markable, considering the temperature was only controlled to ±1° in the present work, that the results are satisfactorily reproducible.

Despite its limitations, the infrared technique gives information which is not available from any other kinetic method. The first advantage of the infrared method is that it allows the simultaneous determination of the monomer and polymer concentrations throughout the reaction, and thus the determination of *both* the rate of disappearance of monomer and the rate of appearance of polymer. By following the disappearance of monomer, it is not necessary to carry the reaction to completion in order to determine the rate as is necessary in the polymer determination of rate. In the particular type of polymerization discussed in this paper, involving N-carboxyanhydrides, the monomers have two carbonyl absorption bands of different intensities. At the monomer concentrations and cell thicknesses used, the disappearance of the less intense band at 1860 cm.$^{-1}$ was followed for the first 80% of the reaction, while the more intense band at 1790 cm.$^{-1}$ was followed for the last 20% of the reaction. This fortunate circumstance allows the accurate determination of the rate of monomer disappearance up to at least 90% anhydride consumption.

The most important advantage of the infrared method is that during the polymerization it is possible to distinguish qualitatively, quantitatively and simultaneously two or more reaction products (if the various products have different infrared spectra). This allows the correlation of reaction rates with reaction products. For example, the gasometric method showed that primary amine initiated polymerizations of NCA's involved two different reaction rates; previous infrared work showed that two different polymers (α and β_L) could be isolated from such reactions. Using the presently described infrared method for determining reaction rates, correlations have been made between the initial slower reaction rate (k_{2a}) and the formation of the low DP (β_L) polymer as well as the later faster reaction rate (k_{2b}) and the formation of high DP (α) polymer.

Finally, it should be noted that, since this infrared method has been applied advantageously to the investigation of NCA polymerizations, within the above specified scope, it also should have general utility in the study of the kinetics of other reactions.

Acknowledgment.—We are pleased to acknowledge the support of this work by the Office of the Surgeon General, Department of the Army, and the valuable assistance of Miss Evelyn DesRoches in the kinetic studies.

BOSTON 15, MASSACHUSETTS

[CONTRIBUTION FROM THE GIBBS LABORATORY, DEPARTMENT OF CHEMISTRY, HARVARD UNIVERSITY]

Polypeptides. XVI. The Polydispersity and Configuration of Low Molecular Weight Poly-γ-benzyl-L-glutamates[1]

BY J. C. MITCHELL, A. E. WOODWARD AND PAUL DOTY

RECEIVED MARCH 21, 1957

Titrations of amine and carboxyl groups in samples of poly-γ-benzyl-L-glutamate prepared by primary amine initiation show that (1) amine groups are nearly preserved throughout the polymerization but are easily lost during polymer isolation and storage, presumably by end group cyclization with the elimination of benzyl alcohol and (2) carboxyl groups are present to the extent of about one per three hundred residues regardless of the molecular weight of the sample. From this value it can be concluded that the Sela and Berger termination step will have a negligible broadening effect on the molecular weight distributions of these low molecular weight polypeptides. In order to estimate the molecular weight distributions the weight average molecular weights of a number of samples in the range of 1000 to 20,000 molecular weight were determined in the ultracentrifuge by means of the Archibald approach-to-equilibrium method. The results show pronounced deviations from the intrinsic viscosity–molecular weight relation established for higher molecular weight samples. The ratios of the weight to number average degrees of polymerization are found to be extraordinarily large[2-11] and suggest striking anomalies in polymerization. It is not surprising, therefore, to find that the samples can be separated into two fractions of widely different molecular weights and having different configurations (α and β) by a variety of means: solvent extraction, paper chromatography and dialysis. The minimum degree of polymerization required for stability of the α-helical configuration in dioxane and dimethylformamide solution is found to be approximately 10.

Early in 1955 end group determinations and intrinsic viscosity measurements on numerous low molecular weight samples of poly-γ-benzyl-L-glutamates clearly suggested that the molecular weight distributions in samples prepared by hexylamine initiation were extremely broad in contrast to the very narrow Poisson distribution generally expected. A rigorous and quantitative proof of this result required, however, the correlation of intrinsic viscosity measurements with weight average molecular weights in the region below 20,000. Since this correlation has only now been achieved, by use of the Archibald approach-to-equilibrium technique in ultracentrifugation, the publication of these results has been delayed. In the interim, however, knowledge of these results and corresponding infrared investigations[2] have motivated kinetic studies of the polymerization in a search for the origin of the observed polydispersity. A preliminary report of the findings has been made[3] and the full reports[4] are being published concurrently with this one.

Another reason for pursuing the absolute es-

(1) For the last papers in this series see XV, M. Idelson and E. R. Blout, THIS JOURNAL **79**, 3948 (1957), and XIV, P. Doty and R. D. Lundberg, *Proc. Natl. Acad. Sci.*, **43**, 213 (1957).

(2) E. R. Blout and A. Asadourian, THIS JOURNAL, **78**, 955 (1956).

(3) P. Doty and R. D. Lundberg, *ibid.*, **78**, 4810 (1956).

(4) (a) R. D. Lundberg and P. Doty, *ibid.*, **79**, 3961 (1957); (b) M. Idelson and E. R. Blout, *ibid.*, **79**, 3948 (1957).

tablishment of molecular weights in this relatively low molecular weight region lies in the desire to determine the critical length of the polypeptide chain required for the α-helical configuration to become the stable configuration. Of course this critical degree of polymerization will depend on the nature of the polypeptide residues and on the solvent. The results presented here identify fairly well this critical chain length in two solvents and, in conjunction with infrared spectra,[2,4b] in the solid state as well.

Following a presentation of experimental details this report takes up the titration of amine and carboxyl groups of poly-γ-benzyl-L-glutamates and the relation these bear to number average molecular weights. The calibration of intrinsic viscosity in dichloroacetic acid solutions against weight average molecular weight is considered next. This then permits an assessment of the magnitude of the polydispersity in the samples. In the final section the problem of helix stability and molecular weight is examined.

Experimental Details

Reagents.—Dimethylformamide and dichloroacetic acid were purified as described elsewhere.[4a] Phenol (Analytical Reagent Grade) was purified according to the method of Waltz and Taylor.[5] Phenol solutions were prepared by dissolving 83 g. of purified phenol in 17 g. of absolute ethanol and stored in the dark.

Sodium methoxide solution was prepared by dissolving 1.0 g. in 1800 ml. of methanol (Reagent Grade) and also stored in the dark. Standardization was made against potassium acid phthalate.

Perchloric acid solutions were made by mixing 2 ml. of 70% perchloric acid (Analytical Reagent Grade) with two liters of methanol. Standardization was carried out conductometrically, as described below, against standard sodium methoxide solution in a mixture of 45 ml. of phenol, 8 ml. of absolute ethanol and 8 ml. of distilled water.

Polypeptides.—Titration studies were made on a number of poly-γ-benzyl-L-glutamate samples prepared by Blout and Karlson[1,6] but only three of these are reported on in detail here. These three samples (no. 862, 834 and 863) were made in dioxane solution by hexylamine initiation using anhydride–initiator mole ratios ([A]/[I]) of 4.0, 8.0 and 20.0, respectively. After the polymerization was 99% complete the product was precipitated by pouring into 20 volumes of diisopropyl ether and dried at 50° *in vacuo*. More recently, three other polypeptides made in dioxane solution in this [A]/[I] range became available for viscosity and molecular weight measurements. Two of these (no. 428 L of [A]/[I] = 4.0 and RL-V202 of [A]/[I] = 20.0) were prepared by R. D. Lundberg and the other (no. 384-10 at [A]/[I] = 10) by M. Idelson and E. R. Blout.[4b]

The samples used in the viscosity–molecular weight study had to be of relatively narrow molecular weight distribution. Consequently fractions and samples prepared in dimethylformamide solution were employed. Details concerning these will be given later.

Titration.—In order to estimate all the groups present, both acid and base titrations were performed. The sample weight was chosen so as to require from 1 to 5 ml. of titrant: this was delivered from 5-ml. micro-burets.

Titrations with 0.01 *N* perchloric acid were carried out conductometrically in the solvent system phenol–ethanol–water[5] with sodium chloride being added, when necessary, to increase the starting conductivity to a measurable value. A dipping type conductivity cell with a constant, *K*, of 1.00 was used. Two conductivity bridges (Industrial Instruments) were employed; one was a Model RC with a maximum reading of 2.5×10^5 ohms, the other a Model RC 16 B with a maximum of 2.5×10^6 ohms.

The polymer samples were dissolved in a given amount of phenol solution in ethanol and then equivalent volumes of ethanol and water were added. The amount of each solvent used depended on how much ethanol and water the polymer would tolerate prior to precipitation while the total volume was kept within about 70 ml.

To check the method, known amounts of *n*-hexylamine and glycylglycine were titrated in 35 ml. of phenol solution, 15 ml. of ethanol and 15 ml. of water. The agreement was found to lie within ±4%. Duplicate titrations of poly-γ-benzyl-L-glutamates were found to agree within ±3% for a polymer of $\overline{DP}_n$ equal to 19 and ± 8% for one with a $\overline{DP}_n$ of 226.

Titrations with 0.01 *N* sodium methoxide were carried out in 25 ml. of chloroform and 10 ml. of absolute ethanol with 0.1% phenolphthalein in ethanol as indicator: the titer of the solvent alone was subtracted from the sample titer. To compare the method with an aqueous titration, an acetic acid solution was titrated in both the chloroform–ethanol system and distilled water; the values obtained agree within 1%. As a further check a low molecular weight polypeptide was titrated in (1) chloroform–ethanol, (2) potentiometrically in 20% water–80% dioxane (volume/volume), (3) in dioxane–water–formaldehyde solution and (4) in benzyl alcohol with phenolphthalein as indicator. The average value of these titrations varied from ±2% for a low molecular weight polymer to ±5% for one of $\overline{DP}_n$ = 550.

Viscosity Measurements.—All viscosity measurements were made at 25° with a modified Ubbelohde type capillary viscometer using dichloroacetic acid as the solvent. The solutions were clarified by passing them through a medium sintered glass filter.

Ultracentrifuge Measurements.—Weight average molecular weights were determined by the Archibald[7] approach-to-sedimentation-equilibrium method following the procedure given by Klainer and Kegeles.[8,9] The essential feature of this method is that the vanishing of the flux of solute across the two cell boundaries, meniscus and bottom, allows the application at these planes of the equation for sedimentation equilibrium

$$(\bar{M}_w)_{app} = \frac{RT}{(1 - \bar{v}\rho)\omega^2}\left[\frac{dc/dx}{xc}\right]_m$$

in which $(\bar{M}_w)_{app}$. is the apparent weight average molecular weight (differing from the true weight average molecular weight by a correction for the non-ideality of the solution), R the gas constant, T the absolute temperature, $\bar{v}$ the partial specific volume of the solute, ρ the density of the solution, ω the angular velocity, c the solute concentration and x the radial distance from the center of rotation. The subscript "m" on the square bracket indicates that the enclosed ratio is evaluated at the air-liquid meniscus.

The measurements were made with a Spinco model E ultracentrifuge equipped with a phase-contrast schlieren diaphragm. A 2-degree centerpiece modified to form synthetic boundaries between solvent and solution according to the suggestion of Kegeles[10] was used in the determination of the concentration at the meniscus.

The angle of the schlieren diaphragm was maintained at 80° in all measurements. Photographs of the schlieren patterns were made on Kodak type II-O and III-O spectroscopic plates through a Wratten #34 A filter and were read using a modified toolmaker's microscope equipped with a projection head[11] as a two-dimensional microcomparator.

The vertical comparator readings were corrected for solvent gradient in the following manner. It was observed that at several speeds the solvent gave only a horizontal schlieren line except for a slight downward curvature, possibly due to diffraction effects, within 0.03 mm. of the meniscus. Thus the average comparator reading of the horizontal (plateau) region in a solution photograph was subtracted from the readings in the curved region, with an appropriate correction for the solvent curvature in the immediate vicinity of the meniscus.

The partial specific volume of poly-γ-benzyl-L-glutamate in dimethylformamide was found to be 0.791 ± 0.006.

(5) J. E. Waltz and G. B. Taylor, *Anal. Chem.*, **19**, 448 (1947).

(6) E. R. Blout and R. H. Karlson, THIS JOURNAL, **78**, 941 (1956).

(7) W. J. Archibald, *J. Phys. Colloid Chem.*, **51**, 1204 (1947).

(8) S. M. Klainer and G. Kegeles, *ibid.*, **59**, 952 (1955).

(9) S. M. Klainer and G. Kegeles, *Arch. Biochem. Biophys.*, **63**, 247 (1956).

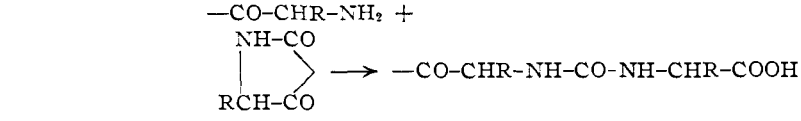
(10) G. Kegeles, THIS JOURNAL, **74**, 5532 (1952).

(11) Available from the Gaertner Scientific Corp., 1201 Wrightwood Ave., Chicago, Ill.

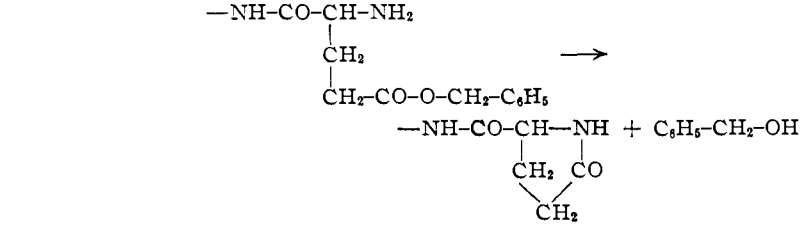
The measurement was made at 25° in a special pycnometer consisting of a volumetric flask with its neck replaced by part of a 0.1-ml. capillary-bore volumetric pipet.

End Group Determinations

Lability of Titratable Groups.—Ideally the only titratable group in a primary amine-initiated poly-γ-benzyl-L-glutamate molecule would be the terminal amine and the determination of amine groups would therefore lead directly to the number average molecular weight. Unfortunately the complications that can arise, particularly in this polypeptide, are numerous. Two of these difficulties may occur during the polymerization itself: the formation of cyclic polypeptides thereby eliminating end groups[12] and the formation of carboxyl groups in a termination step.[13]



After the polypeptide has been formed, two other reactions may occur that would alter the number of titratable groups. These are (1) the loss of amine groups by end group cyclization to form substituted pyrrolidone rings[14] as shown



and (2) the production of carboxyl groups by debenzylation.

Titration of amine groups in reaction mixtures showed at once that the number present was essentially equal to the number added as initiator: consequently significant amounts of cyclic polypeptide formation (and condensation) could be ruled out under the polymerization conditions (25°). However, it soon became apparent that the ordinary procedures of isolation, drying and storage at room temperature as either solid or solution were accompanied by a continuous loss of amine groups. The isolation procedure used for the three samples of the 800 series involving drying at 50° leads to losses of 50 to 70% of the amine groups present in the reaction mixture (Table I). Moreover, 80% loss of amine groups was produced by exposure to a steam-bath for 5 hours. Thus lyophilization of the reaction product (dioxane solution) appeared to be the best way of preserving most of the amine groups. When this was carried out on an [A]/[I] = 4 sample (no. 428 L) over a period of 48 hours the amine content was 85% of that added as initiator. No significant loss of amine groups occurs during storage at −30° over a period of several months. It is of interest to note that no change in intrinsic viscosity was observed during these alterations of amine group concentrations. As a consequence condensation was ruled out here as well and the end group cyclization reaction was accepted as the explanation of the depletion of amine groups.

The carboxyl group concentration of numerous samples was found to be in the same range as for the three polymers listed in Table I. The number of carboxyl groups was essentially unaltered by ordinary isolation procedures and as a consequence it seemed likely that those initially present might arise from the termination reaction of Sela and Berger.[13] However, the carboxyl group concentration could be increased by more drastic treatment. For example it would double upon five hours exposure to 100° presumably due to hydrolysis of the benzyl ester groups.

Discussion

The conservation of amine groups during the polymerization is indicative of the absence of the formation of cyclic polypeptides and condensation reactions, and it clearly indicates that the number average DP of the product is essentially that given by the anhydride–initiator ratio. This conclusion makes use of the observation from kinetic studies[2-4] that all the hexylamine initiator reacts rapidly to produce polymer.

The lability of the amine group due to end group cyclization prevents its assay from being useful as an accurate measure of number average molecular weight. However, it is clear that by lyophilization and cold storage the amine groups can be largely conserved. The loss of amine groups through this reaction is not serious in polymerizations lasting a few hours at room temperature. But polymerization carried out at higher temperatures or for longer times would be expected to show a decay in rate due to this reaction.

The constant carboxyl group concentration found here can be interpreted as arising from a termination reaction. The amount found, about 15×10^{-6} equivalent per gram, upon multiplication by the residue molecular weight gives 0.0033 which, in view of the unknown extent of the debenzylation reaction, would be interpreted as an upper limit for the probability of chain termination occurring each time a monomer adds to the polypeptide chain. More recent work of Lundberg and Doty,[4a] however indicates that this value is considerably too high and that the situation is complicated by the end group cyclization reaction acting as an additional termination step. Nevertheless it can be concluded from these findings that termination steps play a small role in these polymerizations and cannot be held responsible for the unusually wide molecular weight distributions mentioned above.[15]

The Molecular Weight Dependence of Intrinsic Viscosity for Short Polypeptide Chains

In an earlier study[16] of this polypeptide the intrinsic viscosity was related to weight average molecular weights determined by light scattering for degrees of polymerization ranging from 100 to 2000 in two solvent systems, one in which the chain configuration was randomly coiled (dichloroacetic acid) and one in which it was helical (chloroform saturated with formamide). For the purposes of this paper it was necessary to extend this intrinsic viscosity–molecular weight relation down to degrees of polymerization in the neighborhood of 5. The Archibald approach to equilibrium method was used for this purpose. The measurements were made on dimethylformamide solutions of a number of samples prepared either by fractionating the products of polymerizations in dioxane[1,6] or by polymerization in dimethylformamide, a polymerization medium in which it has been discovered that the molecular weight distribution produced is very narrow.[3,4a]

The weight average molecular weights were determined as described briefly in the Experimental Section. In each case the concentration depend-

(12) D. G. H. Ballard and C. H. Bamford, Chemical Society, Special Publication No. 2, 25 (1955).

(13) M. Sela and A. Berger, THIS JOURNAL, **77**, 1893 (1955).

(14) W. E. Hanby, S. G. Waley and J. Watson, *J. Chem. Soc.*, 3239 (1950).

(15) The molecular weight distribution arising in the case of the Sela and Berger termination has been calculated: E. Katchalsky, Y. Shalitin and M. Gehatia, THIS JOURNAL, **77**, 1925 (1955).

(16) P. Doty, J. H. Bradbury and A. M. Holtzer, *ibid.*, **78**, 947 (1956).

TABLE I

CHARACTERISTICS OF LOW MOLECULAR WEIGHT POLY-γ-BENZYL-L-GLUTAMATES PREPARED IN DIOXANE

Sample No.	[A]/[I]	Titrations NaOCH₃	(meq./g.) HClO₄	[A]/[COOH]	[A]/[NH₂]	[η]	DP_w	DP_w/DP_n
862	4	0.018	0.359	250	12	0.08	13	3.3
834	8	.012	.205	380	21	.15	68	8.5
863	20	.014	.088	280	45	.21	128	6.4
428L	4				5	.12	40	10.0
384-10	10					.20	115	11.5
RL V-202	20					.265	170	8.5

ence of the apparent molecular weight as expressed in the second virial coefficient was employed to obtain the actual molecular weight at infinite dilution. The virial coefficient was found to increase substantially with decreasing molecular weight. The magnitude of this effect is surprising and is being investigated further.

The molecular weights thus determined are plotted against the intrinsic viscosity in dichloroacetic acid on a double logarithmic scale in Fig. 1 as open circles. The earlier results for the higher molecular weight range are shown as filled circles. It is seen that deviations from the linearity of the higher molecular region set in at a DP of about 100 and become quite marked below a DP of 50. In this connection it is of interest to note that in the case of polystyrene a somewhat similar study[17] has shown that the deviations set in at a molecular weight of about 5000. Such a molecule of polystyrene has a chain length equivalent to poly-γ-benzyl-L-glutamate of DP 86. Hence the onset of deviation from the Mark–Houwink behavior occurs at roughly the same chain length in these two cases.

Polydispersity and Fractionation of Polypeptides Prepared in Dioxane

The results of the two foregoing sections now permit a quantitative assessment of the polydispersity of the poly-γ-benzyl-L-glutamate samples

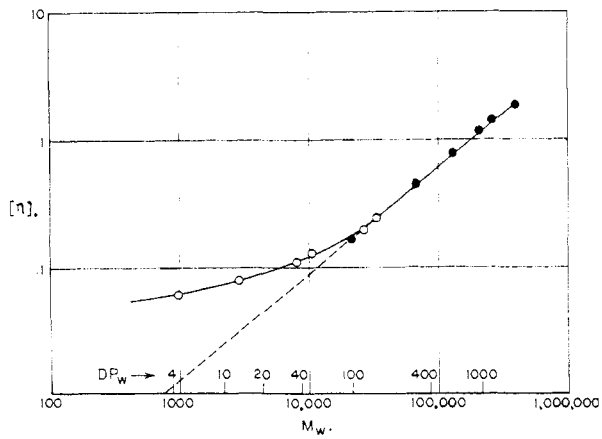


Fig. 1.—Double logarithmic plot of intrinsic viscosity measured in dichloroacetic acid against weight average molecular weight of poly-γ-benzyl-L-glutamate: ●, molecular weights determined by light scattering measurements; O, molecular weights determined by the Archibald ultracentrifugal method.

(17) M. Marzolph and G. V. Schulz, *Makromolekulare Chem.*, **13**, 120 (1954).

of interest. For the hexylamine initiated polypeptides the number average degree of polymerization, DP_n, can be taken as equal to the anhydride–initiator ratio. The weight average value, DP_w, can be assessed from the intrinsic viscosity determinations with the use of Fig. 1. The ratio, DP_w/DP_n, is a direct measure of the polydispersity. For the Poisson distribution it should approach 1 (that is, it should equal $1 + 1/DP_n$) and for the most probable distribution, expected for many polycondensations, it should be equal to 2.

The results on six polypeptides produced in dioxane are assembled in Table I. The polydispersity is seen to be very great indeed. Actually it is so large that the values of DP_w obtained from Fig. 1 are probably somewhat too low because the intrinsic viscosity corresponds to an average slightly lower than the weight average. Measurements on samples with [A]/[I] values greater than 20 show a continual decrease of the polydispersity with molecular weight.[3,6] These results are of course incompatible with the growth of polypeptide chains by the addition of monomer units through a single rate process following rapid initiation. The origin of this broadening of the molecular weight distribution has been found[3] and is described in detail in the accompanying papers.[4]

Fractionation.—With this evidence of very wide molecular weight distributions it was expected that a sample could be fractionated easily into two parts having quite different molecular weights. This has been done in three different ways. The first was fractional extraction with formic acid[2] or hot ethanol.[4b] These liquids appear to dissolve almost exclusively those polypeptide chains that are too short to form stable helical configurations. Consequently, polypeptides such as those listed in Table I were divided in a single step into two parts having intrinsic viscosities in dichloroacetic acid of the order of 0.06 and 0.20. Even if only the earlier viscosity–molecular weight relation were employed this would indicate a difference in molecular weight of a factor of about 5. With the use of Fig. 1 it is seen that this indeed corresponds to differences of about 25-fold in molecular weight.

As a particular example, the two fractions of sample 384–10 prepared by ethanol extraction by Idelson and Blout were examined by both the Archibald method and intrinsic viscosity. The lower fraction (entirely of the β-configuration in the solid state according to the infrared spectrum) had a DP_w of 4 while the higher fraction (entirely of the α-configuration) was of a DP_w of 150. For the unfractionated material the DP_w by the Archibald method was 104. Thus the very great polydispersity is confirmed.

A similarly striking separation into two parts was found in paper chromatograms. Chloroform solutions were applied to strips of Whatman No. 4 filter paper. When dry, the strips were developed in a closed chamber by the descending method using the *n*-butanol–acetic acid–water system. After drying and spraying with ninhydrin two purple spots developed, one at the origin and another at a point described by an R_f value of 0.92 ± 0.02. The chromatograms of fractions showed only one spot: at the origin ($R_f = 0$) for the high fractions and $R_f = 0.92$ for low fractions. Thus it is clear that the short chains not in the helical configuration move in this system while those in the helical configuration remain at the point of application.

The third method of fractionation employed was simple dialysis. A bag made of cellophane tubing and conditioned stepwise to a dioxane–water mixture (25:1) was filled with a 3.4 g./dl. solution in dioxane of a polypeptide with a DP_w of 37. Within a week (two changes of solvent of volume twice that of the bag) the loss from the bag had stopped. The material that had remained inside had a DP_w of 120 and that collected on the outside had DP_w of 4. The two fractions showed only single spots on paper chromatograms. Thus this type of separation has produced results very similar to the other two.

Molecular Weight Dependence of Helix Stability

The onset of helix stability with increasing chain length will be expected to occur rather abruptly[18] and the critical chain length, DP_c, at which it occurs will depend on a number of factors: the composition of the polypeptide, the environment of the polypeptide chain (solid state or solvent) and the temperature. The determination of DP_c for a given set of these factors would be simple if samples of uniform chain length were available. When the actual samples have a distribution of molecular weights the assignment of the value of DP_c is subject to an error proportional to the breadth of the distribution. Thus the accuracy with which DP_c can be specified increases as the molecular weight distribution is narrowed.

For example, sample number 834 was found to be half in the β-configuration (β_L form in Blout's terminology[4b]) and half in the α-configuration as a result of infrared studies on the solid material.[2] Yet it would be misleading indeed to conclude that DP_c was equal to the DP_w of this polypeptide. Since fractionation of this sample[2] produced two parts, one nearly pure β_L and one pure α having DP_w values of 2 and 100, respectively, it is clear that the proportion of α and β configuration shown in the original polypeptide was only a reflection of the amounts of material present having chain lengths below and above DP_c. Thus the value of DP_c must be located within the range of chain lengths present in the sample but it cannot be more definitely fixed.

The fractions produced by formic acid extraction by Blout and Asadourian[2] have somewhat narrower molecular weight distributions and a comparison of their configuration as deduced from infrared spectra with the DP_w values that can now be assigned to them by the use of Fig. 1 permits an approximate estimation of DP_c in the solid state. Their results and our own may be summarized by the statement that peptides containing at least 90% β_L configuration have not been observed with $[\eta]$ greater than 0.075. This intrinsic viscosity corresponds to DP_w to 10. However, the uncertainty in the intrinsic viscosity measurements (± 0.005) and in the possible lack of detection of small amounts of material in the samples place an uncertainty of ± 3 on this estimate. Moreover, since the DP_n of these fractions was not well known the polydispersity of the samples can only be estimated. It is for this reason that the value assigned to DP_c is only an estimate.

In order to obtain a more accurate value of DP_c, particularly in solutions, use was made of the observation that poly-γ-benzyl-L-glutamate samples prepared in dimethylformamide solution were of much narrower molecular weight distribution than those made in dioxane.[4a,19] Two such samples prepared at [A]/[I] of 4 and 10 have been used for the present study. These samples were found to have DP_w values of 5 and 14, respectively. Taking DP_n as equal to [A]/[I] it is seen that the chain length distributions in these samples are very narrow.

The fraction of residues in the helical configuration for dioxane and dimethylformamide solutions of these polypeptides was determined by rotatory dispersion measurements.[20] The results, along with those for a high molecular weight, completely helical sample, are plotted in accordance with Moffitt's equation in Fig. 2. The helical content is deduced from the value of b_0 (obtained from the slopes of the plots). Since the high molecular weight sample was found to have $b_0 = -682°$ in dioxane and −620 in dimethylformamide, the per cent. helical configuration in solution is taken as $100b_0/-682$ and $100b_0/-620$, respectively.

The results are summarized in Table II where it is seen that the [A]/[I] = 4 sample shows no helical content, whereas the [A]/[I] = 10 sample is nearly half helical.[21]

These results permit the assessment of an upper and lower limit to the critical chain length for helix stability, DP_c, in these solvents. If the [A]/[I] = 10 sample were of uniform chain length of 14 and exhibited 50% helical content DP_c would equal 14. However, since some spread in molecular weight distribution exists this can only be taken as an upper limit. It is likely to be several units lower. The lower limit can be found by taking into account the molecular weight distribution

(18) J. A. Schellman, *Compt. rend. trav. lab. Carlsberg, Serie chim.*, **29**, No. 15 (1955).

(19) Current work by J.C.M. is demonstrating that samples prepared in this manner do indeed approach the expected Poisson distribution.

(20) J. T. Yang and P. Doty, THIS JOURNAL, **79**, 761 (1957).

(21) The coefficient of the normal dispersive term, a_0, was found to be quite concentration dependent as the two values in Table II illustrate. This is similar to the behavior already witnessed in chloroform solution[20] and appears to be due to an intermolecularly hydrogen bonded form that breaks up upon dilution to give the solvated β_L chains as first observed by Blout and Asadourian.[2] The use of the high molecular weight value of b_0 to calibrate the scale of helical content may possibly involve some uncertainty in this application since the effect per residue may be somewhat different due to end effects in short helices.

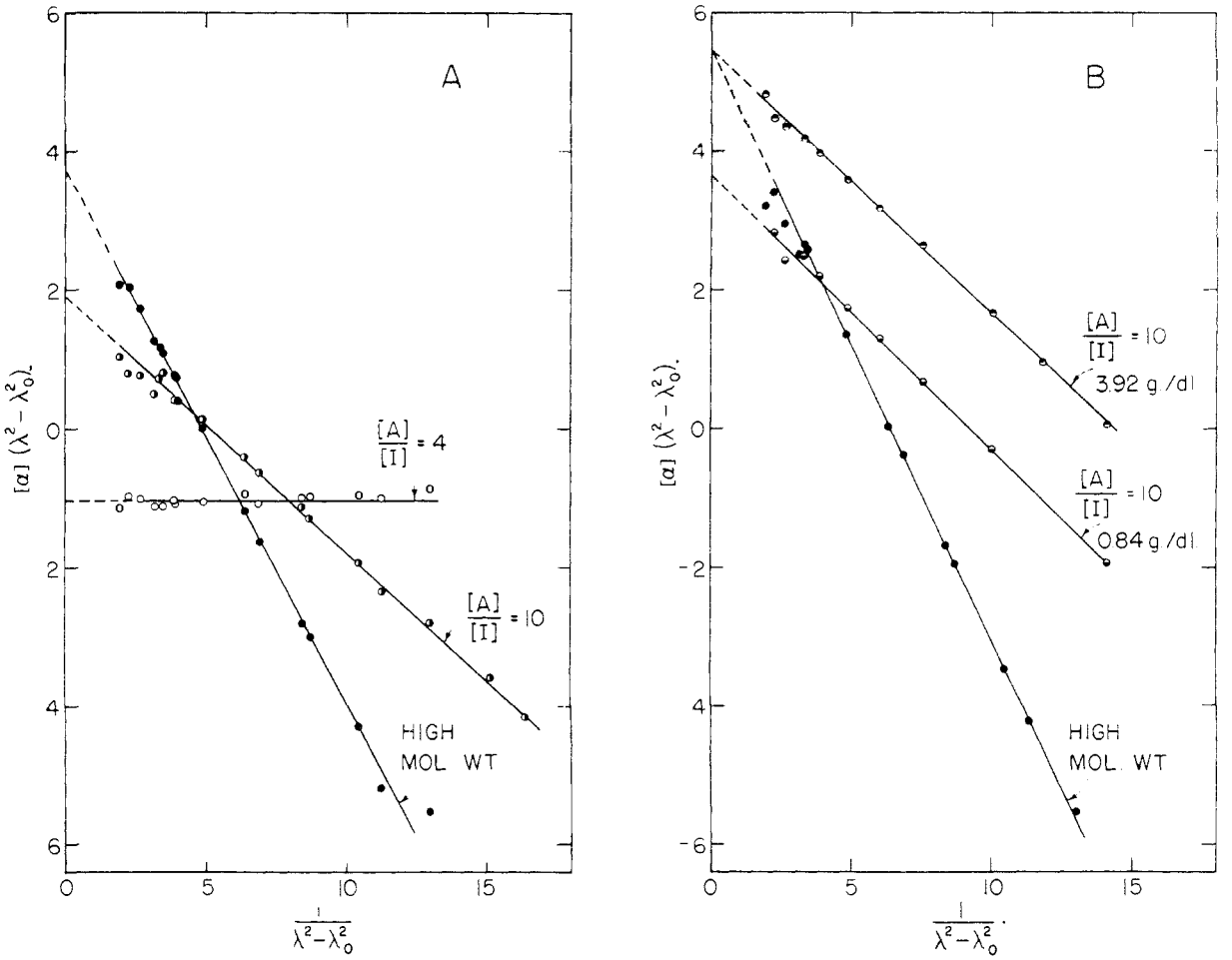


Fig. 2.—Moffitt plots of the optical rotatory dispersion of poly-γ-benzyl-L-glutamate in (A) dimethylformamide and (B) dioxane: λ is in microns; λ_0 is taken as 0.212 μ.

of the [A]/[I] = 4 sample. Since the DP_w is equal to that expected for a Poisson distribution, this distribution can be assumed. In this distribution about 10% of the sample by weight has a DP in excess of 7. If this were helical it could clearly be detected by the rotatory dispersion method. Consequently this forms the lower limit of DP_c. Thus these preliminary studies locate DP_c for these polypeptide–solvent systems between 7 and 14. The average of this range, that is DP of 10, appears as a reasonable estimate of DP_c but further investigation of fractionated samples of polypeptides produced in dimethylformamide will be required to reach the most accurate and precise value of this constant.

TABLE II

THE ROTATORY DISPERSION CONSTANTS[a] AND HELICAL CONTENT OF LOW MOLECULAR WEIGHT POLY-γ-BENZYL-L-GLUTAMATES

Sample No.	[A]/[I]	DP_w	Solvent	a_0	b_0	% Helix
RS-8	4	5	Dimethylformamide	−40	0	0
RL-III 156	10	14	Dimethylformamide	69	−298	44
RK-421	High	High	Dimethylformamide	135	−620	100
RL-III 156	10	14	3.42 g./dl. dioxane	196	−304	44
RL-III 156	10	14	0.84 g./dl. dioxane	132	−318	47
RK-421	High	High	Dioxane	198	−682	100

[a] Uncorrected for the dispersion of the refractive index.

Acknowledgment.—We wish to acknowledge the helpful discussions and occasional assistance provided by Drs. E. R. Blout, R. D. Lundberg, M. Idelson and J. T. Yang throughout the course of this work. We are especially indebted to Professor Gerson Kegeles for informative discussions and for his assistance in obtaining our synthetic boundary cell and to Dr. Blout and Miss Ann Asadourian for the infrared spectral studies. Financial support was provided by the Office of Naval Research (N5Ori-07654) and the American Cancer Society (Institutional Grant).

CAMBRIDGE, MASS.