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VISCOSITY MOLECULAR WEIGHT DETERMINATION OF POLYADENYLIC ACID

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ABSTRACT

In this study viscosity measurements of polyadenylic acid (PolyA) in aqueous solution were carried out under different conditions. In the absence of any additives, the polymer degraded during flow through the capillary of a viscometer or when standing still. Degradation during the former was more severe. The degradation of polyadenylic acid can be prevented by addition of an electrolyte such as KCl to increase the ionic strength. However, in this case the deviation from linearity was still considerable at most ionic strength values. The best fit to the Huggins and Kraemer equations was obtained using a Tris-EDTA buffer solution with a final pH of 7.65. Estimation from intrinsic viscosity and weight-average molecular weight values gave κ and α as 2.04×10^{-5} and 0.89 from the equation $\eta = \kappa M^{\alpha}$. The difference between Huggins (k_1) and Kraemer (k_1') constants was close to 0.50 for all measurements.

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INTRODUCTION

Polyadenylic acid (PolyA) is a homopolymer of RNA containing only adenine as a base unit. The main chain of macromolecules is a linear polymer of nucleotides formed by phosphodiester linkages between the 5′-phosphate of one nucleotide and the 3′-hydroxyl group of the sugar of the adjacent one. Thus, nucleotides in PolyA consist of an adenine base linked to a sugar (ribose) and phosphate esterified to the sugar at carbon 5′ (Fig. 1). Esterification may also occur at carbon 2′ or 3′. In general, most eucaryotic *m*RNAs are terminated by a PolyA tail, 40 to 200 residues in length. The function of the PolyA tail is unknown; however, it may act to stabilize the *m*RNA. Several x-ray diffraction studies of PolyA structure have been reported [1, 2], and the one suggested by Rich et al. [1] is now generally accepted. In this structure, PolyA forms a parallel double helix with pairs of adenine hydrogen bonded via a pair of N—H bonds at pH $\leq$ 6. The free amino protons can form a hydrogen bond with phosphate oxygen from the opposite strand, thereby causing the backbone to be drawn toward the helix axis. Thus, the conformation of PolyA in aqueous solution depends greatly on the solution pH. Witz and Luzzati [3] showed that PolyA molecules appear to be rodlike in shape from pH 5.0 to 7.2. The molecule aggregation is observed to be ionic at pH 5.0 to 7.2. Molecular aggregation is observed at an ionic strength of 0.1 and pH $\leq$ 5 [4]. When the ionic strength is reduced to 0.01, the marked reduction of aggregation shows the competitive formation of helices by an intramolecular process [5]. The radius of gyration decreases under these conditions. The aggregation of PolyA observed with temperature and

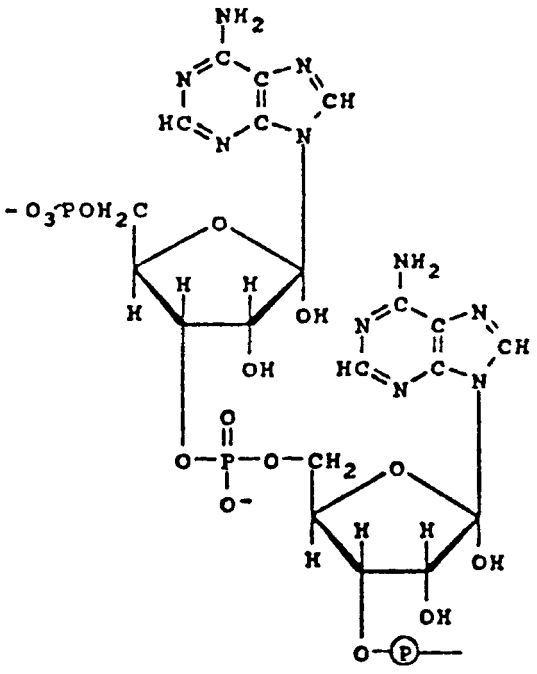

FIG. 1. The repeat unit of PolyA.

ionic strength (e.g., 0.5–4.2 mM Na^+) is attributed to reduced repulsion between phosphate groups and at high temperature to the liberation and reassociation of hydrophobic groups in micelles [6].

Viscosity measurements give very important information about the aggregation, association, and conformation of macromolecules in solution. Therefore, the solution behavior of polyelectrolytes has received much attention for many years [7–13]. In this respect, the study of biopolymers in aqueous solution becomes even more important. Thus, the structure of PolyA described above will determine the characteristic behavior of the viscosity of polyelectrolytes solutions, especially its dependence on added salt concentration, change in solution pH, and the solution concentration of PolyA. For nonassociating systems the viscosity behavior is well represented by

$$\eta_{sp}/c = [\eta] + k_1[\eta]^2 c \qquad \text{(Huggins equation)}$$

$$\ln \eta_r/c = [\eta] + k_1'[\eta]^2 c \qquad \text{(Kraemer equation)}$$

These equations are limiting expressions which should be strictly valid as infinite dilution is approached. The common intercept of both equation is at $c = 0$ and $k_1 - k_1' = 0.5$. However, since the intrinsic viscosity $[\eta]$ is a measure of the shape and size of isolated macromolecules, deviation from linearity is observed for polyelectrolytes under some conditions when η_{sp}/c or $\ln \eta_r/c$ is plotted against c. These conditions may be an association of macromolecules, low ionic strength, adsorption of the polymer onto the walls of the viscometer, etc. In order to obtain linear behavior, the right solution conditions have to be satisfied.

This study is part of our work on the strand-breaking behavior by radiation of PolyA and DNA in aqueous solution. The solution properties of PolyA as determined by viscosity measurements have not been reported before. We intend to evolve a standard procedure for the molecular weight determination of PolyA by a viscosity method and to find the viscosity–molecular weight parameters.

EXPERIMENTAL

Materials

PolyA samples were obtained from Boehringer Mannheim GmbB and Pharmacia. They were used without further purification.

Water was triple distilled by a conventional method using $KMnO_4$ and $K_2Cr_2O_7$.

The buffer solution was a solution of 0.0040 M tris(hydroxymethylaminomethane) and 0.002 M EDTA in triple distilled water. The pH of this solution was 9.65. It was adjusted to 7.8 by addition of glacial acetic acid. The pH of PolyA solutions used for the viscosity measurements was 7.65.

Procedure

The viscosities of PolyA solutions were measured in Tris-EDTA buffer solution at 25°C by using an Ubbelohde-type glass viscometer with a capillary length of 24 cm. The efflux time of the solvent was 179.4 seconds.

Weight-average molecular weights, M_w, were determined with a low angle (6–7°) laser light-scattering photometer (chromatix KMX-6) equipped with a He-Ne laser source ($\lambda = 632.8$ nm). For the molecular weights of the PolyA used in this study, the intensity of the scattered light is independent of the shape of the molecules at the small forward scattering angle used ($\theta = 6$–$7°$). The relationship between M_w and the excess Rayleigh factor, R_θ, is

$$\frac{Kc}{R_\theta} = \frac{1}{M_w} + 2A_2C$$

where c is the polymer concentration in $g \cdot cm^{-3}$, A_2 is the second virial coefficient, and K is the optical constant for the polymer, defined as

$$K = \frac{2\Pi^2 n^2}{\lambda^4 N_A}\left(\frac{dn}{dc}\right)^2 [1 + \cos^2\theta]$$

where n is the refractive index of the solution (1.33) at the incident wavelength λ, dn/dc is the specific refractive index increment, N_A is Avogadro's number, and θ is the angle of scattered light collection. Since for PolyU [14] all single- and double-stranded DNA [15, 16] have the same dn/dc value of 0.166 ± 0.003 M under our solvent conditions, it appears justified to also use this value for PolyA. Furthermore, the molecular weights of PolyA obtained using this value were in excellent agreement ($\pm 5\%$) with those obtained by sedimentation analysis [17]. For the light-scattering measurements, the polymer solutions contained 0.5 M KCl + 1×10^{-2} M tris(hydroxymethylaminomethane) buffer. At the high salt concentrations used, the second virial coefficient, A_2, approaches zero and the plot of Kc/R_θ vs c is parallel to the X axis [17, 18].

RESULTS AND DISCUSSION

The viscosity of PolyA was first determined in a triple distilled aqueous solution without any additives. The pH of the PolyA aqueous solution was 6.9. The flow time against the time interval of measurements is plotted in Figs. 2 and 3. Initially (first 50 minutes) there is a small increase in flow time (Fig. 2), then a sharp decrease up to 150 minutes. The rate of decrease in flow time slows down up to about 200 minutes, then increased again. In a time interval of 235 minutes the flow time decreased from 211.4 to 209.8 minutes when the determination was repeated with the same solution. The results are plotted in Fig. 3. The initial flow time of 200 seconds decreased to 181.8 seconds in 300 minutes. The final flow time of the solvent is 179.4 seconds. Thus, the decrease of flow time is severe when the solution is run through the capillary after 24 hours of solution preparation. This shows a strand break of PolyA in an aqueous solution which contains no stabilizer at a high ionic strength of solution or at other pH values. When the solution is not stirred, degradation is slower (e.g., in 24 hours, the flow time decreased from 209.8 to 200 seconds), but it accelerates with stirring (e.g., in 300 minutes the flow time decreased from 200 to 181.8 seconds). Similar behaviors were also observed for different DNA samples. These behaviors have been reported for other bipolymers (e.g., DNA; see also Ref. 19).

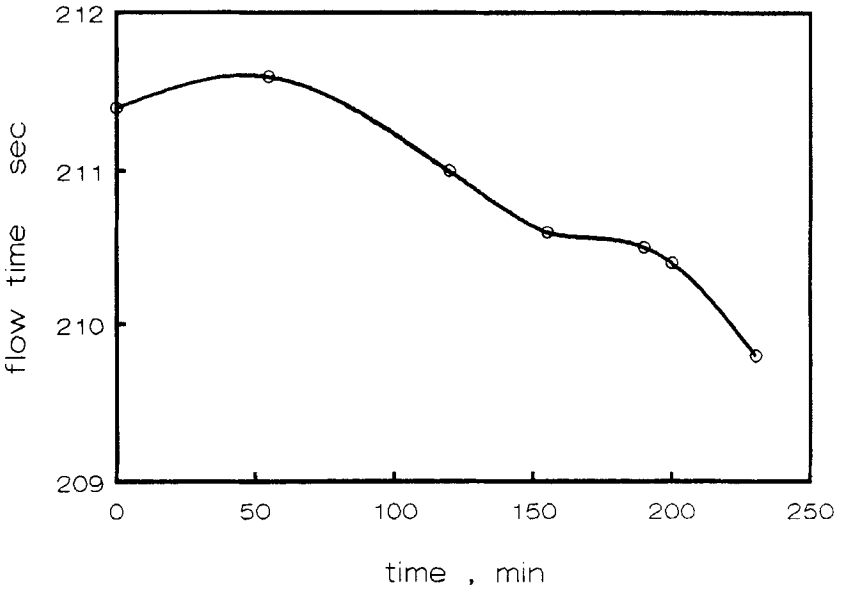

FIG. 2. Flow time of aqueous PolyA solution at 25°C.

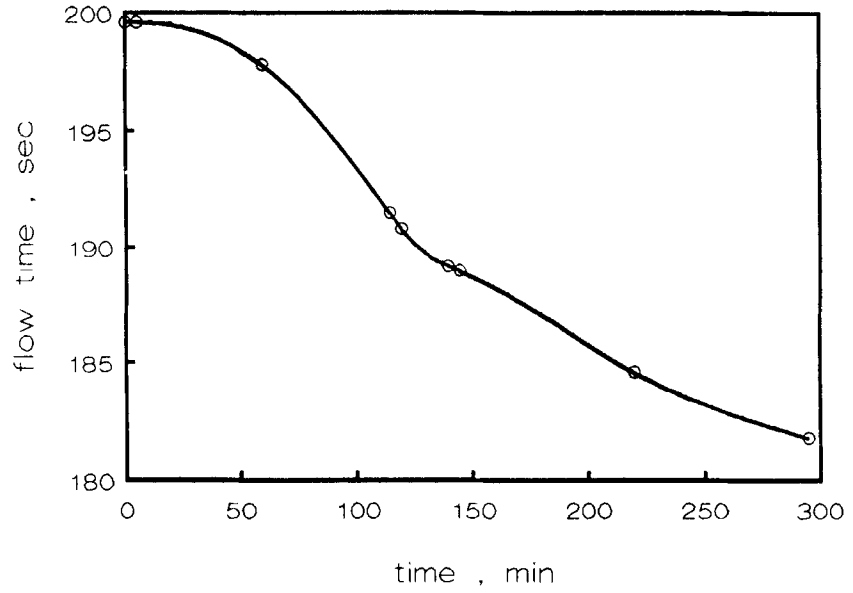

FIG. 3. Flow time of aqueous PolyA solution after 24 hours interval.

In order to avoid chain degradation during viscosity measurements in aqueous solution, some modifications of the experimental conditions were tried. Such modifications as changing the capillary diameter, changing the length of the capillary, and increasing the number of bulbs to four with different sizes on the viscometer did not show much improvement in experimental results.

The next modifications of measurement conditions were made on the ionic strength of the solution and the initial concentration of the polymer. In this case, chain degradation was not observed. The η_{sp}/c against polymer concentration curves are plotted in Figs. 4–6 for initial polymer concentrations of 0.01875, 0.02817, and 0.03750 g/dL and in the presence of KCl with concentrations ranging from 0.05 to 0.30 M. Deviations from linearity are observed for almost all conditions, but they are generally not a function of initial polymer concentration and do not show a regular trend with changes of ionic strength. Although ionic strength has a pronounced effect on intrinsic viscosity values and shows some improvement in the shape of the curves, the viscosity-average molecular weight determination cannot be measured under these conditions.

The last modification in experimental conditions was to change the solution pH in addition to the ionic strengths. After many trial with different buffer systems, the best results were obtained when a Tris-EDTA buffer solution was used for viscosity measurements. Plots of η_{sp}/c against c and of ln η_r/c against c for some standard samples are given in Fig. 7. Huggins and Kraemer constants (k_1 and k_1') are tabulated in Table 1. The weight-average molecular weights determined by the light-scattering method are also given in Table 1. The kinetic energy correction, calculated for viscosity measurements according to a procedure suggested by Craig

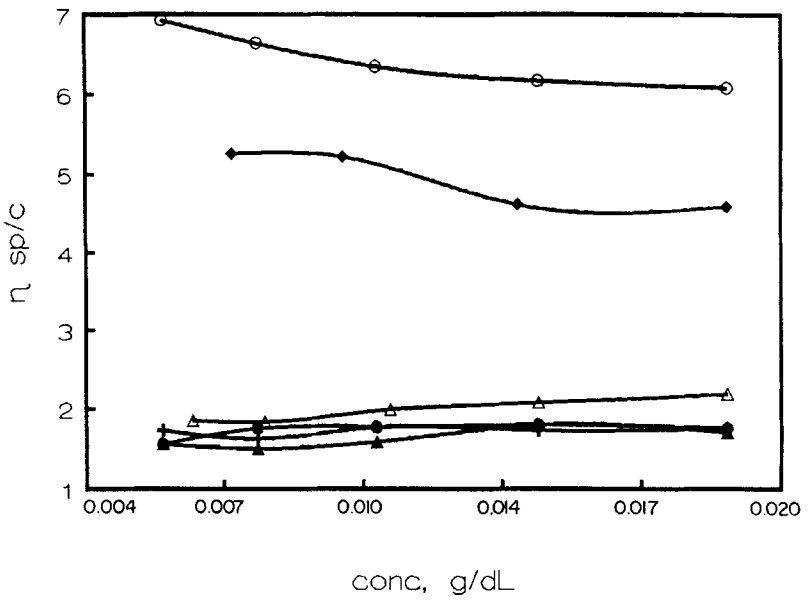

FIG. 4. η_{sp}/c vs c plot in the presence of KCl, [PolyA] = 0.01875 g/L (♦ 0.05 M, △ 0.10 M, ○ 0.15 M, + 0.20 M, ▲ 0.25 M, ● 0.30 M KCl).

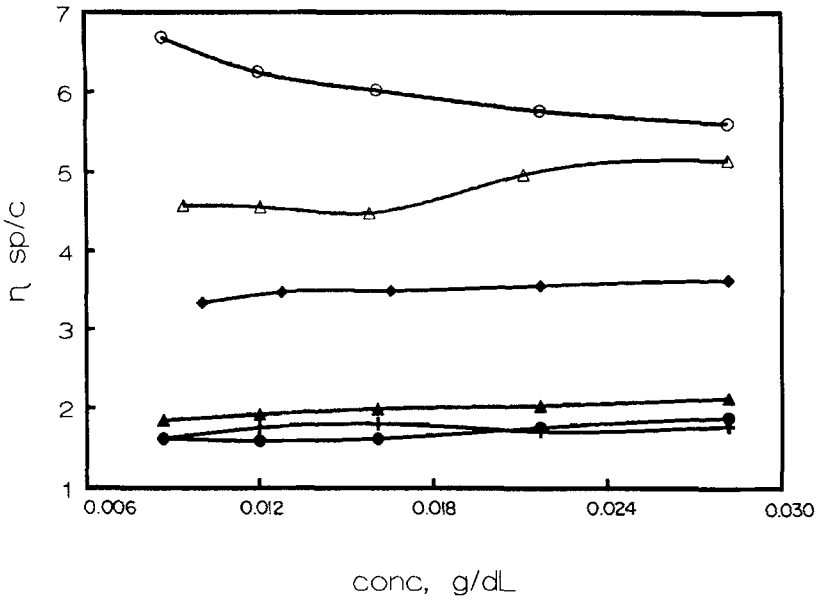

FIG. 5. η_{sp}/c vs c plot in the presence of KCl, [PolyA] = 0.02817 g/L (♦ 0.05 M, △ 0.10 M, ○ 0.15 M, + 0.20 M, ▲ 0.25 M, ● 0.30 M KCl).

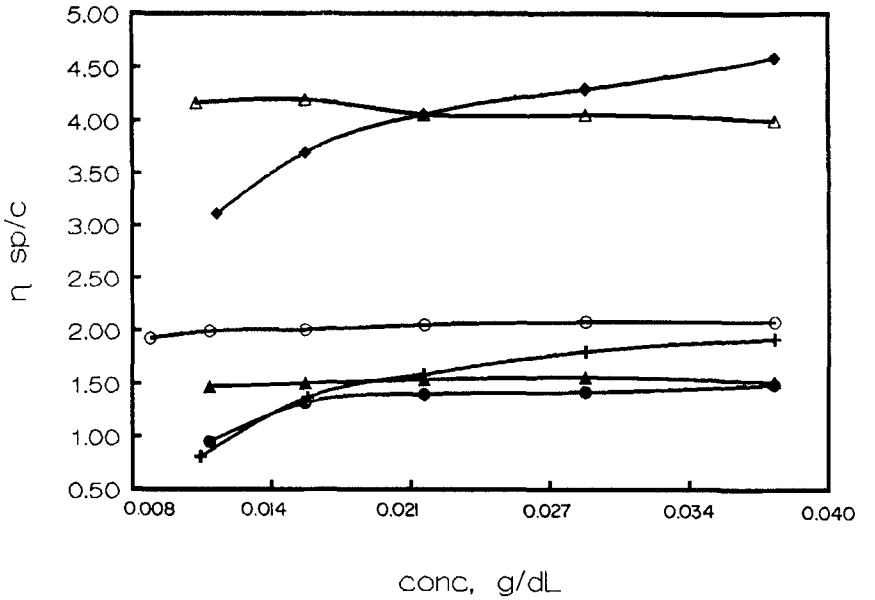

FIG. 6. η_{sp}/c vs c plot in the presence of KCl, [PolyA] = 0.03750 g/L (♦ 0.05 M, △ 0.10 M, ○ 0.15 M, + 0.20 M, ▲ 0.25 M, ● 0.30 M KCl).

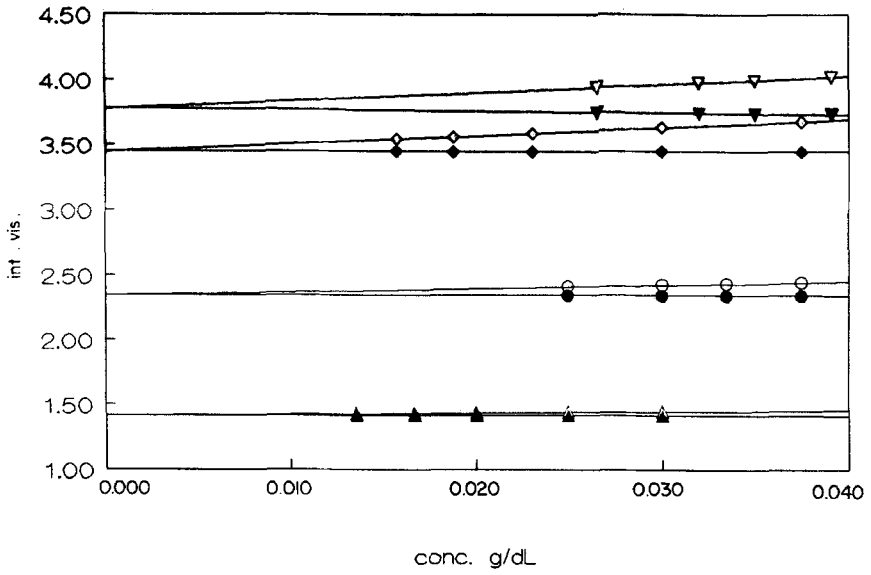

FIG. 7. Plot of η_{sp}/c and ln η_r/c against c for some standard samples:

Sample	Huggin	Kraemer
116	△	▲
106	○	●
130	▽	▼
112	◇	◆

TABLE 1. Molecular Weight, Intrinsic Viscosity, and Huggins-Kraemer Constants for Some PolyA Samples

Sample number	Molecular weight $\times 10^{-3}$ (light scattering)	$[\eta]$	k_1	k_1'	$k_1 - k_1'$
514	140	0.817	0.55	0.04	0.51
116	280	1.415	0.48	−0.03	0.51
106	470	2.346	0.48	−0.04	0.52
104	570	2.736	0.48	−0.04	0.52
112	734	3.444	0.53	−0.01	0.54
130	850	3.776	0.48	−0.05	0.53

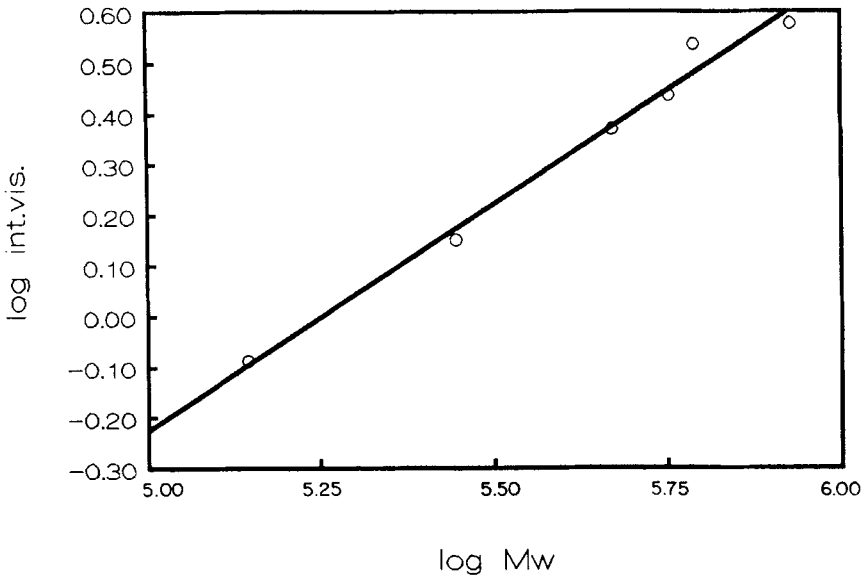

FIG. 8. Log[η] vs log M_w in Tris-EDTA buffer solvent at 25°C.

et al. [20] and Cannon et al. [21], showed that the correction values were well below the limiting values suggested.

Plots of viscosity against concentration for Huggins and Kraemer equations have a common intercept at $c = 0$ for all samples, so that

$$k_1 - k_1' \equiv 0.5$$

Since the values of k_1 are quite close to 0.5, then water is a good solvent for PolyA under the measurement conditions. The approximate value of 0.5 for $k_1 - k_1'$ suggests no associations or aggregations of PolyA by inter- or intramolecular forces in the given solution conditions. Therefore, the viscosity measurements can be considered to have been done at the most suitable conditions.

The plot of log[η] against log M_w for the equation

$$\log[\eta] = \log k + \alpha \log M_w$$

is given in Fig. 8. A linear straight line is obtained. The intercept and slope of the straight line give κ and α as 2.04×10^{-5} and 0.89, respectively. The relationship of [η] in terms of weight-average molecular weight in aqueous solution buffered with Tris-EDTA (pH 7.65) at 25°C is

$$[\eta] = 2.04 \times 10^{-5} M_w^{0.89}$$

CONCLUSION

PolyA degrades in pure water but remains stable when the solution contains a salt that increases the ionic strength. The conformation of PolyA in aqueous solution varies with the ionic strength and the pH of the solution. Viscosity measure-

ments of PolyA in aqueous solution can be measured satisfactorily in the presence of a Tris-EDTA buffer solution at a final pH of 7.65.

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