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Real-Time Spectral Acquisition and Size Exclusion Chromatography Combined To Give Verification of Copolymerization and Analysis of Composition, All as a Function of Molecular Size

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ABSTRACT: A new size exclusion analysis method that uses real-time acquisition of UV as a means of detection has been developed for compositional analysis of copolymer as a function of molecular size. This procedure enables proof of copolymerization. This size exclusion technique depends on distinct ultraviolet absorbance spectra arising from 2 parts of a copolymer to resolve total ultraviolet absorbance of the copolymer into that due to each part. Upon calibration of the detector, copolymer composition plots as a function of molecular weight or molecular size can be made. Absolute percentage error in the method is ±4%. However, this error can be larger with different solvents, different amounts of aggregation, and/or different polymer structures which affect the absorbance spectrum of either copolymer part. Wavelengths at which absorbance of the copolymer parts are monitored must be chosen to maximize the difference in molar absorptivity of the parts. The method has been used to prove graft copolymerization and investigate the composition variation with molecular size in poly(lignin-*g*-(1-amidoethylene-*co*-[1-(*N*-(4-methoxyphenyl)amido)ethylene])).

Introduction

Copolymers are a particularly difficult class of polymers to characterize. They are, as Hamielec describes them,[1] complex polymers having random, block, or graft distribution of their repeat units. Properties of a copolymer depend on molecular composition and internal structure. Composition exerts a major control on physical properties, yet the composition distribution curve, which is the mole fraction of one monomer in the molecule plotted vs. the total weight percent of the sample with that mole fraction or less of monomer, is usually broader than the molecular weight distribution.[2] For random and block copolymers, the composition may not correlate with molecular weight.[3] The composition of graft copolymers generally correlates with molecular weight since the mole fraction of side chain can only increase by increasing molecular weight.

Under the label of internal structure effects, random or block copolymer structural characteristics such as sequence lengths and tacticity control product absorbance and light wavelength absorbed,[4] while graft copolymer structure characteristics, such as number of grafts per backbone molecule and average graft length control molecular size and shape.[5] Since several 1-phenylethylene copolymers have significant commerical importance, there have been extensive efforts to analyze these materials.[6-11] Methods used to analyze these and other copolymers include fractionation, thin-layer chromatography, light scattering, thermal analysis, infrared spectroscopy, and liquid chromatography. Liquid chromatography methods used are based on partition,[12] adsorption,[13] and size exclusion.[14] Size exclusion chromatography is the method used here to analyze complex polymers.

Size exclusion chromatography (SEC) separates the solutes in a solution based on the size or hydrodynamic volume of the molecules. Sorting takes place by passing solution through a column containing a microporous packing. For simple, linear homopolymers, the apparatus and column can be calibrated to relate size, as measured by the volume of solvent eluted between injection and elution of the molecule, to molecular weight. This calibration is performed by measuring elution volume for a series of standards of known molecular weight and holds for the particular temperature and solvent of the calibration experiment.

For complex polymers such as copolymers, molecular size depends on several variables besides molecular weight. These others variables are molecular composition and molecular configuration.[1] Elution volume of a complex polymer is thus often given only as a function of molecular size. Other analyses on the copolymer are possible, however, and several research groups[4,15,16] have studied the composition and configuration of copolymers by size exclusion techniques.

In the following sections, SEC will be used to (i) verify grafting in a graft copolymer, thus proving bonding between the two molecular components, (ii) analyze component distribution in a complex polymer as a function of size, and (iii) provide backbone or side-chain analyses of a copolymer sample as a function of molecular size. This method provides more definitive evidence of grafting than

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Table I
Data for Products from Reaction Mixtures Containing Lignin and 2-Propenamide

A. Terpolymers Containing N-Substituted 2-Propenamide

sample no.[a]	monomer: 2-propenamide g	monomer: 2-propenamide mol	monomer: N-substituted 2-propenamide g	monomer: N-substituted 2-propenamide mol	monomer mol fract[b]	yield g	yield wt %	wt % of reactn prod. sol in SEC buffer (pH 7.4)
1	3.04	0.0428	0.16	0.0009	0.0207	3.15	85.1	36.6
2	2.88	0.0406	0.32	0.0018	0.0426	3.68	99.5	70.9
3	2.72	0.0383	0.48	0.0027	0.0661	3.58	96.8	59.4
4	2.80	0.0394	0.40	0.0023	0.0542	2.85	77.0	37.6
5	2.64	0.0371	0.56	0.0032	0.0785	3.00	81.1	16.1
6	2.56	0.0360	0.64	0.0036	0.0912	2.64	71.4	62.4

B. Copolymer Containing Only Lignin and 2-Propenamide

sample no.	lignin, g	2-propenamide g	2-propenamide mol	$CaCl_2$ wt % of reactn mixture	equiv of 2-hydroperoxy-1,4-dioxacyclohexene present	yield g	yield wt %
7	0.50	3.2	0.045	0.2	0.8	Approx 6×10^{-4}	2.24	60.5

[a] All reactions contained 0.5 g of lignin and 2.0 wt % anhydrous calcium chloride in 20 mL of 1,4-dioxacyclohexane containing 3×10^{-3} equiv of 2-hydroperoxy-1,4-dioxacyclohexane. The hydroperoxide was produced by irradiating oxygen-saturated, 1,4-dioxacyclohexane with a 1000-W Xe lamp. [b] Moles of N-substituted amide/moles, total amide; mole fraction of N-substituted monomer in the reaction mixture.

do classical techniques such as solvent extraction, fractionation, or physical property change. It is also more convenient and versatile than digestion–thin-layer chromatography methods[20] developed by Taga to prove graft copolymerization.

Experimental Section

Methods. Synthesis procedures for poly(lignin-*g*-(1-amidoethylene)) are given in ref 21. The complex polymers used to test this analysis technique were graft copolymers of lignin. Synthesis procedures for the N-substituted 2-propenamide monomer and graft terpolymer containing N-substituted repeat units are given in ref 22. Synthesis of a pure copolymer side chain containing no lignin is described in ref 19. A pure, random-copolymer side chain is used to calibrate the response of the chromatograph detector to side-chain concentration. When synthesized, it is contaminated with iron salts, a part of the initiator. The side chain is purified by dialysis against 0.1 M phosphoric acid of a 1.0 wt % solution of product from the side-chain polymerization in 0.1 M phosphoric acid. The dialysis membrane used is Spectrophore #3, 3500 upper molecular weight limit dialysis tubing. Exterior dialysis fluid is changed and tested daily until a 2-mL sample shows no pink tone when exposed to 3 drops of iron indicator. The indicator is 0.30 wt % 1,10-phenanthroline in 0.01 M HCl. The side-chain solution is then dialyzed against distilled water until the pH of the exterior dialysis fluid no longer decreases from 6 toward more acidic values during dialysis. The side-chain is recovered by freeze-drying. Typical recovery from dialysis is 57 wt % of the original reaction solids.

Preparation of Samples for SEC. The graft and random copolymers used in these assays have, because of their complex structure, complicated solubility properties. Special solubilization procedures are needed to dissolve the polymers.

Solutions of polymer samples 1, 2, and 3 of Table I were prepared by dissolving the concentration of polymer given in Table III in 40 mL of 0.05 M phosphate buffer (pH 7.42), stirring for three days, and centrifuging to remove undissolved material. Solutions of polymer samples 4, 5, and 6 were prepared by first dissolving the concentration of polymer given in Table III in 20 mL of 0.05 N sodium hydroxide solution and then stirring for three days. After the polymers dissolved, sodium hydrogen phosphate was added to each sample to bring the solution pH up to 7.4 and phosphate concentration up to 0.05 M. Each solution was then centrifuged to remove undissolved material.

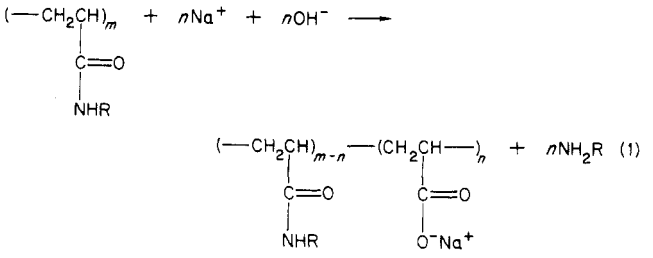
Lignin is dissolved in the same way as samples 4, 5, and 6. This procedure completely solubilizes lignin, and no centrifugation to remove solids is necessary. Poly(1-amidoethylene-*co*-(1-[*N*-(4-methoxyphenyl)amido]ethylene)), the pure side chain for the graft copolymers, is dissolved first in 0.1 M phosphoric acid. After 2 days the solution is diluted with 0.5 M sodium hydroxide and water to produce a solution with 0.05 M sodium phosphate concentration and pH = 7.4.

Aqueous size exclusion chromatography was conducted by using a column packed with diol-bonded Lichrosphere 500.[23] The mobile phase and solvent for all samples was 0.05 M sodium dihydrogen phosphate–disodium hydrogen phosphate buffer with a pH of 7.4. The sample volume injected for all chromatographic analysis was 10 μL, flow rate was 1.0 mL/min, and neither solvent nor samples were degassed. All peaks were detected by absorption of ultraviolet light (190–420 nm). All fluids were filtered through an 8-μm Nucleopore filter before use.

In order to convert the absorbance readings of the ultraviolet detector of the chromatograph to concentrations, a calibration curve was needed. The chromatograph detector was calibrated by pumping, under the same conditions used in analysis, a constant concentration solution of lignin or side chain through the detector for 90 min. Absorbance at 219 nm was measured every 2 min during the last 10 min of each calibration test. Detector and data aquisition settings were the same as those used in the analysis. These last five absorbance values were averaged and used as the absorbance of that concentration of solute in the buffer. A column was not used during this calibration process to avoid adsorption and separation effects. These conditions minimized error in the calibration. The standard deviation of the final absorbance value was found to be random and less that 0.72%.

The side chain of the graft copolymer, poly(1-amidoethylene-*co*-(1-[*N*-(4-methoxyphenyl)amido]ethylene)), absorbs ultraviolet light at 246 nm because of 4-methoxyphenyl groups on some of the amide groups of the copolymer.[24] Thus, to convert absorbance of the side chain to concentration of the side chain, the amount of 4-methoxyphenyl groups in each chromatographed sample has to be determined. This can be done by hydrolysis of the side chain and ultraviolet absorbance assay of the hydrolysis product.

Treatment of primary or N-substituted poly(1-amidoethylene) with base[25] produces either ammonia or an amine, as illustrated in the equation



depending on whether R is hydrogen or an organic group. Hy-

drolysis is a random reaction, producing a randomly hydrolyzed product.

In this work 1-amido-4-methoxybenzene (I) was chemically removed from the copolymer by basic hydrolysis, which was conducted by adding 1.18 g of sodium hydroxide pellets to 10.0 mL of aqueous phosphate buffer copolymer solution. The solution was refluxed in a microrefluxing apparatus for 4.5 h, cooled, and washed 4 times with 7-mL aliquots of diethyl ether. The ether washings were placed in 50-mL volumetric flasks and allowed to dry overnight. The next morning the residues were redissolved with 50 mL of methanol. A sample blank for each quantitative UV analysis was prepared with the same procedure as above except the blank solution contained concentrations of lignin and poly(1-amidoethylene) equal to that of the hydrolyzed copolymer. There were no 4-methoxyphenyl units in the blank.

The concentration of I in methanol was determined from the absorbance of the copolymer digestion solution vs. that of the blank at 235 nm. Absorbance vs. concentration of I in methanol was found to follow Beer's law by preparing a series of solutions of I in methanol and measuring their absorbance.

Reagents. All reagents used were Baker reagent grade and were used without further purification. Lignin used in all experiments was a commerical kraft pine lignin. This material is M_n = 9600, M_w = 22 000 molecular weight lignin with all anionic sites satisfied with hydrogen ions. The ash content of the lignin is 1 wt % or less; iron content is 145 ppm.

Elemental analysis of the lignin is, by weight percent, C, 60.86; H, 5.78; N, 1.09; S, 1.89; O, 28.65.

Instrumentation. The size exclusion separations were performed with a Varian Model 5000 high-performance liquid chromatograph (Walnut Creek, CA) equipped with a Rheodyne 10-μL fixed-loop injector. The column used in this work was a 25 cm × 4.4 mm stainless steel tube packed with 10-μm-diameter 50.0-nm-pore size silica (LiChrosphere: E. Merck, Darmstadt, Germany) covalently bonded with a diol phase. The column packing and synthesis procedures have been described previously.[26] The chromatographic detector used was a Hewlett-Packard Model HP-1040A high-speed spectrophometric detector (Palo Alto, CA) with its supporting computer, the HP-85, containing 16K bytes of additional memory. This detector can perform absorbance measurements at wavelengths from 190 to 600 nm and can detect and store an entire spectrum of the contents of the detector cell over the above wavelength range every second. This capacity allows spectra to be taken at numerous times during the elution of a chromatographic peak and is critical to this polymer-analysis method. In this work, the wavelength range found most useful for the spectra is 200–410 nm.

Qualitative and quantitative spectra for the copolymer hydrolysis product, 1-amino-4-methoxybenzene or, more commonly, *p*-anisidine, were obtained on a Perkin-Elmer, Lambda 3 UV–vis spectrophotometer (Norwalk, CT).

Synopsis of Curve Resolution Software. The multivariate curve resolution software (MCR-2) used in this work was developed by Infometrix Inc., Seattle, WA, and was designed to be used with the Hewlett-Packard 1040A high-speed spectrophotometric detector. The information used by MCR-2 in this analysis is the absorbance at six different wavelengths of the material passing through the detector cell. These data are stored during the elution of the analyte peak and then processed by the MCR-2 software. At any wavelength, the absorbance seen by the detector can be the result of one or more absorbers being present in the detector cell. If more than one absorber is present, detected absorbance is the sum of two or more absorbances, A_1, A_2, ..., each of which is a linear function of the molar absorptivity of the absorber and its concentration

$$A_i = \epsilon_i b C_i$$

If the peak being analyzed contains only one component, every spectra taken during the elution of the peak will be nearly the same, and their differences will be below a preset threshold (1% in this case). The program then reports that only one component is present in the peak.

If more than one component is found, then the program uses multivariate curve resolution techniques[27-29] to resolve the absorbance contribution of each component to the total peak absorbance. Resolution along the peak (time domain) employs a quantitization method developed by Sharaf and Kowalski.[29] MCR-2 can detect up to three absorbers but can only quantitatively separate the total absorbance into that due to one or two absorbers. If two absorbers are present, MCR-2 then reports the fraction of total absorbance at each point along the peak due to each absorber. This allows a chromatogram peak due to two absorbers in the eluent to be separated into contributions from each absorber.

Results and Discussion

It is often difficult to prove that a copolymer has been formed and this is particularly true for block and graft copolymers. Once it has been shown that a copolymer has been formed, it is even more difficult to determine the composition of a copolymer as a function of properties such as molecular weight.

A size exclusion chromatography (SEC) method has been developed that enables both proof of copolymer formation as well as elucidation of copolymer composition as a function of molecular size. Size exclusion chromatography has already been used to identify copolymers.[30-34] When comparing block, alternating, or random copolymers to homopolymers, SEC will separate homopolymer mixtures by size and show two peaks for a mixture instead of a single broad peak for a copolymer. The existence of graft copolymers is suggested when the reaction product's chromatogram shows a pronounced increase in molecular size when compared to the chromatogram of the ungrafted backbone. These are necessary indications of copolymerization but are not compelling proofs.

A method that constitutes far more convincing evidence of copolymerization has now been developed. In this technique, the total spectra of the effluent from a size exclusion column is taken in real time throughout the elution of a chromatographic peak. These spectra are stored and recalled for later processing by computer software (MCR-2) that identifies a particular absorber's contribution to the total peak absorbance. For a copolymer, the absorbance peak measured by the detector is broken down into absorbance contributions from each of the two repeating units of the polymer. The copolymer elution peak is thus broken down into two superimposed peaks that show the absorbance contribution of each repeat unit of the copolymer to each sample fraction of equal molecular size. Further, if the absorbance is quantified with respect to repeat-unit concentration, the two repeat-unit specific absorbance curves can be converted into repeat-unit concentrations for the copolymer as a function of molecular size. For graft or branched copolymers, this will give a plot of weight concentration of each repeat unit in the copolymer as a function of molecular size. For random, alternating, or block copolymers, the size exclusion column can be calibrated to relate molecular size to molecular weight.[3-5] This analysis then produces a plot of copolymer composition as a function of molecular weight.

This new technique has been applied to graft copolymers of the biopolymer and lignin and used to prove grafting. Lignin is a complicated network polymer containing a diverse spectrum of functional groups and repeat-unit configurations.[36] Since it is second only to cellulose in mass of polymer produced by the biosphere, lignin has been used as a basis for grafting by numerous research groups.[37,38] A number of these studies have included synthesis and testing programs, but no proof of copolymer has been offered. Size exclusion chromatography combined with MCR peak analysis have been used to show that recently developed synthesis techniques actually graft lignin.[19,24]

The polymer used in this work, poly(lignin-*g*-(1-amidoethylene)), can be synthesized by hydroperoxide initiation

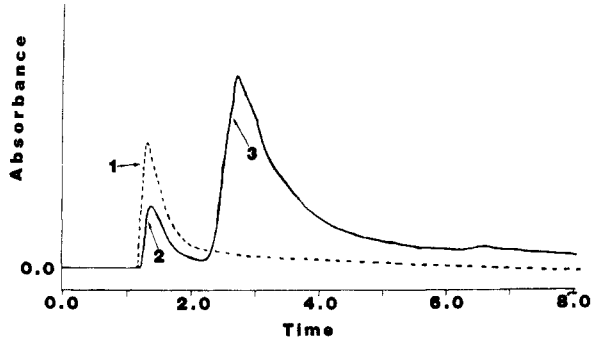


Figure 1. Size exclusion chromatograms of a graft terpolymer (1) and a lignin–poly(1-amidoethylene) mixture (2 and 3).

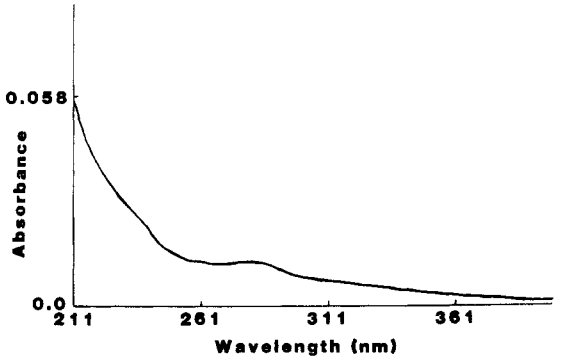


Figure 2. Ultraviolet absorption spectra of graft copolymer sample 7.

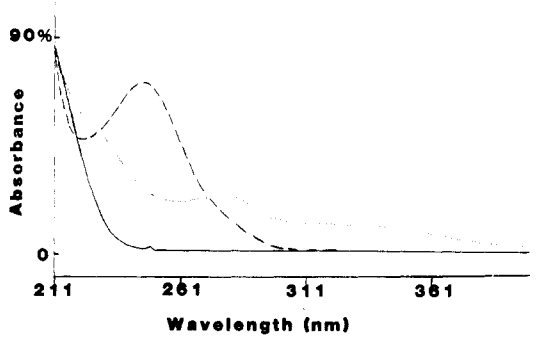


Figure 3. Ultraviolet absorption spectra of poly(1-amidoethylene) (—) (max absorbance = 0.030), poly(1-amidoethylene-*co*-(1-[*N*-(4-methoxyphenyl)amido]ethylene)) (– – –) (max absorbance = 0.093), and lignin (···) (max absorbance = 0.045).

of grafting in a polar organic solvent.[19,41] Synthesis data for sample 7, a poly(lignin-*g*-(1-amidoethylene)) copolymer, tested by SEC, is given in Table I. A chromatogram of this copolymer, run by the procedures given in the methods section, is compared to the chromatogram of a mixture of pure lignin and poly(1-amidoethylene) in Figure 1. These results show (i) that this SEC method can separate mixtures of homopolymer, and (ii) that the reaction product is a much larger molecule than the backbone. Ultraviolet spectra taken during the elution of the copolymer peak 1 and given in Figure 2 show that lignin is present throughout the copolymer peak, since the spectra have the characteristic form of kraft pine lignin absorbance. These SEC results thus show that the grafting reaction sharply increases the size of the lignin-containing molecule and provide strong support for graft copolymerization. However, these data do not prove that the side chain migrates with the backbone since the side-chain absorbance (see Figure 3) is overwhelmed by the backbone absorbance.

To prove that the backbone and side chain are bound together and to quantify the amounts of each present, an

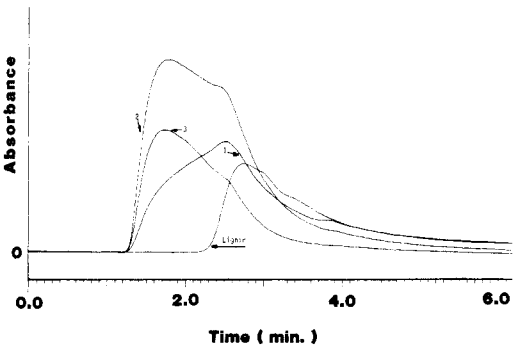


Figure 4. Size exclusion chromatogram of samples 1–3 and lignin (4).

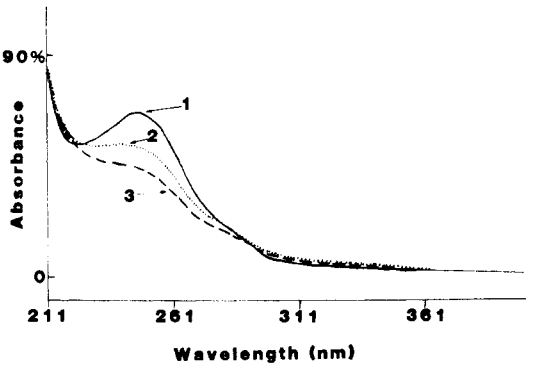


Figure 5. Ultraviolet absorption spectra taken during the elution of sample 1 at 1.845 min (max absorbance = 0.023), sample 2 at 1.644 min (max absorbance = 0.054), and sample 3 at 1.734 min (max absorbance = 0.036).

ultraviolet absorber was introduced into the side chain. The chromophore was a 4-methoxyphenyl unit attached to the nitrogen of 2-propenamide. The resulting monomer, *N*-(4-methoxyphenyl)-2-propenamide, was added to the grafting reaction at amounts equal to 2–8 mole % of all monomer in the reaction. Composition and yield of six reactions performed to synthesize a graft terpolymer are given in Table I. Evidence of monomer structure and synthesis conditions are given in ref 22. A pure ethane-type copolymer of 2-propenamide and N-substituted 2-propenamide was also made by using Fentons reagent as initiator[22] in 70%/30% (v/v) mixture of water and 2-methyl-2-pyrrolidinone. This "pure side chain" showed that the N-substituted amide repeat unit absorbed at 246 nm (Figure 3).

Size exclusion chromatograms of several of these three-component, graft copolymes of Table I are shown in Figure 4. UV spectra, taken during the elution of the peaks (shown in Figure 5), show absorbances at 246 and 287 nm. These absorbances of N-substituted 1-amidoethylene and lignin, respectively, show that the side chain and backbone do migrate together in the size exclusion column and that the reaction product of the lignin reaction is a graft copolymer.

These data, which contain detectable absorbances from both copolymer parts, can be used to quantify the composition of the copolymer throughout the elution peak. The elution peak is detected by monitoring and storing effluent absorbance at 210 ± 2, 220 ± 2, 230 ± 2, 250 ± 2, 280 ± 2, and 300 ± 10 nm. These wavelengths are chosen because they are points of maximum difference in the absorbance spectra of the poly(lignin-*g*-(1-[*N*-substituted]amidoethylene)) parts. In any application of this technique, these analysis wavelengths must be chosen carefully to ensure that the data treatment program,

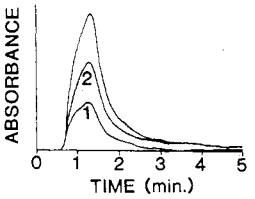


Figure 6. Chromatogram of terpolymer sample 1 from MCR-2 showing the absorbance contribution from N-substituted repeat units (1) and lignin (2) to total absorbance.

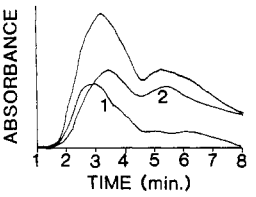


Figure 7. Chromatogram of terpolymer sample 4 from MCR-2 showing the absorbance contribution from N-substituted repeat units (1) and lignin (2) to total absorbance (unlabeled).

MCR-2, can correctly decompose total absorbance into component absorbances.

Once a chromatogram has been run and absorbance as a function of time at all six wavelengths has been sorted for the eluting peak, these data can be processed by MCR-2 to produce a plot of absorbance of each component of the copolymer vs. elution volume. Such component absorbance plots are given in Figures 6 and 7 for samples 1 and 4, respectively. These figures show the absorbance of each polymer component throughout the chromatogram and the relative amounts of each component at any point in the peak scaled in proportion to the component's absorbance.

A peak decomposition into component concentration would be chemically more useful than the peak decomposition into contributing absorbances. Component concentration data as a function of molecular size can be obtained by calibrating the SEC detector. Beer's law, absorbance vs. concentration curves will allow the MCR-2 absorbance plots to be converted to backbone or side-chain concentration vs. molecular size. Calibration techniques for the detector are described in Methods. The side-chain linear calibration curve for side-chain absorbance at 220 ± 2 nm in phosphate buffer at room temperature is A(side chain) = $34.53C_{\text{N-sub}}$ (ppm) + 0.602. The correlation coefficient for this line is 0.985 and $C_{\text{N-sub}}$ is the concentration in ppm of the N-substituted side-chain units in the buffer. The lignin calibration curve at 220 ± 2 nm is A(lignin) = $33.17C_{\text{L}}$ (ppm) + 0.1098. The correlation coefficient of the line is 0.978, and C_{L} is lignin concentration in ppm. Absorbance is in milliabsorbance units (mAu).

The side-chain absorbance calibration curve given above gives the absorbance of N-substituted repeat units vs. the concentration of N-substituted repeat units in phosphate buffer. But, the side chain is a copolymer with N-substituted and unsubstituted repeat units. The concentration of N-substituted repeat units given above is only a fraction of the concentration of side-chain copolymer. To determine the concentration of side chain from absorbance measurements, the fraction of N-substituted repeat units in the pure side chain used in the calibration must be known. The *inverse* of this fraction is the number by which to multiply the N-substituted repeat-unit concentration to get the polymer concentration. Similarly, the concentration of the side-chain copolymer in the graft terpolymer can only be determined if the fraction of side-chain repeat units that are N-substituted is known.

Table II
Hydrolysis Efficiency

soln	N-substituted monomer, g	M[a] of monomer in 50 mL of methanol	A[b] at 235 nm	conc of 1-amido-4-methoxybenzene, M	% yield of[c] hydrolysis
1	0.010 25	0.000 578 6	1.854	0.000 498 70	86.19
2	0.010 40	0.000 587 1	1.857	0.000 499 53	85.08

[a] This is the expected concentration that should result from 100% hydrolysis. [b] Absorbance of methanol solution after being diluted by a factor of 2.50. [c] % Yield = [concentration recovered/concentration expected] × 100.

Table III
Content of N-Substituted Amide in Polymers

sample no.	conc of soln, ppm	amt of 1-amino-4-methoxybenzene groups in polym, wt %	amt of 1-(*N*-(4-methoxyphenyl)amino)ethylene repeat units in polym, wt %
A. Data for Solutions of Graft Terpolymers Described in Table I			
1	747	9.81	14.11
2	1214	2.39	3.44
3	1048	2.19	3.69
4	315	9.19	13.22
5	140	32.24	46.39
6	540	9.95	14.31
B. Data for Poly(1-amidoethylene-*co*-(1-[*N*-(4-methoxyphenyl)amido]-ethylene)) Used to Calibrate Detector[a]			
7	227	7.72	11.17

[a] Monomer mole ratio was 0.0504 (this is the ratio of moles of N-substituted amide repeat units to moles of 1-amidoethylene repeat units in the copolymer).

The reason for this is that unsubstituted repeat units are not seen by the UV detector.

The secondary amide content of these polymers is determined by hydrolyzing the polymers to release 1-amido-4-methoxybenzene (I). The hydrolysis procedure is described in Methods and produces a methanol solution of I that can be analyzed for I concentration by UV spectroscopy. The calibration curve derived for this assay is

$$A_{\text{I}} = 8752C_{\text{I}}\ (\text{M}) + 0.027$$

Here, the absorbance of a methanol solution of I, A_{I}, is in standard units and I concentration, C_{I}, is in molar units.

The hydrolysis reaction is not 100% efficient as shown by tests run on *N*-(4-methoxyphenyl)-2-propenamide. These data, given in Table II, show the hydrolysis to be 85.6 ± 0.6% efficient. The amount of N-substituted repeat units in the side chain is then found by (i) hydrolyzing the polymer, (ii) quantifying the amount of I produced by UV spectroscopy, and (iii) correcting for the efficiency of the hydrolysis. The content of N-substituted repeat units in poly(1-amidoethylene-*co*-(1-[*N*-(4-methoxyphenyl)-amido]ethylene)) used to calibrate the SEC detector and in the graft copolymer dissolved in phosphate buffer for the size exclusion analysis is given in Table III.

Plots of graft copolymer component concentrations as a function of molecular size are given in Figures 8 and 9. Figures 6–9 show that the distribution of components in the solubilized graft copolymer varies from one sample to another. In Figures 6 and 8, both the side chain and backbone are uniformly distributed through the elution peak. Figures 7 and 9, on the other hand, show that the solution of copolymer 4 contains a higher concentration of side chain (1) in the large molecular size zone of the chromatogram. Lignin (2) is concentrated toward the lower molecular size zone of this chromatogram and makes up a larger weight percent of the polymer eluting there.

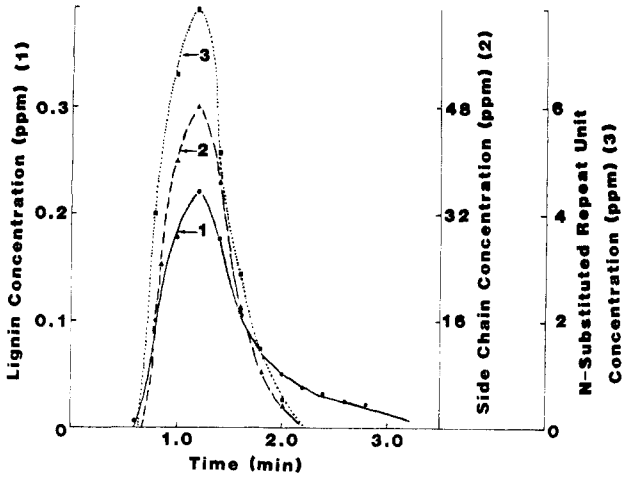


Figure 8. A plot of the concentration of lignin (1), 1-amidoethylene copolymer side chain (2), and N-substituted repeat units of the side chain (3) vs. molecular size as represented by elution time for sample 1.

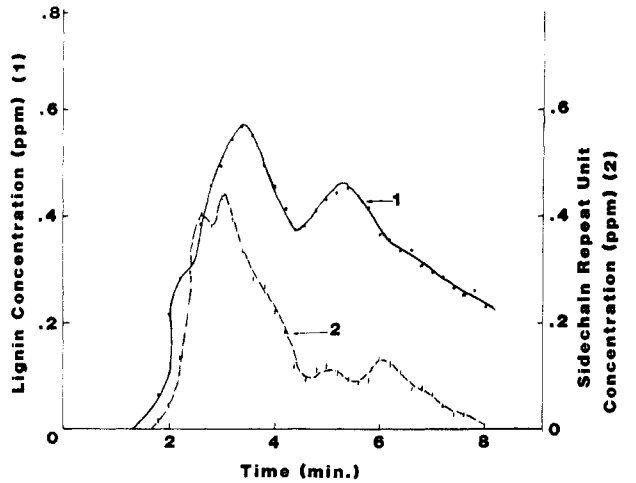


Figure 9. A plot of concentration of N-substituted repeat units (2) and lignin backbone (1) vs. molecular size as represented by elution time for sample 4.

There are indications that the chromatogram (Figures 7 and 9) of sample 4 is not, however, an accurate reflection of the composition of the bulk product.

These N-substituted, graft copolymers are difficult to dissolve in water because the 4-methoxyphenyl group is hydrophobic. Other work has shown that poly(1-amidoethylene-*co*-(1-[*N*-(4-methoxyphenyl)amido]ethylene)) dissolves readily in water with a pH between 0.5 and 3.0. Therefore, hydronium ion binding to the amide side group is probably a very important mechanism in dissolving these polymers. The lignin backbone, to the contrary, dissolves in water by deprotonation of phenol units. In pure form, lignin dissolves best in base and is insoluble in acid.

Different solution methods were used to dissolve portions of samples 1 and 4. All of the copolymer placed in the solvent was *not* dissolved. The approximately neutral solution used to dissolve sample 1 represented an equally accommodating solvent for both parts of the graft copolymer and probably extracted a representative sampling of molecules into solution. The 0.5 M sodium hydroxide solution, however, is a very good solvent for high-lignin-content molecules and a very poor solvent for the partially N-substituted side chain. During dissolution of sample 4, significant amounts of slightly grafted or ungrafted lignin would be extracted from the reaction product by the strong base.

Thus, preferential solution of parts of the product of grafting is the probably reason that the chromatograms of sample 4 are shifted to high lignin content at the low molecular size end of the chromatogram. These results show that selecting a solution process or solvent for a copolymer is like all other aspects of dealing with copolymers. It must be done with great care, or extraneous variables will affect the results.

This analysis technique also shows that rapid accumulation of spectral data across the elution peak of a copolymer allows proof of copolymerization to be established from small amounts of polymer. The amount of copolymer injected into the size exclusion chromatograph in a typical analysis run is 9.5 μg. Further, the spectra-processing techniques possible with the use of MCR-2 curve resolution software allow the solute in the detector[42] to be analyzed for weight percent concentration of two components. This constitutes an analysis on 3 ng of polymer. For a 200 000 molecular weight molecule, this is an analysis of 8 billion molecules.

The technique also enables tests to be performed for the total amount of copolymer eluted or for the adsorption or tailing loss in the chromatography column. By trapazoidal rule integration of the concentration curves vs. time of Figures 8 and 9, a total detection of 6.08 and 3.36 μg of copolymer is obtained. The detection limit of this method is 0.04 μg. These amounts constitute 81.4 and 48.7 wt % of the original material injected, respectively. This is a higher than expected recovery.

Several processes can affect the precision of composition analysis across the chromatographic peak. If one part of the copolymer preferentially aggregates, this can cause a significant shift in that portion's molar absorptivity at a given wavelength.[43] Using a solvent that effectively solubilizes both parts of the copolymer will minimize this effect. Hypochromic shift effects and band shifts can also change the absorbance readings of a copolymer as the molecular tacticity or chain sequence distribution changes.[44] These effects are quite significant with poly(1-phenylethylene)[45] but have not been detected with the materials used here. A critical aspect of this analysis procedure is the selection of wavelengths at which the peak absorbance is to be measured. This must be done to maximize the absorbance difference of the components at the chosen wavelengths or the MCR-2 software will incorrectly distribute the total absorbance between copolymer components.

The precision of this method is the sum of all the uncertainties from the procedures used to obtain the final results. These errors have been summed and plotted about the weight percent side-chain curve shown in Figure 10. This figure plots the total concentration of N-substituted repeat units seen in the elution peak of the terpolymer vs. weight percent of I hydrolyzed from the terpolymer. A datum for sample 6 of Tables I and III is not included in this figure because the sample precipitated during hydrolysis. The reason for the precipitation is unknown.

An absolute relative error of ±4% is found in correlating hydrolysis concentration to I content of the terpolymer.

Conclusions

A method has been developed that allows proof of copolymerization. The technique shows that separate parts of the copolymer, having distinctly different untraviolet spectra, migrate together through a size exclusion column. Separation of the total absorbance of column eluent into contributing absorbances allows copolymer composition plots as a function of molecular weight or molecular size to be made. Upon calibration of the ultraviolet absorbance

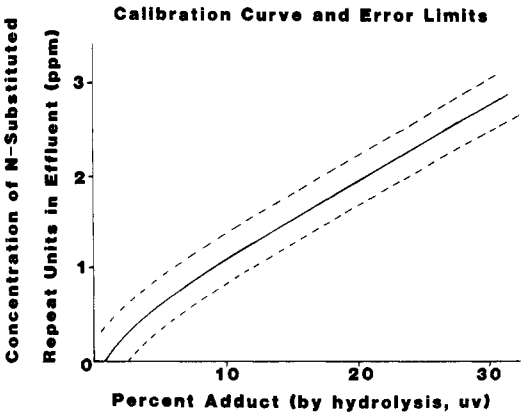


Figure 10. Calibration curve with error limits showing the concentration of N-substituted, side-chain repeat units contained in a solution of a terpolymer that has the listed number percent of N-substituted, repeat units (adduct) in the side chain. The absorbance of each solution presented by this curve is 0.10.

detector, these plots can be converted to graphs of component concentration vs. molecular weight or molecular size. The absolute percentage error in this composition assay is ±4.0%. Changes in solvent, copolymer structure, or coil aggregation can affect the absorbance of the copolymer and thereby increase the error in the composition assay of a copolymer peak. The six wavelengths monitored to gather data for the separation of total absorbance into copolymer part absorbances are critical values that determine the validity and precision of the absorbance decomposition. Wavelengths should be selected such that the copolymer parts have maximum differences in molar absorptivity at these points.

This technique can be used to do compositional analysis on nanogram quantities of copolymer. It has been used to prove free radical, graft copolymerization of 2-propenamide onto lignin and to study the composition change in the copolymer as a function of molecular size.

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Registry No. (Acrylamide)·(*N*-(4-methoxyphenyl)acrylamide (copolymer), 90385-97-0; (acrylamide)·(lignin) (copolymer), 90751-40-9; poly(1-amidoethylene), 9003-05-8; (acrylamide)·(lignin)·(*N*-(4-methoxyphenyl)acrylamide (copolymer), 93082-72-5.

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