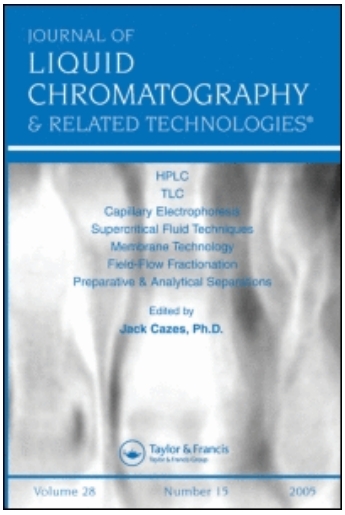

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Effect of the Sulfonation of Sodium Poly(Styrenesulfonate) Compounds on the Elution Behavior in Aqueous Size Exclusion Chromatography

Sadao Mori[a]
[a] Department of Industrial Chemistry Faculty of Engineering Mie University Tsu, Mie, Japan

To cite this Article Mori, Sadao(1998) 'Effect of the Sulfonation of Sodium Poly(Styrenesulfonate) Compounds on the Elution Behavior in Aqueous Size Exclusion Chromatography', Journal of Liquid Chromatography & Related Technologies, 21: 19, 2935 — 2945
To link to this Article: DOI: 10.1080/10826079808006877
URL: http://dx.doi.org/10.1080/10826079808006877

J. LIQ. CHROM. & REL. TECHNOL., 21(19), 2935–2945 (1998)

EFFECT OF THE SULFONATION OF SODIUM POLY(STYRENESULFONATE) COMPOUNDS ON THE ELUTION BEHAVIOR IN AQUEOUS SIZE EXCLUSION CHROMATOGRAPHY

Sadao Mori*,†

Department of Industrial Chemistry
Faculty of Engineering
Mie University
Tsu, Mie 514, Japan

ABSTRACT

Fully sulfonated sodium poly(p-styrenesulfonate) (designated as NaPpSS) was prepared from sodium p-styrenesulfonate monomer and the elution characteristics of NaPpSS and commercially available poly(styrenesulfonate) which was prepared by direct sulfonation of polystyrene (designated as NaPSS) were compared using columns packed with glycerylpropyl group-bonded silica gel and poly(styrene sulfonate) gel by changing ionic strengths of the mobile phase. Plots of log [η]M and retention volumes for NaPpSS, NaPSS and pullulan showed that the retention volumes of NaPpSS and NaPSS increased with increasing the ionic strength of the mobile phase, but the extent of changing retention volume was different between these two types of compounds. Early elution of NaPpSS and NaPSS relative to pullulan of the same molecular size is governed by the ion-exclusion effect and NaPpSS eluted earlier than NaPSS of the same molecular size.

This phenomenon is obvious, because phenyl groups of NaPpSS were completely sulfonated and the ion-exclusion effects for NaPpSS at the low ionic strengths of the mobile phase is much more than that for NaPSS of which phenyl groups were partially sulfonated. Late elution of NaPpSS and NaPSS relative to pullulan of the same molecular size is attributed to hydrophobic interactions. Although NaPpSS samples are less hydrophobic than NaPSS, they still have some small amount of hydrophobicity.

INTRODUCTION

In aqueous size exclusion chromatography (ASEC), it is well-known that elution of ionic polymers is influenced by both the type of stationary phases and the composition of the mobile phases. Since the ionic polymer samples and the stationary phases used for ASEC possess numerous polar groups, mutual interactions between them will lead to non-ideal elution such as early elution or retardation. These mutual interactions may be divided into two secondary effects: ion-exclusion and hydrophobic interactions.[1] Ion-exclusion leads to early elution relative to the retention volume estimated from the hydrodynamic volume of the sample polymer molecule and can be suppressed by the addition of simple electrolytes.[2,3] Hydrophobic interactions lead to the retardation of elution and may be minimized through the proper selection of the mobile phase and the stationary phase,[4] but complete elimination of the interactions was difficult.[5]

In the previous paper,[1] elution behavior of sodium poly(styrenesulfonate) compounds (NaPSS) was compared with that of nonionic linear polysaccharide (pullulan) on several types of ASEC columns by varying ionic strengths of the mobile phase. The divergence of the hydrodynamic volume calibration curves of NaPSS from that of pullulan was observed and retention volumes of NaPSS changed with changing the ionic strength. Early elution of NaPSS than pullulan of the same molecular size at the low ionic strengths of the mobile phase was governed by the ion-exclusion effect, and with increasing the ionic strength, hydrophobic interactions between NaPSS and the packing materials became remarkable. Retention volume of NaPSS was estimated to be governed by the compromise among three effects: size-exclusion, ion-exclusion, and hydrophobic interactions. Intramolecular chain expansion was small and neglected in the present study.

NaPSS samples used in the previous study were purchased from the commercial source and were prepared by the direct sulfonation of polystyrene samples of narrow molecular weight distributions (henceforth referred to as NaPSS). The direct sulfonation into polystyrene limits the complete sulfonation of the polystyrene without excessive decomposition of the polymer and some

amount of unreacted phenyl groups may remain. The degree of sulfonation of the NaPSS samples used in the previous work was between 80 and 90%[6] and the unreacted phenyl groups may be considered to be the main sites of hydrophobic interactions.

In the present work, fully sulfonated NaPSS (sodium poly(p-styrenesulfonate), henceforth referred to as NaPpSS), has been prepared from sodium p-styrenesulfonate monomer and the elution behavior of both sodium poly(styrenesulfonate) samples, fully and partially sulfonated ones, has been compared. If unreacted phenyl groups are only the site of hydrophobic ninteractions, then the fully sulfonated NaPpSS may not show any hydrophobic interactions with the stationary phase. If the ion-exclusion effect of NaPSS is smaller than that of NaPpSS, then it is good evidence that NaPSS has some amount of unreacted phenyl groups. The preliminary report was published elsewhere.[7]

EXPERIMENTAL

Preparation of NaPpSS

Purification of sodium p-styrene sulfonate monomer

Ten grams of sodium p-styrenesulfonate (Nap-SS, reagent grade, Tokyo Chemical Co., Tokyo, Japan) were dissolved in 15 mL of water on a waterbath kept at 90°C. The hot solution was filtered and the filtered solution was cooled to 0°C to precipitate Nap-SS. The precipitate was filtered, washed with cooled acetone, and dried under vacuum at room temperature. This product was again dissolved in water and reprecipitated as above.

Polymerization of Nap-SS

The polymerization procedure of Nap-SS by Wiley, Smith, Ketterer[8] was slightly modified. Five grams of Nap-SS, 0.41 g of sodium sulfite, and 0.28 g of potassium peroxydisulfate (both from Nacalaitesque Co., Kyoto, Japan) were dissolved in 16 mL of degassed water in a 50 mL flask. The latter two reagents were used as polymerization initiators. This solution was stirred at 45°C under reduced pressure for 1 h. The reaction solution was poured into 500 mL cooled acetone dropwise under vigorous agitation. After removing acetone in the solution, another 500 mL of cooled acetone was added and the solution was stirred for 4 h. The precipitated material was filtered and dried under vacuum. This procedure was repeated, again dissolving the product in water and reprecipitating as above. NaPpSS thus obtained is designated as NaPpSS-A.

NaPpSS-B was prepared as the same manner as NaPpSS-A, but the composition of the reaction solution was as follows: 5.0 g of Nap-SS, 0.67 g of sodium bisulfite, 1.34 g of potassium peroxydisulfate, and 50 mL of degassed water.

Fractionation of NaPpSS

Fractionation of NaPpSS according to molecular weight was carried out with slight modification of the procedure of Marshall and Moch.[9] Sodium iodide (NaI) was selected as the salt for the precipitation. 2.6 grams of NaPpSS-A were dissolved in 260 mL of the 4N aqueous NaI solution in a 500 mL flask and the flask was immersed in a constant temperature bath regulated to 20 ± 0.1°C. The 9.1 N aqueous NaI solution was added dropwise to the solution from a burette under stirring until an arbitrarily defined turbidity was observed (63.75 mL). This mixture was stirred for another two hours. The gel-like precipitate was filtered off, redissolved in 3 mL water, precipitated in acetone, washed with acetone, and dried to constant weight under reduced pressure. The fraction is designated as Fl. The fractionation was repeated with the filtered solution by adding another 2.0 - 4.5 mL of the 9.1 N NaI solution and in all, eight fractions, whose total weight accounted for 26.5% of the original sample, were isolated by this technique. Fractionation of NaPpSS-B was carried out in the same manner.

ASEC

ASEC measurements were performed on a high performance liquid chromatograph Model TRAROTAR-V (Jasco Corp., Tokyo, Japan) with a refractive index detector Model SE-11 (Showa Denko, Tokyo, Japan). Columns were Shodex PROTEIN KW-804 (300 mm x 8 mm i.d.) and Shodex Ionpak S-804 (500 mm x 8 mm i.d.)(both from Showa Denko). These columns were used separately. Shodex PROTEIN column was packed with glycerylpropyl group-bonded silica gel and Shodex Ionpak was packed with sodium poly(styrenesulfonate) gel.

The mobile phase was made up from sodium monohydrogen phosphate and sodium dihydrogen phosphate to the desired ionic strength at pH 7.0. Ionic strength was changed from 0.005 to 0.88 M. The flow rate was 1.0 mL/min. The polymer samples were dissolved in the solvent used as the mobile phase in the concentration of 0.05% and the injection volume of the sample solutions was 0.1 mL.

The retention volumes at the exclusion limit (the interstitial volume in the column) were estimated to be 6.0 mL for Shodex PROTEIN KW-804 and 8.81 mL for Shodex Ionpak S-804, which were measured with NaPSS molecular weight 1.2×10^6. The total volume of the mobile phase in the column was

estimated from the retention volume of ethylene glycol. It was 12.9 mL for Shodex PROTEIN KW-804 and 18.5 mL for Shodex Ionpak S-804, respectively.

Samples

Besides fractionated NaPpSS samples, several commercially available NaPSS standards of narrow molecular weight distributions, which have been purchased from Pressure Chemical Co. (Pittsburgh, PA), were used in this experiment and the latter samples are designated as merely NaPSS. Pullulan standards (Showa Denko) were used as nonionic polymer samples. Nominal molecular weights (MW) of NaPSS and pullulan standards obtained as vendor's values were as follows: NaPSS, 4,600; 18,000; 1.0×10^5; 4.0×10^5; 1.2×10^6, pullulan, 5,800; 23,700; 1.0×10^5 ; 3.8×10^5; 8.5×10^5.

Molecular weight averages of NaPpSS fractions were measured by SEC-MALLS (multipul-angle laser light scattering). Column used for the measurement was Shodex Ionpak S-804 and the mobile phase was a phosphate buffer solution of 0.005 M at pH 7.0.

The refractive index increment dn/dC of NaPpSS used in the measurement was 0.200. A MALLS photometer (Wyatt Technology Corp., Santa Barbara, CA) used in this experiment was kindly provided by Showa Denko Co. and Shoko Co. (Minato-ku, Tokyo, Japan).

RESULTS AND DISCUSSION

The main purpose of this paper is to compare retention volumes of NaPSS and NaPpSS of the same molecular size by changing the ionic strengths of the mobile phase. The molecular sizes of the polymers were estimated as the hydrodynamic volumes $[\eta]M$. In order to obtain the intrinsic viscosities of polymers, the assumption has been made that the intrinsic viscosity is independent of the degree of sulfonation and the intrinsic viscosities of these polymers in phosphate buffer solutions at various ionic strengths were calculated from the data in the literature.[10]

To check the validity of the assumption, intrinsic viscosities of the 1.0×10^5 MW NaPSS and the 1.36×10^5 MW NaPpSS at the ionic strength 0.35 M were measured and compared with the calculated ones. The results were as follows: measured intrinsic viscosity of NaPSS was 0.29 and calculated one was 0.30; measured one of NaPpSS was 0.39 and calculated one was 0.37. The difference was small and for this the assumption can be made in this comparison.

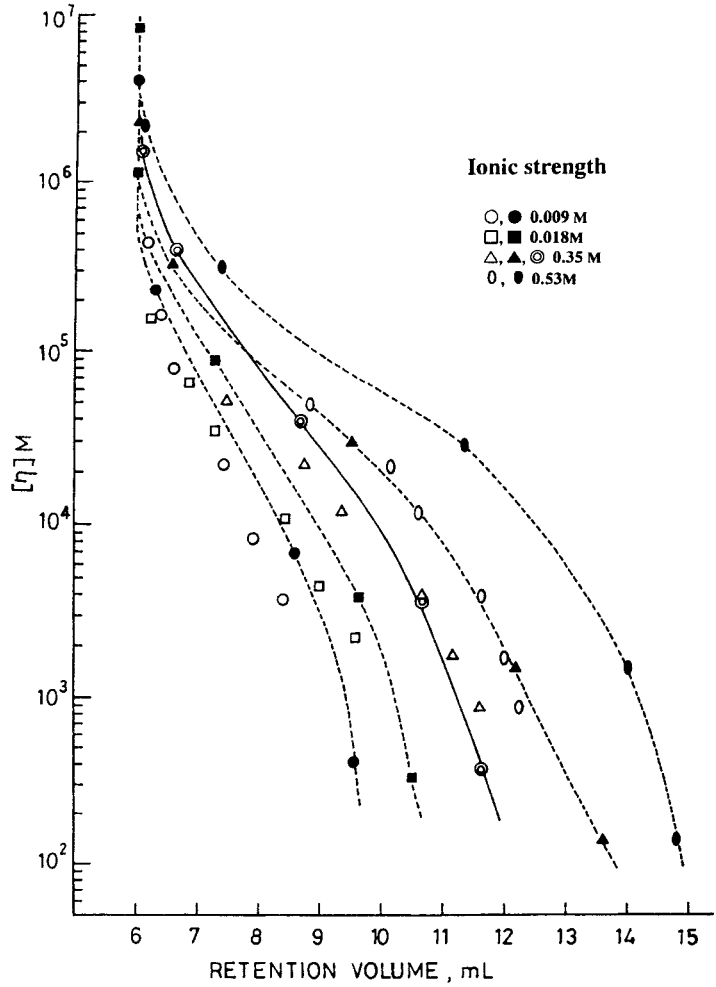

Figure 1. Universal calibration plots for NaPpSS (open symbols), NaPSS (filled symbols), and pullulan (o) on the column of Shodex PROTEIN KW804. A solid line is the pullulan calibration curve, broken lines with filled symbols are the plots for NaPSS, and open symbols are for NaPpSS.

Universal calibration plots for NaPpSS, NaPSS, and pullulan on Shodex PROTEIN KW-804 and Shodex Ionpak S-804 at various ionic strengths are shown in Figures 1 and 2, respectively. A solid line is the pullulan calibration curve and broken lines with filled symbols are the plots for NaPSS.

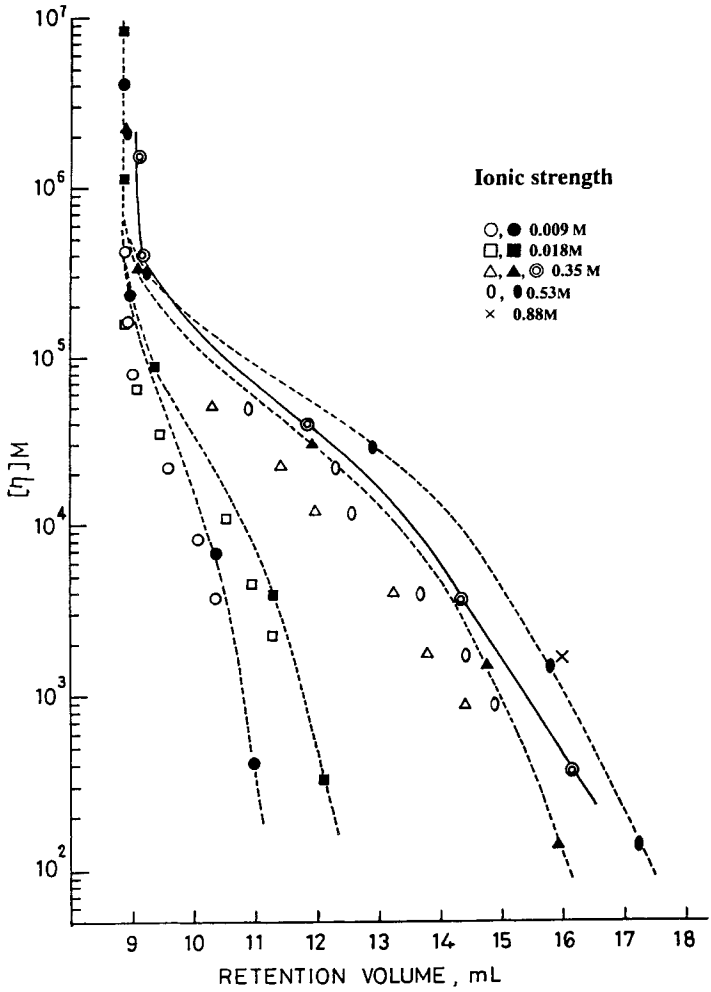

Figure 2. Universal calibration plots for NaPpSS (open symbols), NaPSS (filled symbols), and pullulan (o) on the column of Shodex Ionpak S-804. A solid line is the pullulan calibration curve, broken lines with filled symbols are the plots for NaPSS, open symbols are for NaPpSS, and a symbol × is for NaPpSS.

Open symbols without lines are for NaPpSS. Molecular weight averages of unfractionated NaPpSS-A and NaPpSS-B are as follows: NaPpSS-A, Mw = 4.9 x 10^4; Mn = 3.8 x 10^4, NaPpSS-B, Mw = 1.5 x 10^4; Mn = 1.2 x 10^4. Molecular weight averages of fractionated NaPpSS used for ASEC

Table 1

Molecular Weight Averages of NaPpSS Fractions and NaPSS Standards Determined by SEC-MALLs

Sample	Nominal MW	Molecular Weight Averages $M_W \times 10^{-4}$	$M_n \times 10^{-4}$	M_W/M_n
NaPpSS-A F2		13.9	13.3	1.05
F6		8.53	8.34	1.02
F8		6.01	5.87	1.02
NaPpSS-B F2		3.28	3.03	1.08
F4		2.08	1.83	1.14
F7		1.58	1.43	1.10
NaPSS	1.0×10^5	10.4	10.1	1.03
	4.6×10^4	4.90	4.56	1.07
	1.8×10^4	2.10	1.74	1.21

measurements are listed in Table 1 with those of three NaPSS for comparison purposes. Peak molecular weights for NaPpSS were calculated as (Mw+Mn)/2 and vendor's values were used for NaPSS. Figure 3 is example of chromatograms of NaPpSS. Figure 3(A) is the chromatogram of unfractionated NaPpSS and (B) is the chromatogram of fractionated NaPpSS-AF3. It shows that fractionation is efficient and the chromatogram of NaPpSS-AF3 is symmetrical. Other chromatograms are almost the same shape. As the effect of the ionic strength on retention volume for pullulan was almost negligible, the calibration plot for pullulan was made only for a buffer solution of ionic strength 0.35 M. Secondary effects such as the ion-exclusion effect and the hydrophobic interactions between pullulan and the packing materials were not considered in the present study. The ionic strength 0.88 M is too high to operate the solvent delivery pump adequately, so that only each single sample was used to measure retention volume on Shodex Ionpak S-804: the 18,000 MW NaPSS and NaPpSS-BF4.

Retention volumes of both NaPpSS and NaPSS on both columns increased with increasing the ionic strength of the mobile phase and those of NaPpSS at all ionic strengths were smaller than those of NaPSS having the same molecular sizes. The difference in retention volumes between NaPpSS and NaPSS of the same molecular size increased with increasing the ionic strength. Early elution of the sample polymers relative to pullulan is governed by the ion-exclusion effect,[1] which decreased with increasing the ionic strength of the mobile phase.

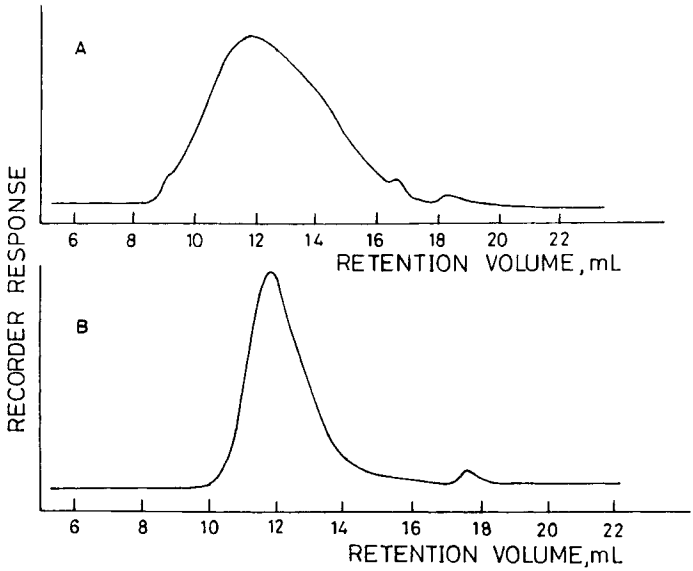

Figure 3. Examples of chromatograms for NaPpSS. (A) Unfractionated NaPpSS, (B) fractionated NaPpSS-AF8. Column: Shodex Ionpak S-804; ionic strength of mobile phase: 0.35 M.

It is obvious that, because of complete sulfonation of phenyl groups of NaPpSS, the ion-exclusion effect for NaPpSS at the low ionic strengths of the mobile phase is much more than that for NaPSS and the former elutes earlier than the latter of the same molecular size.

NaPpSS and NaPSS on Shodex PROTEIN KW-804 eluted from the column earlier than pullulan of the same molecular size at the lower ionic strengths and eluted later at the higher ionic strengths. The extent of the retardation of elution of NaPpSS relative to pullulan of the same molecular size was smaller than that of NaPSS (see Figure 1, ionic strength 0.53 M) and it is attributed to the large ion-exclusion effect and/or the small hydrophobic interactions of NaPpSS to the column packings compared to NaPSS.

Similar elution characteristics were observed on Shodex Ionpak S-804, but the ionic strength where the sample polymers retarded relative to pullulan of the same molecular size was large compared to Shodex PROTEIN KW-804; this indicates that the ion-exclusion effect of the column packings to the sample polymers is large and/or the hydrophobic interactions is small compared to

Shodex PROTEIN KW-804. At the ionic strength 0.53 M, NaPSS eluted later and NaPpSS eluted earlier than pullulan of the same molecular size. At the ionic strength 0.88 M, NaPSS (the 18,000 MW NaPSS) retained in the column and NaPpSS (-BF4) eluted from the column, which means hydrophobic interactions of NaPpSS to the packing materials is small compared to NaPSS.

NaPpSS samples are less hydrophobic than NaPSS as shown in Figures 1 and 2, but they show some small amount of hydrophobicity. All phenyl groups of NaPpSS include sulfonic groups and hydrophobic sites may be phenyl groups themselves, or the backbone C-C linkage. It may also have to consider the electrostatic interactions between the sample polymers and the packing materials.

Shodex PROTEIN KW-804 column still has some amount of unreacted silanol groups on the surface of the packing. Glycerylpropyl (1,2-dihydroxy--3-propoxypropyl) groups, which is bonded on the surface of the packings, are hydrophilic and, therefore, hydrophobic interactions between the support and NaPSS solutes are supposed to be small. However, as reported in the previous paper,[1] late elution of NaPSS relative to pullulan is governed by hydrophobic interactions between NaPSS and the packings. Propyl groups of the packings and unreacted phenyl groups of NaPSS are estimated as main sites of hydrophobic interactions. The packing materials packed in Shodex Ionpak S-804 column have the similar chemical structure to NaPSS and, from the results shown in Figures 1 and 2, the packings are considered to be less hydrophobic than the packing materials in Shodex PROTEIN KW-804.

REFERENCES

† Correspondence address: PAC Research Institute, 4-8, Ibuka, Nagoya, 453-0012, Japan.

1. S. Mori, Anal. Chem., **6**, 530 (1989).

2. C. Rochas, A. Domard, M. Rinaudo, Eur. Polym. J., **16**, 135 (1980).

3. H. G. Barth, F. E. Regnier, J. Chromatogr, **192**, 275 (1980).

4. S. Mori, Anal. Chem., **50**, 745 (1978).

5. P. Rouimeliotis, K. K. Unger, J. Chromatogr., **218**, 535 (1981).

6. Pressure Chemical Co., Certification of Sodium Polystyrene Sulfonate Standards.

7. S. Mori , T. Oosaki, in M. Potschka, P. L. Dubin eds, **Strategies in Size Exclusion Chromatography**, ACS Symposium Series, **635**, 347 (1996).

8. R. H. Wiley, N. R. Smith, C. C. Ketterer, J. Am. Chem. Soc., **76**, 720 (1954).

9. C. A. Marshall, R. A. Moch, J. Polym. Sci., **17**, 591 (1955).

10. P. L. Dubin, M. M. Tecklenburgh, Anal. Chem., **57**, 275 (1985).

Received December 16, 1997
Accepted January 29, 1998
Manuscript 4685