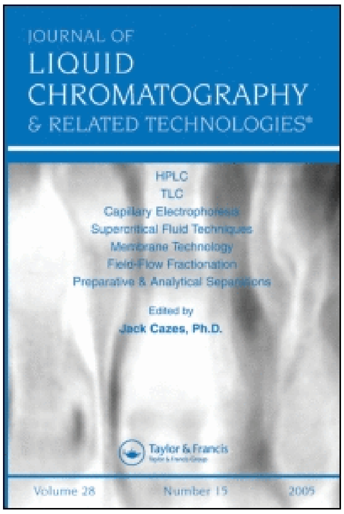

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Applications of High Performance Size Exclusion Chromatography To The Analysis of Oligomers and Small Molecules

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APPLICATIONS OF HIGH PERFORMANCE SIZE EXCLUSION CHROMATOGRAPHY TO THE ANALYSIS OF OLIGOMERS AND SMALL MOLECULES

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ABSTRACT

Size exclusion chromatography of small molecules has been investigated with high efficiency columns of crosslinked polystyrene. Performance evaluations and calibrations have been determined in toluene and in tetrahydrofuran, and the effect of the solvents on solute retention is discussed. Oligomer separations of samples obtained in the thermal degradation of several polymeric materials are reported, and the utility of the technique for polymer degradation studies is clearly evidentiated.

INTRODUCTION

The development of porous packings with small particle size and small pores has led to the availability of very efficient

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column systems for size exclusion chromatography (SEC) of low molecular weight (50-2000) compounds. Such separation systems are ideally suited for the analysis of oligomers and of small molecules which can be present as additives or can be formed through chemical reactions in polymeric materials. Chemical compounds in a mixture can be often resolved as individual peaks and therefore molecular weights can be assigned or substance identification can be performed.

The principal problems in SEC of low molecular weight compounds were early recognized as the following : (i) the molecules are separated according to their molecular volume in solution and not their molecular weight (1); (ii) solute-solvent and solute-gel interactions may influence the retention volumes of the solute (2,3). Many investigations report on the elution behaviour of different organic compounds in SEC. Retention volumes were used to calculate molar volumes of solutes (4), and in other cases correlations were established with dimensions of the molecules as obtained from molecular models (5,6).

The results obtained in the separation of small molecular weight molecules with SEC systems are strongly dependent on the nature of the eluents and of the eluted compounds. In fact, when the solutes are solvated by eluent molecules their effective molar volumes are higher than those calculated for the bulk compounds, and this is reflected in the measured retention volumes (2,3,5,7). Adsorption of the solutes on the porous packings may take place and give higher retention volumes than expected (7). The interactions between solute, solvent and organic gels can be predicted by the solubility parameters, and when the enthalpic contributions to the separation become important the separation mode is seen to shift from pure SEC to adsorption or to partition chromatography (8). Hybrid modes, size-partition and size-adsorption chromatography were recently reviewed (9). A thorough exploitation of the possibilities given by the different separation modes has been made by Sato et al. (10) who performed fractionation by molecular weight of styrene oligomers and then separated individual x-mers into pure diastereoisomers by changing solvent on the same column system.

For the assignement of molecular weights to the compounds fractionated in SEC the calibration of the column system is needed. When dealing with oligomers or generally with low molecular weight compounds, the calibration should be performed on the same eluent by using standards similar to the ones which must be separated, in order to take into account the possible effect of the solvent on the molar volumes. It has been also suggested that such calibrations can be related with those obtained with oligostyrenes or n-alkanes (11,12), which are the most usually employed standards. In some instances the calibration for a given oligomer series is not available, or the structure of the fractionated molecules is not known: due to the very high resolution attainable in the low molecular weight region, the components of a mixture can be separated into individual peaks and collected for further characterization, e.g. by infared spectroscopy, NMR, mass-spectrometry.

For several series of oligomers and small molecules the validity of the "universal calibration" approach, through the use of the parameter $M[\eta]$ proportional to the hydrodynamic volume of the molecules has been also proposed (13-15); an on-line viscosity detector would be needed and it should be carefully checked whether the precision of the measurement of intrinsic viscosity of such low molecular weight molecules is high enough.

In the present work are reported applications of separation by size of small molecules, using very high efficiency columns of microparticulate cross-linked polystyrene (16). Performances and elution behaviour as determined in toluene and in tetrahydrofuran (THF) are shown, and the analysis of samples obtained from polymer degradation studies is discussed.

MATERIALS AND METHOD

The SEC columns were Ultrastyragel type (Waters Ass.,USA), 30 cm x 7.8 mm i.d., with 10^3 A, 500 A and 100 A nominal porosities. A Waters M45 pump was employed, together with a Rheodyne injection valve; flow rate was 1 cm^3/min. Sample injection volumes of 50 or 100 µl were used, depending on the number of columns, and sample concentrations were between 0.2 and 0.5 % (w/v).A Siemens differential refractometer was used as a detector and also, when possible, a Zeiss PM2 UV spectrophotometer at 260 nm wavelength. Measurements were carried out at ambient temperature.

Polystyrene standards from Toyo Soda (Japan) and Arro Laboratories (USA) were used for calibrations, together with n-alkanes (C. Erba, Italy) and aliphatic aldehydes (Fluka, Switzerland).

Polymers used in the degradation studies were either commercial materials (phenol-formaldehyde resin from SIR, Italy; ABS and styrene-acrylonitrile from Montedison, Italy) or synthesized in our laboratory (polystyrenes and poly-p-methyl styrenes).

RESULTS AND DISCUSSION

Performance

The calibration curves of the individual columns, as obtained in toluene with PS standards are shown in Fig. 1. The columns with porosities 500 A and 10^3 A present practically the same separation range and selectivity; it can be noted that also with these two columns fractionation of oligomers down to the size of monomers, dimers, trimers, is still feasible, although with lower resolution than that possible with the 100 A column. Several column combinations were tested by determining the specific resolution R_{sp} for various pairs of n-alkanes according to the equation

$$R_{sp} = \frac{2\,(V_{R,2} - V_{R,1})}{w_1+w_2} \quad \frac{1}{\log\,(M_1/M_2)}$$

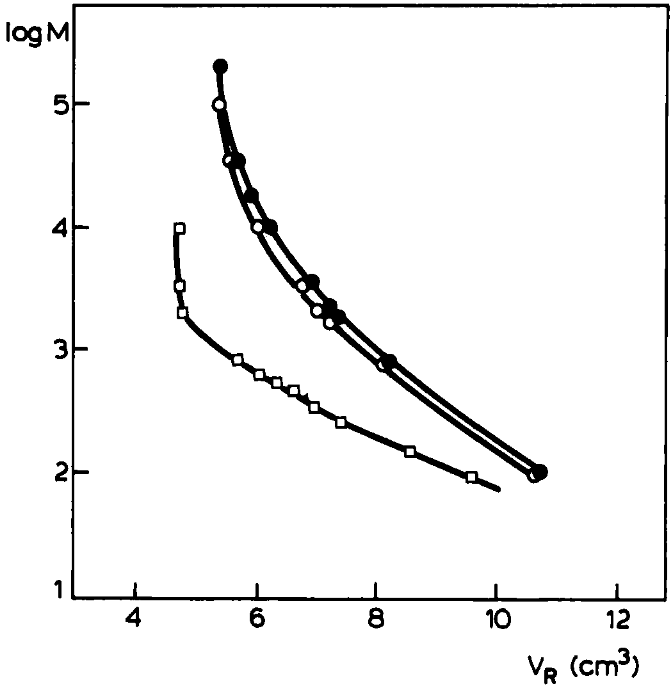


FIGURE 1. Calibrations of individual Ultrastyragel columns with PS standards. Solvent: toluene. □ : 100 A; o : 500 A; ● : 10^3 A.

where $V_{R,1}$ and $V_{R,2}$ are retention volumes, w_1 and w_2 are baseline widths, and M_1 and M_2 are molecular weights of n-alkanes 1 and 2 respectively. In Table 1 are reported the values of R_{sp} obtained with the 100 A column, with the 500+10^3 A, and with the three columns, 10^3+500+100 A, connected in series.

The results for the two-column system can be compared with those obtained by Krishen and Tucker (11) with a 60 cm TSK-2000 H

TABLE 1. Specific Resolution Values, R_{sp}, for Different Pairs of n-Alkanes

	C_1	C_5	C_8	C_{10}	C_{13}	C_{16}	C_{20}	C_{24}	Columns
C_{10}			9.32						100 A
C_{13}			8.66	9.87					
C_{16}			9.72	11.15	9.96				
C_{20}			8.64	9.70	8.63	9.25			
C_{24}			8.47	9.44	8.33	8.90	7.63		
C_{32}			8.05	8.87	7.72	8.17	6.90	6.44	
C_{10}			7.00						10^3 + 500 A
C_{13}			10.82	11.85					
C_{16}			13.46	14.31	16.80				
C_{20}			12.71	12.68	14.83	10.40			
C_{24}			13.24	12.98	15.15	12.87	15.89		
C_{32}			11.55	11.18	12.59	11.14	12.04	10.73	
C_5	7.43								10^3 + 500 + 100 A
C_8	8.54	12.22							
C_{10}	9.74	13.60	14.52						
C_{13}	10.30	14.00	14.80	16.29					
C_{16}	10.70	14.27	15.01	16.52	16.80				
C_{20}	11.11	14.55	15.25	16.78	17.08	17.34			
C_{24}	11.26	14.45	14.99	16.37	16.41	16.20	14.83		
C_{32}	12.54	15.73	16.21	17.79	17.79	17.63	17.02	17.55	

column, whereas the results obtained with the three columns (90 cm) can be compared with the ones determined by Kuo et al. (17) on a 100 cm column bank (TSK-2000 H + TSK-3000 H). In both cases the columns employed in this work give higher values of R_{sp} for almost all the molecular weights covered.

The column system which has been mostly employed for oligomer analysis is the 10^3+500+100 A one. Examples of separations which can be obtained with such columns are shown in Fig.2, where the chromatograms of different low molecular weight PS standards are reported. The oligomers are fractionated into individual components up to degree of polymerization x = 10; the molecular weights of the peaks are easily assigned because the A-500 and A-1000 standards (Toyo Soda Co., Japan) are supplied with a full molecular characterization. The availability of such standards allows for a detailed calibration of the columns in the range of molecular weights 100-1000; the degrees of polymerization of the fractions shown by the standard PS 800 (Arro Laboratories, USA) were assigned according to such calibration. It can be seen that the two standards A-1000 and PS 800 from different producers are very similar but do present some differences in the number and relative amount of the individual x-mers.

Calibration and Effect of the Solvents.

Complete calibrations of the system 10^3 + 500 + 100 A were performed in toluene and in THF with different series of

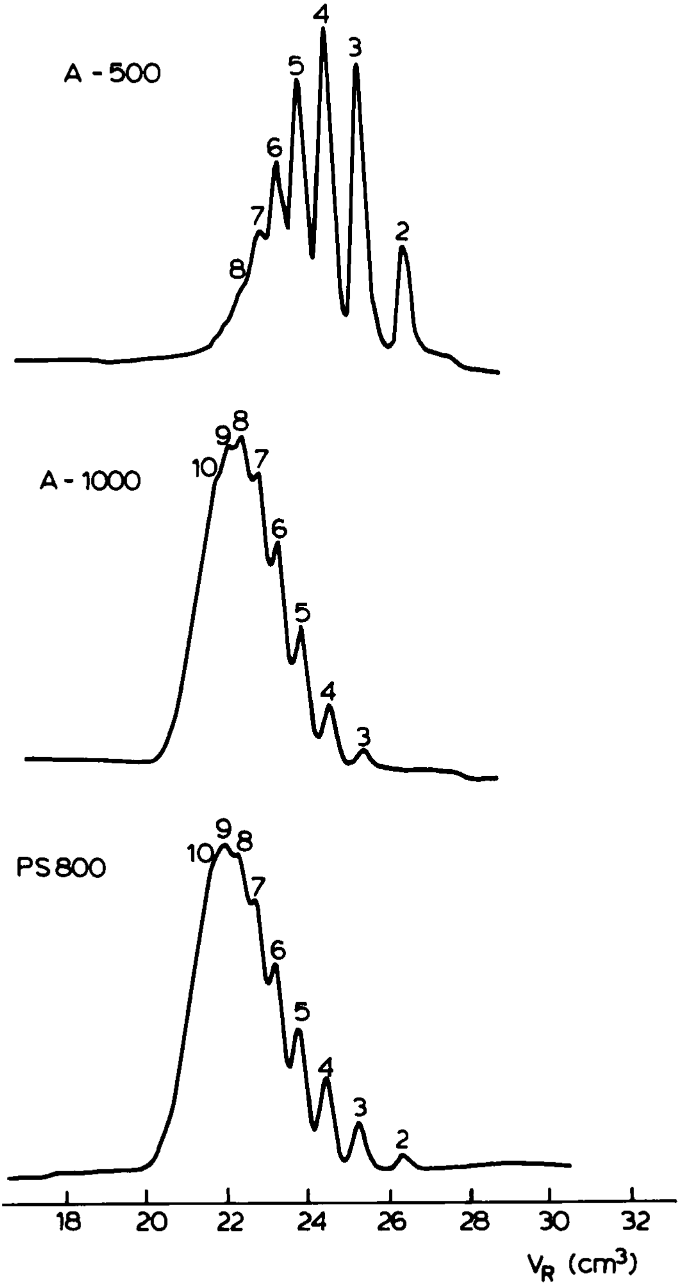


FIGURE 2. Oligostyrenes fractionated on the column system 10^3+500+100 A. Numbers on the peaks indicate degrees of polymerization of the x-mers.

homologous compounds, and are shown in Figs. 3 and 4. Retention volumes V_o for excluded samples were measured with PS standards, whereas the total permeation volume V_t of the columns was given by the peak of methane. The pore volume of the columns, as obtained from the difference $V_t - V_o$, was slightly higher in toluene (18.2 cm^3) than in THF (17.6 cm^3); the different pore volumes in these two solvents were also reflected in the higher number of theoretical plates measured in toluene (32000), compared with that in THF (28600). Even larger differences of gel capacity were reported by Mori (12) comparing elutions in THF and in chloroform with Shodex type columns.

The effect of the solvents is shown not only by the different retention volumes of the n-alkanes and of the PS standards, which are all likely to elute without any specific interaction with the gel or with the solvents, but also by the different elution behaviour of the aliphatic aldehydes, as seen in Figs. 3 and 4. It is evident that in toluene some adsorption phenomena occur, as the retention volume of ethanal is higher that the V_t of the columns; on the other hand, in THF solvation of the solute molecules might be present, similar to that reported for other polar compounds (2,3), and particularly for ketones both in THF (3) and in chloroform (7). The presence of these two effects can be depicted by plotting the logarithm of the molar volumes against the retention volumes of the aldehydes and comparing the results with those of the n-alkanes. The molar

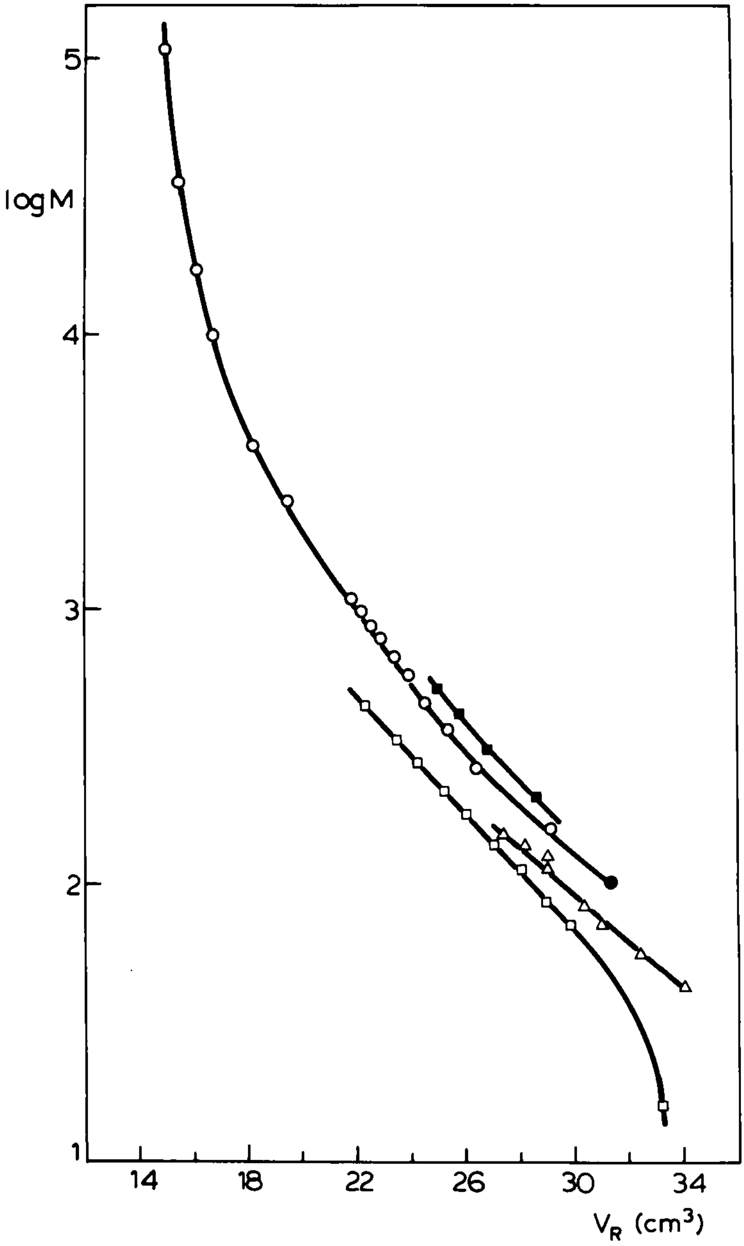


FIGURE 3. Calibration curves of 10^3+500+100 A columns in toluene for different series of homologous compounds.
□ : n-alkanes; △ : aliphatic aldehydes; o : PS standards; ■ : oligomers from PS degradation; ● : styrene.

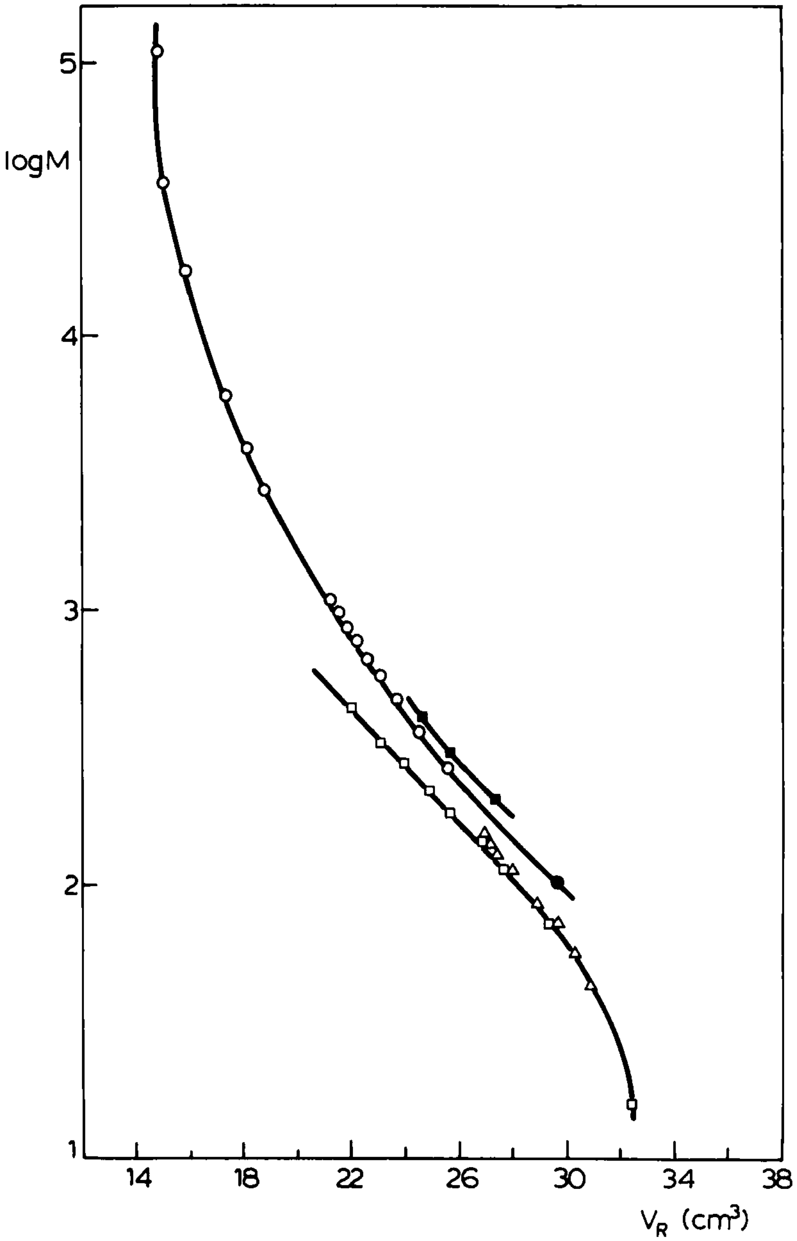


FIGURE 4. Calibration curves of 10^3+500+100 A columns in THF. □ : n-alkanes; △ : aliphatic aldehydes; o : PS standards; ■ : oligomers from PS degradation; ● : styrene.

TABLE 2

Molecular Characteristics of Aliphatic Aldehydes and Retention Volumes in Toluene and in THF for the Column System 10^3+500+100 A.

Sample	MW	Molar Volume (cm^3/mole)	log V_M	$V_R(cm^3)$ THF	$V_R(cm^3)$ Toluene
Ethanal	44.05	56.23	1.750	30.9	34.0
Propanal	58.08	72.08	1.858	30.3	32.4
Butanal	72.12	88.27	1.946	29.7	31.0
Pentanal	86.14	106.41	2.027	28.8	30.3
Heptanal	114.19	134.42	2.128	27.9	29.1
Octanal	128.22	156.16	2.194	27.4	29.0
Nonanal	142.24	172.12	2.236	27.1	28.2
Decanal	156.27	188.28	2.275	26.9	27.4

volumes of the aldehydes were calculated by the ratio of the molecular weights to the density of the samples, and the data of interest are reported in Table 2.

The results are reported in Fig. 5, where it is seen that in THF the aldehydes elute earlier than the alkanes, thus indicating that their effective molar volume is higher than calculated. In toluene, on the contrary, adsorption on the gel leads to retention volumes higher than those of alkanes; in both cases the difference of retention volumes tends to vanish with increasing size of the aldehydes, and practically disappears with decanal.

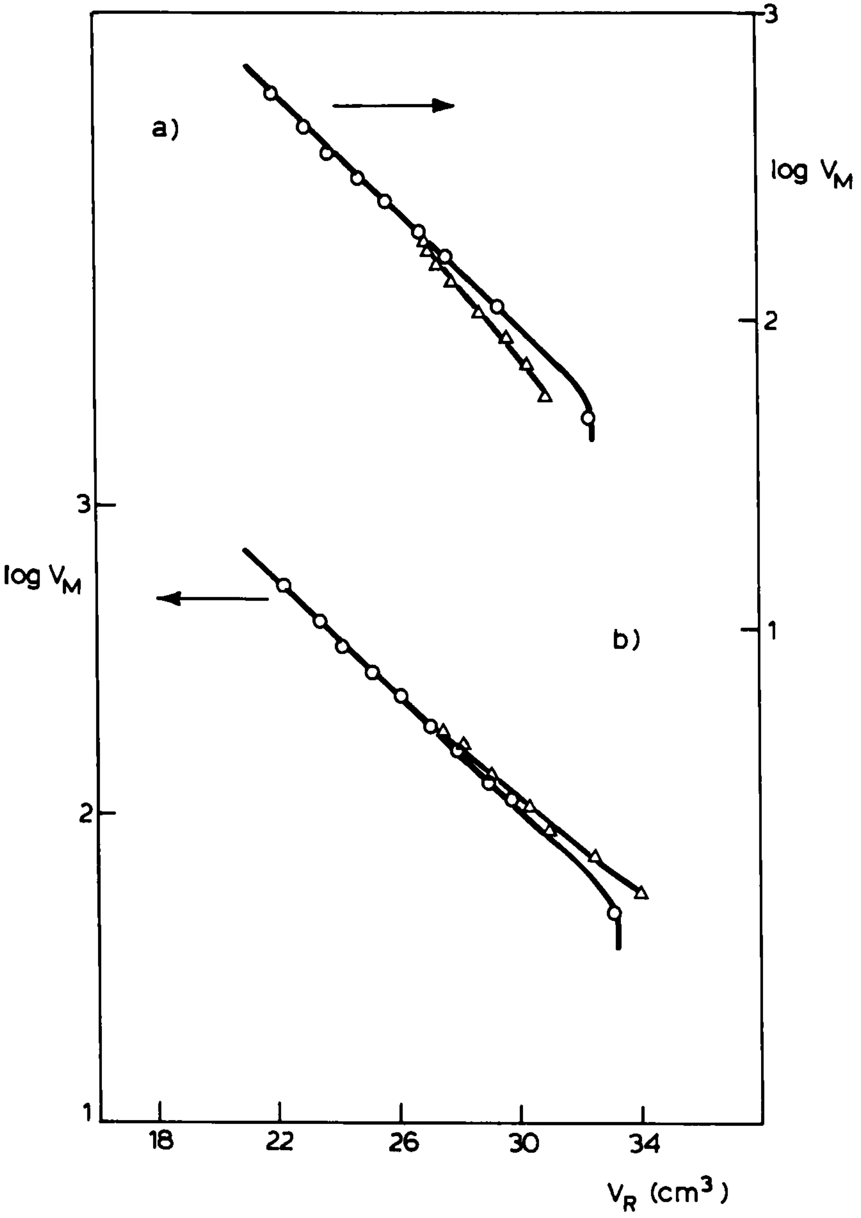


FIGURE 5. Logarithms of molar volumes vs. retention volumes in THF (a) and in toluene (b). o : n-alkanes; Δ : aliphatic aldehydes.

Analysis of oligomers and of degradation products.

Many organic polymers undergo thermal, photo or hydrolytic degradation with production of chain fragments, oligomers and, eventually, monomer or other low molecular weight compounds. Short chain fragments and oligomers can be conveniently fractionated and characterized by SEC. An example is given by the products obtained in the thermal degradation of polystyrene, which are known to be monomer and oligomers up to degree of polymerizarion $x \approx 7$ (18). In an experiment of thermal degradatiom under vacuum of a PS sample, the fraction which is not volatile at the degradation temperatures (300-500 °C) was collected and the chromatogram showed the peaks of oligomers with degrees of polymerizatiom $x = 2 \div 5$. For the molecular weight assignement of these oligomers it is not possible to rely on the calibration obtained with the anionic PS standards because the chemical structure of the latters is different, principally due to the initiator fragments. Dimers and trimers of PS characterized by NMR (19) were used for calibration purposes and their chromatograms are shown in Fig. 6. In Table 3 are reported the molecular characteristics of the different PS oligomers; the calibration relative to the samples from thermal degradation is also shown in Figs. 3 and 4.

Oligomers obtained in the thermal degradation of poly-p-methyl styrene, (PPMS), were as well fractionated on the same columns and the chromatogram in Fig. 7 was obtained. Peak

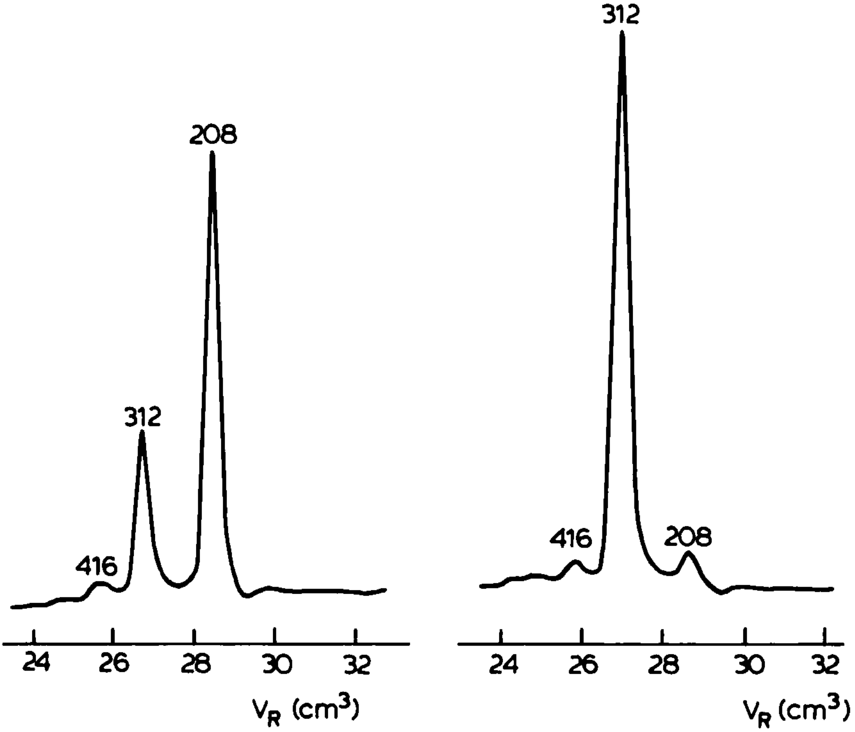


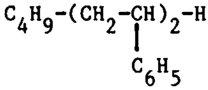
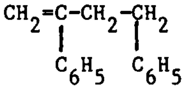
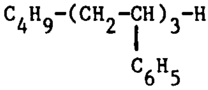
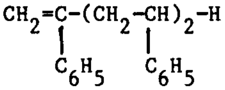
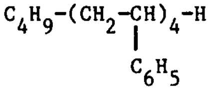
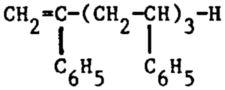
FIGURE 6. Oligomers from PS degradations. Numbers on the peaks indicate molecular weights.

assignement has been done on the basis of the results obtained from mass spectrometry of the fractions (20) and collected in Table 4. The presence of different dimers, having small differences in molecular weights, could be guessed from the shape of the peak having the highest V_R. In cases like this,the separations of isomers and of compounds with almost the same molecular weight are better performed by HPLC (20).

In the thermal degradation under vacuum of phenol-formaldeyde resins it has been reported that the largest

TABLE 3

Molecular Characteristics of PS Oligomers from Anionic Polymerization and from Thermal Degradation.

Oligomer	Anionic Polymerization	MW	Thermal Degradation	MW
Dimer	 $C_4H_9-(CH_2-\underset{C_6H_5}{\underset{\mid}{C}}H)_2-H$	266	 $CH_2=\underset{C_6H_5}{\underset{\mid}{C}}-CH_2-\underset{C_6H_5}{\underset{\mid}{C}}H_2$	208
Trimer	 $C_4H_9-(CH_2-\underset{C_6H_5}{\underset{\mid}{C}}H)_3-H$	370	 $CH_2=\underset{C_6H_5}{\underset{\mid}{C}}-(CH_2-\underset{C_6H_5}{\underset{\mid}{C}}H)_2-H$	312
Tetramer	 $C_4H_9-(CH_2-\underset{C_6H_5}{\underset{\mid}{C}}H)_4-H$	474	 $CH_2=\underset{C_6H_5}{\underset{\mid}{C}}-(CH_2-\underset{C_6H_5}{\underset{\mid}{C}}H)_3-H$	416

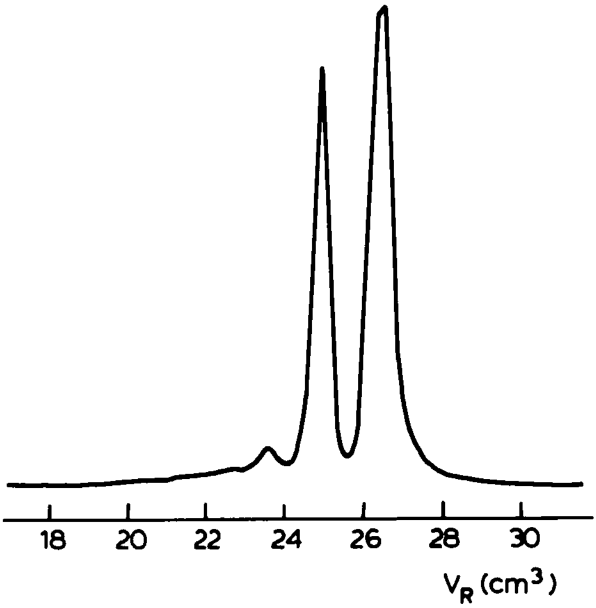
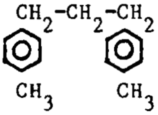
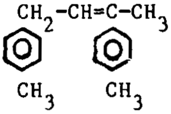
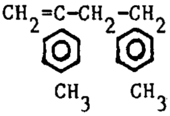
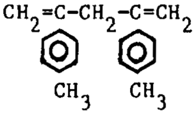
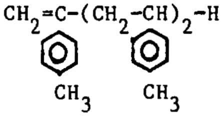
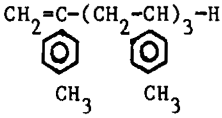


FIGURE 7. Chromatogram of the oligomers obtained in thermal degradation of poly-p-methyl styrene.

TABLE 4

Oligomers Obtained from Thermal Degradation of Poly-p-Methyl Styrene.

Structure	MW	V_R (SEC) (cm^3)
	224	26.5
	236	
	236	
	248	
	354	24.9
	472	23.5

fraction of products which are formed is constituted by several oligomers, originated either by evaporation or fragmentation of the original resin (21). A commercial, novolak type, phenol-formaldehyde resin was submitted to thermal degradation at different temperatures, and the oligomers formed in the process were collected for SEC analysis. In Fig. 8a) is shown the chromatogram of the original resin and in Fig. 8b) the chromatogram of the low molecular weight compounds which evaporate under vacuum at 150 °C: dimer and trimer peaks are found in both samples at 24.8 and 23.7 cm^3 respectively, together with some other compounds (at 26.8 and 25.3 cm^3 retention volumes) which are due to oxidation products of the novolak. In the degradation at higher temperatures (270 and 500 °C) the same oligomers are found (Fig.9), but at these temperatures tetramers (V_R= 22.6 cm^3) and pentamers (V_R= 21.8 cm^3) can also distill from the degradation site, and a different composition of the mixture is obtained.

ABS polymers form an important class of materials which have a wide spectrum of applications. Their thermal behaviour is influenced not only by the composition of the copolymers present, but also by the method of preparation of the material. Styrene-acrylonitrile copolymers, blended with butadiene-acrylonitrile copolymers, are used in some formulations of ABS polymers, whereas in other systems styrene-acrylonitrile copolymers grafted with polybutadiene are present. In the thermal degradation of these products the chain fragments can be

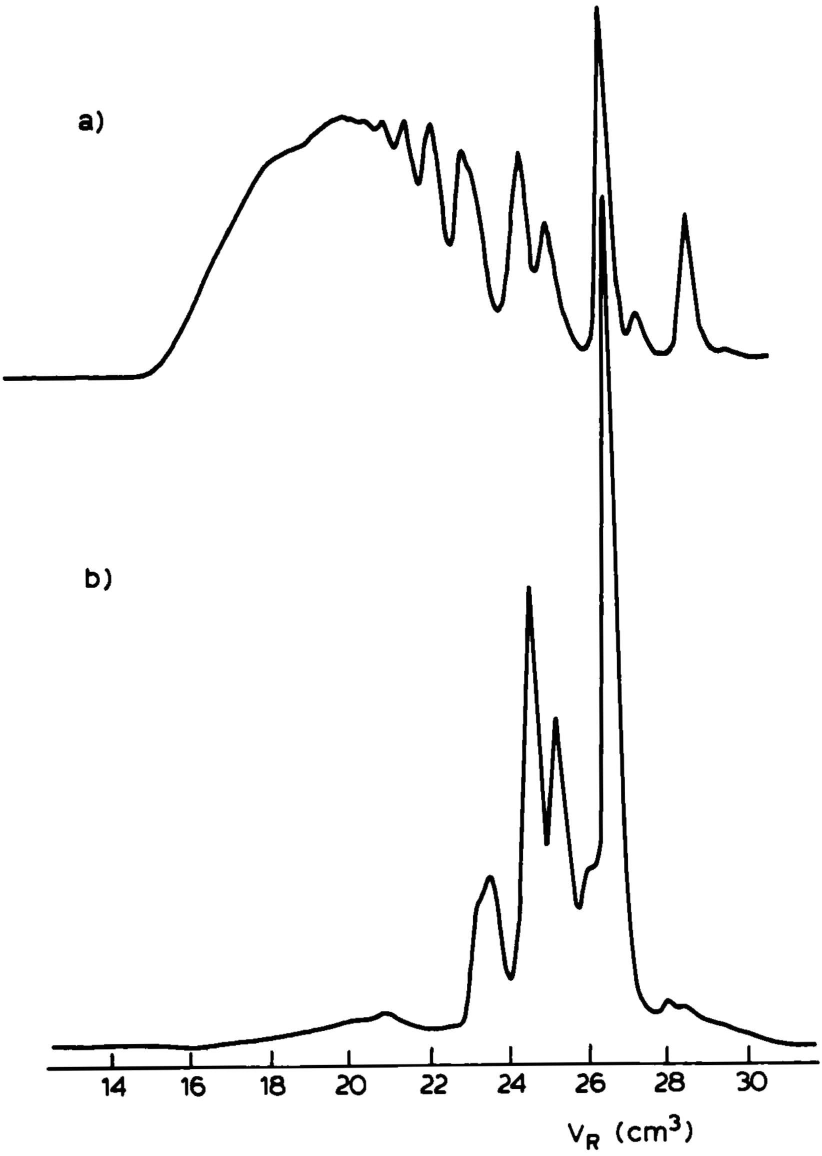


FIGURE 8. Chromatograms of novolak resin (a) and of products(b) obtained after heating at 150 °C.

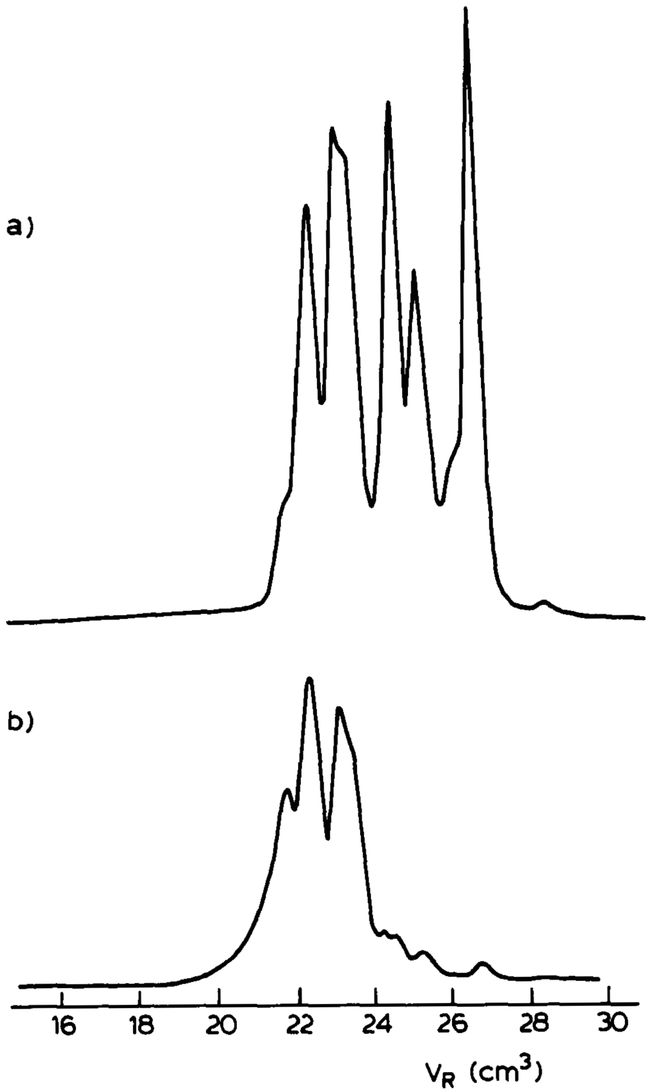


FIGURE 9. Chromatograms of the products of degradation of a novolak resin. Temperatures of degradation : a), 270 °C; b), 500 °C.

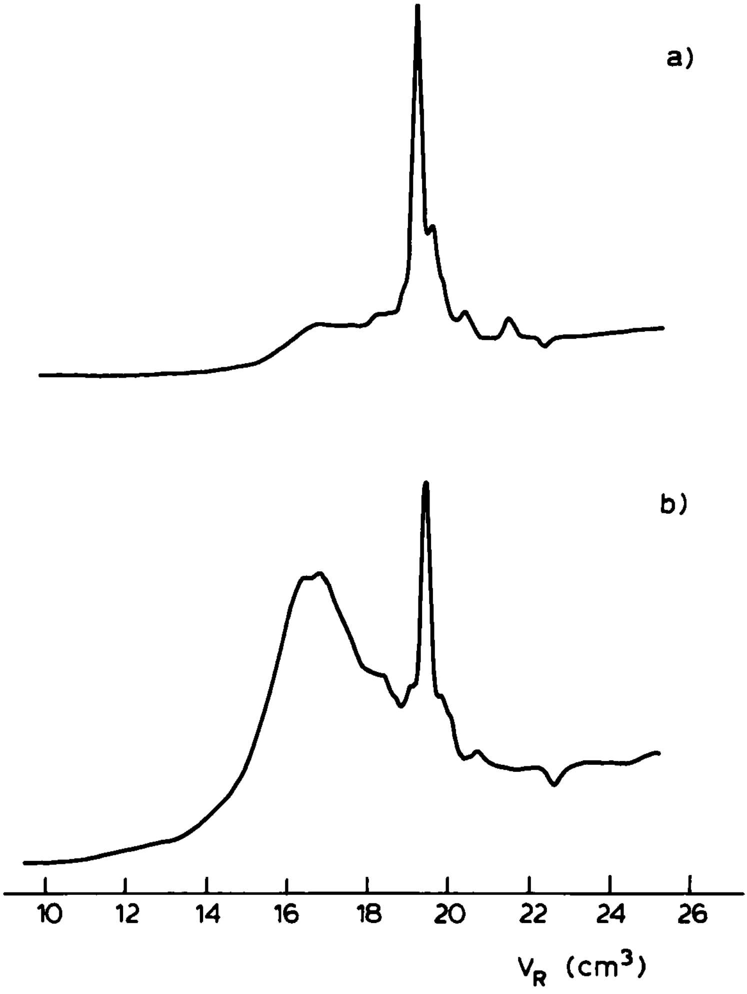


FIGURE 10. Oligomeric products formed in the thermal degradation of a) : styrene-acrylonitrile copolymer; b) : styrene-acrylonitrile-g-butadiene terpolymer.

conveniently analysed by SEC. The interpretation of the results is accomplished by comparing the behaviour, under the same experimental conditions, of the single components which might be present in the ABS. As a matter of example, the chromatograms of the oligomeric products obtained in the thermal degradation of either a styrene-acrylonitrile copolymer or a styrene-acrylonitrile-g-butadiene based ABS resin are shown in Fig. 10 a) and 10 b), respectively. It is known that in the thermal degradation of pure polybutadiene more than 80% of chain fragments are formed (22), whereas in the degradation of styrene-acrylonitrile copolymers the fraction which is not volatile at ambient temperature is mainly constituted by dimers and trimers (23). The broad peak in the higher molecular weight region of Fig. 10 b) can be attributed to the fragments originated from chain scission of polybutadiene, whereas the remaining part of the chromatogram more closely compares with the peaks of Fig. 10 a). By employing a dual detector (UV and DRI) system it should be possible to determine the ratio of styrene to acrylonitrile units in the oligomers which are formed throughout the degradation process, and consequently to establish which is the influence of the butadiene chains on the mechanism of degradation of the styrene-acrylonitrile copolymers. The investigation on such systems is being continued and the results will be subsequently published.

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