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HIGH-PERFORMANCE LIQUID–SOLID ADSORPTION CHROMATOGRAPHY OF SPIROPYRAN-END-LABELLED POLYSTYRENES

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SUMMARY

High-performance liquid–solid adsorption chromatography is used to separate uncoupled and coupled spiropyran-end-labelled polystyrenes from unlabelled polymer chains. Selective separations according to molecular weight, end-group differences, or both are obtained by using adsorbents of 6- and 50-nm pore diameters with mobile phases differing in elution selectivity.

INTRODUCTION

Adsorption chromatography can be used as an alternative to solvent fractionation of oligomers and polymers according to end-group functionality. Thin-layer methods have been used frequently in the past[1], and column separations have been reported recently for end-labelled polystyrenes[2], carboxylic acid-terminated polystyrenes[3] and hydroxyl-terminated poly(methyl methacrylates)[4]. Mansson[3] and Andrews and Vatvars[4] used high-performance, small-pore-diameter, silica adsorbents, and Winnik *et al.*[2] described the use of small-pore-diameter silica in a gravity-feed column. In all of the above examples, polymer chains that contain polar end groups are retained more strongly than chains terminated by the normal process of proton abstraction. Details of effects from adsorbent pore diameter and polymer molecular weight are lacking; however, the normal elution order in polymer adsorption chromatography of low to high molecular weight was reversed in ref. 3, indicating the presence of a mixed size-exclusion and adsorption retention mechanism. Previous studies on the adsorption chromatography of polystyrenes have shown that polymers are generally eluted from small-pore-diameter silicas without dependence on chain molecular weight[5–7], and chain compositional differences (such as end groups) are best detected with delocalizing solvents[8].

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This paper reports on the separation of anionically prepared polystyrenes that exist as a mixture of unlabelled and end-labelled chains that can also couple dimerically. Full utilization of pore-diameter and solvent selectivity effects allows the separation of these mixtures according to molecular weight, end-group differences, or both.

EXPERIMENTAL

Spiropyran-labelled polystyrenes

End-labelled polystyrenes were similar to those used for forced Rayleigh scattering studies[9]. A photochromic spiropyran, 1-(4-iodobutyl)-3,3-dimethylindoline-6-nitrobenzospiropyran, was prepared from 1-(4-iodobutyl)-2,3,3-trimethylindolium iodide, which was obtained on heating 2,3,3-trimethylindoline with diiodobutane, and 5-nitrosalicylaldehyde[10]. The product was purified by fractional crystallization from ethanol–benzene (2:1). Two batches of styrene monomer in benzene were initiated with n-butyllithium and terminated with an equimolar amount of spiropyran in the dark at room temperature (sample No. PS13N-3) and at $-78°C$ (sample No. PS14N-2). End-labelled polystyrene is obtained by nucleophilic substitution at the iodobutyl group by polystyryl anions and has the general structure
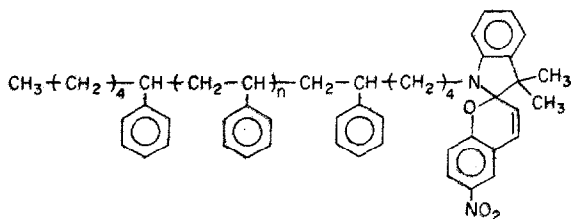
Residual spiropyran reagent was removed by multiple precipitations of the final product with methanol.

Chromatography

A 6-nm nominal pore-diameter, LiChrosorb Si 60 (Merck), 5-μm particle-diameter silica and a 50-nm nominal pore-diameter, LiChrospher Si 500 (Merck), 10-μm particle-diameter silica were used for all separations. Both columns were 250 × 4.6 mm I.D. packed by the stirred slurry method. Eluents were HPLC-grade n-hexane, dichloromethane, and tetrahydrofuran (THF). The solvent delivery system consisted of two M6000A Waters Assoc. pumps and a Waters Model 720 system controller. Samples of 1 mg/20 μl were separated by gradient elution and monitored for UV absorbance at 254 and 350 nm with two LC55 Perkin-Elmer variable-wavelength detectors. Spiropyran end groups absorb at 350 nm without interference from polystyrene repeat units. Molecular-weight distributions were obtained by size-exclusion chromatography (SEC) in THF using 10^6, 10^5, 10^4, and 10^3 Å exclusion-limit μStyragel columns (Waters Assoc.).

RESULTS AND DISCUSSION

Coupling side reactions in the presence of alkyl halides that produce dimeric termination of polystyryl anions[11-14] and living polybutadiene chains[15] have been reported. The coupling mechanism is not entirely clear; it is believed to involve either a metal–halogen exchange or a coupling of polymeric radicals that are generated by a one-electron transfer from polymeric anions to alkyl halide groups[11,15]. Possible products from the end-labelling of polystyryl anions with spiropyran include:proton-terminated polystyrene (P-H); coupled proton-terminated polystyrene (P-P-H); spiropyran-terminated polystyrene (P-spiro); and coupled spiropyran-terminated polystyrene (P-P-spiro). The molecular weight of coupled products will be twice that of uncoupled products[15]. Coupling reactions of polystyrene and polybutadiene were monitored successfully by SEC in previous studies[11-15]. SEC chromatograms of spiropyran-labelled polystyrene (Fig. 1) clearly show formation of coupled product. The molecular-weight distribution of spiropyran-end-labelled chains is monitored at 350 nm and is compared to the total polymer molecular-weight distribution that is monitored by refractive-index detection. Both samples contain more end-labelled material in the uncoupled P-spiro form, but there is more coupled product in the sample deactivated at room temperature (PS13N-3) than in the sample deactivated at $-78°C$ (PS14N-2). SEC clearly shows the formation of coupled products; however, the total amount of P-spiro or the proportion of P-P-spiro to P-spiro cannot be calculated from the refractive-index traces.

Additional information can be obtained from adsorption separation with different eluents on adsorbents differing in pore diameters. The adsorption behavior of narrow-molecular-weight polystyrene standards is dependent on adsorbent pore size (Fig. 2). Gradient elution from the 6-nm pore-diameter adsorbent results in little
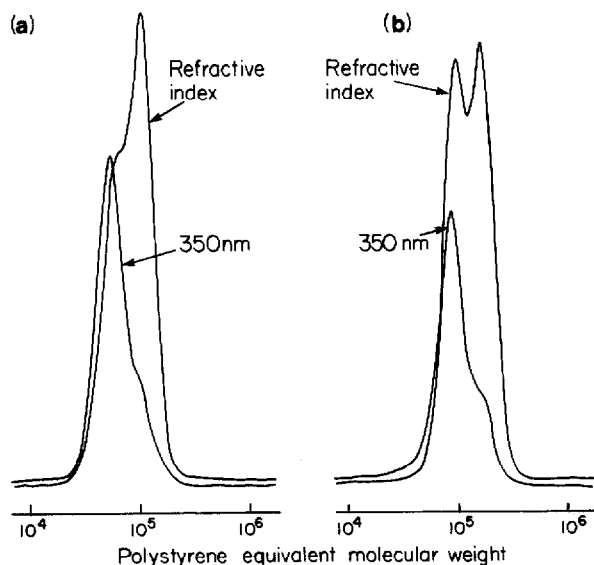

Fig. 1. SEC chromatograms of (a) PS13N-3 and (b) PS14N-2.

molecular-weight dependence above $\bar{M}_w \approx 15,000$ (curves 1 and 2). The dependence of retention volume on molecular weight in the weight range of interest is considerably more pronounced on 50-nm pore-diameter silica (curves 3 and 4). We will refer to separations on 6- and 50-nm pore-diameter silicas as being molecular-weight independent and molecular-weight dependent, respectively. The eluent gradients of Fig. 2 are optimized conditions for the separation of spiropyran-containing polymers and are not necessarily optimum conditions for the separation of polystyrenes according to molecular weight. The slopes of curves 3 and 4 can be increased or decreased simply by changing the gradient rate of increase. However, there are no gradient conditions that will produce a significant molecular-weight dependence in the molecular-weight range examined on the 6-nm pore-diameter adsorbent.

Gradient elution of end-labelled polystyrenes on 6-nm pore-diameter silica with THF produces chromatograms such as Fig. 3. The single sharp peak contains both coupled and uncoupled species and confirms molecular-weight-independent elution on small-pore-diameter silica. A small amount of unidentified polar material is also present in the sample along with residual spiropyran reagent. A similar gra-
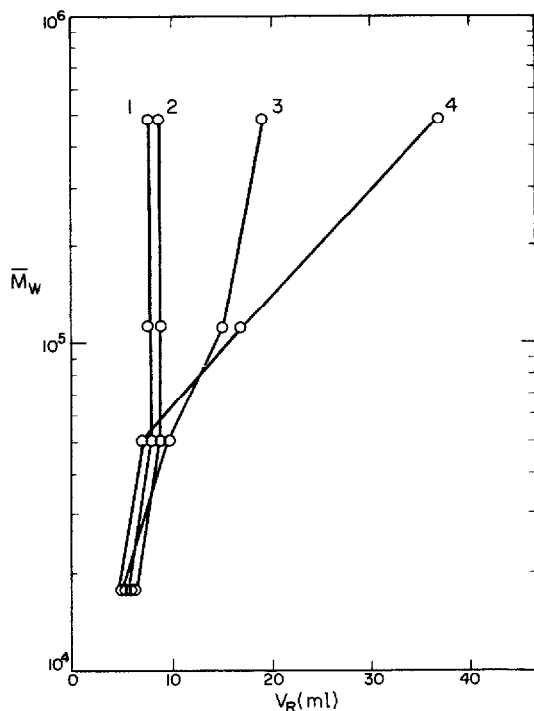

Fig. 2. Molecular-weight dependence of narrow-molecular-weight polystyrenes on adsorption chromatography elution volumes (V_R): (1) LiChrosorb Si 60, gradient elution from n-hexane–dichloromethane (38:62, v/v) at a dichloromethane volumetrically linear rate of increase of 0.43%/ml; (2) LiChrosorb Si 60, gradient elution from n-hexane–THF (65:35, v/v) at a THF volumetrically linear rate of increase of 0.33%/ml; (3) LiChrospher Si 500, gradient elution from n-hexane–dichloromethane (42:58, v/v) at a dichloromethane volumetrically linear rate of increase of 0.20%/ml; (4) LiChrospher Si 500, gradient elution from n-hexane–THF (69:31, v/v) at a THF volumetrically linear rate of increase of 0.20%/ml.
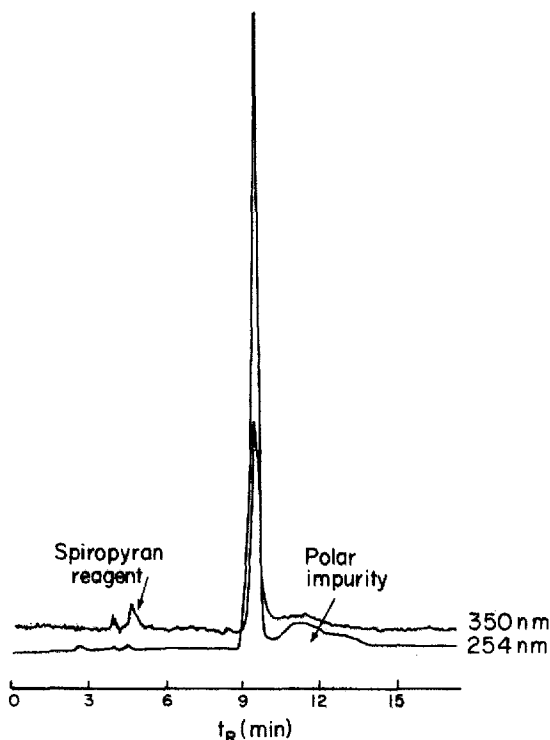
Fig. 3. Elution of spiropyran-end-labelled polystyrene sample PS13N-3 from LiChrosorb Si 60. Conditions given in Fig. 2, curve 2. t_R = Retention time.

dient separation with an eluent containing dichloromethane (Fig. 4) also produces molecular-weight-independent elution but now separates polymer chains according to difference in end groups. Spiropyran-terminated chains, regardless of length, are adsorbed more strongly than proton-terminated chains. The ratio of areas for the two major peaks in the 254-nm trace indicate that most of the sample is proton terminated. The small peak at 350 nm at the retention volume of the unlabelled material is a result of the change in refractive index caused by the large amount of P-H + P-P-H polymers eluted and is not attributed to molecules containing spiro-pyran. Large (1 mg) samples of pure polystyrene readily produce this effect when eluted as a sharp band such as in Fig. 4.

The differences in the solvent selectivities of THF and dichloromethane are related to their adsorption configuration on the silica surface. THF has a preferred, or localized, configuration on the adsorbent surface that results from interaction between surface silanol sites and the THF ether linkage. It generally behaves as a much stronger solvent than delocalized dichloromethane[16]. THF does not differentiate between the large number of polystyrene repeat units and a small number of spiropyran end groups because of the inability of the spiropyran groups to selectively displace localized THF molecules from the adsorbent surface. Displacement of delocalized dichloromethane molecules is a more favorable process and affords the

P-H + P-P-H   P-spiro + P-P-spiro

350 nm

254 nm

0   3   6   9   12   15   18
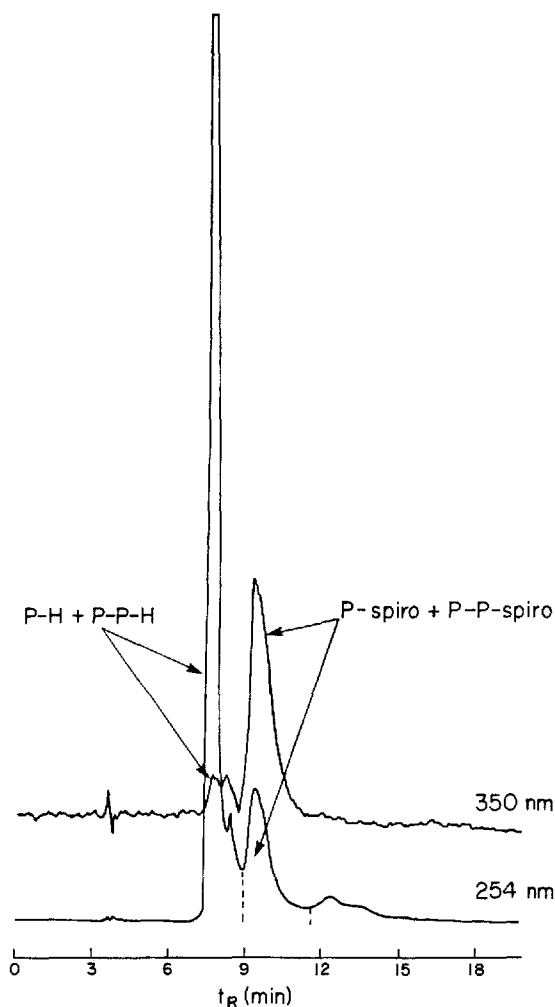
t_R (min)

Fig. 4. Separation of proton-terminated and spiropyran-end-labelled polymers in PS13N-3 on Lichrosorb Si 60. Conditions given in Fig. 2, curve 1.

end-group selectivity of Fig. 4. Further details of solvent selectivity in the adsorption chromatography of polystyrenes have been presented elsewhere[8] and are beyond the scope of this paper.

Molecular-weight-dependent, end-group-independent separations can be obtained with the 50-nm pore-diameter adsorbent and end-group-insensitive eluents such as THF. A considerable improvement in resolution compared to the SEC molecular-weight separation is shown in Fig. 5. The area ratio of total coupled to uncoupled polymer, regardless of end groups, can be easily calculated from the major peak areas of the raw chromatogram. Molecular-weight distributions for each fraction in Fig. 5 were determined by a polynomial fit of the calibration data in Fig. 2. The molecular-weight data of uncoupled and coupled products and the area ratios of uncoupled to coupled polymers are given in Table I.
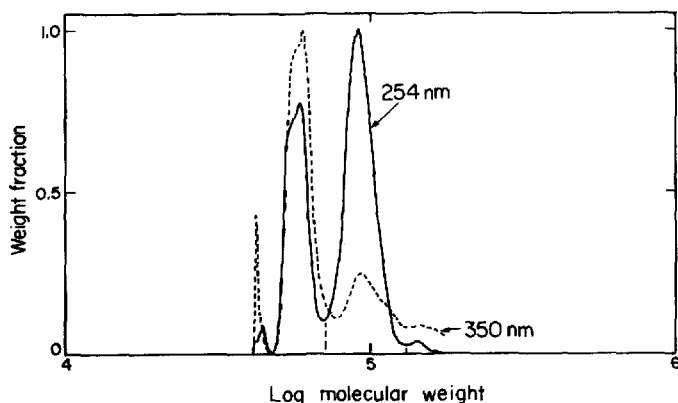
Fig. 5. Molecular-weight separation of PS13N-3 on LiChrospher Si 500. Conditions given in Fig. 2, curve 4.

TABLE I

MOLECULAR-WEIGHT DATA OF SPIROPYRAN-END-LABELLED POLYSTYRENES OBTAINED FROM *n*-HEXANE–THF ADSORPTION CHROMATOGRAPHY

	PS13N-3			PS14N-2		
	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w M_n$	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$
Total uncoupled (P-H + P-spiro)	58,300	57,900	1.01	77,700	77,300	1.01
Total coupled (P-P-H + P-P-spiro)	92,700	91,600	1.01	142,500	141,900	1.00
Area ratio (uncoupled/coupled)	0.60 (254 nm)	3.00 (350 nm)		0.89 (254 nm)	3.05 (350 nm)	

Elution from 50-nm pore-diameter silica with dichloromethane-containing eluents gives separations according to both molecular weight and chemical composition (Fig. 6). P-H and P-P-H chains are separated according to molecular weight and are eluted before the more strongly adsorbed P-spiro and P-P-spiro chains. The amount of P-H material is considerably less than the total amount of uncoupled polymer measured from the areas of Fig. 5, a finding that confirms that most of the end-labelled polymer is in the P-spiro form. Unfortunately, there is no molecular-weight differentiation of the P-spiro and P-P-spiro chains, hence a complete analysis of these samples by a single method such as that used to generate Fig. 6 is not feasible. There is actually a subtle molecular-weight effect on the separation of end-labelled chains. The spiropyran-labelled polymers in the higher-molecular-weight PS14N-2 sample are eluted somewhat sooner than end-labelled chains of the lower-molecular-weight PS13N-3. We have observed this behavior with several end-labelled polystyrenes. The sensitivity of chromatographic retention to end-group differences is apparently dependent on the ratio of the number of end groups to the number of
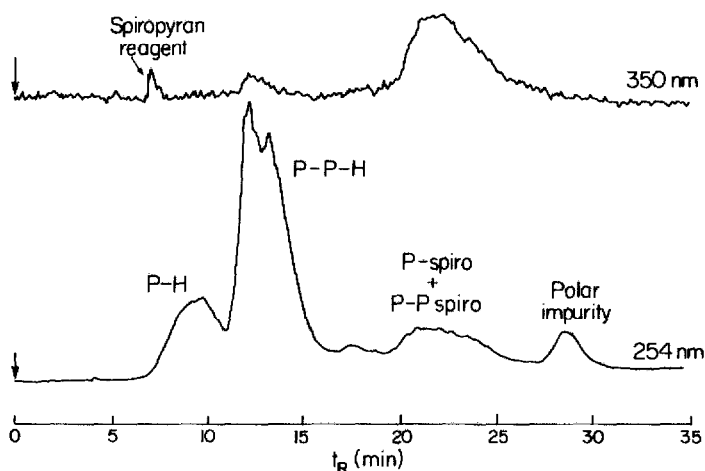
Fig. 6. Molecular-weight and compositional separation of PS13N-3 on LiChrospher Si 500. Conditions given in Fig. 2, curve 3.

repeat units. There is approximately one end group per 560 and 740 repeat units for PS13N-3 and PS14N-2, respectively. The smaller ratio of end groups to repeat units in PS14N-2 results in less retention.

Total analysis of end-labelled samples can be obtained from the area percentage of each component in Figs. 4–6. The percentages of P-H and P-P-H, the total amount of labelled polymer, and the amount of polar impurity can be obtained from the 254-nm response in Fig. 6. The ratio of total unlabelled to total labelled polymer can also be obtained from separations on 6-nm pore-diameter silica with dichloromethane (Fig. 4). The only suitable method for determining the amount and molecular weight of each end-labelled species is by measuring the ratio of areas obtained by detection at 350 nm for THF separations on 50-nm pore-diameter silica (Fig. 5) or by using peak areas from the SEC separation at 350 nm. This ratio yields the amount of P-spiro and P-P-spiro once the total amount of end-labelled material has been determined by the dichloromethane separations described above. Treatment of the data in this fashion gives the area percentage of each component in Table II. Agreement of area measurements for multiple runs and calculations of total end-

TABLE II

QUANTITATIVE RESULTS FOR END-LABELLED POLYSTYRENES

Fraction	Area percentage	
	PS13N-3	PS14N-2
P-H	15.5	22.7
P-P-H	56.0	47.9
P-spiro	15.8	17.8
P-P-spiro	5.3	5.9
Polar impurity	7.4	5.7

labelled material from dichloromethane separations on both 6- and 50-nm pore-diameter silica is $\pm 4\%$ relative. The only significant difference we have found between samples is the lesser amount of P-P-H material obtained at the $-78°C$ deactivation temperature.

A clarifying note should be made about the percentages given in Table II. These area percentages may differ slightly from the actual mass percentages of each component. Some difference in molar absorptivities at 254 nm of spiropyran-terminated and proton-terminated polymer chains is anticipated. This difference results in relatively minor changes in the total absorbance because of the low concentrations of spiropyran end groups compared to polystyrene repeat units. This variation could be determined readily by an independent spectrophotometric study of each dye-labelled component. For demonstration, and also because of insufficient amounts of samples, we have deemed this correction unnecessary.

CONCLUSIONS

High-performance liquid–solid adsorption chromatography can give high-resolution, end-group-selective or molecular-weight-selective separations of polystyrenes when effects of adsorbent pore diameter and solvent selectivity are applied to the separation process. This method is highly suited for the study of products obtained from complex polymer reactions such as the coupling of polystyryl anions presented here. These techniques can provide superior resolution and greater flexibility than SEC; they are rapid and require small samples. For this study we used less than 40 mg of each sample; only a few milligrams are needed once separation conditions have been established.

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