Some Macromolecular Properties of Poly(α-L-glutamic acid) Random Coils[1a]

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ABSTRACT: Light-scattering and viscosity measurements of neutral aqueous solutions of poly(α-L-glutamic acid) (PLGA) as a function of ionic strength and molecular mass show that the fully charged polymer behaves as a typical randomly coiled polyelectrolyte. Extrapolation to infinite ionic strength provides values of the intrinsic viscosity characteristic of the discharged random coil. The intrinsic viscosities are subjected to Stockmayer–Fixman analysis, giving a value for the unperturbed effective bond length of 8 Å. This value is the same as is obtained by a similar analysis of data for poly(γ-benzyl L-glutamate) in dichloroacetic acid and for un-cross-linked protein chains in strongly denaturing media. The viscosity expansion factors for PLGA are calculated from the viscosity data. The perturbed dimensions and thus the mean-radius expansion factors are estimated from the light-scattering data and agree roughly with expectations based on experimental results for other polymer–solvent systems. The second virial coefficients from the light scattering are also extrapolated to infinite ionic strength and show that such an aqueous solution is a "good" solvent for discharged PLGA. The second virial coefficients are shown to be consistent with the unperturbed dimensions deduced from the intrinsic viscosities.

Potentiometric titration curves[2-5] and optical properties[6-10] of poly(L-glutamic acid) (PLGA) indicate that in neutral aqueous solution the polymer is fully charged and that no ordered helical structure is present. In spite of considerable interest in the helix–coil transition,[11,12] particularly as it occurs in aqueous media, in the theory of polypeptide chain dimensions,[13-16] and in *protein* random coils,[17-19] there have been virtually no attempts at thorough experimental macromolecular characterization of the PLGA random coil. Workers have doubtless been deterred by the formidable difficulties that hinder interpretation of experiments on this system: the solutions are very nonideal and the samples presently available are very polydisperse and span a rather narrow range of mean molecular mass.

Although we recognize all the hazards and admit the necessarily tentative nature of the conclusions, we nevertheless feel that such a study can serve a purpose, particularly since the theory of polypeptide chain dimensions is not yet free standing, but requires some input from experiment. Consequently, we have made a start toward such a characterization, and report here light-scattering and intrinsic viscosity measurements of fully charged PLGA at room temperature as a function of ionic strength and mean molecular mass.

To interpret these data, we regard PLGA as a flexible, randomly coiled polyion in nonideal solution. Following procedures developed elsewhere in the study of other polyelectrolytes,[20-22] we eliminate charge effects by suitable extrapolation of the intrinsic viscosities to infinite ionic strength and then estimate unperturbed dimensions by a method[23] whose utility seems to have outstripped the soundness of its theoretical basis.[20-22] The values are compared first to those obtained for several other randomly coiled polypeptides by other investigators, and then with a literature value for PLGA determined by a method different from our own.

As the discussion will show, the presence of polydispersity and of a substantial expansion factor prevents precise, unambiguous determination of the *perturbed* dimensions from the light-scattering data, but does allow determination, at worst, of an upper limit to and, at best, a good estimate of the mean-radius expansion factor. The viscosity and mean-radius expansion factors are compared in the light of recent theoretical and experimental developments.

Finally, we discuss the nonideality of aqueous PLGA solutions, and, again by extrapolation to infinite salt, estimate what portion of this may be attributed to charge effects and what portion to the neutral polypeptide. The consistency of the unperturbed dimensions, deduced from the viscosity data, with the observed nonideality is examined.

Experimental Section

Samples of the sodium salt of PLGA were purchased from Pilot Chemicals. The manufacturer's lot numbers are G-44, G-61, G-72, G-123, and G-128. The degrees of polymerization furnished by the manufacturer are significantly lower than those found and re-

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(2) P. Doty, A. Wada, J. T. Yang, and E. R. Blout, *J. Polym. Sci.*, **23**, 851 (1957).
(3) A. Wada, *Mol. Phys.*, **3**, 409 (1960).
(4) M. Nagasawa and A. M. Holtzer, *J. Amer. Chem. Soc.*, **86**, 538 (1964).
(5) D. Olander and A. Holtzer, *ibid.*, **90**, 4549 (1968).
(6) Y. Cassim and E. Taylor, *Biophys. J.*, **5**, 573 (1965).
(7) Y. Tomimatsu, L. Vitello, and W. Gaffield, *Biopolymers*, **4**, 653 (1966).
(8) W. G. Miller and R. E. Nylund, *J. Amer. Chem. Soc.*, **87**, 3542 (1965).
(9) J. Hermans, *J. Phys. Chem.*, **70**, 510 (1966).
(10) P. Urnes and P. Doty, *Advan. Protein Chem.*, **16**, 401 (1961).
(11) H. Neurath, Ed., "The Proteins," Vol. II, 2nd ed, Academic Press, New York, N. Y., 1964.
(12) G. D. Fasman, Ed., "Poly-α-Amino Acids," Marcel Dekker, New York, N. Y., 1967.
(13) D. A. Brant and P. J. Flory, *J. Amer. Chem. Soc.*, **87**, 2791 (1965).
(14) W. G. Miller and P. J. Flory, *J. Mol. Biol.*, **15**, 298 (1966).
(15) D. A. Brant, W. G. Miller, and P. J. Flory, *ibid.*, **23**, 47 (1967).
(16) W. G. Miller, D. A. Brant, and P. J. Flory, *ibid.*, **23**, 67 (1967).
(17) C. Tanford, K. Kawahara, and S. Lapanje, *J. Amer. Chem. Soc.*, **89**, 729 (1967).
(18) S. Lapanje and C. Tanford, *ibid.*, **89**, 5030 (1967).
(19) W. G. Miller and C. Goebel, *Biochemistry*, **7**, 3925 (1968).
(20) A. Takahashi and M. Nagasawa, *J. Amer. Chem. Soc.*, **86**, 543 (1964).
(21) A. Takahashi, T. Kato, and M. Nagasawa, *J. Phys. Chem.*, **71**, 2001 (1967).
(22) I. Noda, T. Tsuge, and M. Nagasawa, *ibid.*, **74**, 710 (1970).
(23) W. H. Stockmayer and M. Fixman, *J. Polym. Sci., Part C*, **No. 1**, 137 (1963).

ported here. An analysis of the origins of this discrepancy has been made.[24] It suffices to say here that the empirical method used by the manufacturer is rather indirect, and we believe their values are merely nominal.

Water was distilled and passed through a mixed-bed ion-exchange resin column before use. Salts used were reagent grade material. Solvents were 0.1 *M* NaCl, 0.01 *M* sodium phosphate, pH 7.05, in which the ionic strength (*I*) is 0.11 *M;* 0.2 *M* NaCl, no phosphate, pH ~7.0; 0.4 *M* NaCl, 0.01 *M* sodium phosphate, pH 6.8, $I = 0.41$ *M;* 1.0 *M* NaCl, 0.01 *M* sodium phosphate, pH 6.7, $I = 1.01$ *M*.

Solutions of PLGA were prepared by dissolving the sodium salt of the polymer in the designated solvent and dialyzing exhaustively *vs.* the same solvent. For all the above media, titration curves indicate that PLGA has 97–100% of its residues in the ionized form.[5] The concentration of PLGA solutions was determined by pippetting an aliquot of solution into a dialysis bag and then adding to the bag an excess of 0.1 *N* HCl to discharge all carboxyl groups. The bag was sealed and the solution dialyzed exhaustively against water. The bag was then cut open and emptied into a beaker, and then the bag itself was cut up and quantitatively transferred to the beaker. The contents of the beaker were titrated to the phenolphthalein end point with 0.1 *M* NaOH. This method gave polymer concentrations with a reproducibility of ±1% and agreed with a Kjeldahl determination. Polymer concentrations are referred to throughout this study as grams of free acid per cubic centimeter of solution (c) for light scattering and as grams of free acid per deciliter of solution (c'), as is traditional, for viscosity.

Viscometry was done with Size 50 Cannon-Ubbelohde, semimicro, dilution viscometers (Cannon Instruments, State College, Pa.). All solutions measured were at 25.5° and solvent flow times were about 225 sec. The specific viscosity divided by concentration is linear in concentration over the range examined ($c' \simeq 0.1$–0.3%). The intrinsic viscosity ($[\eta]$) is the ordinate intercept of such a plot and is reported in Table I. We also report in that table the parameter k' determined from the slope and intercept using the relation $\eta_{sp}/c' = [\eta] + k'[\eta]^2c'$.

Light-scattering measurements were carried out using a modified Brice-Phoenix Series 1000 light-scattering photometer (Phoenix Precision Instrument Co., Philadelphia, Pa.). The quantity measured is ΔR_Θ, the difference in Rayleigh's ratio between light scattered by a PLGA solution and its dialyzate at an angle Θ from the incident beam. The difference, ΔR_Θ, was measured at 15 angles between 30 and 135° using an erlenmeyer-shaped cell. Calibration of instrument and cell have been described.[25] Filtered light with a vacuum wavelength of 436 nm was used. Polymer solutions were clarified for scattering by filtration through an ultrafine fritted-glass filter. The concentration of polymer solution in the cell was varied by addition of a weighed, filtered quantity of solvent followed by gentle mixing with a small magnetic stirring bar kept in the cell. Further experimental details are available.[24]

The refractive increment at dialysis equilibrium was measured, using light of 436-nm wavelength, with a Brice-Halwer differential refractometer (Phoenix Precision Instruments, Philadelphia, Pa.). The values obtained for the solvent at $I = 0.11$ and at $I = 0.41$ were 0.207 and 0.194 cm³/g, respectively. A value of 0.190 was estimated for the $I = 1.01$ solvent by use of the Gladstone–Dale relation.

Results

(A) Light Scattering. Figure 1 shows typical light-scattering data for an aqueous PLGA solution. The data are plotted according to Zimm.[26,27] The reciprocal angular envelope of light scattered at each concentration of PLGA is concave downward for all samples examined. The lowest four to five

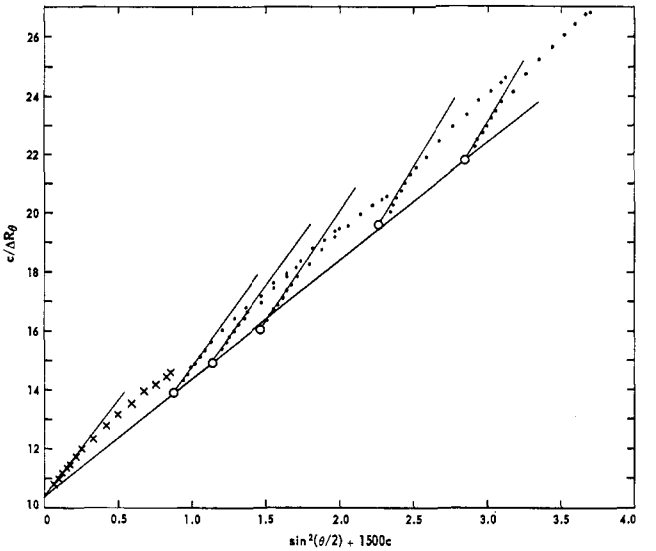

Figure 1. Zimm plot for sample G-61 in 0.4 *M* NaCl, 0.01 *M* phosphate, at pH 6.80. Simple points are data points, circled points are for Θ = 0°, crosses for $c = 0$. See text for description of how extrapolation lines were determined.

points of each envelope define a straight line within experimental error and this line was extrapolated to determine the value of $c/\Delta R_\Theta$ at Θ = 0° ($c/\Delta R_0$). The values of $c/\Delta R_0$ (open circles in Figure 1) fit a straight line very well over the concentration range examined ($c' \simeq 0.05\%$ to $c' \simeq 0.2\%$). The slope and intercept of this line were used along with the appropriate value of the optical constant to determine the weight-average molecular mass (M_w) and second virial coefficient (A_2) for each sample in the usual manner.[28,29] These values are reported in Table I.

Values of $c/\Delta R_\Theta$ for a given nonzero value of Θ do not fit a straight line as well as do the values of $c/\Delta R_0$. If they *are* fit to a straight line, the line has a slope greater than that of the line for $c/\Delta R_0$. The greater Θ, and the greater c, the more the points tend upward from the direction of the $c/\Delta R_0$ line. This is difficult to see in Figure 1 because of the confusion of points from different envelopes at high angles, but is reflected in the steeper slopes of the lines used to extrapolate to zero angle for the higher concentration envelopes. Because of this feature of the Zimm plots, the extrapolation at constant angle to zero concentration (to determine the zero concentration envelope) was done as follows.

The line for Θ = 0° ($c/\Delta R_0$) was established and its slope (α_1) determined. Then, a curve of the form $y = \alpha_0 + \alpha_1 c + \alpha_2 c^2$ was fitted by least squares to the points for each nonzero angle Θ, the constant α_1 being fixed for all Θ at the same value found for Θ = 0°. That is, we assumed that the limiting slope of $c/\Delta R_\Theta$ *vs.* c at $c = 0$ is independent of Θ and equal to that established for Θ = 0°. Zimm's theory of light scattering implies that this assumption is true to the extent that the single-contact approximation used in that theory is valid. The value of α_0 for the fitted line was taken as the limiting value of $c/\Delta R_\Theta$ at $c = 0$, and the series of values thusly obtained define the angular envelope at infinite dilution. The initial slope of this envelope was used in the usual way[28] to obtain the "light-

(24) R. Hawkins, Ph.D. Dissertation, Washington University, St. Louis, Mo., 1967.
(25) T. M. Schuster, Ph.D. Dissertation, Washington University, St. Louis, Mo., 1963.
(26) B. H. Zimm, *J. Chem. Phys.*, **16**, 1093 (1948).
(27) B. H. Zimm, *ibid.*, **16**, 1099 (1948).
(28) E. P. Geiduschek and A. M. Holtzer, *Advan. Med. Phys.*, **6**, 431 (1958).
(29) E. F. Casassa and H. Eisenberg, *Advan. Protein Chem.*, **19**, 287 (1964).

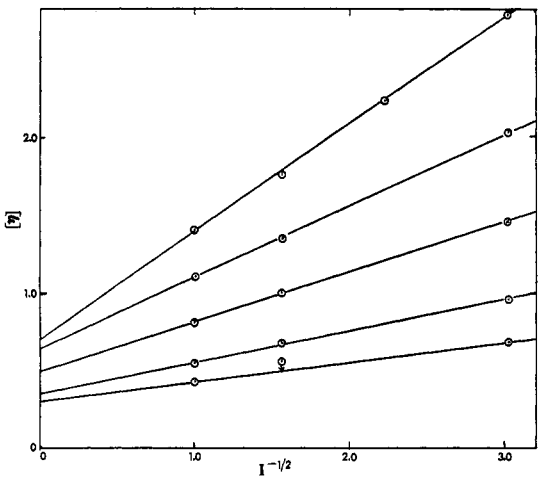

Figure 2. Plot of $[\eta]$ *vs.* $I^{-1/2}$. Samples are, in order of decreasing ordinate intercept, G-61, G-72, G-44, G-123, G-128. Lines are from least-squares analysis.

TABLE I
EXPERIMENTAL RESULTS FROM VISCOSITY AND LIGHT-SCATTERING DATA

Sample	I,[a] M	$[\eta]$, dl/g	k'	$10^{-3} \times M_w$[d]	$10^4 A_2$, (mol cm³)/g²	$[6\langle S^2\rangle_{1s}]^{1/2}$, Å	α_η[e]
G-61	0.11	2.79	0.26	152	31.3	1100	1.94
	0.20[b]	2.23	0.46				1.80
	0.41	1.76	0.34		18.5	872	1.66
	1.01	1.41	0.47		14.5	810	1.54
	∞	0.711					1.23
G-72	0.11	2.03	0.36	117			1.82
	0.41	1.35	0.34		15.2	916	1.59
	1.01	1.11	0.33		13.7	820	1.49
	∞	0.648					1.24
G-44	0.11	1.46	0.34	76.8			1.75
	0.41	1.00	0.38		20.7	860	1.54
	1.01	0.81	0.40				1.44
	∞	0.496					1.22
G-123	0.11	0.965	0.47	55.2			1.61
	0.41	0.680	0.33		21.8		1.43
	1.01	0.550	0.31				1.33
	∞	0.355					1.15
G-128	0.11	0.660	0.42	44.3			1.47
	0.41	0.560	0.46		18.2		1.39
	1.01	0.425	0.21				1.27
	∞	0.300[c]					1.13

[a] In every case but one[b] the solvent is NaCl plus 0.01 *M* phosphate buffer, pH ~7. The phosphate contributes about 0.01 to the ionic strength. [b] 0.2 *M* NaCl, no phosphate, pH ~7. [c] Omitting point at 0.41 = I; see text. [d] Only one value is given per sample. It is the average of several light-scattering experiments, including those performed under conditions not given in this table; *i.e.*, it includes measurements of partially helical samples. We think the given figure is the best one for that sample. [e] From eq 10; see text.

scattering average" root-mean-square radius ($\langle S^2\rangle_{1s}^{1/2}$). These values also can be found from Table I.

(B) Viscosity. In Figure 2, the measured value of the intrinsic viscosity for each PLGA sample in each solvent is shown plotted against the reciprocal of the square root of the ionic strength of the solvent. A linear relation between $[\eta]$ and $I^{-1/2}$ was proposed by Pals and Hermans[30] and has been found to be obeyed by many polyelectrolytes.[22,31] Figure 2 clearly shows the validity of such a relation for PLGA. The linear, least-squares extrapolation to infinite ionic strength is also shown in Figure 2, and we refer to this intercept as $[\eta]_\infty$ for each PLGA sample. We use that quantity as an estimate of the intrinsic viscosity the polymer would have if that part of the polyion expansion due to charge–charge repulsion were diminished to zero by ionic screening.[22] These values are also reported in Table I. Only the data point for the sample of lowest molecular mass at I = 0.41 *M* deviates from the drawn line. This anomalous point was therefore ignored in the extrapolation for that sample.

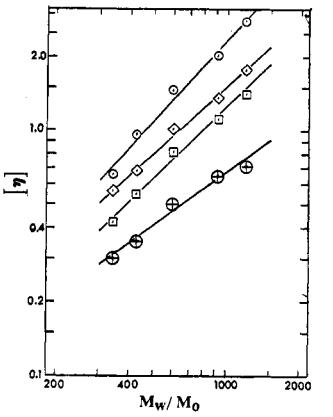

Figure 3. Double logarithmic plot of $[\eta]$ *vs.* (M_w/M_0): (○) I = 0.11, (◇) I = 0.41, (□) I = 1.01, (⊗) I = ∞. Lines are from least-squares analysis.

TABLE II
MARK–HOUWINK PARAMETERS

I	a	$10^5 K$	$10^3 K'$
0.11	1.115	0.474	1.067
0.41	0.923	2.93	2.60
1.01	0.959	1.55	1.64
∞	0.722	13.65	4.58

Figure 3 shows a log–log plot of the intrinsic viscosity *vs.* weight-average degree of polymerization (*i.e.*, the number of glutamic acid residues in a molecule) for each solvent including the infinite ionic strength medium. Table II contains the three parameters K, K', and a from the (least squares) equation for each line, *i.e.*

$$[\eta] = K'(M_w/M_0)^a = KM_w^a \quad (1)$$

The monomer residue mass, M_0, in this case is 129.1 amu. The small range of M_w spanned makes the precise experimental determination of the exponents difficult, and is probably responsible for these being about the same for the 0.41 and 1.01 *M* solvents. The exponent for the 0.11 *M* medium is distinctly greater, as expected for the more expanded coil. Even more striking is the much lower value of the slope for the infinite ionic strength line.

Discussion

(A) Unperturbed Dimensions from $[\eta]$ and M_w. The dependence of $[\eta]$ on molecular mass may be used to estimate the unperturbed effective bond length. This question has been thoroughly investigated for polyelectrolytes.[20–22] It is found empirically that the methods of Stockmayer and Fixman[23]

(30) D. T. F. Pals and J. J. Hermans, *Recl. Trav. Chim. Pays-Bas*, 71, 456 (1952).

(31) R. A. Cox, *J. Polym. Sci.*, 47, 441 (1960).

or of Kurata and Stockmayer[32] are suitable and give similar results. The method used here is that of Stockmayer and Fixman (SF), which requires considerably less computational effort. The basic relation of this procedure for a monodisperse polymer is

$$[\eta]/M^{1/2} = K_\Theta + m_{SF}M^{1/2} = K_\Theta + 0.15\Phi_\Theta BM^{1/2} \quad (2)$$

wherein Φ_Θ is the Flory–Fox viscosity constant[33] for a Θ solvent; its numerical value is somewhat uncertain, and we will employ $\Phi_\Theta = 2.8 \times 10^{21}$ (with everything in cgs units except $[\eta]$, which is in dl/g). The quantity B is a constant for a given polymer–solvent system. The value of K_Θ is defined by the relation[33]

$$[\eta]_\Theta = K_\Theta M^{1/2} \quad (3)$$

where $[\eta]_\Theta$ is the intrinsic viscosity the polymer would have in a Θ solvent at the same temperature. Also

$$K_\Theta = \Phi_\Theta[\langle L_\Theta^2\rangle^{1/2}/M]^3 = \Phi_\Theta[b_\Theta/M_0^{1/2}]^3 \quad (4)$$

wherein $\langle L_\Theta^2\rangle$ is the unperturbed mean-square end-to-end distance of a polymer of molecular mass M and b_Θ is the unperturbed effective bond length, *i.e.*

$$b_\Theta^2 = \langle L_\Theta^2\rangle/(M/M_0) \quad (5)$$

Although there seems little doubt that the intercept of the graph of $[\eta]/M^{1/2}$ *vs.* $M^{1/2}$ (an "SF plot") can give the correct K_Θ,[20-22] theoretical justification is lacking, and serious practical difficulties often intrude.[33b] Nevertheless, it seems to be the best verified experimental route to the unperturbed dimensions that is accessible in the case of PLGA.

There is still less certainty concerning the exact meaning of the slope of the SF plot. It does seem clear, at least, that it is related to the second virial coefficient (A_2) and measures nonideality as A_2 does, *i.e.*, B is zero in Θ solvents and greater than zero in good solvents.

For polyelectrolytes, the slope of the SF plot (m_{SF} in eq 2) may be divided into electrostatic and nonelectrostatic parts.[22] The former is empirically found to be proportional to the reciprocal of the square root of ionic strength[22] and the latter is the slope at infinite ionic strength. Thus, eq 2 becomes

$$[\eta]/M^{1/2} = K_\Theta + [m_\infty + m_e]M^{1/2} = K_\Theta + [m_\infty + b_e I^{-1/2}]M^{1/2} \quad (6)$$

in which m_∞ and b_e are constants independent of both M and I.

In Figure 4, our viscosity and weight-average molecular mass data are plotted for each solvent system in order to show the linear relation given in eq 2. As written, eq 2 refers to monodisperse polymer samples. If eq 2 is to be a valid relation for a polydisperse sample, $[\eta]$ and M must be representative of molecules of the same mass. The value of $[\eta]$ is that characteristic of a molecule with the viscosity average molecular weight (M_v) so M_v is the appropriate value to use in eq 2. If the exponent in eq 1 is unity, then $M_w = M_v$ no matter what the molecular mass distribution. Also, it has been shown that several forms of mass distribution lead to values of M_v very close to M_w even when the exponent is as low as 0.55.[34] Since the exponents encountered here are all nearer to unity than is 0.55 (see Table I), we have assumed $M_v \cong M_w$ for all our data.

(32) M. Kurata and W. H. Stockmayer, *Advan. Polym. Sci.*, **3**, 196 (1963).

(33) (a) P. J. Flory, "Principles of Polymer Chemistry," Cornell University Press, Ithaca, N. Y., 1953; (b) H. Yamakawa, "Modern Theory of Polymer Solutions," Harper and Row, New York, N. Y., 1971.

(34) K. D. Goebel and D. A. Brant, *Macromolecules*, **3**, 634 (1970).

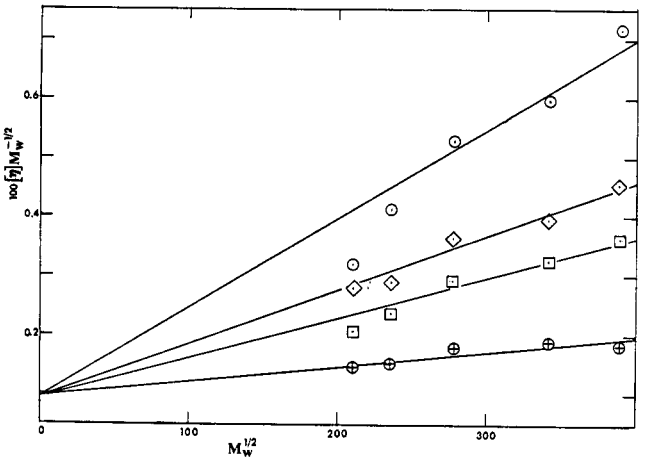

Figure 4. Stockmayer–Fixman plot: (○) I = 0.11 M, (◇) I = 0.41 M, (□) I = 1.01 M, (⊗) I = ∞. Lines determined as described in the text.

Because of the sizable experimental errors, it is difficult to decide exactly which lines through the data on Figure 4 are best. The uncertainty in determination of the slopes and intercepts is very large and the values obtained are significantly dependent on the curve-fitting procedure used. We must, then, describe and justify the particular protocol we employed.

There are three important factors to consider. First, the experimental ionic strengths and intrinsic viscosities are considerably more accurate and more precise than the experimental molecular mass; and, although the reason is unclear, the intrinsic viscosity is empirically found, both elsewhere[22] and here, to be strictly linear in $I^{-1/2}$. Second, eq 2 is better the closer one is to Θ conditions; indeed, it may be a poor approximation in systems sufficiently nonideal so that the exponent in eq 1 is greater than 0.8, the Flory limit for flexible chains. Third, the reduced dependence of $[\eta]/M_w^{1/2}$ on M_w in the case of infinite salt makes the intercept very much less influenced by experimental errors in the abscissa ($M_w^{1/2}$). Given the first factor, one is led to accept the values of $[\eta]_\infty$ obtained from the least-squares extrapolations of Figure 2 as quite reliable; since, furthermore, when these are plotted as on Figure 4, they constitute the set closest to Θ conditions, the second and third factors indicate that they form the best set of numbers we have for determining the Stockmayer–Fixman intercept. Consequently, these infinite salt points were treated by least squares, which showed an intercept (K_Θ) and slope such that

$$[\eta]/M_w^{1/2} = 9.84 \times 10^{-4} + 0.242 \times 10^{-5}M_w^{1/2} \quad (7)$$

from which, using eq 4, we find for the unperturbed effective bond length b_Θ = 8.0 Å. In what follows, then, we will accept 8 Å as the best value of the unperturbed effective bond length so far obtainable from our experiments, but we are obligated to remind the reader once more that it depends heavily on the veracity of our extrapolation to infinite salt, on the "near-ideality" of that medium, on the veracity of eq 2 in describing that near-ideal medium, and on our ability to extrapolate our data accordingly to zero M.

This experimental value of K_Θ also allows calculation (from eq 3) of the viscosity that a sample of given M_w would have at Θ conditions, and thus of the viscosity expansion factor α_η

$$\alpha_\eta^3 = [\eta]/[\eta]_\Theta \quad (8)$$

These values are also given in Table I.

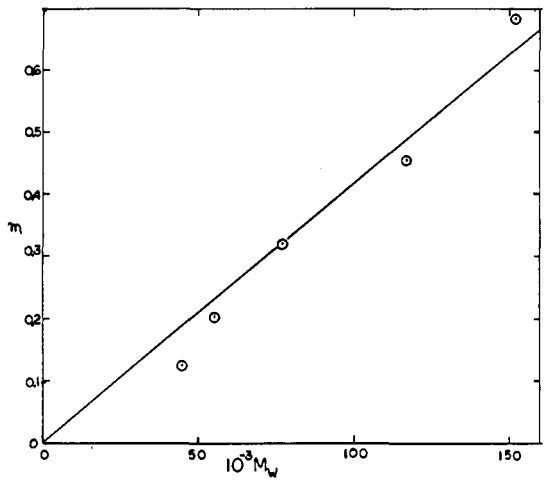

Figure 5. Slopes of the $[\eta]$ *vs.* $I^{-1/2}$ lines (m) plotted *vs.* M_w. Line determined as described in the text.

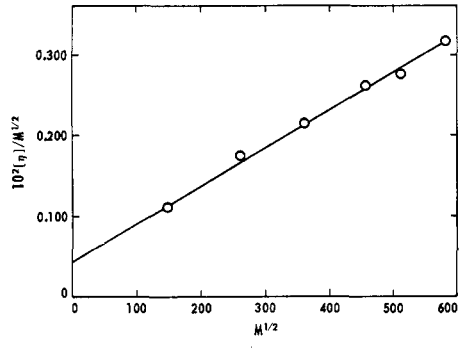

Figure 6. Stockmayer–Fixman plot of data (of ref 38) for PBLG in dichloroacetic acid.

It still remains to describe the procedure used to determine a self-consistent set of Stockmayer–Fixman lines for the data at *finite* salt concentrations shown in Figure 4. That is, it is of interest to inquire if an appropriate value can be chosen for b_e of eq 6 that will fit the data. This quantity is directly related to the slope (m) of the $[\eta]$ *vs.* $I^{-1/2}$ plots of Figure 2, in which, as noted above, we have considerable confidence. A glance at eq 6 shows this relationship to be

$$m = b_e M_w \tag{9}$$

Thus, a plot of the experimental m (from Figure 2) *vs.* the experimental M_w should fit a straight line that goes through the origin and whose slope is b_e. Such a plot is shown in Figure 5. Except for the sample of lowest molecular mass, the fit is seen not to be too bad, and least squares gives, for the best line forced to go through the origin, a value of $b_e = 4.17 \times 10^{-6}$. The change introduced by omitting the deviant point for lowest M_w is immaterial. The lines drawn on Figure 4 to fit the SF plots at the three finite ionic strengths were thus obtained from

$$[\eta]/M_w^{1/2} = 9.84 \times 10^{-4} + [0.242 \times 10^{-5} + 0.417 \times 10^{-5} I^{-1/2}] M_w^{1/2} \tag{10}$$

These lines, while they are not the ones we would draw *a priori*, are seen to fit the data rather well except for the point at lowest M_w and lowest I. The deviation of that point is not surprising, since the points at low M_w in good solvents are known to deviate,[35] and the $I = 0.11$ M medium is the best solvent (*i.e.*, has the greatest B) we have used. If the questionable data point is ignored and the simpler strategy adopted of determining (by least squares) the slope of the best line (at each I) that is forced to the intercept (K_Θ) already determined (from the $I = \infty$ data), the resulting lines are indistinguishable from the ones shown (*i.e.*, the ones obtained from eq 10). Our data thus cannot rule out the Stockmayer–Fixman relation; *i.e.*, an equation of that form does fit. Equation 10, then, whether or not its constants have the significance ascribed to them in the Stockmayer–Fixman analysis, is a concise summary of the molecular weight and ionic strength dependence of the intrinsic viscosity in this system.

The self-consistency of this approach can also be assessed through the quantity α_η given in Table I. The Stockmayer–Fixman treatment implies that $(\alpha_\eta^3 - 1)/M^{1/2}$ is independent of M in a given solvent (*i.e.*, a given ionic strength in the present instance). The Flory theory, on the other hand, says that $(\alpha_\eta^5 - \alpha_\eta^3)/M^{1/2}$ is independent of M in a given solvent. The experimental observations on other polymers and polyelectrolytes favor the Flory theory if the molecular mass is high ($>10^6$), and the Stockmayer–Fixman theory at lower molecular masses, the value of $(\alpha_\eta^5 - \alpha_\eta^3)/M^{1/2}$ increasing with M in that low-M region.[36,37] Our data all fall in the region of lower molecular mass, and the numerical values of Table I do indeed indicate a much more pronounced rise of $(\alpha_\eta^5 - \alpha_\eta^3)/M^{1/2}$ with M than of $(\alpha_\eta^3 - 1)/M^{1/2}$. The latter, is, in fact, almost constant.

It is of interest to compare our data on PLGA to those for other polypeptides. We show in Figure 6 a Stockmayer–Fixman plot using the data for PBLG in dichloroacetic acid previously obtained.[38] The data fit a straight line very well over a considerably wider range of molecular mass than we have available for PLGA. Such a plot yields a value of $b_\Theta = 7.9$ Å. As expected, this is in very good agreement with the value obtained earlier by Kurata and Stockmayer from the same data and their own analysis.[32]

Furthermore, Tanford and his coworkers[17,18] find that randomly coiled *protein* molecules in 6 M guanidine hydrochloride plus 0.1 M β-mercaptoethanol obey a Mark–Houwink law such that

$$[\eta] = 0.00716(M/M_0)^{0.66} \tag{11}$$

where $[\eta]$ is, again, in dl/g. If the data points for the proteins are plotted as in Figure 6 (using $M_0 = 110$), they are badly scattered and a linear relation is hard to discern. However, if eq 11 itself, which smooths the data, is used to determine points for a SF plot, the high molecular weight [$M > 27{,}500$; *i.e.*, $(M/M_0) > 250$] part of the curve defines a line from which we find $b_\Theta = 8.5$ Å. Thus, the random-coil forms of PLGA, PBLG, and protein chains indicate essentially the same b_Θ (near 8 Å) when analyzed in the above manner.

There are very few other estimates of b_Θ for PLGA with which to compare the value found here. We are aware of only one other such study.[39] In that work, measurements were made of the number-average molecular mass, of the second virial coefficient (by osmotic pressure), and of the intrinsic viscosity of three polypeptides, comprising one sample each of PLGA, poly(β-benzyl L-aspartate) (PBLA), and poly(L-lysine) (PLL). Different conditions were used for each

(35) G. C. Berry, *J. Chem. Phys.*, **46**, 1338 (1967).

(36) T. Kato, K. Miyaso, I. Noda, T. Fujimoto, and M. Nagasawa, *Macromolecules*, **3**, 777 (1970).

(37) I. Noda, K. Mizutani, T. Kato, T. Fujimoto, and M. Nagasawa, *ibid.*, **3**, 787 (1970).

(38) P. Doty, J. H. Bradbury, and A. M. Holtzer, *J. Amer. Chem. Soc.*, **78**, 947 (1956).

(39) D. A. Brant and P. J. Flory, *ibid.*, **87**, 2788 (1965).

polymer, PLGA being examined in aqueous 0.3 M sodium phosphate at pH 7.85 and 37°, PBLA in m-cresol at 100°, and PLL in aqueous 1 M sodium bromide at pH 4.54 and 37°. The same data from the literature on PBLG in dichloroacetic acid at 25° as used here[38] were also included in the earlier analysis. The value of b_Θ was found to be ~11.5 Å for all. These workers were thus led to the rather far-reaching conclusion that the unperturbed dimensions of all polypeptides having unbranched β-carbon atoms ("alanine-like" polymers) are the same regardless of solvent, temperature, and the details of side-chain structure.

The numerical value of b_Θ (8 Å) obtained here for PLGA differs significantly from this earlier one. Our value could be wrong; however, the practical difficulties, mentioned above, that intrude when the SF plot is used, generally give rise to values of b_Θ that are too high.[38a] The correct value must then be less than 8 Å, bringing us further from agreement with the earlier value of 11 Å.

It is not impossible that both are correct and simply reflect the difference in temperature and solvent composition employed in the two studies of PLGA. We would then be led to abandon the earlier conclusion that b_Θ is independent of temperature and solvent for all alanine-like polypeptides. It is also possible, however, that the discrepancy arises from differences in the methods employed to derive b_Θ from the experimental data. Indeed, we consider this the most likely possibility, since exactly the same data for PBLG were used in both studies with discordant results. We are thus led to examine closely the interpretive scheme used in the earlier investigation.

In the study in question,[39] b_Θ was obtained from measurements of A_2, $[\eta]$, and molecular weight through use of the following relations

$$[\eta] = \Phi b_\Theta M_v^{1/2} \alpha^3 \tag{12}$$

$$A_2 M/[\eta] = 188 \ln [1 + 0.886(\alpha^2 - 1)] \tag{13}$$

in which Φ was given the value 2.1×10^{-21} (with $[\eta]$ in dl/g). Recent experimental work indicates that eq 13 does not agree with experiment in systems for which α is greater than about 1.5, and that it gives an underestimate of α for large α.[36,37,40–42] In the procedure under examination, this would lead to an *over*estimate of b_Θ and could explain the discrepancy.

Furthermore, eq 13, like (2) above, refers to a monodisperse system, and application to a polydisperse system requires that all the quantities used in its left-hand side refer to the same molecule. Since A_2 varies very slowly with molecular mass, it follows that the M used should be M_v, and, as discussed above, this means that the appropriate choice for M is very near M_w. Examination of the calculations, of ref 39, however, shows that M_n was used to compute b_Θ = 11.5 Å for PLGA. The value of M_w was not measured in that study, so it is not possible to recalculate b_Θ from the earlier data. However, since M_w must be substantially greater than M_n, the corresponding value of b_Θ must be lower than 11.5 Å. Thus, the agreement of b_Θ for PLGA with the other three systems studied in the earlier work is called into question. In later work from the same laboratory,[16] b_Θ is given a range of values from 8.9 to 11.5 Å, but the choice of M (in eq 13) and the solvent conditions used are not given. If we calculate b_Θ from *our* data using eq 12 and 13, the values decrease systematically from 10 to 8 Å with decreasing goodness of solvent (increasing ionic strength) and with decreasing M_w, whereas they should be constant.

Thus, the constancy of b_Θ found in the older study for the different polypeptides may be fortuitous. We conclude that the constancy of b_Θ for alanine-like polypeptides *may* hold; indeed we believe it does, but should be regarded as experimentally verified only at room temperature for PLGA in aqueous medium, PBLG in dichloroacetic acid, and non-cross-linked protein chains in 6 M guanidine hydrochloride. The constancy of b_Θ for all alanine-like polymers plays a central role in the theory of polypeptide chain dimensions, and clearly more experimental work is called for.

Establishment of the correct numerical value of b_Θ is important not only as a test of the theory of polypeptide chain dimensions but as an essential ingredient in that theory. Theoretical calculations of unperturbed dimensions rely on available data for peptide bond angles, distances, and moments; and on atomic van der Waals radii and torsional barriers to rotation. At its present stage of development, the theory cannot produce numerical results *a priori;* particular choices of certain molecular interaction parameters have to be made so as to bring computed dimensions into agreement with whatever experimental value is considered acceptable.[13–16] Particularly important choices are those of effective dielectric constant for the dipolar interaction between neighboring amide units, of van der Waals radius for the β-carbon methylene group, and of the presence or absence of an attractive branch in the van der Waals interaction potential. Our results indicate that a readjustment of these parameters should be made to bring the theory into agreement with the new value of $b_\Theta \simeq 8.0$ Å. The computations of Miller and Goebel[19] on protein chains seem to indicate that such an adjustment will result in better agreement between calculated and observed values of unperturbed protein random coil dimensions at 25°.

(B) Perturbed Dimensions from Light Scattering. We have assumed that in neutral solutions PLGA consists of polydisperse, non-Gaussian, flexible chains. The proper analysis of light-scattering data from such a system is still a matter of debate. The determination of the "light-scattering average" of the root-mean-square radius, $\langle S^2 \rangle_{1s}^{1/2}$, from the data is, however, independent of model,[28] and we have reported these values [as $6\langle S^2 \rangle_{1s}^{1/2}$] in Table I. There are two difficulties in interpreting this quantity. First, it is unclear how to deduce the root-mean-square end-to-end distance $\langle L^2 \rangle_{1s}^{1/2}$ from $\langle S^2 \rangle_{1s}^{1/2}$ for non-Gaussian coils. This, we believe, is not serious, since the ratio $\langle L^2 \rangle_{1s}/\langle S^2 \rangle_{1s}$ is probably close to its Gaussian value of six in any case. Second, and more unfortunately, the difference in averaging for $\langle L^2 \rangle_{1s}^{1/2}$ and $(M_w/M_0)^{1/2}$ makes their ratio of little molecular significance. Since $\langle L^2 \rangle_{1s}^{1/2}$ for both Gaussian coils and rods is appropriate to a molecule of higher degree of polymerization than the weight average, the ratio in the case of expanded coils must be an upper limit to the true perturbed effective bond length.

To obtain a better estimate of the perturbed effective bond length from the light-scattering data, one is forced to less certain ground. We therefore choose to accept the suggestion that the particle scattering factor $[P(\Theta)]$ for expanded coils has the same functional form as for unperturbed coils, but with the perturbed radius replacing the unperturbed radius.[43,44] If that is true, then $P(\Theta)$ for expanded coils

(40) T. Norisuye, K. Kawahara, and H. Fujita, *J. Chem. Phys.*, **49**, 4330 (1968).
(41) K. Kawahara, T. Norisuye, and H. Fujita, *ibid.*, **49**, 4339 (1968).
(42) G. Tanaka, S. Imai, and H. Yamakawa, *ibid.*, **52**, 2639 (1970).
(43) P. J. Flory and R. L. Jernigan, *J. Amer. Chem. Soc.*, **90**, 3128 (1968).
(44) P. J. Flory, "Statistical Mechanics of Chain Molecules," Interscience, New York, N. Y., 1969, pp 344–348.

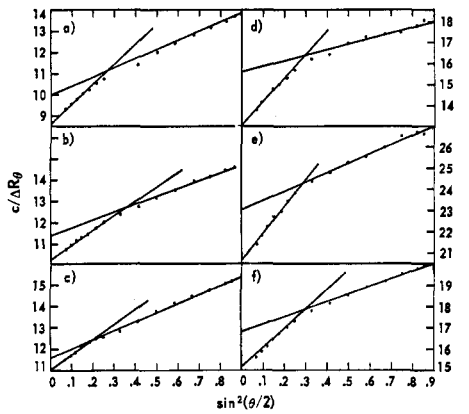

Figure 7. Light-scattering envelopes at infinite dilution. Lines indicate low-angle limit and asymptote for each, as described in text. Samples are: (a) G-61, I = 0.11 M; (b) G-61, I = 0.41 M; (c) G-61, I = 1.01 M; (d) G-72, I = 0.41 M; (e) G-44, I = 0.41 M; (f) G-72, I = 1.01 M.

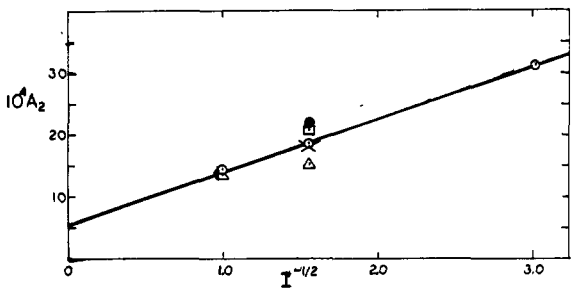

Figure 8. Ionic strength dependence of the second virial coefficient: (○) G-61, (△) G-72, (□) G-44, (●) G-123, (×) G-128.

TABLE III
QUANTITIES FROM THE LIGHT-SCATTERING ASYMPTOTE

Sample	I	In_s	Sl_s	b, Å	$\alpha_s = b/8.0$, Å	α_η[a]
G-61	0.11	10.0	4.2	17.5	2.19	1.94
G-61	0.41	11.4	3.6	15.2	1.90	1.66
G-61	1.01	11.6	4.2	16.1	2.01	1.54
G-72	0.41	15.5	2.8	13.4	1.68	1.59
G-72	1.01	16.8	3.6	14.9	1.86	1.49
G-44	0.41	23.1	4.3	16.5	2.06	1.54

[a] From Table I.

should approach an asymptote at high angles just as for Gaussian coils. The importance of this asymptote is that, as long as one is neither indiscriminate nor injudicious,[45] its slope provides a value of the effective bond length that is independent of polydispersity.

All of the angular envelopes at c = 0 display strong linearity at high angles as well as at low, as seen from Figure 7. The intercept (In_s) and slope (Sl_s) of each of these putatively asymptotic lines are given in Table III. Accepting the previous paragraph and assuming that the high-angle experimental data are indeed asymptotic, we have calculated the perturbed effective bond lengths and report these, along with the corresponding value of the mean-radius expansion factor ($\alpha_s \equiv \langle S^2\rangle^{1/2}/\langle S_\Theta^2\rangle^{1/2} \cong b/b_\Theta$), calculated by using the unperturbed effective bond length that we determined as above from our viscosity data. These are recorded in Table III. Needless to say, b and therefore α_s are molecular-mass dependent, and it is still unclear to which molecular mass the aforesaid numerical values refer. However, considering the large errors in determining both quantities and the modesty of their dependence on molecular mass, the weight average is probably a sufficiently close estimate. In any event, the experimental values of α_s in Table III show no clearly defined trend with either M_w or I. The only conclusion that can be drawn is that $\alpha_s \cong 2$ for PLGA in the range of ionic strength and molecular mass employed in this study.

(C) Comparison of α_η and α_s. Since the measurements of α_η are of lower error and can be made over a much wider range of M and ionic strength than those of α_s, it is of interest to inquire whether better values of α_s can be derived from our values of α_η. Unfortunately, there is no very reliable theoretical or experimental route to follow. The Flory–Fox theory[38] assumes $\alpha_s = \alpha_\eta$, but this aspect of their analysis is not borne out by experiment[36,37,40–42] and is certainly not expected to be true here. The Yamakawa theory[42] leads to $\alpha_s = \alpha_\eta^{1.25}$. Some recent experiments on uncharged polymers[40,41] give $\alpha_s = \alpha_\eta^{1.5}$. Most closely analogous to our study, however, are polyelectrolyte experiments[21] that provide the quantity Φ/Φ_Θ *vs.* α_η over a very wide range of α_η. Using the well-known relation $\alpha_s = [\Phi_\Theta/\Phi]^{1/3}\alpha_\eta$, getting the numerical values of the square bracket from Figure 8 of ref 21 at the values of α_η in Table I above, we can calculate the corresponding values of α_s. The result of calculation of α_s from our α_η is essentially the same whether Yamakawa's theory, the nonelectrolyte experiments, or the polyelectrolyte experiments are used as the basis: all of the values of α_s *calculated from our* α_η *values* for the samples of Table III are, within experimental error, about 2.0. (There is one possible exception, G-61 in 0.11 M solution, which is a bit higher, *i.e.*, 2.3–2.9.) Since this agrees with the value deduced from our light-scattering data, we conclude that the approximation suggested by Flory and Jernigan[43,44] (which we were forced to use to interpret these data) concerning the light scattering from a non-Gaussian coil seems to be borne out, at least in the present instance.

(D) The Nonideality of PLGA Solutions. Since the dependence of the second virial coefficient on molecular mass is extremely mild, one does not expect any to be apparent in our experiments, in which the error is large and the range small. Indeed, the data of Table I do not show any trend of A_2 with M_w. The dependence of A_2 on ionic strength is, however, pronounced. It has been found for other polyelectrolytes[21] that A_2 is linear in $I^{-1/2}$. The appropriate plot is shown in Figure 8. Clearly, our data for PLGA are well described by the best line through the points, which is

$$10^4A_2 = 5.5 + 8.5I^{-1/2} \qquad (14)$$

This immediately allows us to conclude that, in the absence of charge–charge interactions (*i.e.*, at $I = \infty$), PLGA's in the available range of molecular mass would have a second virial coefficient at 25° of 5.5 × 10^{-4} (mol cm^3)/g^2. Although more data are clearly called for, it seems unlikely that they could alter the line on Figure 8 so as to produce anything but a substantial, positive ordinate intercept. Thus, water is a "good" solvent for uncharged PLGA random coils.

This result is at first sight surprising, since the acid (uncharged) form of PLGA is well known to be insoluble in water. Of course, the experimental discharge of the random coil is accompanied by its transformation to helix, but this does not extricate us from the dilemma. The equilibrium between acid-form helix and acid-form coil lies overwhelm-

(45) A. R. Shultz and W. H. Stockmayer, *Macromolecules*, 2, 178 (1969).

ingly on the helix side. Water has thus clearly expressed its preference for helix over coil in these media, and the solubility of the same solid phase must therefore be even greater if helix is the solution phase. Thus, it must be true that the aqueous medium is an even better solvent for the acid form of the helix than for the acid form of the coil. Yet, uncharged PLGA helices are insoluble. It is possible that there are important differences in solvent interaction between a –COOH group and a –COO group, *i.e.*, between a carboxyl and a hypothetical *discharged* carboxylate. The A_2 we obtain at $I = \infty$ is for a polymer with the latter as side chain, not the former. We deem it much more likely, however, that the crystallinity of PLGA precipitates lies at the root of the difficulty. The term "good solvent" in polymer parlance compares the dissolved polymer with an amorphous precipitate. A solvent that is "good" to the tune of $A_2 = 5 \times 10^{-4}$ (mol cm^3)/g^2 may still not be good enough to prevail against the blandishments of crystallinity. The partially crystalline character of PLGA is well known, and has thus far foiled attempts to find a Θ solvent for this substance.[39]

Our estimated value of A_2 for infinite ionic strength solutions of randomly coiled PLGA may be compared to the measurements[46] of a related, nonionic water-soluble polypeptide, poly(N^5-(3-hydroxypropyl)-L-glutamine). It is found that at 25° this polymer has $A_2 = 2.8 \times 10^{-4}$, 3.0×10^{-4}, and 7.5×10^{-4} for samples with M_w equal respectively to 333×10^3, 39.1×10^3, and 18.7×10^3. The helix content of these samples under the indicated conditions decreases with decreasing molecular weight, being respectively 27, 20, and 13%. This would indicate that helix formation decreases A_2[47] and that A_2 is substantially positive for the uncharged random coil in water, as we find for PLGA.

The question arises of the consistency of our interpretation of the data on second virial coefficients with our analysis above of the molecular dimensions as obtained through the viscosity. The close connection between the two has been known, at least qualitatively, since the pioneering work of Flory.[33] Clearly, if water at infinite ionic strength is a good solvent (*i.e.*, $A_2 > 0$), then the expansion factor is greater than unity, and the intrinsic viscosity must be greater than it is in a Θ solvent. That is, we should find $[\eta]_\infty > [\eta]_\Theta$. Combining eq 3 and 4, we note that

$$[\eta]_\Theta = K_\Theta M^{1/2} = \Phi_\Theta b_\Theta{}^3 M^{1/2}/M_0{}^{1/2} \qquad (15)$$

Using the value of K_Θ reported above (the value giving $b_\Theta \simeq 8$ Å), we indeed always find from eq 15 that $[\eta]_\infty > [\eta]_\Theta$. If an appreciably larger value of b_Θ (~10 Å) is tried, however, the $[\eta]_\Theta$ values are sometimes above and sometimes below the experimental values of $[\eta]_\infty$. If a value as high as 11.5 Å is used, the calculated values of $[\eta]_\Theta$ are in all cases larger than $[\eta]_\infty$. We therefore feel that the lower value of b_Θ (8 Å) is supported by the observed second virial coefficients.

(46) K. Okita, A. Teramoto, and H. Fujita, *Biopolymers*, **9**, 717 (1970).

(47) The sterically excluded volume [estimated according to B. Zimm, *J. Chem. Phys.*, **14**, 164 (1946)] would lead to a second virial coefficient for PLGA random coils that is over four times that expected for the α helix. The experimental second virial coefficient, however, is a result of a manifold of interactions in addition to the sterically excluded volume.

Statistics of Random Copolymers Containing Blocks of One Component

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ABSTRACT: When long sequences (blocks) of one component occur in the molecules of a copolymer system, the blocks may segregate into domains. For a copolymer of A and O, we present a formula for the weight fraction of macromolecules which contain exactly i A blocks of length k or greater. A Monte Carlo estimate of this weight fraction and an analytical determination of the corresponding mole fraction have been given by Frensdorff. We employ an extension of the latter technique.

Frensdorff[1] has recently made an important contribution to the statistical theory of copolymers of A and O containing blocks (sequences) of one component. He addresses himself to the determination of the fraction of macromolecules which have exactly i A blocks of length k or greater. The problem is of interest because[1] "copolymer properties can be profoundly influenced by the presence of blocks ... of one of the constituent monomers, especially if these blocks are long and numerous enough to segregate into separate phases of glassy or crystalline domains." A number of specific techniques have been developed to synthesize macromolecules with just such blocks. However, long sequences may form to a greater or lesser extent in random copolymers, too, depending on the kinetics of the addition process.

Frensdorff[1,2] has used a clever modification of the Markov transition matrix to determine a generating function. From this the (mole) fraction of macromolecules with i A blocks of length $\geq k$ has been analytically calculated. He notes, though, that a more significant quantity may be the corresponding weight fraction. The magnitude of the latter he has determined by a Monte Carlo procedure for a few values of the parameters. In the present paper the weight fraction of macromolecules with i A blocks of length $\geq k$ is expressed in algebraic form.

The notation of Frensdorff has been followed rather closely. For completeness, required quantities are briefly redefined but the reader is advised to peruse ref 1 for a deeper understanding.

(1) H. K. Frensdorff, *Macromolecules*, **4**, 369 (1971).

(2) H. K. Frensdorff and R. Pariser, *J. Chem. Phys.*, **39**, 2303 (1963).