

## Poly( $\gamma$ -*p*-nitrobenzyl-L-glutamate): Synthetic Aspects, Sedimentation, and Viscosity Studies

J. FEYEN, K. DE GROOT, A. C. DE VISSER, and A. BANTJES,  
*Polymer Division, Department of Chemical Technology, Twente University  
of Technology, Enschede, The Netherlands*

### Synopsis

A series of poly( $\gamma$ -*p*-nitrobenzyl-L-glutamates), PNBG, has been synthesized by the polymerization of *N*-carboxyanhydride (NCA) derivatives of  $\gamma$ -*p*-nitrobenzyl-L-glutamate, NBG, using triethylamine as an initiator. We studied the influence of (a) the solvents dioxane, nitrobenzene, dimethylformamide (DMF), and DMF-1,2-dichloroethane mixture and (b) the anhydride-initiator ratio (A/I) for the polymerization in nitrobenzene (A/I varying from 50 to 750) on the properties of the polymers obtained. In order to improve its synthesis, NBG, was prepared by three different methods. Ten samples of PNBG, ranging in  $M_w$  from 10,000 to 50,000, were examined viscometrically in DMF and dichloroacetic acid (DCA) and by ultracentrifugation in DMF. The data for  $[\eta]$  and  $S_0$  (limiting sedimentation coefficient) as functions of  $M_w$  for PNBG in DMF were utilized, applying theories of Kuhn and Kuhn,<sup>13</sup> Schachman,<sup>14</sup> and Perrin,<sup>15</sup> for the estimation of the length per monomeric residue  $h$ . Viscosity data gave a  $h$  value of about 2.3 Å, whereas sedimentation yielded 1.5 Å. Treating viscosity and sedimentation data for poly( $\gamma$ -benzyl-L-glutamate), PBLG, in the same way leads to somewhat higher  $h$  values (2.4 Å and 1.7 Å, respectively).

Although a nitroaromatic effect was shown to be absent for PNBG in DMF, it can be concluded that in this medium PNBG has a somewhat more compact structure than PBLG.

### INTRODUCTION

Recently, many investigations of the synthesis and physicochemical behavior of a variety of poly( $\gamma$ -alkyl glutamates)<sup>1-7</sup> have been reported. It is well known that the stability of the secondary structures of these synthetic polypeptides depends on the nature and sequence of the  $\alpha$ -amino acid components and the solvent environment.<sup>8,9</sup>

Goodman et al.<sup>8</sup> have investigated homo- and copolymers of  $\beta$ -*p*-nitrobenzyl-L-aspartate and  $\beta$ -benzyl-D- (and -L-) aspartate as well as of  $\gamma$ -*p*-nitrobenzyl-L-glutamate, NBG, and  $\gamma$ -benzyl-L-glutamate, BLG.

In chloroform there is reversal from the left-handed helical sense of poly( $\beta$ -benzyl-L-aspartate) to the right-handed sense of poly( $\beta$ -*p*-nitrobenzyl-L-aspartate) when 26-32 mole % of the latter is present in the copolymer. In dimethylformamide (DMF) there is a transition from the random conformation of poly( $\beta$ -benzyl-D- (or -L-) aspartate) to the right-

handed helical sense of poly( $\beta$ -*p*-nitrobenzyl-L-aspartate). In the glutamate series however, no helix reversal was observed in chloroform or DMF. The enhanced rotatory properties in the case of poly(nitrated aspartates) were attributed to the occurrence of a side-chain helix (nitroaromatic effect).

In DMF or chloroform solutions no such effect was observed in the glutamate series.

Fraser et al.<sup>10</sup> measured the helix stability of a series of substituted poly( $\gamma$ -benzyl-L-glutamates) in ethylene dichloride–dichloroacetic acid (EDC–DCA) mixtures. In the case of poly(*o*-, *m*-, and *p*-nitrobenzyl-L-glutamates) a nitroaromatic effect was demonstrated by the magnitude of the change, in the optical rotatory dispersion parameter  $b_0$ .

These authors have also carried out x-ray diffraction studies to establish the conformation in the solid phase of substituted poly( $\gamma$ -benzyl-L-glutamates). The effect of substituents on the axial rise per residue  $h$  and the angular separation between residues ( $\phi$ ) was determined. It was found that a *p*-nitro substituent caused an increase in the  $h$  value (1.505 to 1.525 Å) without a compensating decrease in  $\phi$  (1.74 radians).

Although the hydrodynamic properties of poly( $\gamma$ -benzyl-L-glutamate), PBLG, have been studied extensively by Fujita et al.,<sup>11</sup> Doty et al.,<sup>3</sup> and Spach et al.,<sup>12</sup> no such investigation was carried out for substituted poly( $\gamma$ -benzyl-L-glutamates). The aim of the present work is twofold. In the first place we wish to establish in which way the introduction of a *p*-nitro substituent in PBLG changes its hydrodynamic behavior in solution. We therefore have carried out viscosity and sedimentation studies in DMF using poly( $\gamma$ -*p*-nitrobenzyl-L-glutamate), PNBG, samples with varying molecular weights. Secondly, the aim is to investigate whether these methods can shed some light on the nitroaromatic effect as proposed by Goodman et al.<sup>8</sup>

Two new efficient routes for the synthesis of NBG were developed. The effect of the solvent (dioxane, DMF, EDC–DMF, and nitrobenzene) and anhydride–initiator ratio (A/I) on the polymerization of NBG–NCA using triethylamine as an initiator were studied.

## EXPERIMENTAL\*

### Materials

All melting and boiling points are uncorrected. Infrared spectra were obtained on a Unicam S.P. 200. PMR spectra were determined on a Varian A-60 instrument, using tetramethylsilane (TMS,  $\delta$ 0.0) as internal standard.

#### *$\gamma$ -p-Nitrobenzyl-L-glutamate, NBG*

(a) **Via Goodman's Procedure.**<sup>8</sup> NGRB was obtained in 25% yield; m.p. 173–176°;  $[\alpha]_D^{25}$  19.7° ( $c = 0.46$ , in aqueous acetic acid, 94%). The

\* Microanalysis was carried out in the analytical section of our department under the supervision of Mr. W. Potman.

infrared spectrum showed the characteristic nitro peaks at 1530 and 1350  $\text{cm}^{-1}$ .

**(b) Via the Reaction of L-Glutamic Acid, *p*-Nitrobenzyl Alcohol, and *p*-Toluenesulfonic Acid.** L-Glutamic acid (7.3 g, 0.05 mole), *p*-nitrobenzyl alcohol (7.65 g, 0.05 mole), and *p*-toluenesulfonic acid monohydrate (9.5 g, 0.55 mole) were stirred and refluxed in benzene for 3 hr, while water was removed by azeotropic distillation. After cooling, ether (500 ml) was added and a light yellow solid (20.1 g, m.p. 125–145°) could be isolated after 15 min stirring.

This compound was stirred in chloroform (200 ml) for 10 min. After filtration 14.7 g (m.p. 150–156°) of a solid was obtained. Infrared and PMR spectra indicated that this solid was the *p*-toluenesulfonic acid salt of NBG. Decomposition of this salt was performed by the addition of triethylamine (4.61 ml, 0.032 mole) to a chloroform (60 ml) mixture of the salt at 0–5°C. After 15 min stirring, ether (400 ml) was added and the resulting solid was collected and crystallized from water (500 ml) using decolorizing charcoal. Almost pure NBG (6.1 g (43%), m.p. 170–171°C) was obtained.

Calculated values for  $\text{C}_{12}\text{H}_{14}\text{N}_2\text{O}_6$ : C 51.06; H 4.96; N 9.93. Values found: C 50.1; H 5.1; N 9.9.

Thin-layer chromatography, using *n*-butanol–acetic acid–water (4:1:5) as eluent, revealed the presence of a small amount of  $\alpha$ -ester.

Purification of the  $\gamma$ -ester was carried out by heating the ester (1.4 g, 5 mmole) in water (50 ml) with copper(II) acetate (1.2 g, 6 mmole) at 80°C for 15 min. A violet precipitate was formed and the resulting mixture was filtered by suction. The violet copper complex (1.5 g, dec. 209–210°C) was decomposed by treatment with boiling ethylene diaminetetraacetic acid (EDTA) solution in water (100 ml, pH 4.5). Filtration of the resulting solution and cooling gave a crystalline solid. After filtration and drying, the pure  $\gamma$ -ester (1.0 g, 71%), m.p. 177–178°C, Rf value 0.64 (*n*-butanol–acetic acid–water 4:1:5),  $[\alpha]_{\text{D}}^{25}$  20.5° ( $c = 0.63$  in aqueous acetic acid, 94%) was isolated.

Chromatography of the unpurified ester mixture furnished also the pure  $\alpha$ -ester (m.p. 165–166°C), Rf value 0.73 (*n*-butanol–acetic acid–water 4:1:5)  $[\alpha]_{\text{D}}^{25}$  11.1° ( $c = 0.2$  in aqueous acetic acid, 94%). The infrared spectrum of this compound showed peaks at 1530 and 1350  $\text{cm}^{-1}$  ( $\text{NO}_2$ ), 1720 and 1740  $\text{cm}^{-1}$  (C=O, ester and acid).

**(c) Via the Reaction of L-Glutamic Acid with *p*-Nitrobenzyl Alcohol and Concentrated Sulfuric Acid.** L-Glutamic acid (10.0 g, 0.068 mole) was dissolved in a solution of *p*-nitrobenzyl alcohol (10.4 g, 0.068 mole) and sulfuric acid (8.4 g, 80%) at 70°C. The solution was stirred for 3 hr. After cooling a crystalline mass was obtained. This mixture was dissolved in water (100 ml) and sodium bicarbonate (11.0 g) was added. The pH of the mixture was adjusted to 7. The resulting solid was filtered by suction, stirred with ether (500 ml), filtered by suction, and recrystallized from water (300 ml) using decolorizing charcoal. A light yellow solid (3.7 g, 19%) m.p. 173–175°,  $[\alpha]_{\text{D}}^{25}$  19.2° ( $c = 0.73$  in aqueous acetic acid, 94%)

was obtained. Thin-layer chromatography did not show the presence of any  $\alpha$ -ester. Concentration of the ether layer yielded *p*-nitrobenzyl alcohol (4.5 g).

Literature values for yields and physical constants of NBG are given here: 20% yield, corrected m.p. 171°,  $[\alpha]_D^{25}$  19.52° ( $c = 1.0$  in glacial acetic acid);<sup>8</sup> overall yield 27%, m.p. 171–172°,  $[\alpha]_D^{21}$  19.2° ( $c = 0.8$  in glacial acetic acid).<sup>16</sup>

#### *$\gamma$ -p-Nitrobenzyl-L-glutamate-N-carboxyanhydride, NBG-NCA*

In a three-necked flask equipped with a reflux condenser, a gas inlet tube, a magnetic stirrer, and a sodium hydroxide trap system,  $\gamma$ -*p*-nitrobenzyl-L-glutamate (3.0 g, 0.011 mole, m.p. 177–178°C, method (b)) was suspended in dioxane (60 ml). The system was kept at 40°C and phosgene was passed through the mixture. After 1 hr the mixture became clear and nitrogen was passed through for 3 hr. The solution was concentrated at 40°C/14 mm and the remaining light yellow oil was dissolved in ethyl acetate. This solution was stirred for 20 min with decolorizing charcoal, filtered, and *n*-hexane was added. A light yellow solid was obtained. The reprecipitation procedure was repeated twice.  $\gamma$ -*p*-Nitrobenzyl-L-glutamate-N-carboxyanhydride (2.1 g, m.p. 105–107°, dec) was isolated in 62% yield. The same experiment was carried out with  $\gamma$ -*p*-nitrobenzyl-L-glutamate (m.p. 173–176°, method (a)) giving a yield of 63% (m.p. 100–101°C dec).

Literature values: 63.9% yield, corrected m.p. 103–105°C (dec);<sup>8</sup> 91% yield, m.p. 104–106°C (dec).<sup>16</sup>

#### *Polymers*

The NBG-NCA (method (b)) having a m.p. 105–107° was used for the polymerization reaction in different solvents (samples N<sub>1</sub>–N<sub>5</sub>, Table I). The NCA derivative with m.p. 100–101° (method (a)) was used for the polymerization in nitrobenzene, with varying anhydride-initiator (A/I) ratios (N<sub>6</sub>–N<sub>10</sub>, Table I). Further experimental conditions are given in Table I.

Polymers N<sub>1</sub>, N<sub>2</sub>, and N<sub>5</sub> were obtained by filtration of the reaction mixture, N<sub>3</sub>, N<sub>4</sub>, and N<sub>6</sub>–N<sub>10</sub> by precipitation in ethanol and filtration or centrifugation of the resulting mixture. Purification was carried out either by dissolution in DMF and precipitation in ethanol or by treatment with ether and warm ethanol. The polymers were dried for several days at room temperature in a vacuum desiccator (0.05 mm).

#### *Solvents and Initiator*

The solvents were dried and purified as follows: Dioxane was purified according to Fieser's method,<sup>17</sup> b.p. 101°; nitrobenzene was dried over P<sub>2</sub>O<sub>5</sub>, b.p. 94.5/15 mm; DMF distilled from CaH<sub>2</sub>, b.p. 47°/14 mm; 1,2-dichloroethane, EDC, distilled from CaH<sub>2</sub>, b.p. 84°; dichloroacetic acid, DCA, was furnished by Schuchardt, b.p. 194°; ethanol was supplied by

TABLE I  
Experimental Conditions, Molecular Weights, and Results of Sedimentation and Viscosity Studies on PNBG Samples

Sample code	Polymerization solvent	Reaction time, hr	Reaction Conc NCA, mg/ml	A/I	Yield, %	$M_w$	$M_z$	$M_z/M_w$	$[\eta]^a$ in DCA	$[\eta]^a$ in DMF	$S_0^{20}$ in DMF <sup>a</sup>
N <sub>1</sub>	Dioxane	72	46.6	50	64	49,600	79,000	1.6	0.325	0.480	4.6
N <sub>2</sub>	Dioxane	65	35.0	50	64	44,000	67,000	1.5	0.228	0.246	4.1
N <sub>3</sub>	Nitrobenzene	72	46.6	50	77	38,000	71,000	1.9	0.284	0.391	3.5
N <sub>4</sub>	DMF	120	35.0	50	69	22,000	38,000	1.7	0.179	0.130	2.9
N <sub>5</sub>	EDC-DMF	65	13.3	50	69	43,400	38,000	0.9	0.185	0.284	4.0
N <sub>6</sub>	Nitrobenzene	150	33.3	50	70	38,100	33,000	0.9	0.243	0.282	3.5
N <sub>7</sub>	Nitrobenzene	150	33.3	100	60	27,000	33,000	1.2	0.192	0.200	3.7
N <sub>8</sub>	Nitrobenzene	150	33.3	200	25	15,300	20,400	1.3	0.146	0.110	—
N <sub>9</sub>	Nitrobenzene	150	33.3	400	55	11,000	14,000	1.3	0.114	0.055	2.0
N <sub>10</sub>	Nitrobenzene	150	33.3	750	25	10,000	21,000	2.1	0.091	0.049	—

<sup>a</sup>  $[\eta]$  expressed in dl/g,  $S_0^{20}$  in Svedberg units.

Merck, b.p. 78–79°; triethylamine was refluxed with sodium for 8 hr and distilled from sodium, b.p. 89°.

Two initiator (triethylamine) solutions in dioxane were used: for  $N_1$ – $N_5$  0.25 mmole/ml and for  $N_6$ – $N_{10}$  0.05 mmole/ml. Among solvents as benzene, ethanol, chloroform, carbontetrachloride, anisyl alcohol, acetone, nitrile, dimethylsulfoxide, dimethylformamide, nitrobenzene, and dichloroacetic acid only the last three can be used for the dissolution of PNBG.

### Methods

**Viscosity.** Measurements were performed in Ubbelohde capillary-type viscometers at  $25 \pm 0.01^\circ\text{C}$  using DCA and DMF solutions.

**Ultracentrifugation.** Molecular weights ( $M_z$ <sup>18</sup> and  $M_w$ <sup>19</sup>) were determined by sedimentation diffusion equilibrium methods using short columns in a Spinco Beckman Model E ultracentrifuge, equipped with Schlieren optics and electronic speed control. Measurements were carried out using DMF as a solvent at  $20^\circ\text{C}$ . The partial specific volume  $\bar{v}$  of PNBG in DMF at  $20^\circ\text{C}$  was  $0.732 \pm 0.005$  ml/g determined pycnometrically.

The  $S$  values at zero concentration ( $S_0$ ) were obtained by extrapolating  $1/S$  to zero concentration ( $c$ ), according to the equation

$$1/S = 1/S_0 (1 + k_s c) \quad (1)$$

where  $k_s$  is a parameter independent of  $c$ . Measurements were made at  $20 \pm 0.01^\circ\text{C}$ .

## RESULTS

### Synthesis of Poly( $\gamma$ -*p*-Nitrobenzyl-L-glutamate), PNBG

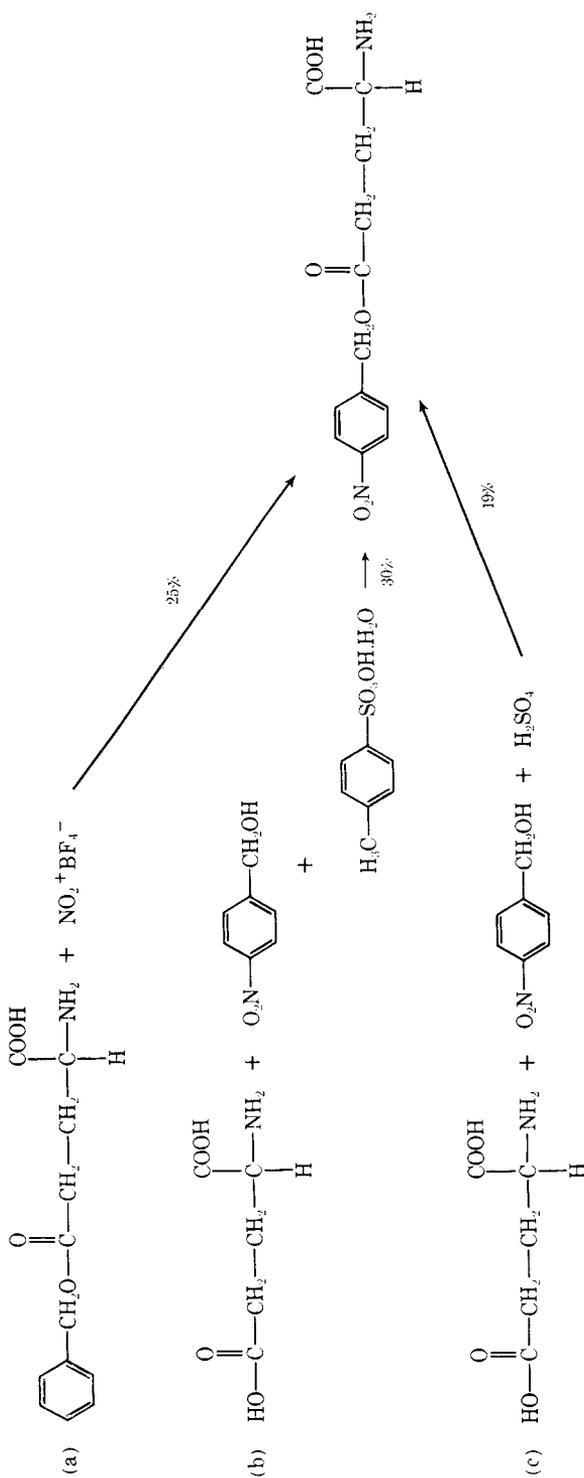
$\gamma$ -*p*-Nitrobenzyl-L-glutamate (NBG) was synthesized via Goodman's procedure<sup>8</sup> (a) and via to new routes (b) and (c) as depicted in scheme I. The crude product obtained via method (b) was purified via the copper complex. A small amount of  $\alpha$ -ester (m.p. 165–166°,  $[\alpha]_D^{25}$  11.1° ( $c = 0.2$  in aqueous acetic acid, 94%) was isolated and characterized.

Method (b), a one-step synthesis, turned out to be more attractive for the preparation of NBG than the procedures reported by Goodman et al.<sup>8</sup> and Ledger et al.<sup>16</sup> NBG (m.p. 177–178°,  $[\alpha]_D^{25}$  20.5°,  $c = 0.63$  in aqueous acetic acid, 94%), was obtained in an overall yield of 30%. NBG, synthesized via methods (a) and (b) was treated with phosgene in dioxane in the usual way to give the *N*-carboxyanhydride (NCA) derivative. NBG–NCA was polymerized using triethylamine as an initiator under the conditions given in Table I.

Depending on the solvent and anhydride–initiator ratio (A/I) used, weight-average molecular weights ( $M_w$ ) varying from 10,000 to 49,600 were obtained.

When  $\log A/I$  was plotted versus  $\log M_w$ , we obtained a straight line (Fig. 1) according to the equation

$$\log A/I = -2 \log M_w + 10.9 \quad (2)$$



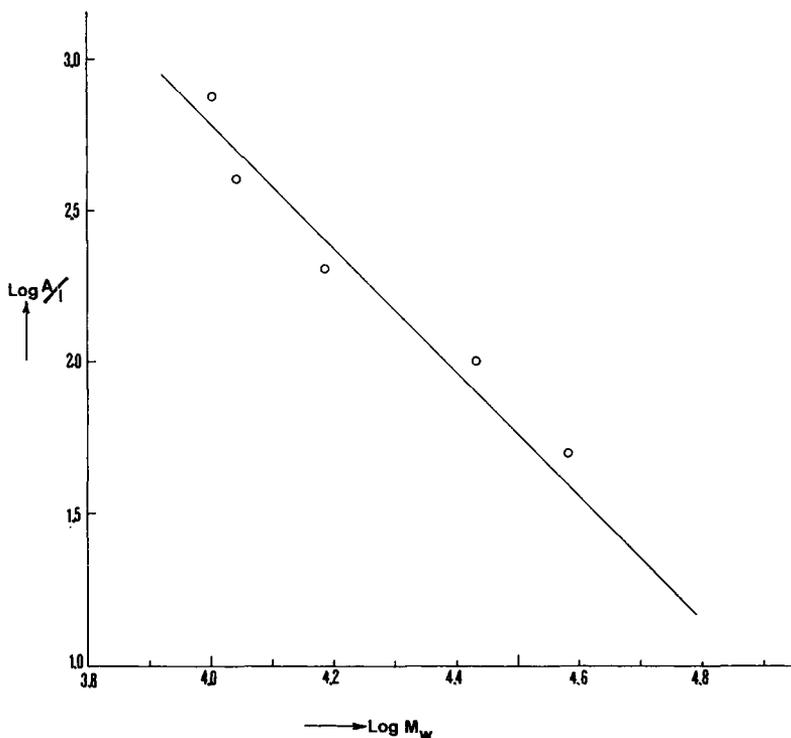


Fig. 1. Plot of  $\log A/I$  versus  $\log M_w$ .

### Viscosity

The results from the viscosity measurements are summarized in Table I. Values of  $[\eta]$  were obtained by plotting  $\eta_{\text{spec}}/c$  against  $c$  and extrapolating the resulting curve to infinite dilution, where  $\eta_{\text{spec}}$  is the specific viscosity and  $c$  is the polymer concentration in grams per deciliter.

Figure 2 shows the double logarithmic plot for  $[\eta]$  of PNBG in DCA at 25°C against  $M_w$ . The relation

$$[\eta] = 1.15 \times 10^{-4} M_w^{0.72} \quad (3)$$

holds over the range of  $M_w$  investigated.

A plot for  $\log [\eta]$  of PNBG in DMF at 25°C against  $\log M_w$  according to the relation

$$[\eta] = 10^{-6.76} \times M_w^{1.36} \quad (4)$$

has been given in Figure 3.

A double logarithmic plot for  $[\eta]$  of PNBG in DCA at 25°C against the values for  $[\eta]$  in DMF (Fig. 4) gave a straight line, indicating that the viscosity values obtained are quite consistent.

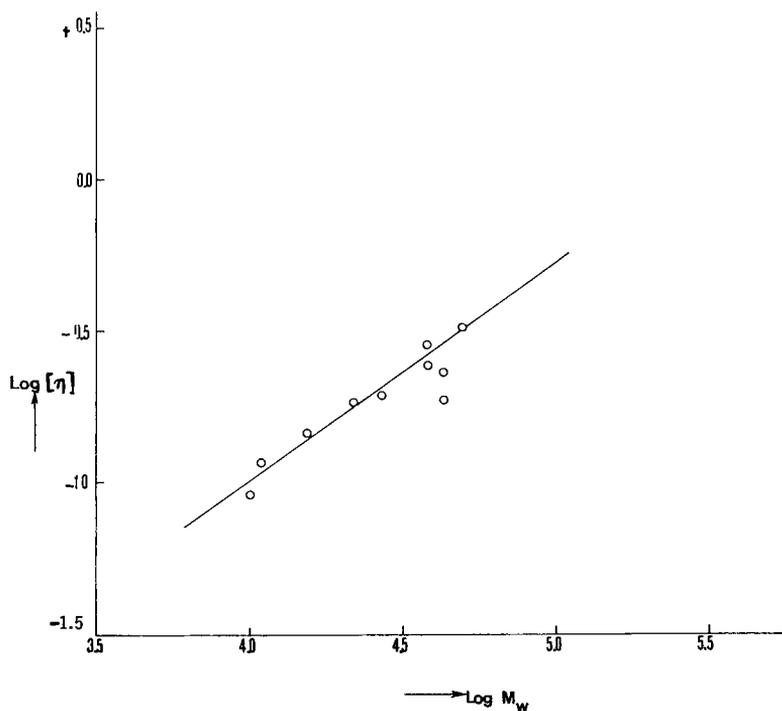


Fig. 2. Plot of  $\log [\eta]$  ( $[\eta]$  in dl/g) in DCA versus  $\log M_w$  of PNBG.

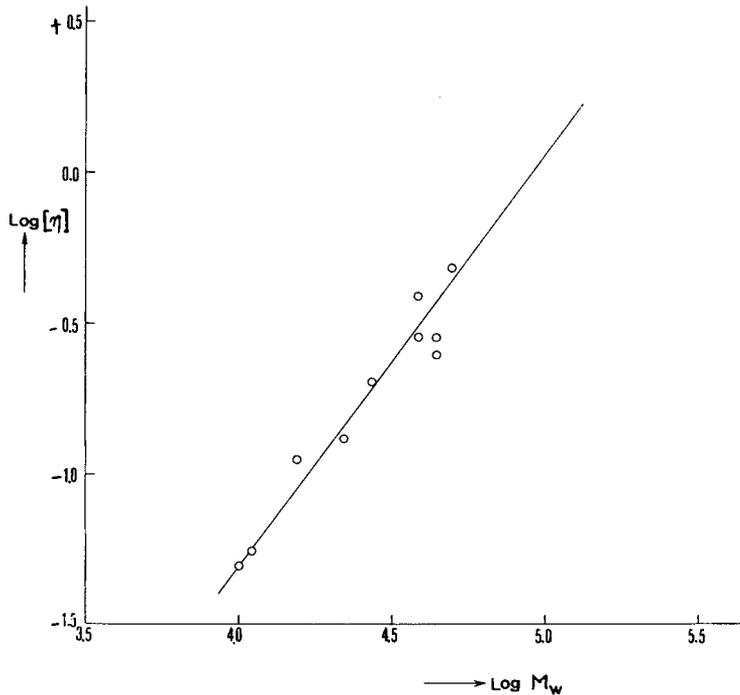


Fig. 3. Plot of  $\log [\eta]$  ( $[\eta]$  in dl/g) versus  $\log M_w$  of PNBG, both measured in DMF.

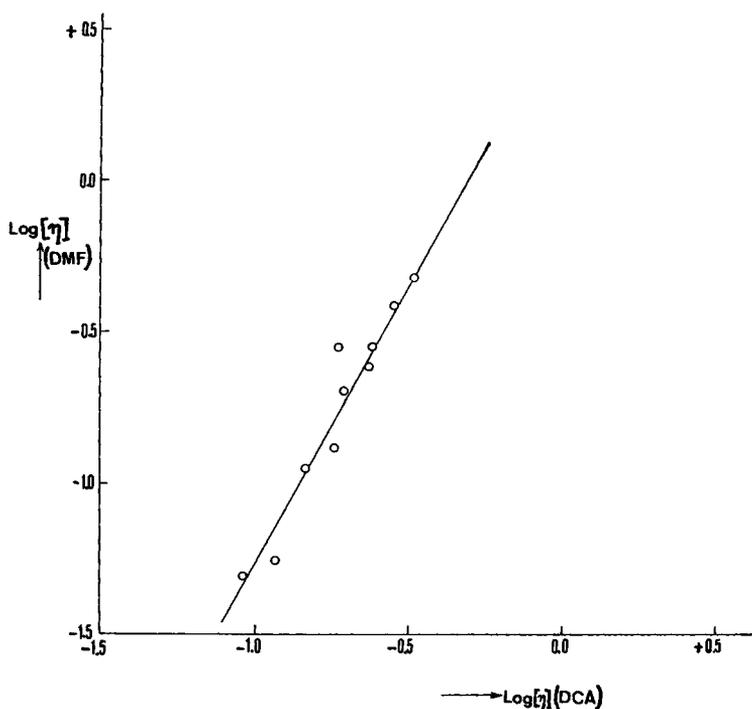


Fig. 4. Plot of  $\log [\eta]$  in DMF versus  $\log [\eta]$  measured in DCA of PNBG,  $[\eta]$  being expressed in dl/g.

### Sedimentation

The data for  $S$  as a function of  $c$  fitted the empirical relation

$$1/S = (1/S_0) (1 + k_s c) \quad (1)$$

where  $k_s$  is a positive parameter independent of  $c$ . The  $S_0$  values are given in Table I.

$S_0$  varies linearly with  $\log M_w$  (Fig. 5) according to the equation

$$S_0 = 3.53 \log M_w - 12.3 \quad (5)$$

where  $S_0$  is expressed in Svedberg units.

Pressure effects were assumed to be neglectable according to Fujita et al.<sup>11</sup>

## DISCUSSION

### Synthesis

From Table I we can conclude that it is possible to obtain PNBG by polymerization in dioxane with  $M_w$ 's up to 49,600. The highest  $M_w$  reported in the literature<sup>12</sup> reached a value of 19,000.

The effect of the A/I ratio on the  $M_w$  of PNBG was studied by the polymerization of PNBG-NCA in nitrobenzene, keeping the anhydride concentration (33 mg/ml) constant and varying the amount of initiator. The plot of  $\log A/I$  versus  $\log M_w$  is a straight line (Fig. 1) having a negative slope. A similar effect was observed by Ballard and Bamford<sup>20</sup> in the polymerization of DL-phenylalanine-NCA in nitrobenzene using tri-*n*-butylamine as an initiator. These authors proposed a mechanistic scheme and derived complex kinetic equations for this polymerization reaction.

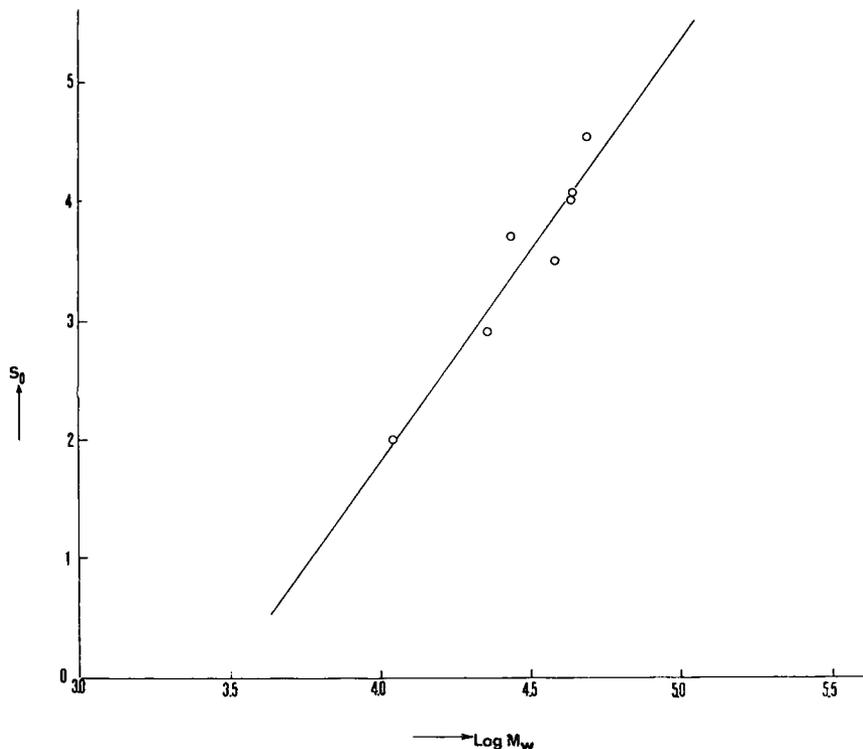


Fig. 5. Plot of  $S_0$  versus  $\log M_w$  both measured in DMF;  $S_0$  in Svedberg units.

The fall-off in molecular weight at increasing A/I ratios, starting from A/I = 45, was attributed to the formation of 3-hydantoinylacetic acid derivatives. These acids are responsible for the neutralization of the initiator, leading to conversions less than 100%, and the inhibition of initiator catalyzed coupling reactions. In the case of PBLG, Blout and Karlson<sup>21</sup> found  $M_w$  to be nearly independent of the A/I ratio, although for A/I ratios higher than 200 also a slightly decrease in  $M_w$  could be observed for the polymerization in dioxane using triethylamine as an initiator.

For a quantitative treatment of these phenomena, however, more detailed kinetic studies of the influence of different types of termination reactions in various solvents are needed.

### Hydrodynamic Properties

Equation (3) was obtained by the use of viscosity data for PNBG in DCA given in Table I and Figure 2.

A similar relation for PBLG was obtained by Doty et al.<sup>3</sup>

$$[\eta] = 2.78 \times 10^{-5} M_w^{0.87} \quad (6)$$

As can be seen this relation cannot be used for the determination of  $M_w$ 's up to  $10^5$  of *substituted* poly( $\gamma$ -benzyl-L-glutamates) from viscosity data.<sup>22</sup> The exponent of  $M_w$  in equation (3), being 0.72, indicates that just as in the case of PBLG, PNBG exists as a random coil in DCA.

When PNBG is dissolved in DMF, however, the exponent of the similar equation (4) becomes 1.36. We therefore expect the shape of a PNBG molecule in DMF to be rodlike. In accordance with Scheraga and Mandelkern<sup>23</sup> we approximate this cylindrical shape by a rigid prolate ellipsoid of revolution. In that case we may apply theoretically derived equations for the  $[\eta]$  as functions for the axial ratio  $p$ , defined as the ratio between the major semi axis  $a$  and the minor semi axis  $b$  of this prolate ellipsoid.

Kuhn and Kuhn's<sup>13</sup> equations for the relation between  $[\eta]$  and  $p$  read:

$$\frac{100}{\bar{v}} [\eta] = 2.5 + 0.4075 (p-1)^{1.508} \quad (1 \leq p < 15) \quad (7)$$

$$\frac{100}{\bar{v}} [\eta] = 1.6 + \frac{p^2}{5} \left[ \frac{1}{3(\ln 2p - 1.5)} + \frac{1}{\ln 2p - 0.5} \right] \quad (p > 15) \quad (8)$$

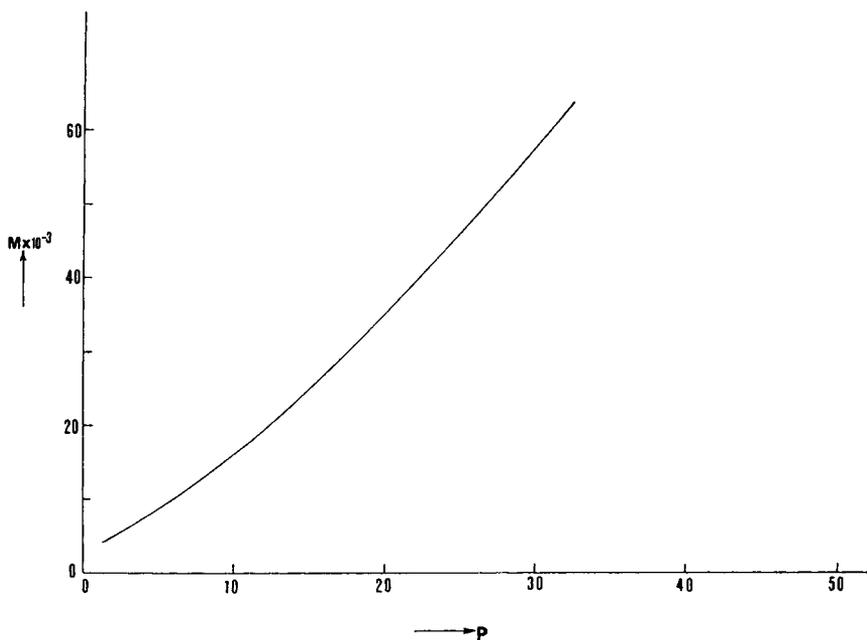


Fig. 6. Plot of  $M_w$  versus the axial ratio  $p$ , from viscosity data in DMF.

where  $[\eta]$  is expressed in dl/g, and  $\bar{v}$ , the partial specific volume of the polymer, in  $\text{cm}^3/\text{g}$ .

These relations were used because they also hold in the case of not very elongated ellipsoids ( $1 < p < 15$ ). Equations (7) and (8), relating  $[\eta]$  to  $p$ , together with our experimentally found relation between  $[\eta]$  and  $M_w$  (equation (3)), define  $M_w$  as a function of  $p$ .  $M_w$  as a function of  $p$ , thus calculated for PNBG, is shown in Figure 6.

We see that for large values of  $p$  ( $p > 10$ ), the value of  $M/p$  reaches a constant value ( $M/p = 2100$ ). For rodlike particles this indicates that the diameter is independent of the length.

With the relations:

$$\frac{h}{M_0} = \frac{2b}{M/p} \quad (9)$$

and

$$M = \frac{4}{3} \pi b^3 p \frac{1}{\bar{v}} N_A \quad (10)$$

(where  $M_0$  = molecular weight of monomer residue,  $M$  = molecular weight of polymer,  $N_A$  = Avogadro's number), we may calculate the semiminor axis  $b$  and the length per monomeric unit  $h$ , when  $M/p$  is known. The results for  $b$  and  $h$  for PNBG are presented in Table II. We have applied the same procedure to viscosity data for PBLG, reported by Fujita et al.<sup>11</sup>

Contrary to these authors we have not assigned arbitrary values to  $M/p$  in order to calculate  $h$ , since handling the data in our way leads straightforwardly to both  $h$  and  $M/p$ . The values thus found for  $a$ ,  $h$ , and  $M/p$  are also given in Table II.

According to Benoit et al.<sup>24</sup> the accuracy in the value of  $h$  obtained viscometrically is about 15%, provided that the samples used are monodisperse. Since this is not the case we expect the experimental value of  $h$  to deviate from the real one.

TABLE II  
Comparison of Dimensions of PNBG and PBLG\*

	PNBG	PBLG
Experimental equation for sedimentation	$S_0 = 3.5 \log M_w - 12.3$	$S_0 = 1.7 \log M_w - 5.8$
$b$	11.1 Å	9.2 Å
$h$	1.5 Å	1.7 Å
$M/p$	4,000	2,350
Experimental equation for viscosity	$\log [\eta] = -6.76 + 1.36 \log M_w$	$\log [\eta] = -8.72 + 1.69 \log M_w$
$b$	9.0 Å	7.5 Å
$h$	2.3 Å	2.4 Å
$M/p$	2,100	1,330

\* As calculated from results reported by Fujita et al.<sup>11</sup>

In the case of sedimentation, Perrin<sup>16</sup> has derived the following equation for  $S_0$  as a function of  $p$ :

$$S_0 = 0.222 \frac{1 - \bar{v}d}{\bar{v}\eta} b^2 \frac{p}{\sqrt{p^2 - 1}} \ln (p + \sqrt{p^2 - 1}) \quad (11)$$

where  $\eta$  is the viscosity (in poise) and  $d$  the density of the solvent (in g/cm<sup>3</sup>). When  $p > 3$ , we may replace this equation according to Schachman<sup>14</sup> into

$$S_0 = 0.222 \frac{1 - \bar{v}d}{\bar{v}\eta} b^2 \ln 2p \quad (12)$$

with an error in  $S_0$  of less than 3%. According to Fujita et al.,<sup>11</sup> equation (12) still holds if the difference in polydispersity of individual samples is not too great (Table I shows this is indeed the case).

Writing this equation in the form:

$$S_0 = 0.222 \frac{1 - \bar{v}d}{\bar{v}\eta} b^2 \left( \ln M - \ln \frac{M}{2p} \right) \quad (13)$$

and comparing with our experimentally found relation between  $S_0$  and  $M$  (eq. (5)) allows the calculation of both  $b^2$  and  $\log M/p$ . Since the error in the values of  $b^2$  and the  $\log M/p$  thus found is about 10%, we expect to find a rather accurate value for the semi minor axis  $b$ , but only an order of magnitude of  $M/p$ . The value of  $b$ , being known however, we may use equation (10) to calculate a more reliable value for  $M/p$ . From equation (9) we obtain the value of  $h$ .

This value of  $h$  is only slightly sensitive to heterodispersity and we therefore expect it to approach the real value.

Similar results for PBLG were obtained in the same way using experimental data found by Fujita et al.<sup>11</sup> From Table II we can see that the  $h$  values for PBLG and PNBG from viscosity data are significantly higher than those obtained from sedimentation measurements. One of the reasons for this discrepancy might be the degree of heterodispersity of the samples used. The degree of heterodispersity of our samples is on the same order as Fujita's samples.

The  $h$  values obtained from viscosity data are not much different (PNBG 2.3 Å and PBLG 2.4 Å).

In the case of sedimentation we can see that the introduction of a *p*-nitro substituent in PBLG increases the diameter  $2b$  from 18.4 to 22.2 Å. As mentioned in the introduction the  $h$  value of PNBG in the solid phase increases slightly as compared to PBLG. However, comparing the  $h$  values in DMF solution shows an opposite effect;  $h$  for PNBG 1.5 Å and for PBLG 1.7 Å.

Both viscosity and sedimentation measurements show that there is a tendency of decreasing  $h$  going from PBLG to PNBG in DMF solution. It seems therefore that in DMF, PNBG has a somewhat more compact structure than PBLG. In the case of the aspartate series the tendency of poly-( $\beta$ -benzyl-D- (or -L-)aspartates) to be in the helix configuration in DMF increases with increasing nitro substitution.<sup>8,25,26</sup> No definite explanation for this feature has been given in the literature.

Fraser et al.<sup>10</sup> have reported that in the glutamate series the PNBG helix is less stable than the PBLG helix in EDC-DCA mixtures. A nitroaromatic effect was demonstrated when PNBG was dissolved in EDC-DCA mixtures. However, this effect was absent for PNBG in DMF.

It should be most interesting to study the influence of solvents on the order of stabilities of helices going from PBLG to PNBG. If in analogy with the aspartate series in DMF, the PNBG helix should be more stable than the PBLG helix, we can conclude that an increase of stability is accompanied by a decrease in  $h$  value. Since a nitroaromatic effect has been shown to be absent for PNBG in DMF solution, we then can also conclude that the occurrence of a nitroaromatic effect cannot be correlated with changes in  $h$  values and stabilities in a straightforward way.

### References

1. C. H. Bamford, A. Elliott, and W. E. Hanby, in *Synthetic Polypeptides*, Academic Press, New York, 1956, p.55.
2. E. Katchalski and M. Sela, *Adv. Protein Chem.*, **13**, 243 (1958).
3. P. Doty, J. H. Bradbury, and A. M. Holtzer, *J. Am. Chem. Soc.*, **78**, 947 (1956).
4. E. R. Blout and A. Asadourian, *J. Am. Chem. Soc.*, **78**, 955 (1956).
5. D. W. Clayton, G. W. Kenner, and R. C. Sheppard, *J. Chem. Soc.*, **1956**, 371.
6. St. Guttman and R. Boissonas, *Helv. Chim. Acta*, **41**, 1852 (1958).
- 7a. J. Noguchi, Japan Pat. 13976 (1962).
- 7b. T. Hayakawa, H. Nishi, J. Noguchi, S. Ikeda, T. Yamashita and T. Isemura, *Nippon Kagaku Zasshi*, **82**, 601 (1962).
- 7c. S. Sugai, K. Kamashima, S. Makino and J. Noguchi, *J. Polymer Sci.*, [A-2], **4**, 183 (1966).
8. M. Goodman, A. M. Felix, C. M. Deber, A. R. Brause, and G. Schwartz, *Biopolymers*, **1**, 371 (1963).
9. D. A. D. Parry and A. Elliott, *J. Mol. Biol.*, **25**, 1 (1967).
10. R. D. B. Fraser, B. S. Harrap, R. Ledger, T. P. Macrae, F. H. C. Stewart, and E. Suzuki, *Biopolymers*, **5**, 797 (1967).
11. H. Fujita, A. Teramoto, T. Yamashita, K. Okita, and S. Ikeda, *Biopolymers*, **4**, 781 (1966).
12. G. Spach, *Compt. Rend.*, **249**, 543 (1959).
13. W. Kuhn and H. Kuhn, *Helv. Chim. Acta*, **28**, 97 (1945).
14. A. R. Peacocke and H. K. Schachman, *Biochim. Biophys. Acta*, **15**, 198 (1954).
15. F. Perrin, *J. Phys. Radium*, **7**, 1 (1936).
16. R. Ledger and F. H. C. Stewart, *Austral. J. Chem.*, **18**, 1477 (1965).
17. L. F. Fieser, *Experiments in Organic Chemistry*, 2nd ed., D. C. Heath & Co., Boston, Mass., 1941, p. 361.
18. K. E. van Holde and R. L. Baldwin, *J. Phys. Chem.*, **62**, 734 (1958).
19. O. Lamm, *Arkiv. Mat. Astron. Fysik*, **21B**, no. 2 (1929).
20. D. G. H. Ballard and C. H. Bamford, *J. Chem. Soc.*, **1956**, 381.
21. E. R. Blout and R. H. Karlson, *J. Am. Chem. Soc.*, **78**, 941 (1956).
22. R. Ledger and F. H. C. Stewart, *Austral. J. Chem.*, **19**, 1729 (1966).
23. H. A. Scheraga and L. Mandelkern, *J. Am. Chem. Soc.*, **75**, 179 (1953).
24. H. Benoit, L. Freund, and G. Spach, in *Poly- $\alpha$ -amino acids*, Series I, G. D. Fasman, Ed., Marcel Dekker, New York, 1967, p. 105.
25. D. F. Bradley, M. Goodman, A. Felix, and R. Records, *Biopolymers*, **4**, 607 (1966).
26. M. Hashimoto and S. Arakawa, *Bull. Chem. Soc. Japan*, **40**, 1698 (1967).

Received February 16, 1971

Revised April 29, 1971