[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, PURDUE UNIVERSITY]

The Subfractionation of Amylose and Characterization of the Subfractions by Light Scattering[1,2]

BY WILBUR W. EVERETT[3] AND JOSEPH F. FOSTER

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Potato amylose was separated into seven subfractions by fractional precipitation from dimethyl sulfoxide with absolute ethanol at constant temperature. The fractions were studied by light scattering and their weight-average molecular weights and Z-average radii of gyration evaluated from Zimm plots of the scattering data. All fractions were studied in dimethyl sulfoxide and the molecular weights were shown to range from approximately 1.5×10^6 to 2.2×10^6 and the radii of gyration from 330 to 940 Å. Light scattering measurements were also made on the highest molecular weight fraction in two other solvents, 0.5 N aqueous KCl and 1.0 N KOH. The molecular weights in these solvents agreed within experimental error with the value in dimethyl sulfoxide. It is concluded that aggregation is absent in any of the solvents employed. The second virial coefficient was found to be very small in aqueous KCl and it is concluded that the Θ temperature in this solvent is near 25°. The highest molecular weight fraction was also examined in aqueous KCl in the ultracentrifuge using the Archibald method and the results shown to be in agreement with the light scattering data. From this result one may infer that contamination by high molecular weight amylopectin is negligible.

Introduction

In the course of a study of the physical properties of amylose in solution, it became desirable to obtain subfractions of amylose. These subfractions should each exhibit a fairly small distribution of molecular weights, and the range of molecular weights from the lowest to the highest molecular weight subfraction should be rather large. It was also desirable to characterize these subfractions as to certain physical properties, such as molecular weight and molecular shape.

The subfractionation of amylose has been carried out in various ways by several investigators [4-7] In this paper a method of subfractionation is reported, involving the precipitation of amylose from dimethyl sulfoxide with ethanol at constant temperature. It will be shown that this gives a series of amylose subfractions with a wide range of molecular weights.

The molecular weights of these subfractions were determined by measuring the angular distribution of light scattered from dilute solutions of the subfractions. The weight-average molecular weights were obtained by treating the data in the manner developed by Zimm.[8] The Z-average radii of gyration and the second virial coefficients were also obtained by this procedure.

It is interesting to note here that the weight-average molecular weights presented are the first reported molecular weights determined on amylose from light scattering measurements by treating the data in the manner developed by Zimm.[8] Foster and Paschall[9] have reported apparent molecular weights determined by 90° scattering, but these were not corrected for the dissymmetry of the angular scattering. Foster and Sterman[10] reported a molecular weight for a corn amylose which molecular weight had been corrected for dissymmetry assuming the amylose to be a rod for the purpose of obtaining $1/P_{90}$.

In some respects it is surprising that more light scattering studies of amylose have not been reported. The explanation doubtless resides in two well known facts: (1) the presence in starch of the very high molecular weight branched polymer, amylopectin, and the difficulty of freeing amylose completely from this material; and (2) the fact that amylose is notoriously unstable in aqueous solution, tending to precipitate spontaneously from solution (retrogradation). The latter fact, of course, should not serve as any deterrent to light scattering experiments in more powerful solvents. Evidence is presented in this paper that potato amylose can be freed of amylopectin to an extent that meaningful light scattering measurements are possible. Further it is shown that the fractions employed are stable and free of aggregation even in aqueous solution for a period such that scattering experiments can be conducted with ease.

Experimental

Sources and Preparation of Materials.—The potato amylose was prepared by R. L. Smith (potato amylose fractions $VA_I + VA_{II}$) by precipitation of the amylose as the butanol complex.[11] This material was used without further recrystallization. The dimethyl sulfoxide used for the physical measurements was dried by shaking with calcium oxide for at least 24 hours or by refluxing over CaO in a dry system. The dried dimethyl sulfoxide was distilled in a closed system under reduced pressure, and the middle portion of the distillate was retained. Care was exercised to see that the dimethyl sulfoxide at no time came into contact with rubber, as this introduced fluorescent impurities into the solvent. This procedure gives a product whose refractive index agrees well with the value reported in the literature.[12] The dimethyl sulfoxide was stored in a glass stoppered flask in a desiccator over anhydrous calcium chloride until used.

Absolute ethanol and 99.9% dimethyl sulfoxide were used in the fractionation without purification.

Distilled water was used in preparing all aqueous solvents, and analytical grade inorganic reagents were used unless otherwise specified. U.S.P. iodine was resublimed for use in potentiometric iodine titrations.

Fractionation.—Trial precipitation studies were first carried out in dimethyl sulfoxide by precipitating various

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(2) Presented in part before the Division of Carbohydrate Chemistry, American Chemical Society, Chicago, Ill., September, 1958.

(3) Predoctoral fellow of the National Science Foundation, 1956–1958. Present address: National Institutes of Health, Baltimore City Hospitals, Baltimore, Md.

(4) S. Lansky, M. Kooi and T. J. Schoch, THIS JOURNAL, **71**, 4066 (1949).

(5) R. W. Kerr, *ibid.*, **67**, 2268 (1945).

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(9) E. F. Paschall and J. F. Foster, *J. Polymer Sci.*, **9**, 73, 85 (1952).

(10) J. F. Foster and M. D. Sterman, *ibid.*, **21**, 91 (1956).

(11) R. L. Smith, Ph.D. Thesis, Iowa State College, 1953.

(12) W. Strecker and R. Spitaler, *Ber.*, **59**, 1754 (1926).

amylose samples with absolute ethanol or ethylene dichloride and following the optical rotation. This gave a relation between percentage precipitated and volume percentage of precipitant such as is shown in Fig. 1 for whole corn amylose and two subfractions thereof.

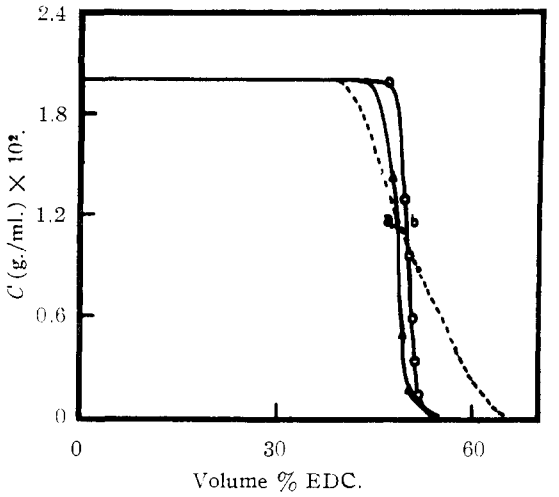

Fig. 1.—Precipitation curves for subfractions of corn amylose and whole corn amylose (dotted curve). Fractions a and b had limiting viscosity numbers 165 and 94, respectively. Concentrations were obtained from optical rotation on the supernatant and are plotted *versus* volume per cent. of the precipitant, ethylene dichloride. The solvent was dimethyl sulfoxide.

The actual fractionation was carried out at 4° in a cold room. Approximately 25 g. of PAS was dissolved in 3.5 liters of dimethyl sulfoxide, and 1 liter of absolute ethanol was added; this solution was then passed through a Sharples supercentrifuge several times to remove any undissolved material. Then 1.2 liters of absolute ethanol was added, and the trace of precipitate formed was removed by centrifuging. This precipitated material was discarded and is presumed, on basis of evidence to be presented, to have contained most of the amylopectin contaminant in the original amylose. The concentration at this point was determined by optical rotation measurements to be 0.482%. Appropriate amounts of ethanol were then added, and the fractions precipitating were removed by centrifuging in the Sharples supercentrifuge using continuous flow. This procedure gave six subfractions of amylose. These were designated as AF I, II, III, IV, V and VI.

The percentage precipitated was determined by optical rotation measurements on the solution after removal of each subfraction. The amount recovered was less in each case than the amount precipitated due to mechanical losses of some of the precipitate. The amount precipitated in each case was approximately AF I 32, II 12, III 13, IV 16, V 16 and VI 14%. The total amount recovered was 75% of the original material.

AF I was refractionated into two fractions by the same procedure as used for the initial fractionation. These two fractions were designated as AFIA and AFIB.

All precipitated fractions were washed repeatedly with absolute ethanol and dried in a vacuum oven at 55°.

AF IV, which had a low iodine binding value and an anomolously high molecular weight, was recrystallized once from 1% aqueous NaCl solution with butanol.

Sample Dispersion.—The amylose was dispersed in dimethyl sulfoxide by placing a suspension of the amylose in dimethyl sulfoxide in a desiccator over anhydrous calcium chloride and allowing it to stand for at least 24 hours. At times it was necessary to stir the amylose–dimethyl sulfoxide mixture for a short time to complete the dispersion. All solutions were prepared in glass stoppered flasks.

The amylose was dispersed in aqueous KOH by stirring under nitrogen for about two hours. The nitrogen used was dry high purity nitrogen, and this was passed through a vanadyl sulfate train in an effort to remove the last traces of oxygen. In order to avoid degradation during dispersion in aqueous KOH it was necessary to first sparge the oxygen from the aqueous KOH with the nitrogen, and then the amylose sample to be dispersed was added. The mixture was sparged with nitrogen for 15 minutes while stirring with a magnetic stirrer, and the flask was then sealed and the stirring continued until solution was complete. This usually took about 2 hours.

The neutral aqueous KCl solutions were prepared by neutralizing an alkaline solution, prepared as described above, with 1.0 N HCl. The titration was followed by means of a glass electrode *p*H meter.

Light Scattering Procedure.—A Brice–Phoenix Universal light scattering photometer, manufactured by the Phoenix Precision Instrument Co., Philadelphia, Penna., was used without modification. The narrow slit system (Cat. No. K342) was installed, enabling the use of the cylindrical cell (Cat. No. C-101) and the semi-cylindrical partitioned Doty cell (obtained from Pyrocell, Inc.). Checks with dilute fluorescein solution showed the instrumental scattering envelope to be constant within 1% from 30 to 140° for the cylindrical cell and from 45 to 135° for the Doty cell. The angular scattering measurements were usually made at angles from 35 to 135° for the cylindrical cell and from 45 to 135° for the Doty cell. Most runs in dimethyl sulfoxide were carried out in the Doty cell because of the small volume of solution required. All runs in either alkaline or aqueous solutions were made in the cylindrical cell.

The constant relating measured scattering intensity with the narrow beam and a given cell to the instrumental calibration with the wide beam and the square cell (opal glass reference standard) was 1.46 for the cylindrical cell and 1.59 for the Doty cell, as determined with several Ludox (colloidal silica manufactured by du Pont Co., Wilmington, Del.) solutions of different concentrations. Absolute calibration of the instrument on the basis of the opal glass reference standard was assumed to be correct, and further checks with two standard dextran samples (supplied through the courtesy of Dr. F. R. Senti of the Northern Utilization Research Branch, U.S.D.A.) and with crystalline bovine plasma albumin by other workers in this Laboratory indicated this calibration to be correct within ±3%.

Clarification of solvent and solution was accomplished by centrifugation for about two hours at 20,000 × G. in a Servall angle centrifuge using stainless steel tubes. The loss in concentration during centrifugation was not greater than 2% in any case, as determined by optical rotation measurements on the solutions before and after centrifuging. In the case of dimethyl sulfoxide solutions, it was found that a highly fluorescent impurity was introduced into the solution and solvent if even one drop of dimethyl sulfoxide came into contact with the rubber gaskets of the centrifuge tube covers. In order to prevent this the gaskets were not used when centrifuging dimethyl sulfoxide.

The solvent was transferred to the scattering cell by use of a modified pipet clamped above the centrifuge tube; the tube was not removed from the centrifuge head until after transfer by pipet. The solvent scattering was checked at all angles to be used and, if satisfactory, portions of the relatively concentrated solution were added from the modified pipet in small increments to form a concentration series.[13] This procedure minimizes the effect of the possible accumulative contamination which can be very serious in a dilution series procedure. Concentrations were determined on the stock solutions in all cases by optical rotation and on each concentration by direct weighing of the initial amount of solvent and of the amount of each increment of stock solution added. A final check of the concentration of the most concentrated solution was made by optical rotation after completion of the scattering measurements. The specific rotations at the sodium D line and 25° used in concentration determinations were determined in most cases in this Laboratory. The values used were: for amylose in 1 *N* KOH, 156, in 0.5 *N* KOH, 174; in aqueous KCl solution, 200; and in dimethyl sulfoxide, 171°.

Refractive increments in dimethyl sulfoxide were determined by use of the analytical ultracentrifuge (Spinco model E). This was accomplished by running a solution of known concentration in the ultracentrifuge at low speeds until a peak pulled well away from the meniscus. The area under

(13) P. Doty and B. Boyce, THIS JOURNAL, **74**, 5029 (1952).

this peak is related to the concentration and refractive increment by

$$C_0 = k \tan \theta \frac{A_t}{(dn/dc)} \left(\frac{X_t}{X_m}\right)^2 \quad (1)$$

where k is the characteristic of the instrument, θ is the angle of the Schlieren bar, A_t is the area under the peak and $(X_t/X_m)^2$ is a correction for radial dilution. The value of $A_t \cdot X_t/X_m)^2$ was found by the z-scale method of Trautman.[14] The refractive increment of sucrose obtained in this way checked within better than 1% the value given in the literature.[15] The value obtained for amylose in dimethyl sulfoxide at 436 mμ was 0.0676 ± 3% cm.^{3}/g. and for 546 mμ was 0.0659 ± 3% cm.^{3}/g. These are averages from runs on two different samples (AFIA and a corn amylose) at various bar angles. For hygroscopic solvents this method has the advantage over the differential refractometer that changes in the bulk refractive index due to impurities do not appreciably affect the value of the refractive increment determined in the ultracentrifuge. The values obtained by use of the differential refractometer may be appreciably in error due to traces of impurities such as moisture.

The value of (dn/dc) used for amylose in 1 N KOH was 0.146 cm.^{3}/g.[10] and in 0.5 N KCl was 0.156 cm.^{3}/g. (obtained for amylopectin in water).[15] No attempt was made to check these constants in this study.

Values of C/R^*_θ were plotted as a function of $\sin^2 \theta/2 + 1000\ C$ in the usual manner.[8] Here R^*_θ contains the factor $\sin \theta/(1 + \cos^2 \theta)$ where $\sin \theta$ is a correction term for the scattering volume viewed by the photomultiplier tube at different angles and the $(1 + \cos^2 \theta)$ is necessary because of the use of unpolarized light. According to the equation

$$KC/R^*_{\theta,c} = 1/MP_\theta + 2BC \quad (2)$$

the intercept at $\theta = 0$ and $C = 0$ is equal to $1/MK$ since $P_0 = 1$. Here $K = (2\ \pi^2 n^2_0/N_0\ \lambda^4)\ [dn/dc]^2$ and $R^*_\theta = (i_\theta r^2/I_0) \sin \theta/(1 + \cos^2\theta)$; P_θ is the so-called particle scattering factor. Because of the large angular dependence, it was necessary to apply the reflection correction.[16] To check the extrapolations, measurements were made at both 430 and 546 mμ for most of the reported runs. The second virial coefficient B was obtained by multiplying $^1/_2$ of the slope of the zero angle extrapolation by K. The mean-square Z-average radii of gyration were determined from the initial slopes of the zero concentration lines divided by the intercepts times a constant.

The depolarization of 90° scattering was determined for amylose in dimethyl sulfoxide. Extrapolation to zero concentration and a crude extrapolation to zero slit width showed the depolarization to be less than 2%. This was neglected in calculating R_{90}.

The dimethyl sulfoxide solutions of amylose were checked for fluorescence with filters which did not pass light of the incident wave lengths. No fluorescence could be detected by this method.

Determination of Molecular Weight in the Ultracentrifuge. The molecular weight of AFIA was determined in the ultracentrifuge using the Archibald[17] procedure as modified by Klainer and Kegeles[18] and by Trautman.[14] The sample was run in 0.5 N aqueous KCl solution at a concentration of approximately 0.5 g./100 ml. in the Spinco model E ultracentrifuge. Runs were made at speeds of 4197, 8210 and 12590 r.p.m., and pictures were taken of the boundary at appropriate time intervals. At the speed of 12590 r.p.m. the peak was allowed to break completely away from the meniscus before the final picture was taken.

The data were treated in the manner described by Trautman.[14] This involves plotting a function of the intercept at the meniscus *versus* a function of the concentration. The procedure used here has been described in detail by Erlander and Foster[19] in a paper from this Laboratory.

Results and Discussion

Figures 2, 3 and 4 show typical Zimm plots obtained on AFIB in dimethyl sulfoxide and AFIA

(14) R. Trautman, *J. Phys. Chem.*, **60**, 1211 (1956).
(15) B. A. Brice and M. Halwer, *J. Opt. Soc. Amer.*, **41**, 1033 (1951).
(16) H. Sheffer and J. C. Hyde, *Can. J. Chem.*, **30**, 817 (1952).
(17) W. J. Archibald, *J. Phys. Chem.*, **51**, 1204 (1947).
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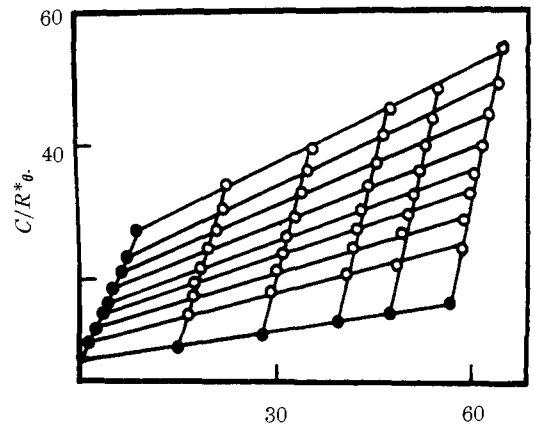

Fig. 2.—Light scattering results for fraction AFIB in dimethyl sulfoxide at 4360 Å.

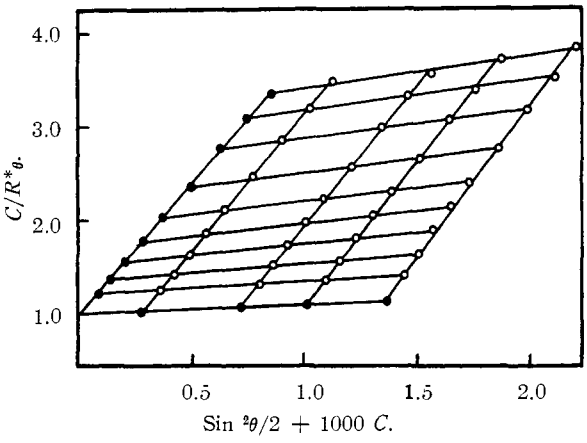

Fig. 3.—Light scattering results for fraction AFIA in 0.5 N aqueous KCl at 4360 Å and temperature approximately 28°.

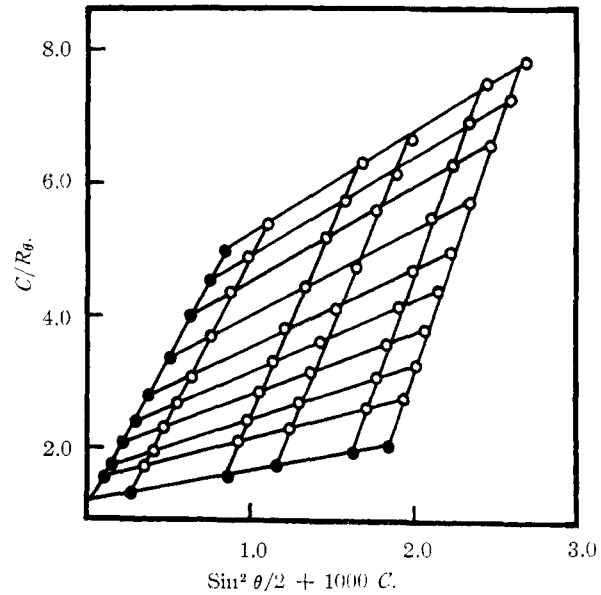

Fig. 4.—Light scattering results for fraction AFIA in 1.0 N aqueous KOH at 4360 Å.

in 0.5 N KCl and 1 N KOH at 436 mμ. The Zimm plots obtained on all fractions in all of the solvents were of this same general nature. The lines were straight in all cases within experimental error. If there was an appreciable amount of high molecular weight amylopectin present one would expect a marked downward curvature of the zero concentration line. This was not observed for any of the fractions.

The data obtained in the fractionation and the data obtained by light scattering and iodine titrations on the fractions are reported in Table I. It may be seen from this table that the molecular weights obtained at the two wave lengths on a given fraction are in fairly good agreement. It is very difficult to obtain good results at the green wave length in dimethyl sulfoxide because of the very low scattering. Values obtained from the radii of gyration at 546 mμ agreed within experimental error with those obtained at 436 mμ.

TABLE I

RESULTS OF SUBFRACTIONATION, LIGHT SCATTERING MEASUREMENTS AND IODINE TITRATIONS

Fraction	Precipitated in fractionation, %	Mg. I_2 bound 100 mg. amylose	Solvent[a] for light scattering	$\bar{M}_w \times 10^{-5}$ 436 mμ	$\bar{M}_w \times 10^{-5}$ 546 mμ	Radius of gyration 436 mμ
AFIA	20	19.2	DMSO	22.2	...	935
AFIA	..	...	1 N KOH	23.4	24.7	912
AFIA	..	...	0.5 N KCl[b]	24.4	25.1	763
AFIA	..	...	0.5 N KCl[c]	24.4	...	745
AFIB	12	19.3	DMSO	13.5	14.8	724
AF II	12	...[e]	DMSO	10.5	...	656
AF III	13	20.0	DMSO	8.47	8.70	610
AF IV	16	17.6[d]	DMSO	5.52	6.00	543
AF V	16	18.3	DMSO	2.70	2.67	425
AF VI	14	...	DMSO	1.46	1.52	334

[a] DMSO = dimethyl sulfoxide. [b] Carried out at about 31°. [c] Carried out at about 28°. [d] Before recrystallization with 1-butanol. [e] AF II was not titrated.

The molecular weights of AFIA in d methyl sulfoxide, 1 N KOH and 0.5 N KCl all agree within experimental error. The major part of the variation is probably due to error in the refractive increments of the amylose in the various solvents, as this quantity enters into K as a squared term. The agreement obtained in the three solvents rather conclusively rules out the occurrence of any significant amount of aggregation in any of these solvents.

The values obtained for the virial coefficient B in dimethyl sulfoxide varied from about 1–3 $\times 10^{-4}$. The values generally increased with decreasing molecular weight. All of the light-scattering experiments were not carried out at the same temperature, however, so no quantitative information can be gained from the virial coefficients in dimethyl sulfoxide. The virial coefficient for AFIA in 1 N KOH was 8.9×10^{-5}, and the virial coefficient in aqueous 0.5 N KCl at about 31° was 2.89×10^{-5} and at about 28° was 1.41×10^{-5}. From the low values of the second virial coefficient in aqueous 0.5 N KCl it would seem that the θ point for amylose in aqueous 0.5 N KCl must be about 25°. This is not surprising in view of the fact that the second virial coefficient of amylopectin in water has been reported to be zero.[20] The θ point has not been determined exactly as the light scattering instrument employed does not permit thermostating of the scattering cell.

Table I also gives the Z-average radii of gyration of the amylose fractions in the various solvents. The radii of gyration in dimethyl sulfoxide decrease with decreasing molecular weight as they should, and the value of the radius of gyration of AFIA in water is smaller than that for AFIA in 0.5 N KOH. The amylose has a higher second virial coefficient in dimethyl sulfoxide than in aqueous 0.5 N KCl, in qualitative agreement with the fact that the radius of gyration is larger in dimethyl sulfoxide than in 0.5 N KCl.

The quantitative correlation of the molecular weight with the radii of gyration and the angular dependence of the light scattering data will be discussed in a later paper with reference to their relation to the conformation of the amylose molecule in solution.

A cumulative weight distribution curve was obtained for the fractionation by plotting the cumulative percentage precipitated *versus* the molecular weight, the values of which are given in Table I. The differential distribution curve given in Fig. 5 was obtained by differentiating the cumulative curve just described.

The dashed curve was calculated assuming a bifunctional linear condensation polymerization by the relationship

$$W_x = (1 - P)^2 P^{x-1} \qquad (3)$$

where W_x is the weight fraction of chain length X and P is the fraction of monomer converted to polymer in the polymerization process[21]; X at the peak of the weight distribution curve is approximately equal to the number-average X of the sample, designated X_n. Then P may be calculated by the equation

$$X_n = 1/(1 - P) \qquad (4)$$

using X at the peak of the experimental curve in Fig. 4 which is 1670. The theoretical distribution for a bifunctional condensation was calculated using a value of P of 0.999 obtained by use of equation 4. The calculation of W_x was carried out for values of X ranging from 100 to several thousand by equation 3; W_x was then plotted on the ordinate of Fig. 5, using an appropriate scale, *versus* $XM_x = \bar{M}_n$.

Only in the case where the fractions from which the experimental curve is obtained are very narrow would the experimental curve be expected to agree with the theoretical curve. Since in this fractionation the ratio of $\bar{M}_w$ to $\bar{M}_n$ could not be expected to be much lower than 1.5, the difference in the high molecular weight end of the experimental curve as compared to the theoretical curve is probably not significant. However, the fact that the curves are of the same general shape, and that there is only one maximum in the experimental

(20) C. J. Stacy and J. F. Foster, *J. Polymer Sci.*, **20**, 56 (1956).

(21) P. J. Flory, "Principles of Polymer Chemistry," Chapter VIII, Cornell University Press, Ithaca, N. Y., 1953.

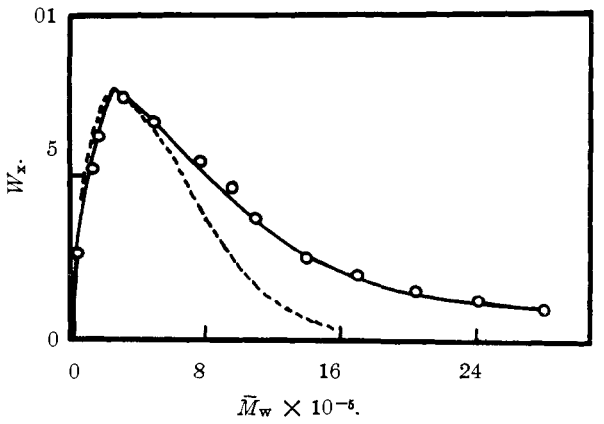

Fig. 5.—Weight fraction distribution curve from data on amylose subfractions. The dotted curve is theoretical as calculated in the text.

curve would seem to show that the sample used in the fractionation was a continuous homologous series of linear polyglucose chains. It would also seem that no substance of radically larger molecular weight than that of the main part of the sample was present. Some higher molecular weight material had been removed from AF IV by butanol fractionation before the data for this curve were obtained. Also as mentioned earlier a small initial precipitate, presumed to contain amylopectin, was discarded. There seemed to be no measurable quantity of this contaminant in the other fractions.

The iodine-binding capacities of most of the fractions were determined in an effort to detect any non-amylose impurity that might be present. The values obtained for the fractions studied are reported in Table I. The binding capacity of AF IV is seen to be somewhat low indicating appreciable contamination with amylopectin. The molecular weight obtained on AF IV was initially out of line with the rest of the samples. A reasonable value for $\bar{M}_w$ was obtained on AF IV which had been recrystallized from a dilute 1% NaCl solution of AF IV by butanol precipitation as shown in Table I. The iodine-binding value was not redetermined after recrystallization. Iodine binding values obtained on amylose samples which have been many times recrystallized with butanol are usually about 19.0–19.5 mg. I_2/100 mg. amylose under the conditions used here.

Other evidence for the absence of any appreciable amount of amylopectin in these fractions has been obtained by ultracentrifugation. Only one symmetrical peak has been observed in these samples during ultracentrifugation at various speeds in several solvents at varying concentrations. Any amylopectin of molecular weight appreciably greater than that of the main fraction would be expected to have a higher sedimentation constant than the fraction and would therefore be expected to show up as a separate peak.

The weight-average molecular weight was also determined for AFIA in 0.5 N KCl by application of the Archibald method in the ultracentrifuge. Figure 6 is a Trautman plot[14] of Y^*, a function of the height of the intercept of a pattern at the meniscus, *versus* X^* which is a function of the amount of substance which has sedimented out from the meniscus. The slope of the chord of this curve is the initial $(\overline{S/D})_w$, and therefore the molecular weight may be calculated from: $\bar{M}_w = RT/(1-\bar{V}\rho)(\overline{S/D})_w$, where R is the gas constant, T is the absolute temperature, $\bar{V}$ is the partial specific volume of the solute in the solvent employed, and ρ is the density of the solution. The experiment was carried out at 25° by use of the thermistor temperature control (RTIC) on the Spinco model E ultracentrifuge; $\bar{V}$ was assumed to be the same as amylopectin in water which was determined in this Laboratory to be 0.65.

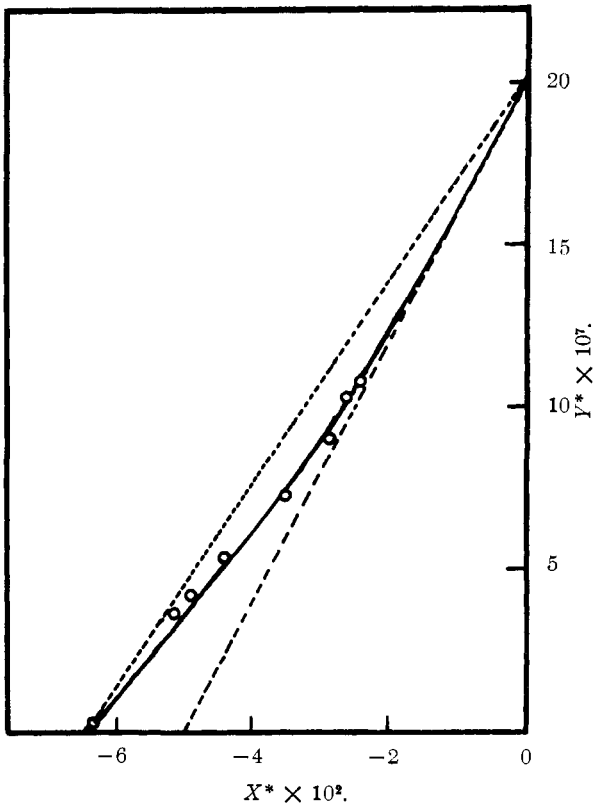

Fig. 6.—Trautman plot of sedimentation data on fraction AFIA in 0.5 N aqueous KCl at 25.0°. For significance of dotted lines see text.

The solid curve drawn in Fig. 6 is rather arbitrary but is a reasonable estimate of the expected curve for a polydisperse polymer of weight average molecular weight 2.44 × 10^6, the value obtained for this fraction by light scattering. The chord drawn corresponds to the result which would be obtained with a completely homogeneous polymer of this molecular weight. The initial tangent to the Trautman curve should correspond to the initial $\bar{M}_z$ of the polymer.[19] The tangent drawn is highly arbitrary and corresponds to an $\bar{M}_z$ of 3.1 × 10^6. Clearly a straight line could be drawn through the experimental points; the slope of such a line would correspond to an $\bar{M}_w$ of 2.08 × 10^6. In that case the presence of a discrete component of much higher molecular weight would have to be assumed to account for the light scatter-

ing molecular weight. While this possibility cannot be ruled out it seems much less likely than the first interpretation. It is concluded that the Archibald data are in complete accord with the interpretation that this fraction consists of a reasonably normal distribution of polymer species of $\bar{M}_w$ 2.44 × 10^6 and $\bar{M}_z/\bar{M}_w$ of the order 1.3.

It must be pointed out that the above discussion is based on the assumption that the second virial coefficient of amylose in 0.5 N aqueous KCl is zero at 25°. This must be essentially true as shown by the virial coefficients at 28 and 31° given earlier in this paper. Any concentration dependence will induce a downward curvatuve into the plot and would give anomalously low molecular weights and erroneously narrow molecular weight distributions.

LAFAYETTE, IND.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, PURDUE UNIVERSITY]

The Conformation of Amylose in Solution[1,2]

BY WILBUR W. EVERETT[3] AND JOSEPH F. FOSTER

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The limiting viscosity numbers of subfractions of amylose were determined in dimethyl sulfoxide, 0.33 N aqueous KCl and 0.5 N aqueous KOH. These results were correlated with the weight-average molecular weights previously reported for these fractions by means of log–log plots and constants in the modified Staudinger equation were determined. The results showed the polymer to be in a coiled conformation in each of the solvents used. Correlation of the radii of gyration from light scattering with the weight-average molecular weights and plots of the angular dependence of scattering intensity also agreed with a coiled conformation. Aqueous KCl solution has been shown to be a θ solvent for amylose near 25°. Under such conditions the exponent in the Staudinger viscosity equation was found to be 0.50 and current theories for unperturbed random coils were shown to be applicable to the data. Some implications of the results with regard to the behavior of aqueous solutions of amylose were discussed.

Introduction

Very few attempts have been made to determine the molecular shape of amylose in solution. Foster and Hixon[4] reported constants in the modified Staudinger equation for unsubstituted amylose in ethylenediamine and for amylose acetates in chloroform. They obtained results which indicated that the amylose behaved essentially as a rod in these cases; however, this work was not carried out on sub-fractionated polymer. Dombrow and Beckman[5] carried out sedimentation and diffusion studies on amylose triacetates in chloroform and concluded that the results were compatible with a helical configuration. Goodison and Higginbotham[6] carried out viscosity and molecular weight studies on subfractions of amylose. These subfractions were acetylated, and the number-average molecular weights and limiting viscosity numbers of the acetates were determined in nitroethane. The values determined for the exponent a in the modified Staudinger equation were for sago amylose acetate 0.44, for tapoica amylose acetate 0.65, and for maize amylose acetate 0.87. The large variation in these values was explained by assuming the presence of branching in at least two of the amyloses. These values would suggest a coiled conformation in these cases.

Others have evaluated the constants for the modified Staudinger equation on fractionated and unfractionated amyloses in aqueous KOH and amylose acetates in chloroform; however, no attempt was made in these cases to interpret the results as to molecular shape, as the values lay in the range where unambiguous conclusions concerning conformation are impossible.

This lack of information concerning the shape of unsubstituted amylose in various solvents renders difficult any interpretation of various observations made on the behavior of amylose in solution. This is particularly true in respect to the spontaneous precipitation of amylose from aqueous solutions (retrogradation). Since it is quite well known that amylose may exist in several conformations in the solid state, depending on the conditions under which the amylose is precipitated from solution, it is possible that it could exist in either a coiled form or alternatively as a rod (helix) in solution. In order to decide between these possibilities, the subfractionation of a potato amylose sample into seven subfractions has been achieved and the molecular weights and viscosities of these subfractions have been studied in 0.5 N KOH, dimethyl sulfoxide, and aqueous KCl solutions. The constants in the modified Staudinger equation were determined, and these constants were compared with theoretical constants for models of different molecular shape. The conformation of the amylose in the various solvents is deduced from these comparisons. Correlation of various data obtained from light scattering lead to conclusions which agree with those obtained from viscosity–molecular weight relations.

Experimental

Materials.—The amylose subfractions used here are those described in a previous paper.[7] The solvents used are those

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(3) Predoctoral Fellow of the National Science Foundation, 1956-1958. Present address: National Institutes of Health, Baltimore City Hospitals, Baltimore, Md.

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