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Chemical Analysis of Macromolecular Particles Using Two-Dimensional High-Performance Liquid Chromatography (HPLC), Illustrated by Polymeric Automobile Direction Indicator Lenses (DILs)

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ABSTRACT: Through the combined application of size-exclusion and reversed-phase liquid chromatography (off-line coupling), it is possible to analyze fragments of automobile direction indicator lenses (DILs) of differing origin for their content of low-molecular-weight substances or their subpolymeric components.

Because of their two-dimensional nature and the application of in-series detector combinations, the chromatograms have a high resolution and contain a host of analytically relevant data.

The material required is in the microgram range.

KEYWORDS: forensic science, chromatographic analysis, polymers, automobile direction indicator lenses, forensic chemistry, two-dimensional high-performance liquid chromatography (HPLC), polymer analysis, road accidents

The examination of trace evidence of macromolecular structure is gaining in significance because synthetic and partially synthetic polymeric products are increasingly widely used [1,2].

Aside from the use of optical [3],² physical [4–7],³ or chemical [8,9] techniques for analyzing polymeric materials, instrumentation-aided methods have increasingly been applied in forensic science laboratories [10–13]. These methods are primarily used for characterization of the macrostructures and microstructures of the polymeric matrix. However, the analytical methods currently in use, particularly in investigations of chemically related polymers, still fall short of ideal levels of discrimination [14].

Chromatographic methods provide a valuable basis for the identification of polymeric substances. Aside from characterizing the major polymer, they also allow low-molecular-

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²See also the DDR Standard, Prüfung von Plasten, Bestimmung der Brechungsahl und der Dispersion (Examination of Plastics: Determination of the Refractive Index and the Dispersion), TGL 16768, May 1963.

³See also the DDR Standard Prüfung von Elastomeren, Bestimmung der Dichte (Examination of Elastomers: Determination of Density), TGL 14370, Sept. 1962.

weight substances to be analyzed. These substances derive from the nature of the synthesis auxiliary agents and specific additives.

Preference is often given to pyrolysis gas chromatography [15,16]; however, even when this is combined with other methods, the reproducibility and interpretation of such analyses require a great deal of effort [17,18].

Much has been reported on the use of size-exclusion liquid chromatography (SEC) for analyzing polymeric substances in forensic science [19–21]. This method, after prior calibration with standard substances, can provide data on the molecular weight (MW) or MW distributions of the substances analyzed. However, because of the restricted distribution coefficients ($0 < K_d < 1$), the resolving power is limited. The separation mechanism involved causes substances with similar MWs (hydrodynamic volumes) to appear at the same elution volume.

The use of a second chromatographic technique is particularly appropriate in those cases where SEC separation efficiency is insufficient or where detailed analytical findings on certain components are necessary. Because of its high performance, reliability, reproducibility, and resolution, reversed-phase chromatography (RPC) is often the method of choice. The use of an SEC/RPC combination for detecting low-molecular-weight constituents and separating them from the macromolecular components has been reported [22,23].

When unknown and reference samples are compared, the characteristic "fingerprints" will usually identify the material so that in most cases it is possible to do without a detailed characterization of the individual components. Through this two-dimensional approach, chromatographic information on the relative molecular size (SEC) and the molecular character (RPC) of individual components is generated.

This paper deals with the two-dimensional SEC/RPC technique used for examining automobile direction indicator lenses (DILs).

Experimental Materials and Methods

Instrumentation

Examinations were performed using a 1090 A type high-performance liquid chromatography (HPLC) instrument (Hewlett-Packard, Germany) with a multichannel integrator (DPU), built-in diode array detector (DAD), and an external refractive index (RI) detector (1037 A).

Solvents

The chromatographic solvents listed below were twice distilled in a 60-cm Vigreux column and then filtered on a 0.5- μm Millipore filter: dichloromethane (DCM), reagent grade (RG); methanol RG (MeOH); ethanol RG (EtOH); i-propanol RG (i-PropOH) (Laborchemie Apolda, Germany); and deionized water (W).

Off-Line SEC/RPC Combination

To detect the low-molecular-weight sample constituents, the SEC eluates of the individual samples were cut in the range of $4.0 < t_R < 6.0$ min and the fractions (fractionating volume, 2 mL) were collected in the quartz vessels depicted in Fig. 1. Evaporation to 100 μL was performed by attaching the condensing tube at 30°C and 10^5 Pa. Typically, 20 μL (see Table 2) of this solution can be injected onto the RPC column.

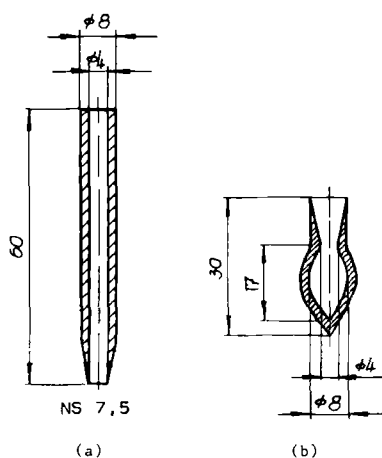


FIG. 1—Backflow arrangement for SEC fraction evaporation (units in millimetres): (a) quartz condensing tube, (b) quartz evaporation vessel (fraction collector), capacity approximately 2 mL.

Samples and Sample Preparation

Fragments of the DILs listed in Table 1 were used for this study. The DIL samples in Table 1 were chromatographed after 50 mg of each was dissolved in 1 mL of dichloromethane (DCM).

Chromatographic Conditions

The chromatographic conditions for SEC and RPC DIL analyses are given in Table 2.

TABLE 1—Origin and destination of the DILs.

Automobile Manufacturer	Model	Code	Country of Origin	Polymer Type ^a
Wartburg	1987	WA	GDR ^b	PMMA
	1985	TR 1		
	1982	TR 2		
Trabant	1982	TR 3	GDR	SAN
	1982	TR 4		
	1982	TR 5		
	1980	TR 6		
Lada	1975	LA	USSR	PMMA
Volga	1978	WO	USSR	PMMA
Moskvich	1982	MO	USSR	ABSM
Zaporozhets	1984	ZA	USSR	ABSM
Skoda	1968	SK	Czechoslovakia	SAN
Dacia	1985	DA	Romania	PC
Mazda	1982	MA	Japan	SAN
VW Golf	1978	VW	FRG ^c	PMMA

^aKey to abbreviations:

SAN = styrene acrylonitrile copolymer.

PMMA = polymethyl methacrylate.

ABSM = acrylonitrile butadiene styrene methyl methacrylate copolymer.

PC = polycarbonate.

^bGDR = German Democratic Republic.

^cFRG = Federal Republic of Germany.

TABLE 2—Chromatographic conditions for SEC and RPC.

	SEC		RPC
Samples	WA, TR1–TR6, LA, VO, MO, ZA, SK, DA, MA, VW	WA	TR1–TR6
Separation column	LiChrogel PS 1 (Merck, FRG) (250 by 7 mm ID)	LiChrosorb RP-18 (Merck, FRG) (250 by 4.6 mm ID)	LiChrosorb RP-18 (Merck, FRG) (250 by 4.6 mm ID)
Particle size	10 μm	5 μm	5 μm
Sample concentration	50 mg/mL (DCM)	SEC fraction evaporated to 100 μL (2 runs), preparative mode (off- line)	SEC fraction evaporated to 100 μL , prepara- tive mode (off-line)
Solvent A	...	W	W
Solvent B	...	MeOH/i-PropOH, 60:40 (v/v)	MeOH/EtOH, 50:50 (v/v)
Solvent C	DCM
Gradient	...	35–45% B/3 min 45% B/3 min 45–35% B/2 min	40–100% B/15 min 100% B/2 min 100–40% B/2 min
Injection volume	5 μL (250 μL), an- alytically 25 μL (1.25 mg), preparatively	20 μL of the SEC fraction (off-line)	20 μL of the SEC frac- tion (off-line)
Flow	1 mL/min	1 mL/min	1 mL/min
Column temper- ature	30°C	40°C	40°C
UV signals, wavelength/ band width	254/20 280/20	254/20	254/20 280/20
RI signal, range, atten- uation	2×10^{-5} (30°C); 2°

Results and Discussion

Examination of DILs of Various Automobile Types

Figure 2 shows the SEC chromatograms of visually similar DILs from different automobile models. The chromatograms were recorded using ultraviolet (UV)/RI detectors in series.

In this case, the SEC data allow clear differentiation, with the automobile model being identifiable through DIL fragment analysis. (For the sake of clarity, the same sensitivity and scale were applied to all samples.) In addition, the difference among the individual detection signals provide data on the polymers, displayed at the exclusion limit ($t_R = 3.5$ min).

Examination of DILs of Automobiles of the Same Type

Figure 3 shows the results of the chromatographic examinations of similar DIL fragments (SAN) from two different Trabant (TR) cars.

The SEC chromatograms suggest differences in the MW distribution. The subsequent RPC separation of the SEC fractions, which contain all low-molecular-weight compounds, enables a clear distinction to be made. The chromatograms recorded simultaneously at two different wave lengths (254 and 280 nm) contain a large amount of information, which should be sufficient for clear differentiation.

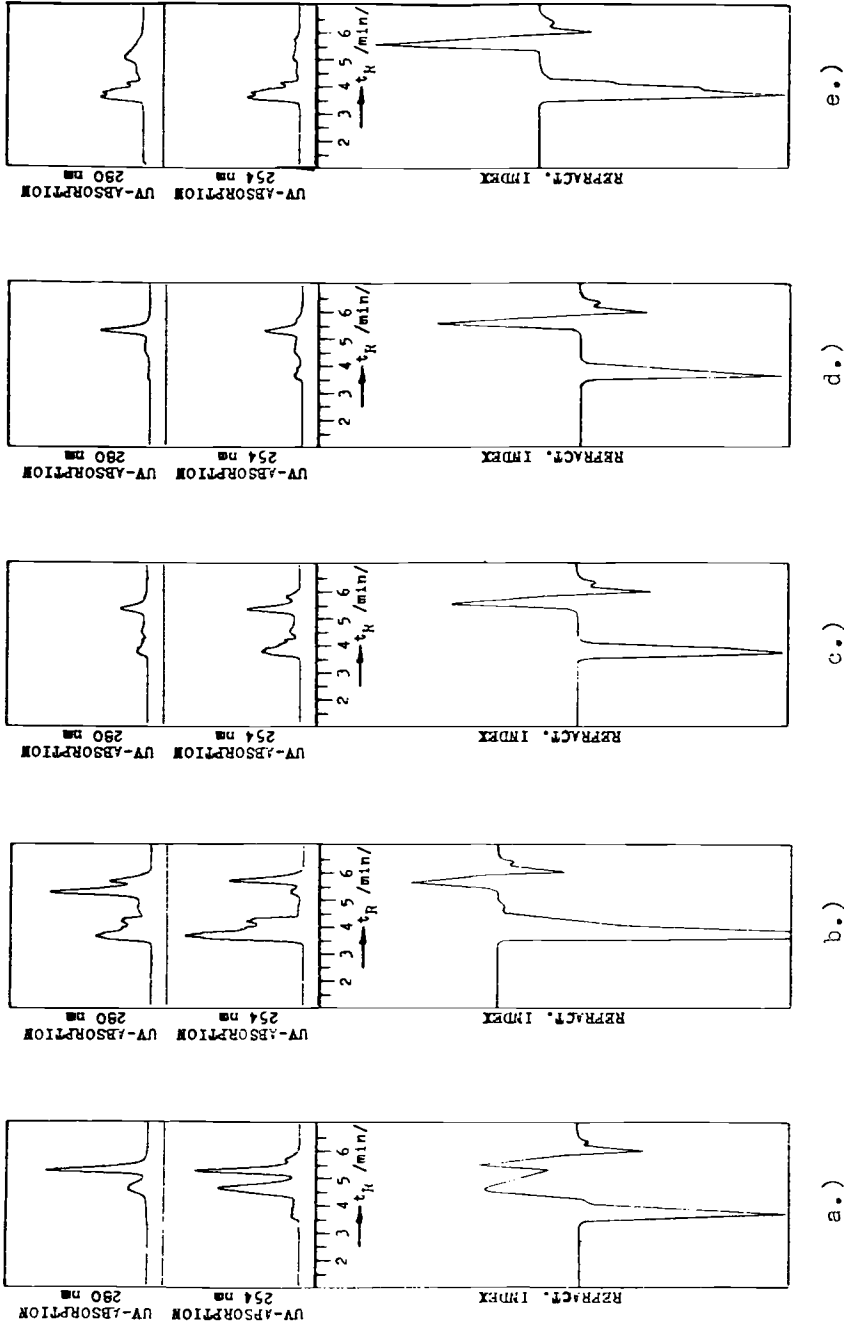


FIG. 2—SEC analyses: (a) WA (PMMA); (b) TR 3 (SAN); (c) LA (PMMA); (d) VO (PMMA); and (e) MO (ABSM).

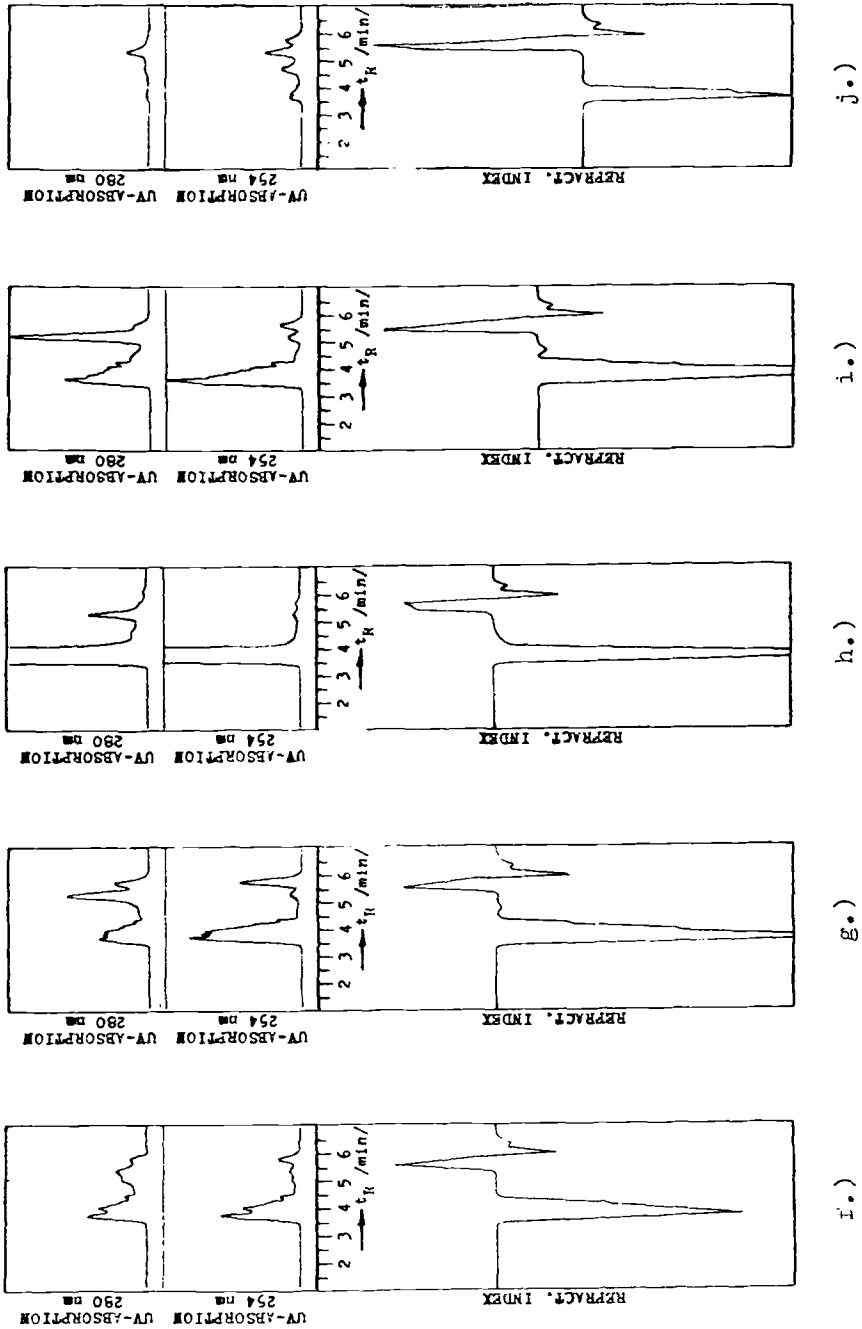


FIG. 2—Continued: (f) ZA (ABSM); (g) SK (SAN); (h) DA (PC); (i) MA (SAN); and (j) VW (PMMA).

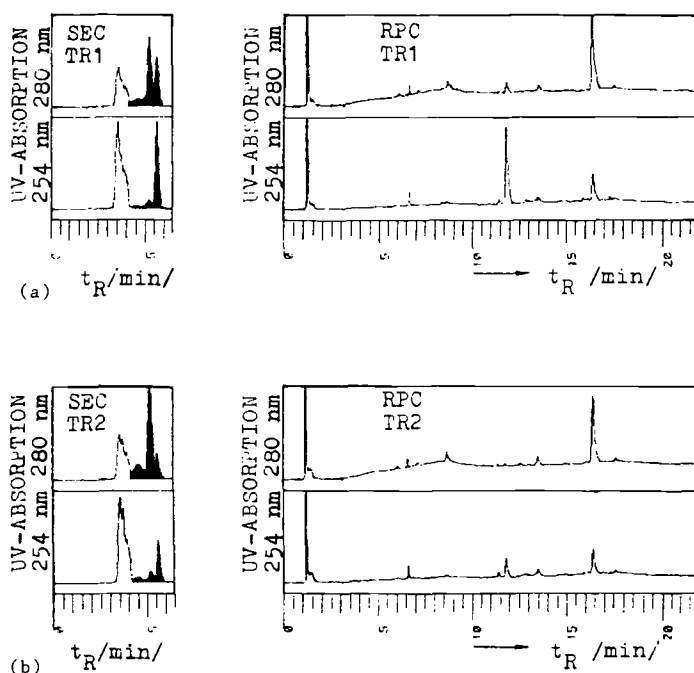


FIG. 3—SEC/RPC off-line combination for identical TR DILs of different ages (polymer, SAN): (a) SEC/RPC of the TR 1 sample (1985) and (b) SEC/RPC of the TR 2 sample (1982).

Table 3 summarizes the chromatographic data from a number of major components of the TR samples, which were recorded at 254 nm using SEC/RPC off-line coupling.

We suggest that the detected quantitative differences in the polymer composition may be mainly due to deviations during manufacturing or processing operations (batch differences) or, alternatively, to different weathering conditions.

The reproducibilities obtained from SEC/RPC offline coupling for some selected peaks of the TR 4 sample after seven runs are given in Table 4.

The SEC/RPC-chromatograms obtained for DIL fragments of Sample WA (polymethyl methacrylate [PMMA]) are shown in Fig. 4.

Summary

The combined use of size-exclusion liquid chromatography and reversed-phase chromatography (off-line), paired with the use of detector combinations in series, enables fragmented automobile direction indicator lenses to be analyzed for their low-molecular-weight constituents and macromolecular components.

The results obtained allow fragments of polymeric materials to be assessed in forensic science terms.

The high degree of differentiation achieved is mainly due to the quantitative recording of low-molecular-weight additives whose presence depends on a multitude of factors (including production, storage, external influences, and other factors).

TABLE 3—SEC/RPC data for identical SAN copolymers (TR samples) of different ages, peak t_R , in minutes.

TR Sample	SEC Data, area %				RPC Data, area %						
	3.5-4.0	4.55	5.16	5.58	6.66	8.68	11.46	11.85	13.55	16.45	17.61
TR 1	61.75	2.44	4.26	31.55	3.54	2.54	3.12	57.28	6.74	22.72	3.05
TR 2	72.79	4.79	5.49	16.94	8.24	4.45	5.84	23.63	12.49	38.16	6.55
TR 3	72.20	3.09	4.50	20.21	6.51	4.29	5.86	30.27	9.22	39.74	2.29
TR 4	68.90	3.18	4.31	23.61	6.67	2.53	4.59	40.24	8.00	30.03	7.28
TR 5	69.33	3.06	4.24	23.38	7.11	3.76	6.05	26.88	16.30	31.60	7.93
TR 6	67.74	4.06	6.23	21.97	6.11	2.27	5.21	49.68	9.30	23.56	3.29

TABLE 4—Retention time and peak area reproducibility (detection 254 nm, 280 nm, and 300 nm) for the TR 4 sample with SEC/RPC off-line combination).

SEC				RPC			
t_R , min		Area, %		t_R , min		Area, %	
X	SD_R	X	SD_R	X^a	SD_R	X	SD_R
4.55 ^a	0.03	3.18	0.68	6.66	0.08	6.67	5.43
5.16 ^a	0.06	4.31	1.04	11.46	1.02	4.59	3.16
5.58 ^a	0.05	23.61	0.35	11.85	1.10	40.24	2.09
4.58 ^b	0.05	6.74	0.44	13.55	0.09	8.00	2.73
4.65 ^c	0.07	11.60	0.50	16.45	0.09	30.03	2.17
				17.61	1.11	7.21	3.98

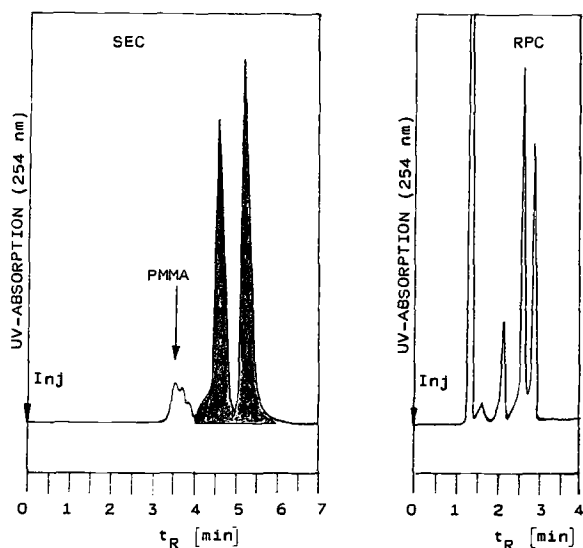
^aDetection, 254 nm.^bDetection, 280 nm.^cDetection, 300 nm.

FIG. 4—Two-dimensional SEC/RPC analysis of sample WA (polymer, PMMA).

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