CHARACTERIZATION OF ORGANOMETALLIC POLYMERS BY SIZE EXCLUSION CHROMATOGRAPHY ON PRECONDITIONED COLUMNS

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SUMMARY

Tin-bearing organometallic polymers (OMPs) having tributyltin substituents on pendant carboxylic acid groups are in current, extensive use as marine biocides. Fractionation of the substituted polymer by size exclusion chromatography (SEC) on polystyrene cross-linked with divinylbenzene (PS–DVB) is complicated by surface adsorption of cations even on this relatively inert packing material. This paper describes chromatography performed successfully on PS–DVB columns following pretreatment with an organometallic cation to establish a positively charged surface.

Tin-specific SEC illustrates the methodology. The column packing is preconditioned with an organic solution of tributyltin-bearing cation derived from monomer esters, to prevent cleavage of the tin-bearing moieties from the OMP. Mass-sensitive differential refractive index and element-specific graphite furnace atomic absorption spectroscopy detectors in tandem give self-consistent values for key molecular parameters (molecular weight, molecular weight dispersion, and the distribution of tin in high- and low-molecular-weight fractions). Samples prepared on a laboratory scale or on a commercial scale show no substantial differences in products prepared by two substantially different reaction pathways. The modified procedure is recommended as a critical method for comparing different organometallic polymers in organic solution.

INTRODUCTION

Tin-bearing organometallic polymers (OMPs) are highly effective, controlled release, long-lived molluscides[1] increasingly used on ocean-going ships as well as buoys and private craft exposed to marine microorganisms. A method for the laboratory scale preparation of OMP employs the free-radical induced copolymerization of methyl methacrylate (MMA) and tri-*n*-butyltin methacrylate (TBTM) at the temperature of refluxing benzene[2]. The suggested product formula is depicted in Fig. 1. The polymer molecular weight (MW) may range from 35 000 to 65 000 or more[3]. Our experimental objectives for the characterization of this polymer by size exclusion chromatography (SEC) on columns packed with polystyrene cross-linked with divinylbenzene (PS–DVB) were discussed in earlier papers[4,5]. We used SEC coupled with
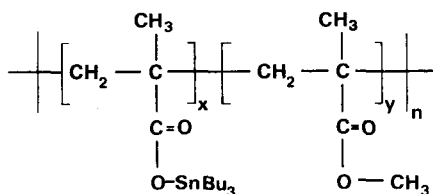
Fig. 1. Suggested formula for OMPs, showing pendant esters along a polymer backbone chain. No definite values have been attributed to x, y, or n. Bu = n-butyl.

a graphite furnace atomic absorption (GFAA) spectrometer to determine from chromatograms polymer MW, molecular weight dispersion (MWD, or continuous distribution of the polymer peak into MW fractions) and the extent and kinetics of reactions[6] by which tin-bearing monomers are converted to polymer, *i.e.*, the final ratio of reacted high-molecular-weight tin species to low-molecular-weight tin species, or the "conversion". Conversion and tin distribution are critically important parameters for predicting mechanical properties of polymers and the useful lifetime of OMPs.

These early studies involved repeated injections of OMP without intervening column regeneration, but more recently we have found that tin-bearing moieties along the polymer chain were adsorbed on PS–DVB packing materials from tetrahydrofuran (THF) solutions of OMP and could then be desorbed by trace amounts of acetic acid in THF[7]. This implies that dissociation of the OMP on PS–DVB may occur on unmodified column packing.

The mechanism by which cations are adsorbed on PS–DVB is the subject of a recent article concerning aqueous solutions containing inorganic salts, where ionic species form a charged surface on the neutral layer[8]. Non-coulombic forces are thought to be responsible for the initial adsorption of cations. In organic solutions of OMP, there are no added salts. If the behaviour of organic and inorganic solutions is analogous, the only cations here available are the tributyltin substituents, owing to dissociation of either polymer or monomer esters.

In the present experiments, we precondition our columns with the same cationic tin-bearing species that is associated with the polymer, inject much larger samples of OMP than formerly[5] and collect fractions for off-line analysis. A corollory objective is the comparison of OMP samples prepared by two different pathways: by copolymerization of MMA and TBTM to yield the polymer tin ester (Fig. 1); or by prepolymerization of MMA and methacrylic acid (MAA) followed by esterification with bis(tributyltin) oxide (TBTO). Our data successfully show that both reaction pathways yield an equivalent product, indicating that the latter, commercially developed pathway provides OMP molecularly similar to the material previously prepared on a laboratory scale and successfully tested in the field for period up to five years[2]. Moreover, the method is suggested as a standard procedure for evaluating the major molecular parameters.

A companion papers[9] will describe complementary analyses of polymers and prepolymers by Fourier transform high-resolution nuclear magnetic resonance (FT-NMR) showing complete configurational sequencing information at the triad level, which is similar for both types of OMP.

EXPERIMENTAL*

Chemicals

Benzene, THF, methanol, methylisoamyl ketone, acetic acid, and other common solvents all were of reagent grade or higher purity. The Stoddard solvent (mineral spirits) serving as a solvent for various OMPs reportedly was of commercial grade. A sample of TBTM used for conditioning chromatographic columns had been stored at − 15°C for one year before use, with probable degradation. This sample was dissolved in THF and used without purification.

Polymers

The free-radical induced polymerization of MMA and TBTM[2] may employ a MMA:TBTM ratio of 1:1 (OMP-2) or 2:1 (OMP-8). Samples prepared on a laboratory scale, here are designated OMP-2N or OMP-8N. In a process proposed for commercial scaled-up production, a "prepolymer" of MMA and MAA is prepared in the 1:1 or 2:1 ratio by free-radical copolymerization. The pendant free acid groups are then esterified by TBTO. These samples are designated OMP-2P and OMP-8P respectively.

Instrumental methods

The SEC–UV/ΔRI–GFAA system has been described in detail[5]. The mobile phase consisted of THF. In some of the experiments, discrete quantities of THF (50 μl or 500 μl) containing 1.0% (v/v) acetic acid were injected at measured intervals after injection of the polymer solutions to elute completely the adsorbed tin[7].

GFAA measurements were taken both on-line[5] and off-line. For off-line measurements, discrete volumes (0.5–1.0 ml) of effluent were collected in vials during a chromatographic run for subsequent quantitation. Individual specimens were diluted with appropriate, measured volumes of THF to assure that GFAA readings were taken within the scale of linear response.

Tin recovery was calculated by comparing the amount of signal, summed over all of the discrete, collected volumes, with that of a sample injected into the sample loop and collected before entering the SEC column.

Excepting one experiment, the SEC columns consisted of μStyragel (Waters Assoc., Milford, MA, U.S.A.) having a nominal average pore size of 100 Å, 1000 Å, or 10 000Å. Columns of these different pore sizes were used in several combinations.

Several experiments were performed to test whether a dissociation mechanism results from exposure of the tributyltin-bearing polymer to aromatic groups in the solid phase. One of these experiments employed one, two, or three columns in series, following regeneration by treatment with acetic acid as described above to remove all traces of tin before the next injection. A second experiment used two sets of three columns pretreated individually with a solution of organotin species (OMP or TBTM) in THF. Tables and figures summarizing experimental results also outline the individual experimental conditions.

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* Certain suppliers of chemicals and equipment are identified by name in order to specify the experimental conditions adequately. This does not imply endorsement or recommendation by the National Bureau of Standards nor does it imply that the particular brands of chemicals and equipment named are necessarily the best for the purpose.

For molecular weight estimation, columns were calibrated with a series of standard samples of polystyrene in THF (Arro Labs, Joliet, IL, U.S.A.)[3].

Treatment of data

The determination of number- and weight-average MW by SEC–GFAA has been described in detail[3]. In the present work we used a manual procedure[10] for calculating MW from SEC–ΔRI chromatograms. This consisted simply of tracing a peak onto graph paper and then cutting out, and weighing 0.5-cm strips of paper subtending the SEC peak. The MW assigned to each strip was estimated at its center for the present experiments. For low-molecular-weight species, usually present in much smaller amounts, the MWs were estimated from their elution volumes, determined at the intersection point of lines tangent to the sides of the peak. Number- and weight-average MW values were calculated from the same formulae (eqns. 1 and 2) for both SEC–GFAA and SEC–ΔRI.

$$M_w = \frac{\Sigma h_i m_i}{\Sigma h_i} \tag{1}$$

$$M_n = \frac{\Sigma h_i}{\Sigma h_i / M_i} \tag{2}$$

Each h_i represents either the height of a GFAA peak or the weight of a 0.5-cm segment of the peak cut out along its base (ΔRI).

The extent of polymer conversion was calculated from eqn. 3

$$\Delta P(\%) = \frac{(P)}{(P + M)} \cdot 100 \tag{3}$$

where ΔP is the degree of conversion, P depends on the polymer content, and M depends on the proportion of monomer or low-molecular-weight species. It is assumed that areas in the chromatograms represent actual weight fractions of high-molecular-weight (P) or low-molecular-weight (M) species in the unfractionated mixture.

RESULTS AND DISCUSSION

SEC evidence supporting a cleavage mechanism

Earlier SEC–GFAA results on OMPs tested in this laboratory employed columns that had been injected repeatedly with OMP solutions, and showed tin distributed in three distinct fractions: a high-molecular weight fraction, a low-molecular weight fraction and a cationic fraction absorbed on PS–DVB. The last was readily desorbed by 1.0% (v/v) acetic acid in THF, with nearly quantitative overall tin recovery[7].

In Table I are listed results of the chromatographic separations of six individual samples of an OMP dissolved in THF, with THF as the eluent. Desorption of tin species was effected by injecting 1.0% solutions (50 μl) of acetic acid in THF

TABLE I

SUMMARY OF SEC–GFAA FOR OMP-2N

Column: μStyragel 1000 Å, one. Mobile phase, THF; flow-rate 0.5 ml min^{-1}. Injected polymer, 0.38 mg in 50 μl of THF. 1% Acetic acid in THF injected 16 min after the polymer, and at 5-min intervals there after. $n = 6$.

	Polymer (%)*	Monomer (%)*	Adsorbed species (%)*	Recovery (%)**
	7.54	0.94	91.5	79.7
	5.39	0.86	93.7	80.3
	4.36	0.22	95.4	88.2
	8.03	0.66	91.3	61.5
	4.31	0.52	95.2	69.2
	4.48	0.48	95.0	72.8
Average	5.69	0.61	93.7	75.3
S.D.	1.68	0.26	1.9	9.4

* Percent of total tin observed.
** Percent of total tin injected.

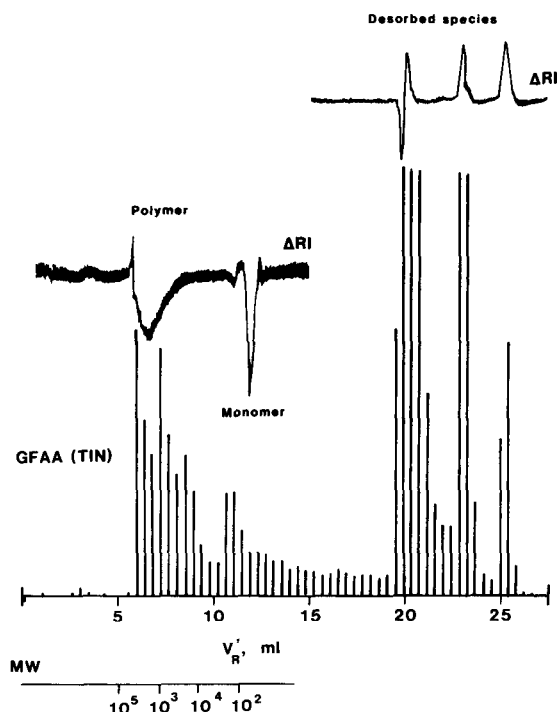Fig. 2. SEC–ΔRI–GFAA chromatograms of OMP-2N. Solvent, THF. Column, μStyragel (one), average pore size 1000 Å. Mobile phase, THF. Flow-rate, 0.5 ml min^{-1}. Detector, Perkin-Elmer 360 equipped with Sn-specific EDL lamp operating at 224.6 nm. Injected sample volume, 50 μl; concentration, 0.38 mg per 50 μl. 1% Acetic acid in THF injected (50 μl) 18 min after polymer, and at 5 min intervals thereafter. ΔRI sensitivity, $1 \cdot 10^{-6}$ ΔRI for full scale deflection, decreased to $5 \cdot 10^{-6}$ ΔRI at 15 ml.

18 min after the polymer, and at 5-min intervals thereafter. We now include ΔRI chromatograms to indicate the fate of polymer. Fig. 2 shows in the ΔRI chromatogram a much larger proportion of polymer mass than monomer, in direct contrast with the GFAA chromatogram, now indicating that polymer is successfully eluted by THF. Again it is clear that tin species are held back, in conjunction with earlier experiments in the SEC–GFAA of OMPs[3,6], the current data indicate that column conditioning is the critical prerequisite of successful SEC separations, to avoid cleavage of esters of the undissociated polymer. A set of experiments was devised to verify that cleavage will occur on columns treated with 1.0% acetic acid in THF with all traces of tin removed and not preconditioned with a tin-bearing cation.

Demonstration of cleavage

Varying length of column. A 500-μl solution containing 6.5 mg of OMP in THF was injected into either one, two, or three 30-cm 1000-Å PS–DVB columns previously treated individually or in tandem with 1.0% acetic acid in THF, using THF as the mobile phase. Polymer fractions and the monomer fractions were collected consecutively for off-line tin determination. The adsorbed species were then desorbed with 1.0% acetic acid in THF. The data (Table II) clearly demonstrate dissociation of tin-bearing species from the polymer chain on either one, two, or three columns. This occurs primarily on the initial column since the quantity of polymer-associated tin is not affected by the number of additional columns used.

A relatively high proportion of monomer tin was eluted by THF from the single column, undoubtedly because the quantity of tin injected with and extracted from the single polymer injection was sufficient eventually to saturate the single column, reducing adsorption. In contrast, less than 3.0% coeluted with the low-molecular-weight species from two or three columns were used. All of the remaining tin-bearing species was adsorbed. We considered two suggested explanations: (a) that equilibria are established between active column sites and polymer sites, with the $(C_4H_9)_3Sn^+$ moiety exchanging between them[4]; or (b) that a small fraction of tin-to-polymer bonds were exceptionally stable, possibly owing to molecular rearrangements incorporating tin into the polymer chain. The former is supported by existing evidence. A smaller sample (0.68 mg, Table III) eluted through three columns gave a smaller proportion of polymer-associated tin, with higher adsorption, almost en-

TABLE II

ANALYTICAL SEC OF OMP-2P ON REGENERATED PS–DVB COLUMNS OF VARIED LENGTH

The injected solution volume contained 6.8 mg of unfractionated polymer per 500 μl. Column: 1000-Å μStyragel, 7.8 mm I.D.

Column length (cm)	Polymer tin (%)	Monomer tin (%)	Adsorbed tin (%)	Recovery* (%)
30	7.7	18.1	74.1	99.9
60	9.9	2.5	87.7	100.1
90	7.9	2.0	90.1	100.0

* Fraction of total tin recovered.

TABLE III

ANALYTICAL SEC OF OMP-2P ON 90-cm 3-PS–DVB COLUMNS: DISTRIBUTION OF AD-
SORBED TIN SPECIES

Tin recovery: 67.0% of total tin injected with 50 μl of solution containing 0.68 mg of polymer.

Fraction	Location	Percentage of total tin observed (%)
Polymer	Eluted	2.69
Monomer	Eluted	0.67
Adsorbed species*	Column 1	95.1
Adsorbed species*	Column 2	0.78
Adsorbed species*	Column 3	0.77

* Desorbed with aid of 1.0% acetic acid in THF.

tirely on the first of three columns. Prior molecular rearrangement should have re-
sulted in a constant proportion of polymer tin, regardless of polymer concentration.
This phenomenon does not occur. Moreover, we have not as yet detected C–Sn–C
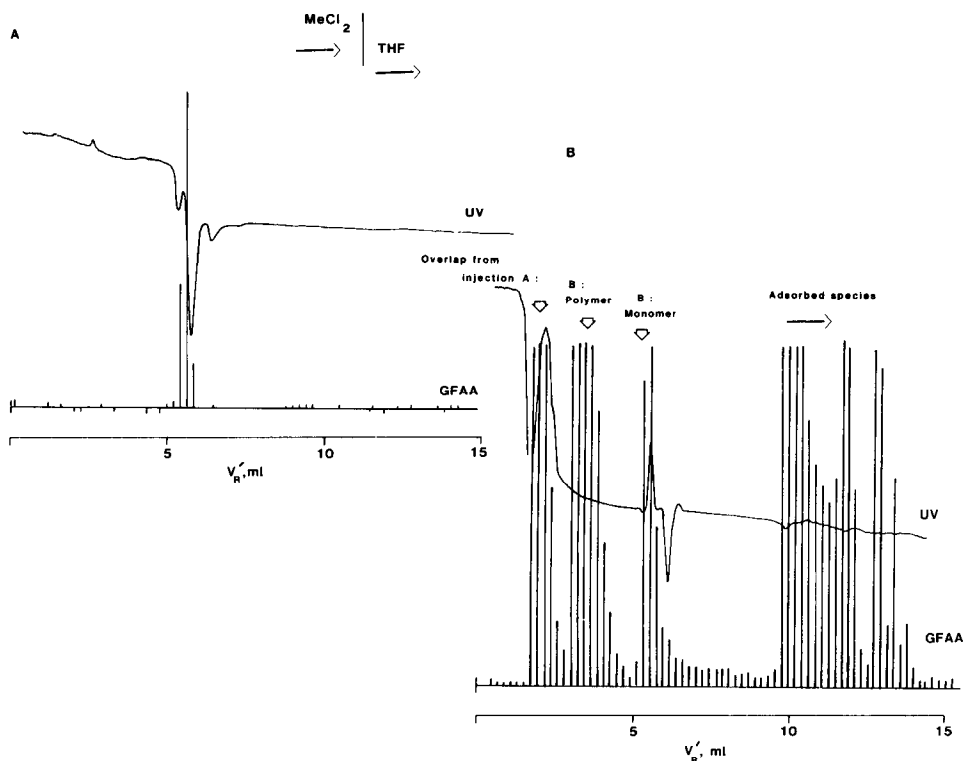bonds by FT-NMR in any of the OMPs.

Fig. 3. SEC–GFAA of OMP-2P. (A) Solvent, methylene chloride. Mobile phase, methylene chloride
(MeCl$_2$) for the first 25 ml, then THF. Flow-rate, 0.5 ml min^{-1}. Injected sample, 0.45 mg per 50 μl. UV
sensitivity, 0.0125 a.u.f.s. Column the same as for Fig. 2. (B) Solvent methylene chloride (MeCl$_2$), eluent
THF. Other conditions as above. Acetic acid injected 16 min after the polymer solution and at 2–5 min
intervals thereafter.

Methylene chloride as mobile phase on μStyragel. Methylene chloride was substituted for THF as the mobile phase to reduce the possibility that the mobile phase might act as a solvating agent promoting dissociation of tin cations. Following injection of OMP dissolved in methylene chloride, only one peak was eluted from a single column, with a retention volume of 6 ml (Fig. 3A). Introducing 100% THF as the eluent resulted in the appearance of a monomer peak having a retention volume of about 6 ml, that appears as the first peak in Fig. 3B. A solution of OMP dissolved in methylene chloride but injected into a mobile phase of THF, gave polymer and monomer peaks with elution volumes identical to those obtained for OMP dissolved in THF (second and third peaks in Fig. 3B). Thus it is evident that methylene chloride, although a good solvent, is not a satisfactory mobile phase for OMPs.

Propyl modified silica as solid phase

Chromatography on this packing material gave a single peak with subsequent prolonged bleeding (Fig. 4). In view of the absorption problem, no additional experiments were undertaken using these columns.

Prior conditioning with OMP

Three selected 1000-Å μStyragel columns were conditioned individually by injecting three 5.0-mg samples of an OMP in 500 μl of THF prior to the run on which data were obtained. Although previously used for analysis of oil specimens, the columns had not been regenerated, *e.g.* by treatment with one percent acetic acid in THF. A plate count showed them to be individually and in tandem in good condition, having 20 000 theoretical plates per meter.

A sample of OMP fractionated on these columns gave the SEC–ΔRI and the SEC–GFAA chromatograms shown in Fig. 5. Coelution of tin-bearing species and polymer mass indicates that: (1) the triorganotin species indeed is bonded to polymer prior to chromatography, and (2) that it remains undissociated after traversing these
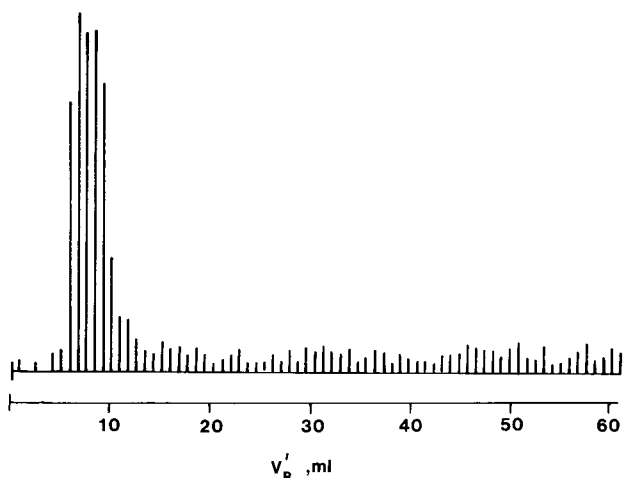

Fig. 4. SEC–GFAA of OMP-2N. Columns, propyl substituted silica gel (two). Average pore size 100 and 500 Å; dimensions 250 × 4.6 mm I.D. Other conditions the same as for Fig. 2, omitting acetic acid.
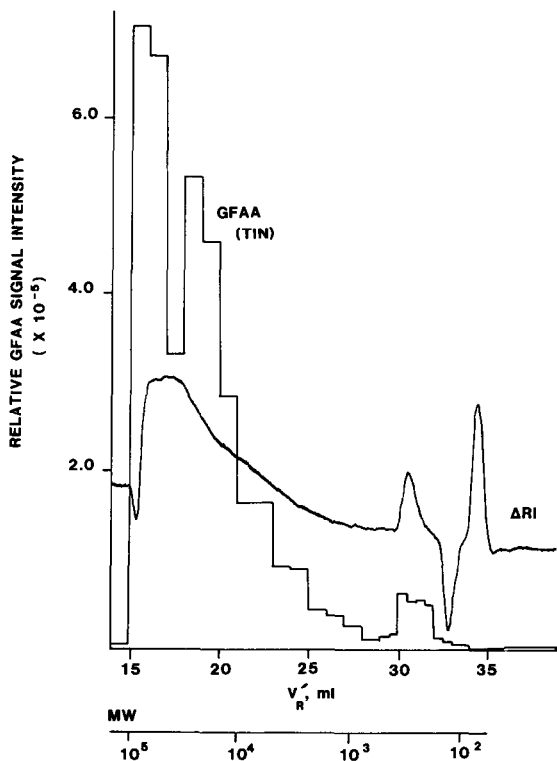
Fig. 5. SEC–ΔRI, GFAA chromatograms of OMP-2N. Columns μStyragel (three), pore size 1000 Å. Solvent and eluent THF. Flow-rate, 1.0 ml min^{-1}. Injected sample volume 5.0 mg per 500 μl; 0.5-ml fractions collected for off-line GFAA. ΔRI sensitivity, 1 · 10^{-6} ΔRI unit per full scale deflection.

TABLE IV

SEC–ΔRI CHROMATOGRAPHY OF OMPs

Columns: three μStyragel, 10 000, 1000 and 100 Å, in series. $n = 3$.

Sample	M_w (daltons)	M_n (daltons)	MWD	Mass conversion (%)*
OMP-8N	46 000	31 300	1.49	96.7
OMP-8P	50 000	28 400	1.75	96.3
OMP-2N	52 300	32 600	1.56	96.2
OMP-2P	63 700	40 900	1.56	96.6
OMP-2P	69 700	36 900	1.88	95.7
OMP-2P	62 800	33 900	1.85	95.8
OMP-2P				
Average	65 400	37 200	1.76	96.0
S.D.	3750	3500	0.18	0.5

* Conversion of low- to high-molecular-weight species.

TABLE V

SEC–GFAA CHROMATOGRAPHY OF OMPs

Columns: three μStyragel, 10 000, 1000 and 100 Å. $n = 2$.

Sample	M_w (daltons)	M_n (daltons)	MWD	Conversion* (%)	Recovery* (%)
OMP-8N	35 300	13 900	2.53	89.9	104.0
OMP-8P	37 800	15 000	2.52	95.9	93.4
OMP-2N	41 600	18 500	2.25	90.0	94.1
OMP-2P	65 000	28 500	2.28	92.5	111.0
OMP-2P	56 900	24 900	2.29	92.1	97.3
OMP-2P					
Average	61 600	26 700	2.28	92.3	104.2
S.D.	5700	2500	0.01	0.3	9.7

* Conversion of low-molecular-weight tin-bearing species to polymer.

columns. Assymetry in the SEC–ΔRI high polymer peaks shows that a fraction of the polymer is totally excluded, requiring SEC columns of greater pore size.

Prior conditioning with TBTM

Fig. 6 includes SEC–UV–ΔRI, SEC–UV, SEC–GFAA chromatograms of
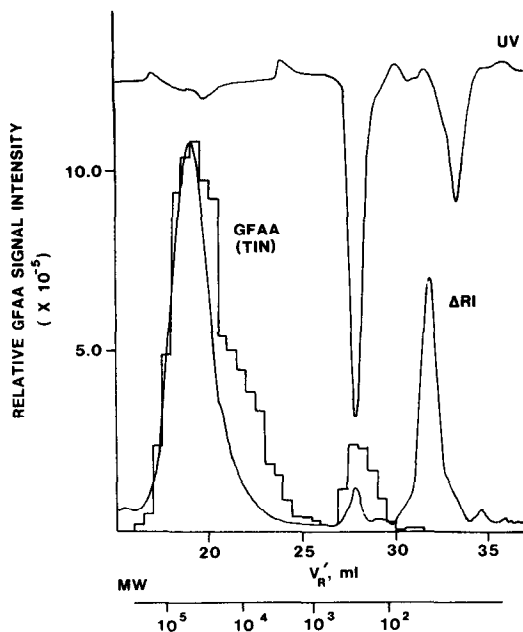

Fig. 6. Sample OMP-2N. Columns, μStyragel (three), pore size 10 000, 1000 and 100 Å, consecutively. Solvent and eluent THF. Flow-rate, 0.5 ml min^{-1}. Injected sample, 4.6 mg per 500 μl; 0.5-ml fractions collected for off-line GFAA. UV sensitivity, 0.1 a.u.f.s. ΔRI sensitivity, 1 · 10^{-6} ΔRI unit for full scale deflection.
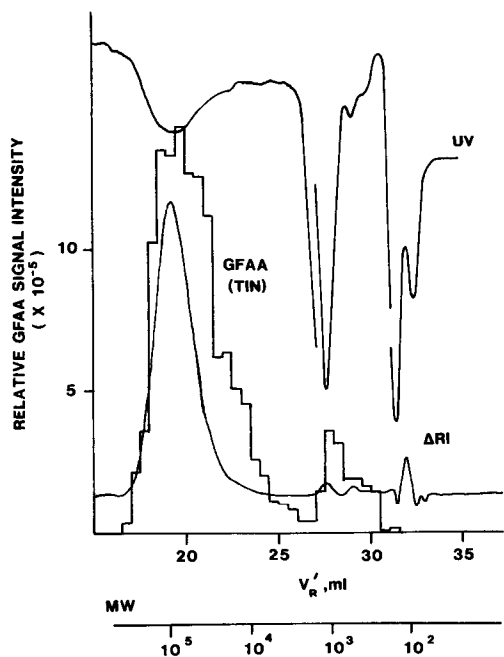
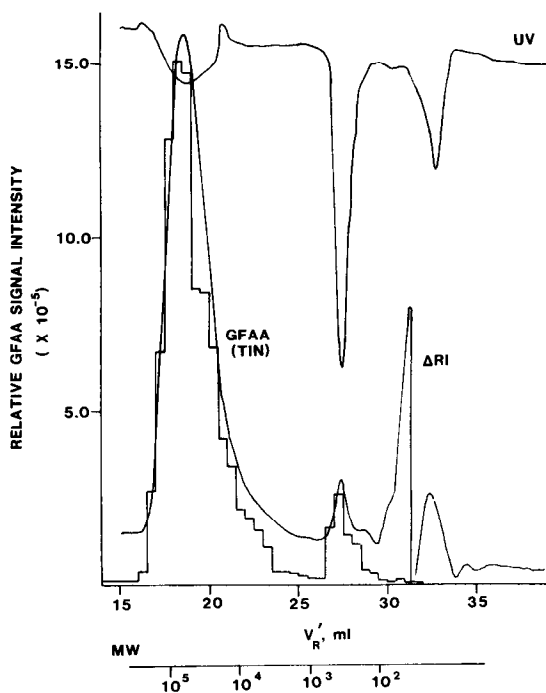Fig. 7. OMP-8N. All conditions the same as Fig. 6. Injected sample, 5.8 mg per 500 μl.

Fig. 8. SEC–UV–ΔRI, GFAA chromatograms of OMP-2P. All conditions the same as Fig. 6. Injected sample, 4.2 mg per 500 μl.

OMP-2P (commercial). Three columns were used in tandem, of pore size 10 000, 1000, and 100 Å, respectively. The column calibration is indicated below the chromatograms. Each column was deactivated with a 500 μl injection containing 2.0% (w/v) of TBTM. The data for this polymer and four other preparations dissolved in mineral spirits all are presented in Tables IV and V, with regard to mass (ΔRI) and tin (GFAA) chromatograms, respectively. The high values for mass conversion are not quite matched by those for tin conversion. Tin recovery, however is complete, indicating that adsorption of tin-bearing cations on the pre-conditioned packing is no longer a problem.

The similarity of both laboratory and commercial preparations (OMP-2N and OMP-8N, Figs. 6 and 7, OMP-2P and OMP-8P, Figs. 8 and 9) extends to MW determinations (Tables III and IV) obtained by either mass or tin measurement, and reproducible for either system within ±10%.

The fact that a UV peak nearly coincides with both the ΔRI and the GFAA peaks strongly indicates that chromophoric species are bonded to the tin-bearing low-molecular-weight species (650–1000 daltons). The MW suggests a compound or complex of tri-*n*-butyltin (or a degradation product) bonded to a chromophoric organic acid such as benzoic acid, or MAA. A specification requiring, for example, that total conversion exceed 89% with respect to either mass or tin would be met by all of the samples tested.
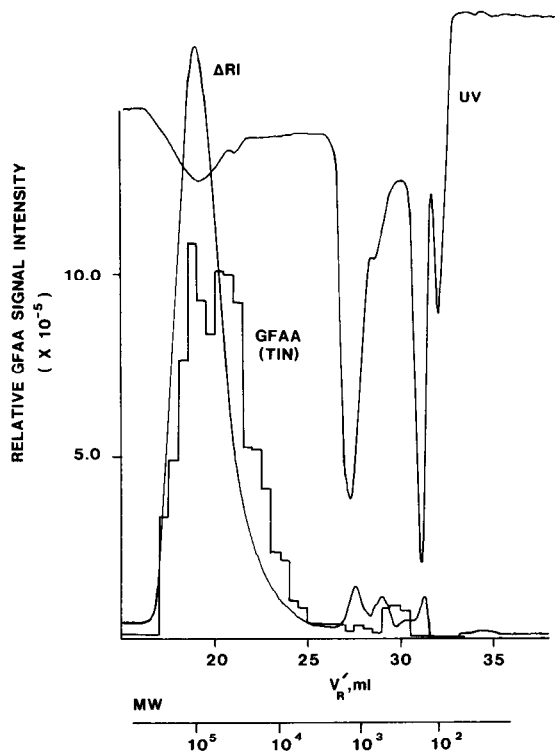

Fig. 9. OMP-8P. All conditions the same as Fig. 6. Injected sample, 4.9 mg per 500 μl.

CONCLUSIONS

SEC coupled with multiple detectors can provide powerful criteria for the characterization of organotin vinyl copolymers, for which formal criteria are needed to specify molecular properties, provided that columns are preconditioned with tin-bearing cations. A self-consistent measure of MW and MWD results, based on the distribution of either tin (GFAA) or mass (ΔRI) among fractions of different MWs. The strong correspondence between the distribution of mass and of tin is most important because it is known that a high proportion of tin in the low-molecular-weight material adversely affects field performance. The lack of chemically identical standards means that it is not possible to determine absolute MWs by measuring the elution volumes of a set of standards. Nevertheless, so long as materials of similar composition are being compared, the relative MWs provide valid criteria for comparing samples polymerized under non-equivalent conditions.

Direct comparison of samples prepared by esterification of prepolymer (OMP-P series) and samples prepared by copolymerization of TBTM and MMA (OMP-N series) shows essentially the same chromatograms (SEC) and spectra (NMR)[9].

ACKNOWLEDGEMENTS

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