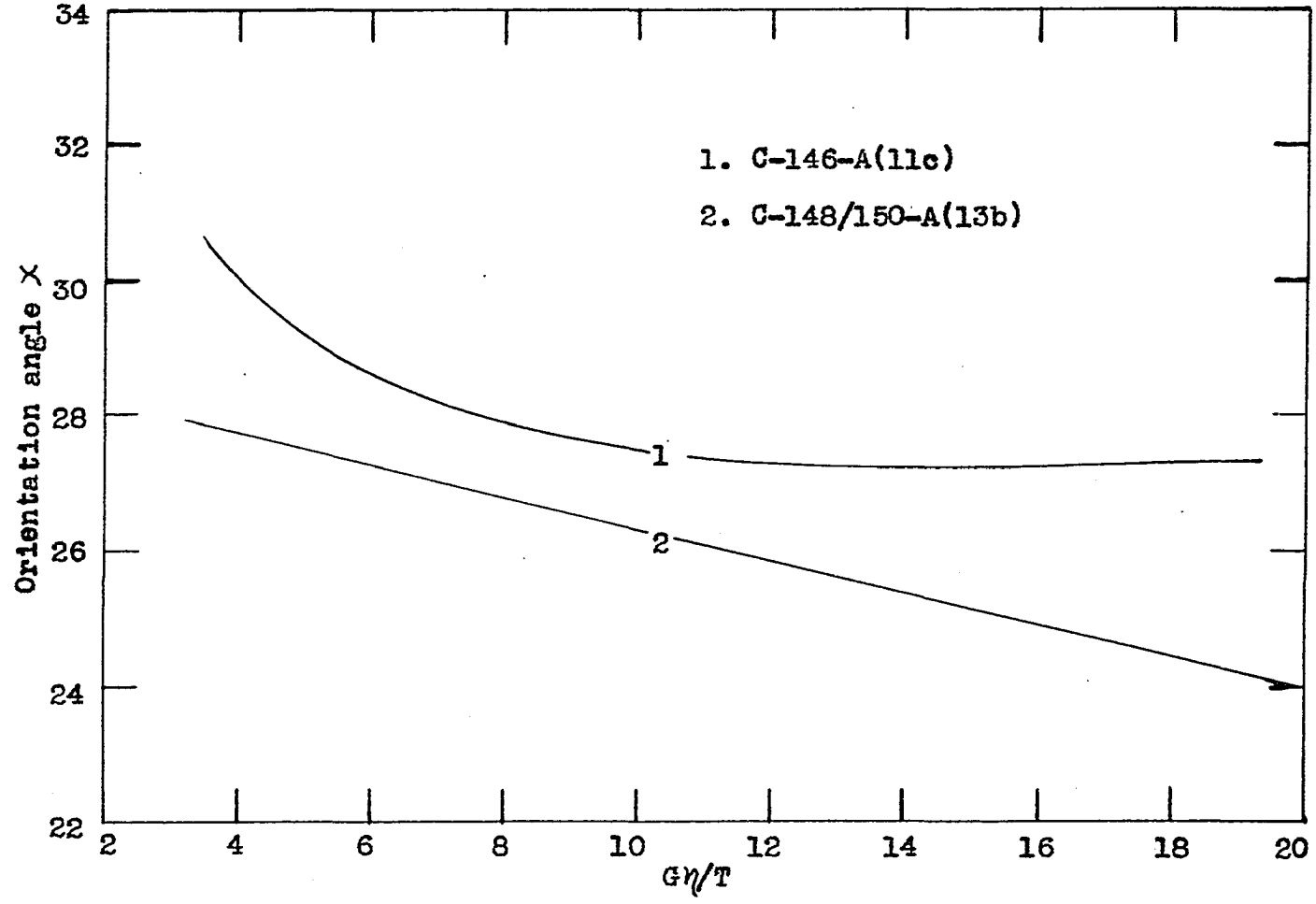


Fig. 29. χ vs. $G\eta/T$ for the iodine complexes of corn amyloses. Experimental curves only.

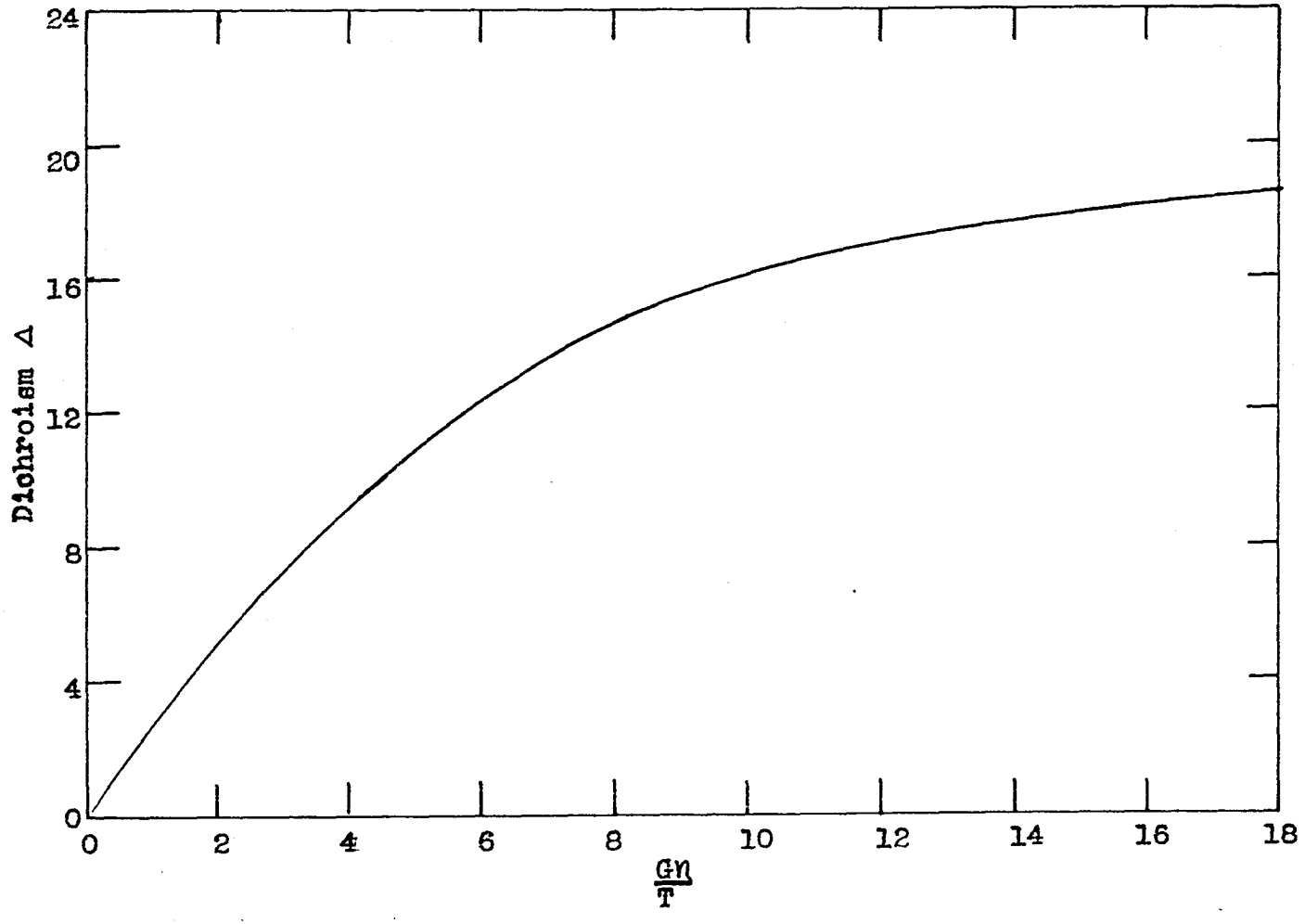


Fig. 30. Δ vs. Gη/T for the iodine complex of Hassid's amylose.

DISCUSSION

Birefringence Results

Molecular interaction

Concentration effects. From Fig. 14 it can be seen that there is apparently some interaction between the molecules of a solution of potato amylose P-5/6-A(7b) in glycerol and ethylenediamine. Apparently the effect is much greater at high concentrations of the amylose, the particles appearing to be longer than at low concentrations. However, no such effect was found with amylose sample C-148/150-A(13b) in the concentration range 0.2% to 0.8% (Fig. 15) in which range the potato amylose showed a slight but definite change in observed length. The difference may be due to the fact that the corn amylose has a lower average molecular weight, and the shorter molecules may be more independent of neighboring molecules than the longer potato amylose molecules. Also, the concentration effect may be due partially or entirely to amylopectin in the amylose samples. In this case no predictions could be made without knowing the amylopectin concentrations in these samples.

Investigations of the concentration effect with a

series of amylose samples essentially free of amylopectin would be very valuable, but only one such sample (Hassid's) was available, and it was too limited in quantity to be used for such a study.

The equations used in the theoretical work were developed on the basis of each molecule of amylose being isolated in the solution, surrounded only by pure solvent. The other molecules were assumed too far away to have any effect. The nature of the intermolecular action when the molecules are not far apart is not known; however, when they are not so close together as to be in more or less continuous contact, and not close enough, also, for van der Waals and other such forces to have an appreciable effect, then one might suspect the effect to be primarily one of viscosity. The individual molecule might still be considered to be moving all by itself in the solution, but in a more viscous solvent. If this were the case, the viscosity of the solution should be used instead of the viscosity of the solvent in the Peterlin-Stuart and other equations.

Qualitatively at least, this procedure would be correct. For a given experimentally determined χ value, the corresponding value of $G\eta/T$ would be larger at higher amylose concentrations. This would give a shorter average length for the molecules, a change in the right direction.

The corn amylose, which showed no concentration effect in the range examined, has a lower intrinsic viscosity than the potato amylose, consequently the solution viscosity was no doubt more nearly equal to the solvent viscosity.

Precipitation in the machine. A precipitate developed in all amylose solutions when run at the high gradients obtained at low temperatures. One such solution was centrifuged, and the decantate and the original sample (C-148/150-A(13a)) were run in the usual manner. From the shapes of the χ vs. $G\eta/T$ curves (Fig. 18) for both cases, it appears that some unusually long particles were developed at moderate to high gradients.

The decantate from the machine fractionation shows the effect of long particles much more than the original sample, though not as much as those samples in which the precipitate was developed but not removed. There are apparently aggregates formed which are much longer than the original molecules, but not large enough to be visible or to be removed by an ordinary centrifuge.

Polydispersity

Length vs. $G\eta/T$. All the graphs of length vs. $G\eta/T$ show that the average lengths as measured by streaming orientation become shorter at high gradients. This does

not mean that the molecules become shorter, but rather that the weighting of the average changes. At low gradients, only the longer molecules are oriented sufficiently to contribute appreciably to the birefringence, consequently the observed orientation angle is essentially that of the longer particles. At higher gradients, the shorter molecules become more oriented, and the birefringence, hence the measured orientation angle, becomes a function of all the molecules rather than only the longer ones.

Empirical curves for χ vs. $G\eta/T$. A little later, curves for χ vs. $G\eta/T$ for various hypothetical mixtures will be discussed. Sadron's equation (equation 21) for was used for calculating these curves, and the mixtures were selected to fit the experimental curves obtained by dichroism. They are shown in Figs. 24-28. To determine the effect of a small amount of a large molecule such as amylopectin (which has no effect on curves obtained by dichroism), one of these curves (Fig. 27), was selected, and the effect of 1% of a material with a particle of 10,000 Å was incorporated into the calculations for the curve. The resulting curve is shown in Fig. 31. Two other curves are also shown, illustrating the effect of material of 4,000 Å length. For comparison, the curve without the added long component and curves for four monodisperse systems are included.

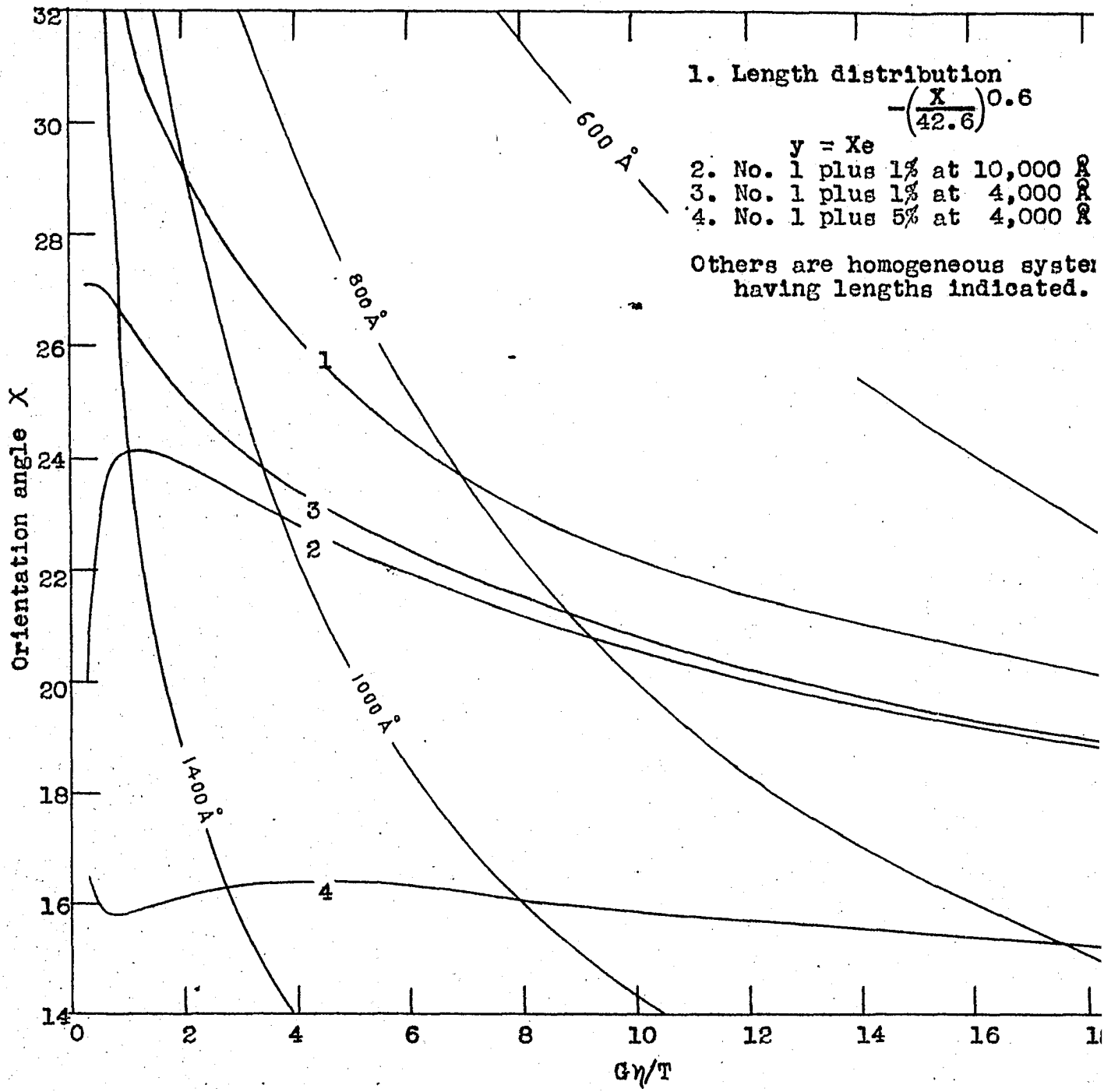


Fig. 31. X vs. $G\eta/T$ for hypothetical systems of rigid molecules.

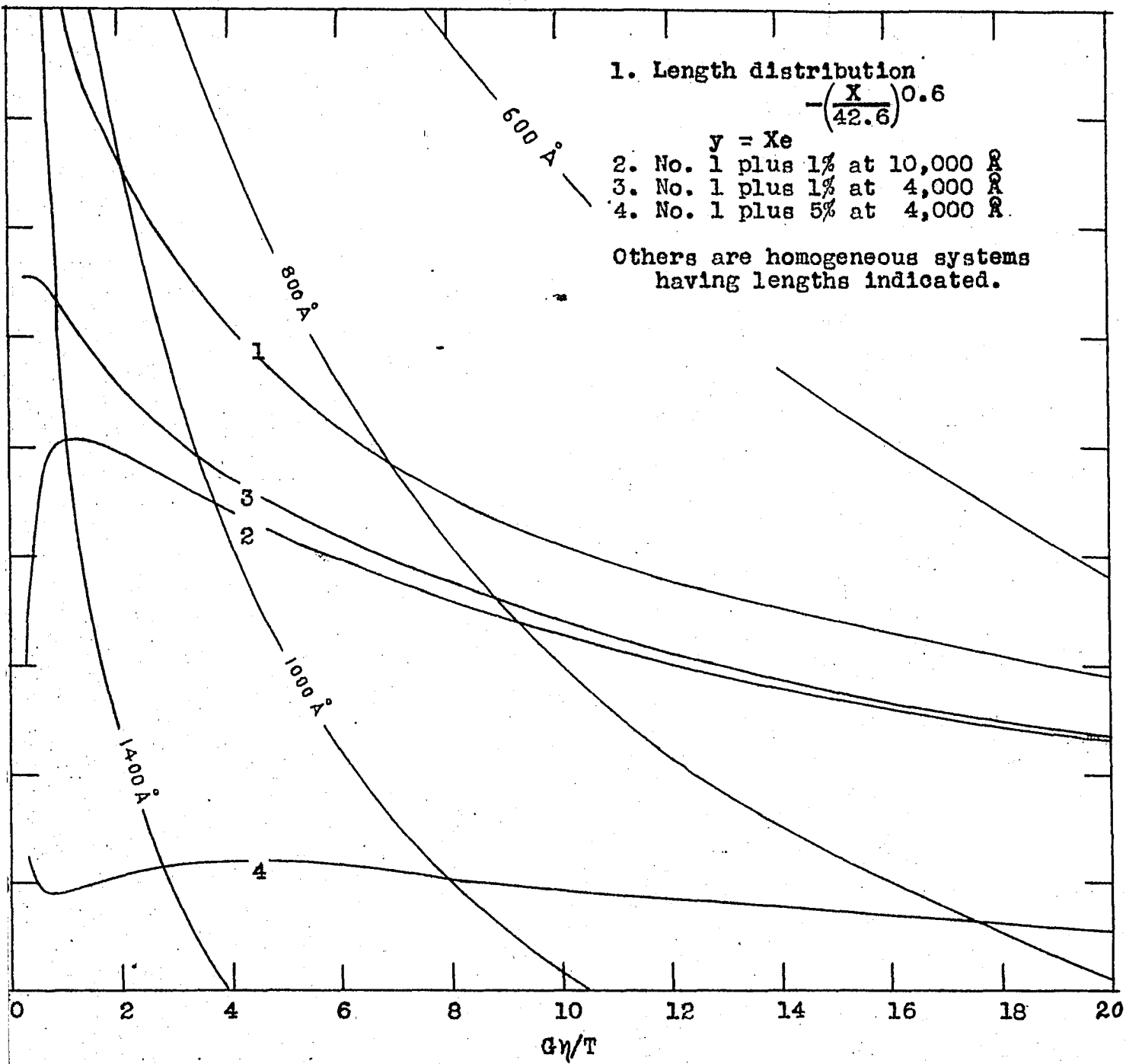


Fig. 31. X vs. $G\eta/T$ for hypothetical systems of rigid molecules.

None of the above curves agrees closely with the experimental curves for the orientation angle as determined by birefringence. Nevertheless, there is a similarity between the theoretical and experimental curves which indicates that some long material was present in the amylose sample and caused the increase of χ with gradient at low gradients. Perfect agreement cannot be expected with any conceivable length distribution, because it is probable that neither amylose nor amylopectin is rigid, hence lengths will increase with gradient. Also, the theoretical data used was extrapolated beyond the range given by Scheraga, et al., and was not very dependable.

Curves for f vs. $G\eta/T$ for mixtures. Using equation 22 for f, and the data of Scheraga, et al., for monodisperse systems, several curves for f vs. $G\eta/T$ for hypothetical mixtures of rigid molecules of different lengths were prepared. These are shown in Fig. 32, together with the curve for several homogeneous substances. It must be emphasized that these f values are the values for these mixtures, not Scheraga's values for a monodisperse system. Thus, if the molecules are rigid, the f values given should be proportional to Δ values actually measured for a system with the stated distribution.

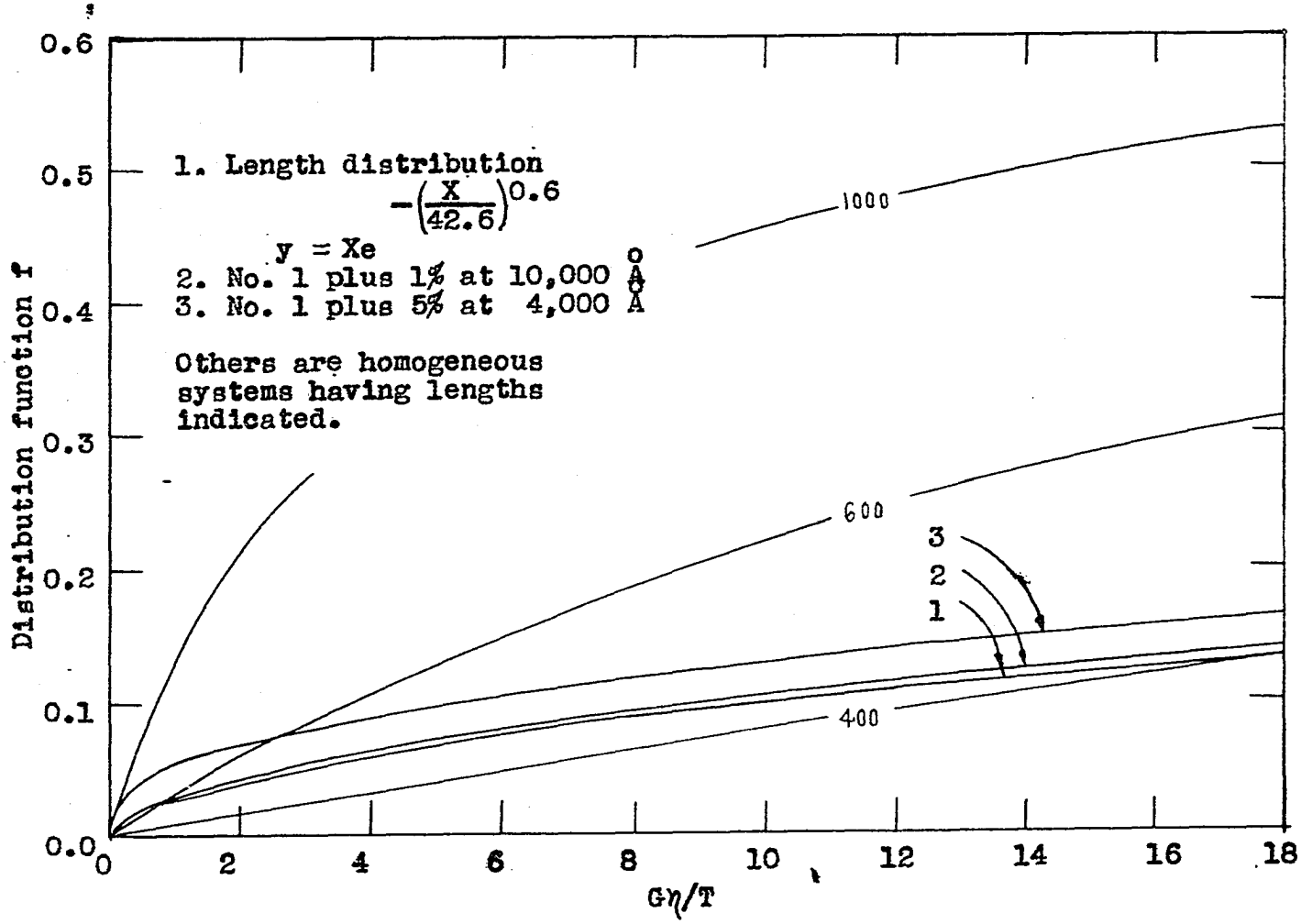


Fig. 32. f vs. Gη/T for hypothetical systems of rigid molecules.

Scheraga's data were calculated on the basis of $f = 1$ at infinite gradients; that is, at infinite G and $G\eta/T$. It is obvious from Sadron's equation that the same limit is reached in the case of mixtures.

Elongation of molecules. As stated before, the values of f obtained from Scheraga's data (and Sadron's equation, if dealing with a mixture) and experimentally determined χ values should be proportional to the measured values of Δ for the sample; that is,

$$\frac{\Delta}{f} = \text{a constant} \tag{27}$$

The quantity Δ is proportional not only to f, but also to the absolute birefringence of the molecules, the concentration of the solution, and the length of the light path through the solution. The latter is constant for any given machine, so equation 27 may be written

$$\frac{\Delta}{f} = kc(n_1-n_2) \tag{28}$$

or

$$\frac{\Delta/c}{f} = k(n_1-n_2) \tag{29}$$

where (n_1-n_2) is the birefringence per gram of the individual molecules, and c is the concentration in grams per 100 ml.

If the proper values of f are used in equation 29, and if the value of (n_1-n_2) so calculated is not constant, a change in the molecules is indicated. The most probable change is a stretching effect due to the flow gradient. If the value of (n_1-n_2) were found to increase with gradient, this might be considered additional experiiental evidence for elongation because a molecule of given length would certainly be more birefringent when stretched out than when partially coiled.

A change in the value of (n_1-n_2) as calculated from equation 29 does not necessarily mean a change in the actual birefringence of the amylose molecules if Scheraga's table of f vs. α for monodisperse systems is used in the equation. It has been shown that this value of f is not the true value for a mixture, and consequently the values of (n_1-n_2) will be in error. This is illustrated in Fig. 33, where values $k(n_1-n_2)$ for several polydisperse systems are calculated.

To determine these curves, χ values at various values of $G\eta/T$ were taken from the curve of Fig. 31. Scheraga's tables alone were used to find f from χ (that is, f was found as though the system were monodisperse). Values of Δ/c, on the other hand, were found for this mixture by using Sadron's equation and assuming the measured Δ/c to be proportional to the calculated f from the Sadron equation

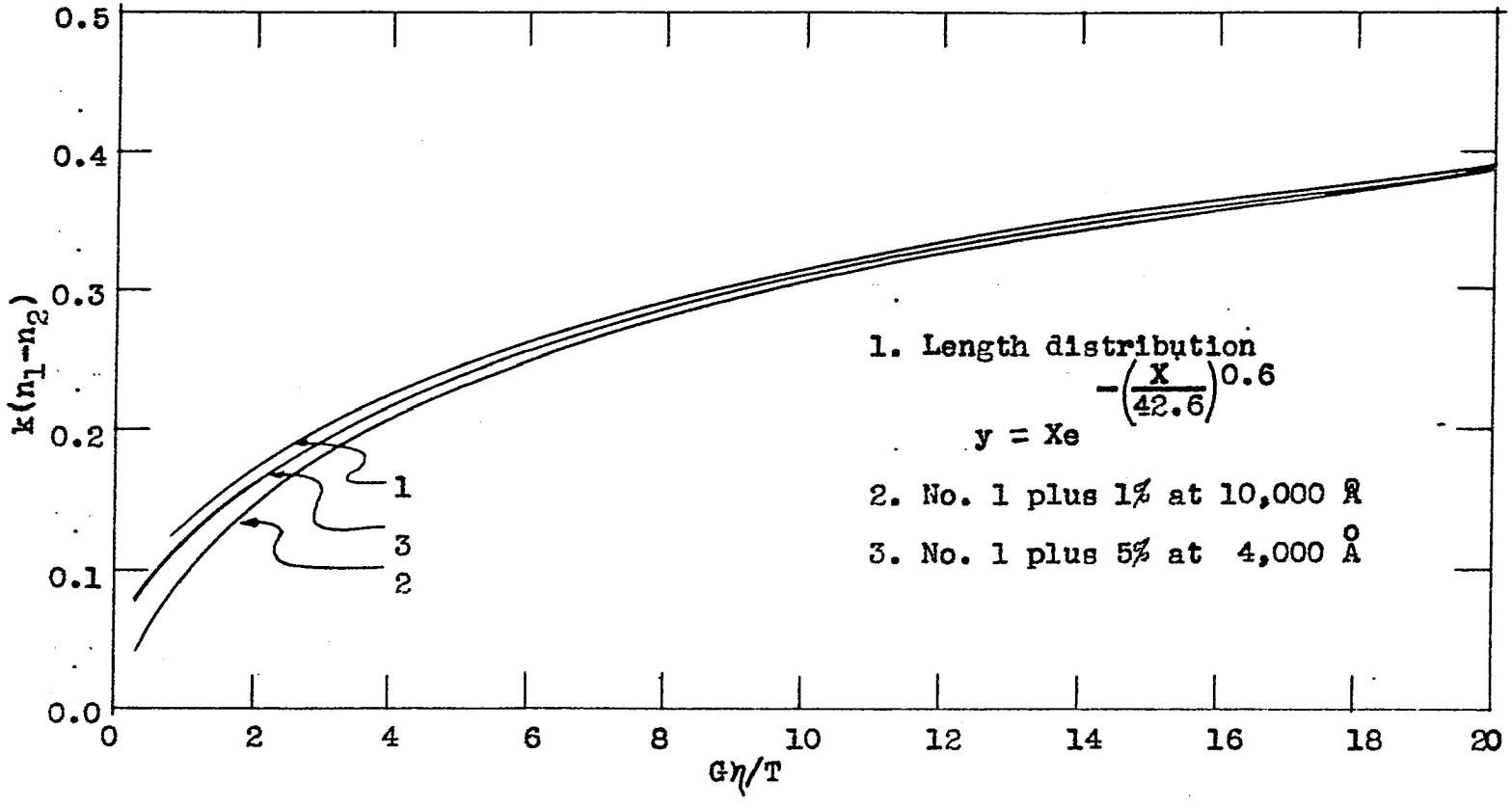


Fig. 35. Apparent absolute birefringence $k(n_1-n_2)$ vs. $G\eta/T$ for hypothetical mixtures calculated as monodisperse systems.

(Fig. 32). The values of $k(n_1-n_2)$ were found from these values using equation 29.

Since no accurate knowledge of the distribution of lengths was available for the amylose samples used in birefringence, the values of $k(n_1-n_2)$ for these samples were calculated using the data for f for a monodisperse system. As one would predict from the preceding paragraph and the known polydispersity of the samples, the values were not constant, but increased with increasing gradients. This is shown in Fig. 34 for the potato amylose P-5/6-A(7b), the sample whose iodine complex appeared to have the length distribution required by curve 1, Fig. 31 and 32. In Fig. 34 the experimental value for $k(n_1-n_2)$ for the potato amylose is compared with curve 2 of Fig. 33, the latter being normalized to the same value as the former at $G\eta/T = 2$.

The experimental curve is a much more linear curve than the theoretical, and comparison with Fig. 33 shows that the other theoretical curves in this figure would either have even more curvature, or would not extrapolate to zero birefringence at zero gradient as the experimental did. This means that either the potato amylose is much more polydisperse with respect to long particles than any of the theoretical length distributions tried, or that the birefringence per molecule was actually increasing at higher

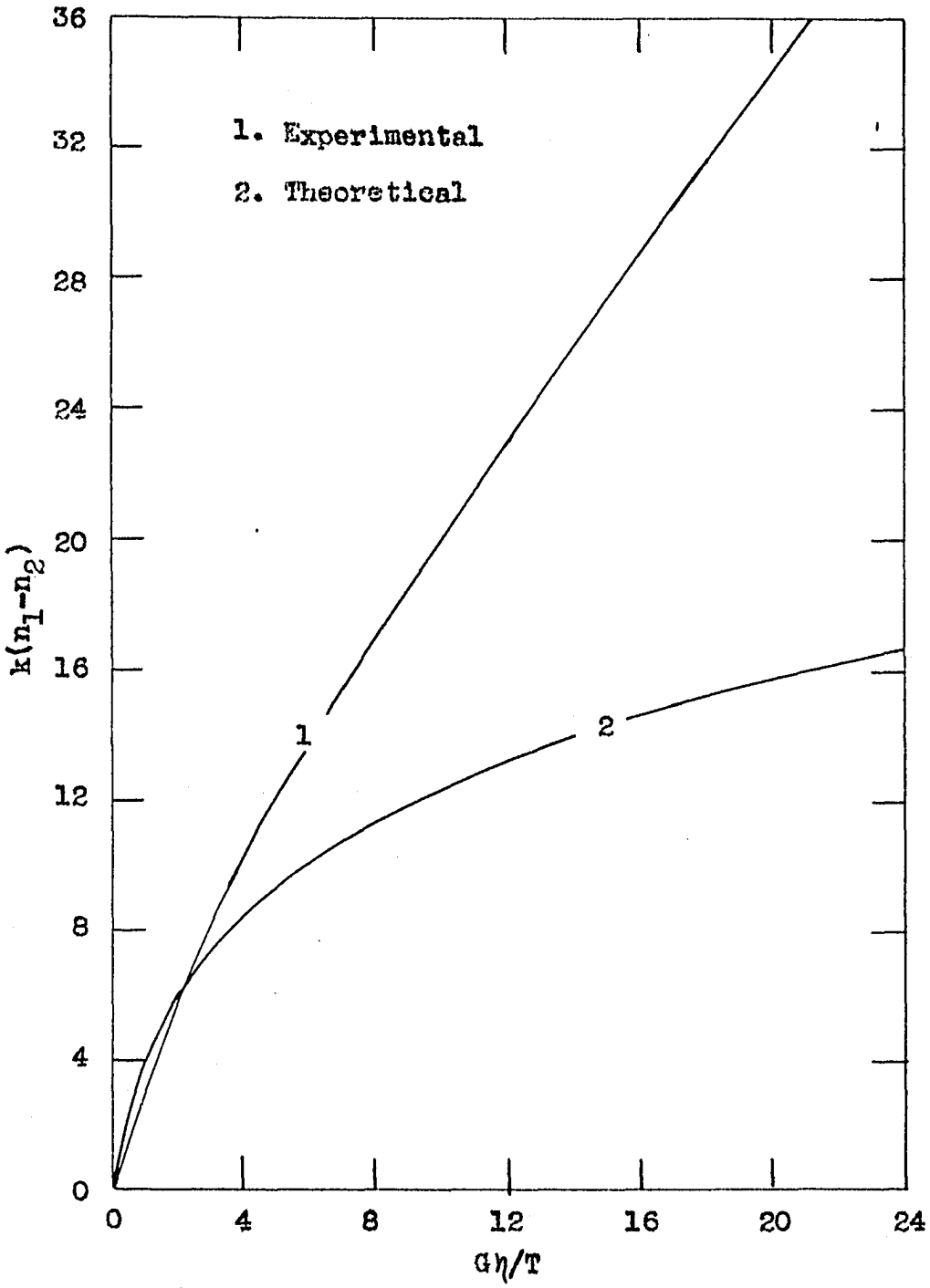


Fig. 34. The birefringence, $k(n_1-n_2)$, vs. $G\eta/T$ for potato amylose P-5/6-A(7b).

gradients. The latter would indicate stretching of the molecule.

A comparison of Fig. 17 and 25 shows that while Hassid's amylose appears to have a somewhat shorter average length than its iodine complex at low $G\eta/T$, the two become equal at about $G\eta/T = 7$, and above this value the uncomplexed amylose is apparently longer than the complex. This indicates elongation of the molecules present. It is not obvious whether the effect is due to stretching of the amylose or of the trace of amylopectin present. However, it is believed that the quantity of amylopectin present is very small, and would not greatly effect the results. Large quantities changed the shapes of the χ vs. $G\eta/T$ curves so greatly that comparison became difficult.

All other samples run by both birefringence and dichroism had longer average lengths at all gradients when in the uncomplexed state. One corn amylose, C-146-A(11c), showed a greater difference at high and low gradients than at moderate gradients. The relatively greater lengths at low gradients were probably due to amylopectin; the greater length at high gradients may have been due to elongation. However, too much significance should not be attached to birefringence results of samples other than Hassid's, because of the effects of amylopectin.

Birefringence vs. intrinsic viscosity

According to Kuhn (40), $\Delta/G\eta c$ should be proportional to the d.p. at low values of $G\eta$. Since intrinsic viscosity, according to the Staudinger equation, is also proportional to d.p., a plot of $[\eta]$ vs. $\Delta/G\eta c$ should give a straight line. Such plots are shown in Fig. 35 for two values of $G\eta/T$. Data for these graphs were taken from Table 2.

It can be seen from Fig. 35 that Kuhn's statement applies to the birefringence of amylose in ethylenediamine-glycerol reasonably well at $G\eta/T = 10$, though not so well at $G\eta/T = 5$. Since both $[\eta]$ and $\Delta/G\eta c$ are proportional to the first power of d.p., according to this theory, polydispersity of the amylose should affect both terms equally and cannot account for the fact that they are not quite proportional. However, since these samples contain varying amounts of amylopectin, which may be expected to affect both terms, but not necessarily equally, minor deviations from proportionality are to be expected.

Dichroism Results

Molecular interaction

Aggregation. The evidence obtained indicated strongly that aggregation was of little or no importance in

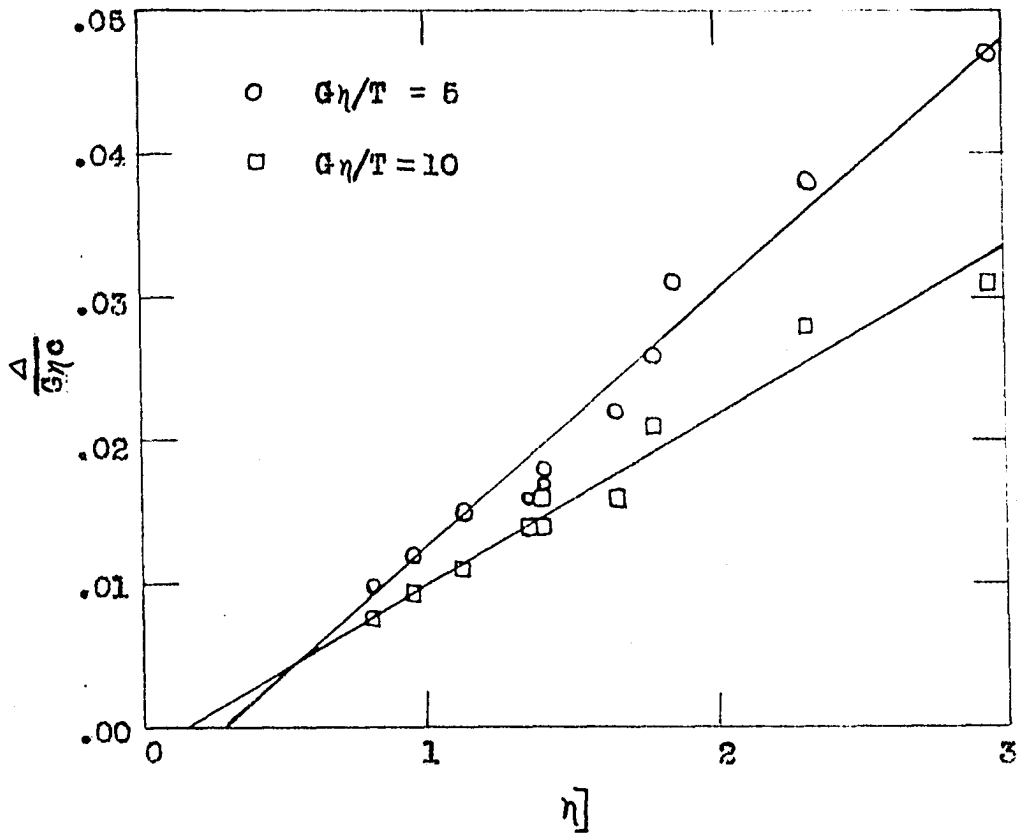


Fig. 35. $\Delta/G\eta c$ vs. $\eta]$ for representative samples of Schoch's series of amylose preparations.

work with the iodine complex when proper precautions were observed. For best results the amylose samples used were dissolved in 15% (by volume) pyridine at 80-90°, cooled, complexed fairly promptly with excess iodine, and added to glycerol. If not cooled before iodine addition, the samples were apparently degraded, aggregated, or in some other manner rendered unreliable. When such samples were cooled after addition of iodine, the blue color of the complex developed to a greater or less extent. The fact that this color appeared at relatively high temperatures with long chain tapioca amyloses, but at much lower temperatures with the shorter corn amyloses, indicates that perhaps the pyridine concentration used would be too high for very short chain molecules. Conversely, one might use a higher concentration for very long chain amyloses, which might be desirable to prevent aggregation.

When 15% pyridine was the initial solvent, it was apparently not necessary to cool the hot solution and complex in the shortest possible time, 5 or 10 minutes delay not being serious. However, a visible precipitate appeared in every sample after a few hours at room temperatures, so it is obvious that aggregation begins fairly soon. The precipitate will not form an iodine complex even on long standing, so it might not interfere. This, however, is not certain.

It was also found that delays of 5 to 10 minutes between forming the complex and adding to glycerol were not serious. Such a delay is unnecessary, however, and this result is of interest only in showing that very likely there was no aggregation during the short complexing time ordinarily used.

Long range interactions. Concentration studies such as were carried with amylose preparations, using flow birefringence, would have been valuable in the case of the dichroism of the iodine complex. However, the concentration range over which good results could be obtained was so limited that such studies were impractical.

Lack of evidence concerning long range interactions was not considered a serious matter, since the concentrations used were so low (of the order of 0.002% as amylose) that interaction was probably negligible.

Polydispersity

Empirical curves for χ vs. $G\eta/T$. Sadron's equations were used with Scheraga's data to calculate curves for χ vs. $G\eta/T$ for a number of hypothetical systems of various length distributions. Eventually length distributions were found to match all the dichroism results except those for the corn samples. The empirical curves are shown with the matching experimental curves in Figs. 24-28.

Equations of the form

$$y = Xe^{-\left(\frac{X}{X_1}\right)^a} \qquad (30)$$

were found to fit all but the corn samples. In these equations, y is the weight fraction of the sample which has the length X in Ångstrom units, and X_1 is a constant. By taking the derivative of y with respect to X and setting equal to zero, one finds that at the maximum value of y,

$$X = X_1\left(\frac{1}{a}\right)^{\frac{1}{a}} \qquad (31)$$

For the same value of a, a higher X_1 corresponds to a higher average length, but in general X_1 is not the most probable length as it is in the Maxwell equation.

The higher the value of a, the more homogeneous is the sample. This term might be considered a "homogeneity index". The significance of this term, if any, is not at present known.

Length distributions according to the Flory equation

$$w_n = np^{n-1}(1-p)^2 \qquad (32)$$

were tried, but were not disperse enough to fit the experimental results. In this equation w_n is the weight fraction

of polymer of d.p. = n. For amylose, $p = 1-1/\bar{n}$, where $\bar{n}$ is the numerical average d.p. for the mixture. The Flory equation was derived on the assumption of completely random polymerization, and one would expect less, not more dispersity in the fractionated amyloses used. The results actually found are therefore rather surprising, and have not been explained. A χ curve calculated from a length distribution according to the Flory equation is shown in Fig. 27.

It is possible that distribution according to the Flory equation could be combined with small amounts of rather long material to fit the experimental data with curves having more theoretical justification than the empirical ones from equation 30.

Average d.p. Weight and numerical averages were calculated for a sample whose length distribution on a weight basis is given by the equation

$$y = Xe^{-\left(\frac{X}{13}\right)^{0.5}} \qquad (33)$$

The weight average was 130, and the numerical average was 78. It is probable that there are no very short molecules present in the amyloses studied, because water solubility would be expected to eliminate them during the processing

of the sample. Since molecules of lengths under 100 Å contribute practically nothing to the χ vs. $G\eta/T$ curves, the length distributions used to calculate the curves of Figs. 24-28 could be cut off below 100 Å to allow for water solubility, and the χ vs. $G\eta/T$ curves calculated from the resulting distributions would remain unchanged. The weight average length would be increased somewhat, and the number average length would be changed very greatly.

Since the exact length distribution of the shortest molecules would be practically impossible to determine, and since these have such a great effect on the averages, particularly the number average, it is not practical to attempt to establish any exact average d.p. However, it appears that the numberical average d.p. is not more than a few hundred.

The χ vs. $G\eta/T$ curve calculated from equation 33 fitted the experimental curve for the iodine complex of Hassid's amylose quite well. According to Hassid, this sample had a numberical average d.p. of about 900, much greater than indicated by the data obtained. The discrepancy may indicate aggregation of the sample used by Hassid for his molecular weight determination. The difference could also be due to incomplete complexing of the ends of the amylose molecules.

Rigidity

It is to be expected that a molecule of the amylose-iodine complex would be rather rigid. If so, k_1-k_2 should be constant. This value is determined by equation 36, modified for incomplete orientation,

$$k_1-k_2 = \frac{-\log_e \tan(45-\Delta)}{cfl} \qquad (34)$$

In this equation c is the concentration of dichroic material and l is the length of the light path through the solution. It is necessary to use the values of f for the proper polydisperse system.

A graph of k_1-k_2 vs. $G\eta/T$ is shown* in Fig. 36 for amylose P-5/6-A(7b). For this graph, the proper values of f were found by using equation 22 and the length distribution equation 30. The Δ values were those actually measured for this sample. The dichroism was found to be essentially constant, indicating a rigid molecule.

*The absolute value of k_1-k_2 was not determined, since only the shape of the curve was desired. The light path l was taken as unity.

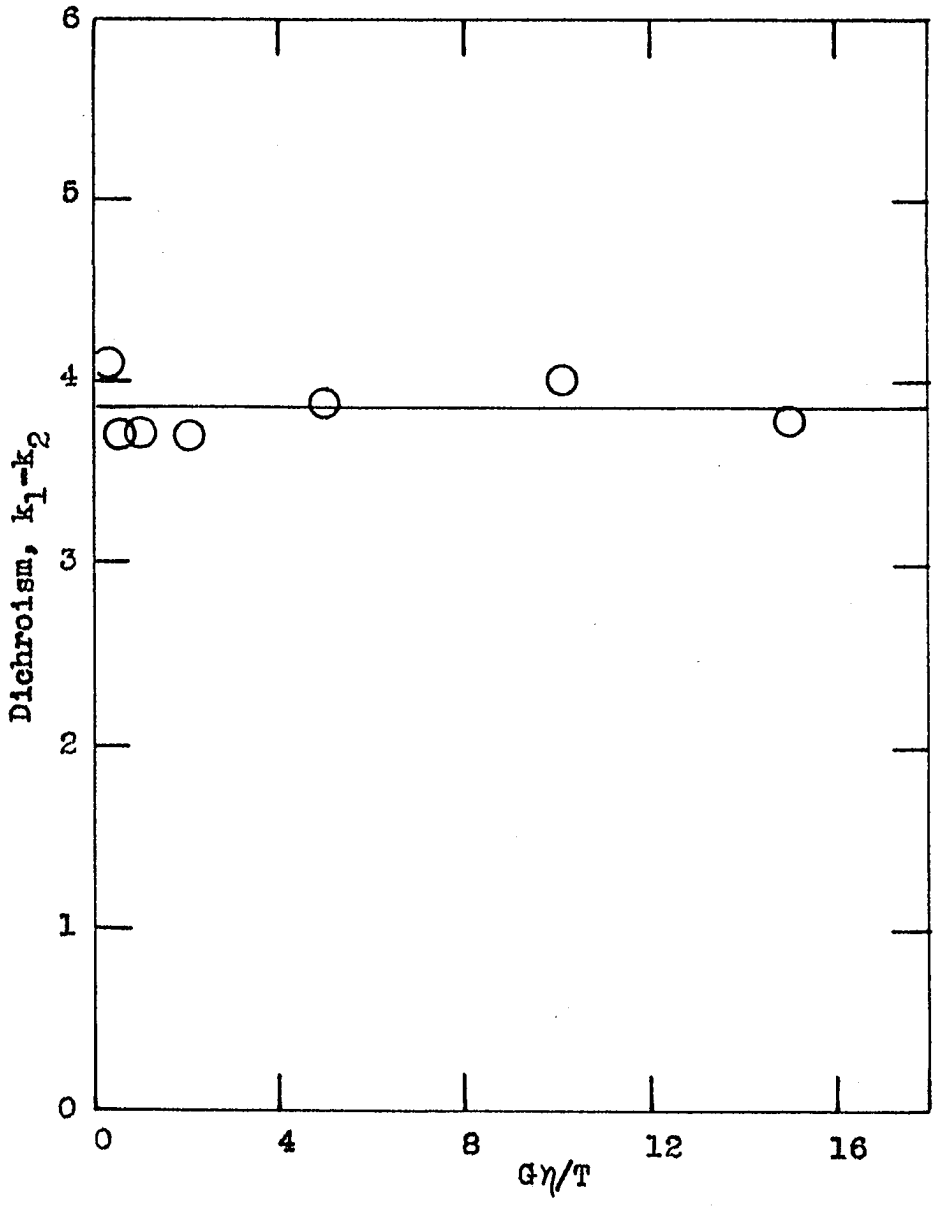


Fig. 36. Absolute dichroism per gram vs. $G\eta/T$ for the iodine complex of potato amylose P-5/6-A(7b).

SUMMARY AND CONCLUSIONS

1. Methods for the study of amylose-iodine complexes by flow dichroism were developed. It was found best to dissolve the amylose and prepare the complex in 15% pyridine. Glycerol was used to increase the viscosity of the solution, and also stabilize the complex, reducing the rate of aggregation.
2. The conventional birefringence apparatus was modified by the addition of a photoelectric measuring circuit designed for dichroism studies. It made possible the detection of small variations in light intensity, which is necessary for locating maxima and minima when investigating dichroic solutions.
3. The use of the iodine complex in amylose studies permitted work at very low concentrations, less than 0.002% being the usual value. This was very desirable since intermolecular attractions are much less in dilute solutions. Other techniques in general require much higher concentrations, therefore are more subject to error.
4. Aggregation effects in the amylose-iodine complexes were shown to be probably absent under the conditions established as most suitable. This was considered a most important feature in the application of dichroism

of the complex.

5. Theoretical curves for χ vs. $G\eta/T$ based on empirical length distributions were found to fit most of the experimental curves for the amylose-iodine complexes. From the fact that the same length distribution functions yielded orientation distribution functions f which were found to be proportional to the experimentally determined $\log_e \tan(45 - \Delta)$, it was concluded that the individual molecules of the amylose-iodine complex are essentially rigid.
6. Amylopectin was found to have practically no effect when the amylose-iodine complex was studied under the proper conditions. This was an important advantage over other methods for investigating amyloses, since it is difficult to free amylose of amylopectin completely.
7. A number of samples, including most of those studied by the dichroism of the iodine complex, were investigated by the established techniques of streaming birefringence. Some of the results obtained were compared with similar results obtained by flow dichroism.
8. Undesirable concentration effects were found when amyloses were studied in ethylenediamine-glycerol solutions, but these effects could be made small by using solutions of less than 1% amylose. The average lengths of the

amyloses appeared longer at high concentrations than at low, and precipitates developed in the solutions. At higher concentrations the solutions became turbid enough to make observation difficult.

9. Some evidence, by no means conclusive, was found pointing to a lack of rigidity of amylose when dissolved in an ethylenediamine-glycerol solution. The curve for χ vs. $G\eta/T$ of the purest amylose showed the sample to have about the same average length at low values of $G\eta/T$ when in the uncomplexed form as in the form of the iodine complex, but the lengths of the uncomplexed form were greater at higher $G\eta/T$. A comparison of experimental and theoretical values of absolute birefringence also suggests the possibility of non-rigidity.

10. Amylopectin was found to have a pronounced effect on birefringence results, especially at low $G\eta/T$.

11. The average lengths for a given sample were not usually the same when determined by birefringence and by dichroism, the birefringence results generally indicating longer molecules. This probably resulted from the amylopectin present.

12. Insofar as the data obtained is valid, it indicates that the d.p.'s are much lower than those obtained for the same samples by other methods.

13. It was concluded that the blue amylose-iodine complex is essentially molecular in nature and does not require micelle formation as has been assumed by some workers.

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