in the poly(1NMA-*co*-BMA) may therefore be represented by a combination of Schemes I and II.

Assuming that all quantum yields derived from Schemes I and II refer to the local terms in any poly(1NMA-*co*-BMA), we rewrite eq 9 and 10 as

$$\Phi_M = \frac{k_{FM}k_D}{k_{SM}k_D - k_Mk_{SD}}\left(\Phi_{cs} + \frac{k_{SD}}{k_D}\right) \quad (9')$$

$$\Phi_D = \frac{k_Mk_{FD}}{k_{SM}k_D}\left(\frac{k_{SM}}{k_M} - \Phi_{cs}\right) \quad (10')$$

Kinetic parameters in eq 9′ and 10′ are known except for k_{FD} and k_{SD}. The rate constants for excimer fluorescence (k_{FD}) and for main-chain scission from the excimer singlet state (k_{SD}) can be obtained by comparing eq 9′ and 10′ with the experimental eq 6 and 7. The rate constant for excimer internal quenching (k_{ID}) can be determined finally from the definition $k_D = k_{FD} + k_{ID} + k_{SD}$ (eq 21).

Table III summarizes a set of kinetic parameters for poly(1NMA-*co*-BMA) in deaerated benzene solution. From Table III a conclusion concerning the effect of intramolecular excimer formation on the photodegradation can be drawn as follows: The main-chain scission from the excited singlet state of the 1-naphthyl chromophore ($k_{SM} = 6.9 \times 10^6$ s^{-1}) is comparable to the monomer fluorescence emission ($k_{FM} = 7.3 \times 10^6$ s^{-1}). Since the rate constant for intramolecular excimer formation ($k_{DM} = 3.8 \times 10^8$ s^{-1}) is approximately 4 times that for monomer internal quenching ($k_{IM} = 0.96 \times 10^8$ s^{-1}), the quenching of the fluorescence state of the 1-naphthyl chromophore in the excimer sites is highly efficient compared with the case in the nonexcimer sites. The lower rate constant for excimer dissociation ($k_{MD} = 3.7 \times 10^7$ s^{-1}), which is 1 order of magnitude below k_{DM}, shows that the excimer dissociation to regenerate the monomer fluorescence state is negligible relative to the association into the excimer fluorescence state. It is particularly noteworthy that main-chain scission from the excimer fluorescence state makes little contribution to the photodegradation of poly(1NMA-*co*-BMA) because of its extremely small rate constant ($k_{SD} = 8 \times 10^3$ s^{-1}). Thus the intramolecular excimer formation is responsible for the photostabilization of poly(1NMA-*co*-BMA).

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Determination of Molecular Weight Distribution of Synthetic Flexible-Chain Polyelectrolytes by Polyacrylamide Gel Electrophoresis[1]

Jang-Lin Chen and Herbert Morawetz*

Polymer Research Institute, Polytechnic Institute of New York, Brooklyn, New York 11201.
Received February 16, 1982

ABSTRACT: Polyacrylamide gel electrophoresis was found to yield high resolution in the determination of chain length distributions of synthetic polyelectrolytes. Data are reported for poly(styrenesulfonate) obtained from polystyrene with narrow molecular weight distributions and for unfractionated poly(acrylic acid).

Although gel permeation chromatography (GPC) has proved to be a powerful method for the determination of the molecular weight distribution (MWD) of high polymers, its application to water-soluble polymers has en-

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Table I
Characterization of Poly(styrenesulfonates)

sample	$\bar{P}_w$ of parent PS	degree of sulfonation
PSS-1	89	0.90
PSS-2	196	0.88
PSS-3	371	0.89

countered a number of problems.[2] Application of GPC to polyelectrolytes has been reported from a number of laboratories.[3] It is complicated by electrostatic interactions between the stationary phase and the solution phase and by Donnan equilibrium effects.

Polyacrylamide gel electrophoresis (PAGE) has been used for a number of years for the estimation of the molecular weight of proteins in detergent solutions. Detergents such as sodium dodecyl sulfate (SDS) form a complex with polypeptide chains,[4] which thus acquire a charge density almost independent of the nature of the protein. The electrophoretic mobility of such chains through a gel decreases with increasing chain length, and gel electrophoresis provides a high resolution of chains with different molecular weights.[5] This high resolving power of PAGE has proved particularly valuable in the development of methods for the sequencing of DNA,[6] where chains differing by a single nucleotide residue could be distinguished for chains containing up to 300 nucleotides. This high resolving power, far superior to what is achievable in GPC, is probably due to several factors. In PAGE the macromolecular chain moves in a homogeneous medium, minimizing zone spreading. In addition, fractionation efficiency is aided by focusing the samples into a very thin starting zone prior to electrophoretic separation.[7]

With the spectacular success of the application of PAGE to the resolution of biological ionized chain molecules, it seemed appropriate to evaluate the usefulness of this technique for the characterization of synthetic polyelectrolytes. In this report we describe results obtained with relatively monodisperse poly(styrenesulfonates) and with unfractionated poly(acrylic acid) samples.

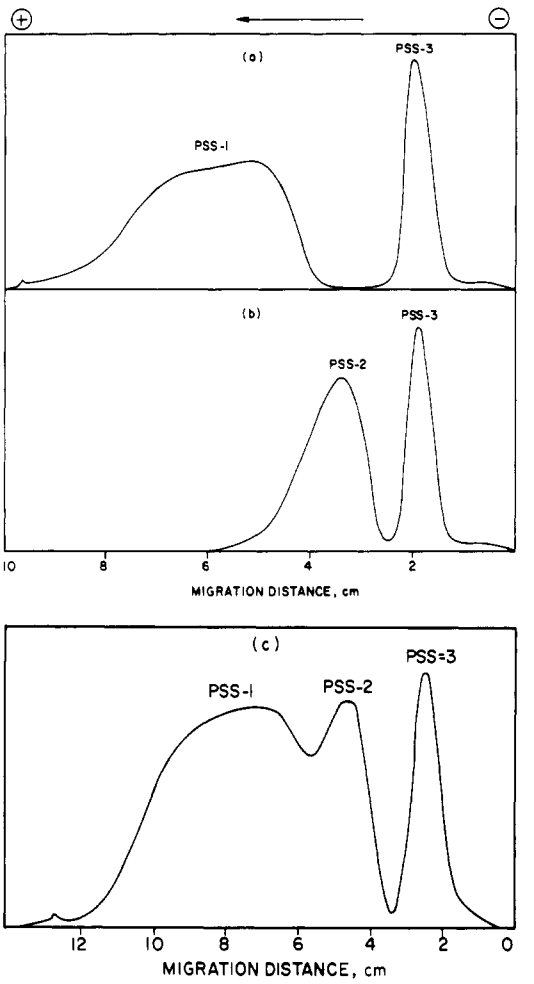

Figure 1. Densitometer traces of PSS after electrophoresis: (a) PSS-1 + PSS-3, time of electrophoresis 140 min; (b) PSS-2 + PSS-3, time of electrophoresis 140 min; (c) PSS-1 + PSS-2 + PSS-3, time of electrophoresis 180 min. In all experiments the gel concentration was 112 mg/mL and the current was 2 mA per tube. A 15-cm tube was used in experiment c.

Experimental Section

Polymers. Three samples of poly(styrenesulfonate) (PSS) prepared by sulfonation of polystyrene with narrow molecular weight distribution were purchased from Pressure Chemical Co. Table I lists the weight-average degrees of polymerization, $\bar{P}_w$, of the parent polystyrene and the degrees of sulfonation of these samples. With the sulfonation carried out under conditions avoiding chain scission and cross-linking,[8] it was assumed that $\bar{P}_w$ of the PSS was the same as for the polystyrenes from which they were derived. Poly(acrylic acid) was prepared by AIBN-initiated polymerization of acrylic acid in methanol at 60 °C. The molecular weight was controlled by the initiator concentration or by addition of butanethiol chain transfer agent. The viscosity-average molecular weight, M_η, was determined from the intrinsic viscosity in 1.25 M aqueous NaSCN at 30 °C using the relation of Soda and Kagawa:[9] $[\eta] = 121 \times 10^{-5} M^{0.5}$ (dL/g).

Equipment. A 12-tube gel electrophoresis cell DE-102 and dc power supply PS-500 (500 V, 125 mA) were manufactured by Hoeffer Scientific Instruments. The diffusion destainer and rubber grommets for holding glass tubes in the buffer chamber were purchased from Polysciences, Inc. A Jayce-Loebl MKIIICS double-beam microdensitometer equipped with a GE BXT projector lamp was used to scan the gel pattern.

Procedures. Rodbard and Chrambach's[10] multiphasic buffer system A (separation pH 9.45, $I = 0.015$) was used, and the disk electrophoresis procedure outlined by Davis was generally followed.[11]

Separation gel solutions of various concentrations were prepared by dissolving calculated amounts of acrylamide and methylenebis(acrylamide) in Tris-HCl buffer. The weight percentage of cross-linking agent to total monomers was kept constant at 5% throughout this study. After exposure of the solution to reduced pressure to eliminate atmospheric oxygen, 0.02 mL of 1% ammonium persulfate and 1 μL of *N,N,N′,N′*-tetramethylethylenediamine per milliliter were added. Polymerization to a gel was carried out in 12-cm-long glass tubes of 5-mm i.d., 7-mm o.d., at 25 °C for 1 h. The highly porous spacer gel was prepared according to Davis. A sample solution (10–50 μL) of 1 μg of polymer/μL of buffer solution containing 10% sucrose was layered on the top of the spacer gel before covering with electrode buffer solution. For each run, 12 gels were studied simultaneously.

Electrophoresis was performed at 25 °C under a constant current of 2–5 mA per electrophoretic tube. The separation time was varied from 30 min to 3 h depending on gel concentration and the voltage applied, as well as on the resolution required. After electrophoresis, the inner glass wall was rinsed with water injected through a 26-gauge hypodermic needle, and pressure was applied to remove the gel. The gel was stained with 0.2% methyl green (Eastman Chemicals) in a stirred sodium acetate buffer solution (pH 5.0, $I = 0.1$) for 2 h. Destaining to reveal the polyion zones was accomplished in a diffusion destainer, with distilled water frequently changed. The distribution of the stained polyanions was recorded as a microdensitometer trace.

Results and Discussion

PAGE of Poly(styrenesulfonates). While PAGE of proteins yields sharp boundaries since one deals with monodisperse macromolecules, a distribution of mobilities will necessarily be observed with synthetic polyelectrolytes,

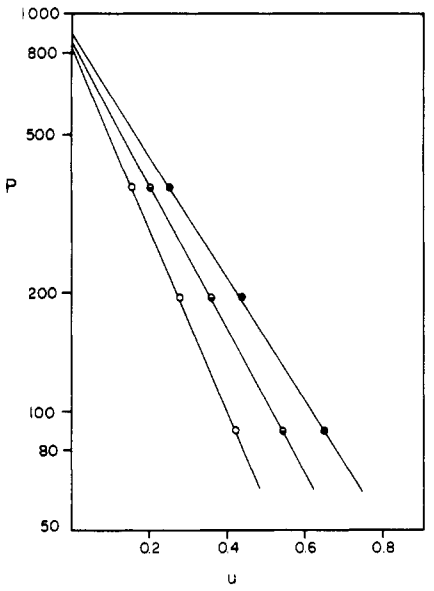

Figure 2. Relation between the degree of polymerization P and the relative electrophoretic mobility of PSS using bromophenol blue as a standard of reference. Gel densities: (●) 100 mg/mL; (⊖) 112 mg/mL; (○) 128 mg/mL. The maximum in the densitometer trace was assumed to correspond to the weight-average degree of polymerization of the PSS.

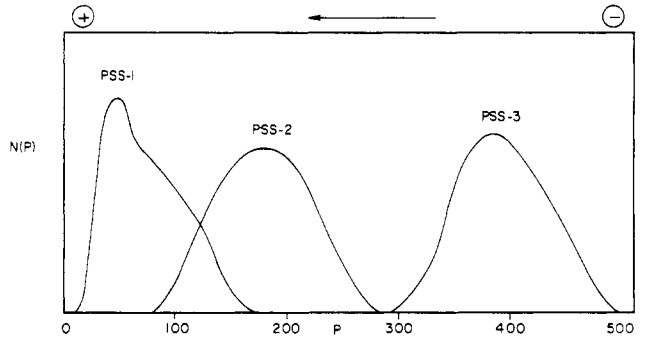

Figure 3. Chain length distribution of PSS.

even if they were prepared under conditions yielding narrow distributions of chain lengths. To test the resolution of the method, we carried out electrophoreses of mixtures of PSS samples that had been derived from polystyrenes prepared by the anionic polymerization technique.[8]

Densitometer traces of mixtures of PSS after electrophoresis are shown in Figure 1. It may be seen that PSS-1 and PSS-2 are well resolved from PSS-3, but PSS-1 and PSS-2 are only partially resolved from each other.

Studies of proteins in SDS solution[5] and of nucleic acids[12] showed that the electrophoretic mobility u is linear in the logarithm of the molecular weight

$$\log M = A - Bu \tag{1}$$

over a broad range, with the constants A and B depending on the concentration of the gel phase. It may be noted that relation 1 implies zero electrophoretic transport for $\log M > A$, but because of the distribution of pore sizes in the gel, plots of $\log M$ against u tend to flatten out as this limit is approached. Figure 2 shows that for our PSS samples, $\log P$ is linear in the relative mobility,[15] with the slope of the plot increasing with an increasing gel density. (It should be noted that in free electrophoresis in the absence of a gel, the mobility of polyions is independent of chain length.[13] The physical cause for this surprising result has found a convincing interpretation.[14])

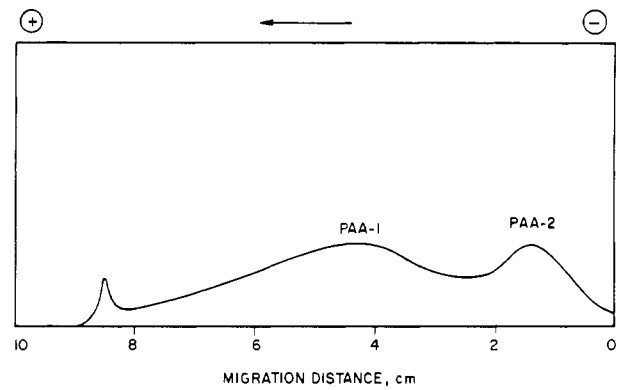

Figure 4. Densitometer trace of a mixture of equal weights of poly(acrylic acid) with M_η = 8600 (PAA-1) and M_η = 56000 (PAA-2); gel density 128 mg/mL, current 4 mA, time of electrophoresis 60 min.

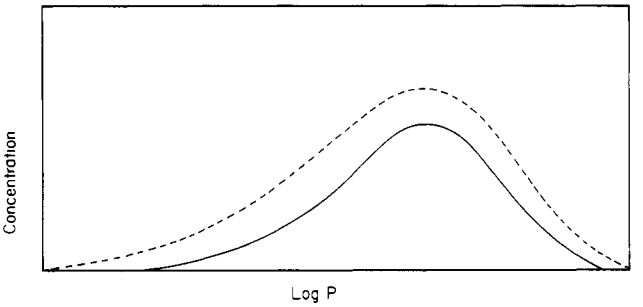

Figure 5. Comparison of densitometer trace after electrophoresis (—) with mobility distribution expected for a sample with a normal distribution of chain length (- - -).

On dividing the distribution of migration distances in the upper two traces of Figure 1 by the migration distance of bromophenol blue (9.5 cm), we obtain a distribution of relative mobilities. We may then use the line in Figure 2 obtained for the same gel density to convert the distribution of relative mobilities into distributions of the degrees of polymerization. These are shown in Figure 3. It is clear that sample PSS-1 has a bimodal distribution of chain length. We should like to note that the GPC chromatogram provided by the supplier did not reveal this feature, indicating the superiority of the PAGE analysis. The ratio of the weight-average to the number-average degree of polymerization, $\bar{P}_w/\bar{P}_n$, corresponding to these distributions is 1.20 for PSS-1, 1.05 for PSS-2, and 1.01 for PSS-3. It should also be noted that the distribution of electrophoretic velocities was found to remain constant over a threefold variation in PSS concentration.

PAGE of Poly(acrylic acid). Figure 4 represents a densitometer trace of a mixture of two poly(acrylic acid) samples, $\bar{M}_\eta$ = 56000 and $\bar{M}_\eta$ = 8600, after electrophoresis. These unfractionated polymers are only partially resolved. The small narrow peak with high mobility corresponds to a low molecular weight fraction that is not significantly retarded by the gel. In Figure 5 we have compared the distribution of P deduced from the distribution of mobilities observed with the lower molecular weight PAA with the distribution expected for a normal weight distribution of degrees of polymerization given by

$$W(P) = P\epsilon^2 \exp(-\epsilon P) \tag{2}$$

where $\epsilon = 1/\bar{P}_n$. Assuming $\bar{P}_n$ = 180, we find that there is excellent agreement between the two distributions.

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(15) The relative mobility is here defined using the mobility of the "tracking dye" bromophenol blue as a reference.

Structure of Benzoyl Peroxide Initiated Polystyrene: Determination of the Initiator-Derived Functionality by ^{13}C NMR

Graeme Moad,* David H. Solomon, Stanley R. Johns, and Richard I. Willing

Division of Applied Organic Chemistry, CSIRO, Melbourne, Victoria, Australia 3001.
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ABSTRACT: The benzoate groups incorporated into polystyrene prepared with benzoyl-*carbonyl*-^{13}C peroxide initiator have been evaluated by NMR spectroscopy. This provides, for the first time, a direct measure of the amounts of initiation by head and tail addition of benzoyloxy radical to styrene as well as a means of determining the extent of transfer to benzoyl peroxide and primary radical termination. For example, polystyrene prepared by bulk polymerization at 60 °C using 0.04 M benzoyl peroxide contains ca. 1.7 benzoate end groups per molecule, 87% of which derive from initiation by tail addition to styrene, 5% by head addition, and 8% by termination through transfer to initiator or primary radical termination. The method also shows the dependence of these processes on reaction conditions.

Introduction

There is an extensive literature on the reaction of benzoyl and other aroyl peroxides with styrene and on the use of these initiators in the preparation of polystyrene. Several studies have been concerned with evaluating details of the polymerization mechanism.[1-11] These investigations concentrated on counting the number of benzoyloxy and phenyl end groups by various techniques and on analyzing the kinetics of the polymerization reaction. Some attempt has been made to correlate these data with what is known about termination. The various effects of "abnormal" termination reactions (transfer to initiator, primary radical termination) have been considered. However, the possibility of nonselectivity in the initiation step has generally been ignored.

Recently, we reported the results of radical trapping experiments that enabled us to outline the pathways by which benzoyloxy and phenyl radicals react with styrene.[12-14] We showed that benzoyloxy radicals react with styrene to afford products from tail addition, head addition, and aromatic substitution (79:6:14) while phenyl radicals give tail addition and aromatic substitution (99:1). In the present paper we show how these pathways are reflected in the structure of polystyrene prepared with benzoyl peroxide as initiator. This has been achieved by using peroxide in which the carbonyl carbon is 90% enriched in ^{13}C and employing NMR to identify and quantify the various types of benzoate groups in the polymer.

Experimental Section

General Procedures. NMR spectra were recorded with a Bruker WM250 or a Varian CFT20 spectrometer. Deuteriochloroform was used as solvent and chemical shifts are reported in parts per million from internal tetramethylsilane. HPLC was performed with a DuPont Model 850 liquid chromatograph that was coupled to an LDC 308 computing integrator. An Altex Ultrasphere ODS column 10 mm × 25 cm was employed, with ethanol/water as eluant. Gel permeation chromatography (GPC) was carried out with a Waters instrument that could be coupled to a Chromatix KMX-6 light scattering photometer and a Chromatix LDS-2 data system. The programs MOLWT and GPCL (Chromatix) were used for data acquisition and reduction. A set of five Waters μ-Styragel columns (10^6-, 10^5-, 10^4-, 10^3-, and 500-Å pore size) was employed and tetrahydrofuran was used as the eluant. Mass spectra were obtained with a Finnigan 3300 spectrometer.

Benzoic-*carboxy*-^{13}C Acid. Benzoic acid enriched in ^{13}C was obtained commercially (Merck Sharpe and Dohme). The degree enrichment was shown by mass spectrometry to be 87 ± 2%.

Benzoyl-*carbonyl*-^{13}C Peroxide. Benzoic-*carboxy*-^{13}C acid (900 mg) was treated with thionyl chloride (1.1 mL), and the mixture was heated at 100 °C for 1 h. The excess thionyl chloride was then evaporated and the residue distilled to give benzoyl-*carbonyl*-^{13}C chloride: 940 mg, 90%; bp 170 °C (100 mmHg).

This product (900 mg) was slowly added to a solution of sodium peroxide (400 mg) in water (7 mL) and benzene (1 mL) at 5 °C and the mixture stirred for 90 min. The mixture was then extracted with benzene, and the extract was washed with water, dried ($MgSO_4$), and evaporated. The crude peroxide was dissolved in chloroform and precipitated with methanol at 0 °C to give benzoyl-*carbonyl*-^{13}C peroxide: 650 mg, 84%; ^{13}C NMR δ 163.1 (C=O).

Polymerizations. Freshly distilled styrene was degassed and then distilled into an ampule containing the appropriate amount of benzoyl peroxide on a vacuum line operating at 10^{-6} mmHg. The ampule was degassed by the freeze–thaw technique, sealed, and immersed in a constant-temperature bath at 60.0 ± 0.1 °C. Polystyrene samples A, B, C, D, and E were prepared by using 0.01, 0.04, 0.1, 0.1, and 0.1 M benzoyl peroxide and reaction times

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