Journal of Chromatography, 389 (1987) 273–278
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 208

Note

Simultaneous separation of oligomeric and polymeric ethylene glycols (degree of polymerization 1–110) using reversed-phase high-performance liquid chromatography

GÜNES BARKA*
Dr. H. Knauer Wissenschaftliche Geräte KG, Heuchelheimerstr. 9, D-6830 Bad Homburg (F.R.G.)
and
PER HOFFMANN
Deutsches Schiffahrtsmuseum, Van-Ronzelen-Str., D-2850 Bremerhaven (F.R.G.)
(First received July 1st, 1986; revised manuscript received October 27th, 1986)

For several decades polyethylene glycols (PEGs) have been employed for the conservation of waterlogged wooden archaeological finds. The Viking ships in Denmark, *e.g.*, the Gustavus Adolphus' "Wasa" in Stockholm, and mediaeval "Bremen Cog" in Bremerhaven have been treated with this substance. Infiltration of the old timbers with PEG can considerably reduce the enormous shrinkage that normally occurs on drying such heavily degraded wood.

Stabilization treatments can only be optimized when some important parameters are known: the penetrability of PEGs of different molecular size into woods of different species and with various degrees of degradation; the diffusion velocity of various PEGs; and the dependence of the dimensional stabilization achieved on the amount of PEG incorporated into the wood. Investigations into these questions depend strongly on the quantitative determination of PEG differentiated by molecular size (*i.e.*, degree of polymerization) in wood or wood extracts.

The separation of PEGs using reversed-phase (RP) chromatography has been described extensively by Melander *et al.*[1]. An earlier isocratic separation was performed by Eisenbeiss and Ehlerding[2] with a refractometer as a detector. Lai *et al.*[3] used a phenyl-bonded silica phase with UV detection at 190 nm. Detection at wavelengths shorter than 210 nm proved to be difficult. The noise caused by the absorption of the eluent made the sensitive detection of peaks impossible, and an increase in acetonitrile concentration during elution caused a strong baseline drift. Berry[4] described the addition of sodium azide to water and Van der Wal and Snyder[5] used 5 ppm nitric acid to compensate for the baseline drift. These workers[4,5] also mentioned "ghost peaks", which were thought to arise from impurities in the acetonitrile, but which could not be eliminated by treating this solvent with aluminium oxide. These peaks always appeared during gradient elution at 40% acetonitrile concentration.

In this paper we present an improved method for separating oligomeric and polymeric PEGs (MW 62–4858) in aqueous extracts from PEG-treated archaeological wood according to their individual degrees of polymerization (DP) by reversed-phase high-performance liquid chromatography (HPLC) with UV detection.

0021-9673/87/$03.50

EXPERIMENTAL

The system used consisted of two type 64.00 HPLC pumps, a type 87.00 variable-wavelength monitor, a type 50 B high-pressure gradient programmer, a dynamic mixing chamber and a two-channel recorder from Dr. H. Knauer KG (Bad Homburg, F.R.G.) a type RH 7125 injection valve from Rheodyne (Cotati, CA, U.S.A.). The HPLC columns (Dr. H. Knauer KG) were packed with LiChrosorb RP-8 (7 μm, 100 Å) and Hypersil MOS WP (10 μm, 300 Å).

Mono-, di-, tri- and polyethylene glycols were of commercial grade from Hoechst (Frankfurt, F.R.G.), the average molecular weights of the polymers being given as 200, 400, 1500, 3000 and 4000.

All other chemicals and eluents were of pro analysi or LiChrosolv quality from Merck (Darmstadt, F.R.G.).

Special treatment was applied to the water used. Tap water was purified by ion exchange, then potassium permanganate was added and the water was slowly distilled over a 100-cm column filled with Raschig rings. The first third of the distillate was discarded. The purified water so obtained was suitable for working at 190 nm.

Sample preparation

For dimensional stabilization, a 4-cm thick bottom plank from an 1800-year-old Roman river barge, excavated in 1980 in Cologne, was treated with PEG 1000. The heavily degraded, waterlogged oak was submerged for 5 years in an aqueous solution the PEG concentration of which was gradually increased to 65%. Following this immersion treatment, the surface was painted six times "wet in wet" with a 60% solution of PEG 3000 to give additional hardness to the soft surface. PEG 3000 is much harder then PEG 1000. The wood was then left to air dry.

From the dry plank a plug was bored from surface to surface and split into seven layers, each 5 mm thick. These samples were cut into shavings and extracted for 24 h with hot water under reflux. The extracts were diluted to equal volumes. No further purification was attempted.

Analytical conditions

The separation of PEGs with a very wide molecular weight range requires two columns with different pore widths. For our work we used modified silica gels with pores of 100 and 300 Å. The 100 Å reversed-phase column was connected directly with the injection valve with the 300 Å column behind it.

To avoid drifting of the baseline during gradient elution at 190 nm, sodium azide was added to the water until the water had the same absorption as the acetonitrile used. All analyses were carried out at room temperature at a flow-rate of 1 ml/min.

RESULTS

In most previous work[1–3,5–8], only PEGs of low (MW < 1500) and relatively narrow spans of DP have been separated by reversed-phase chromatography. Work dealing with gel permeation or size-exclusion chromatography (GPC, SEC) gave separations of these polymers only into approximate groups such as PEG 1500, 3000 or 4000[5–6,8].

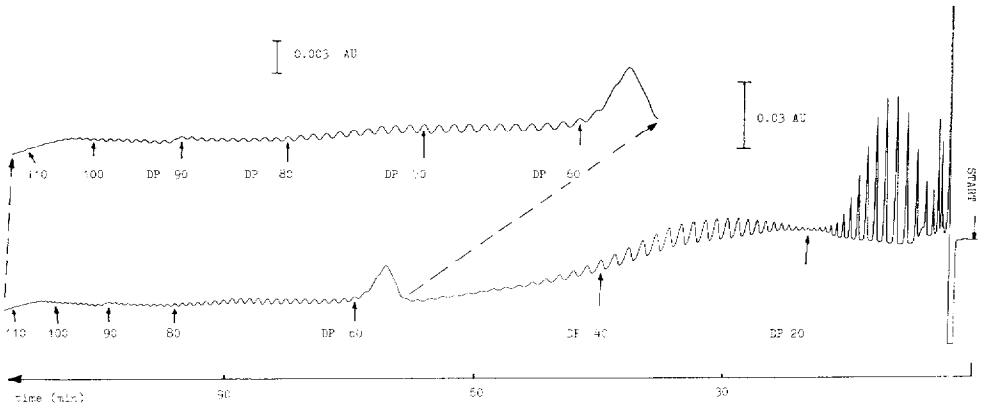

Fig. 1. Separation of a standard PEG mixture with DP from 1 to 110. Two analytical columns (250 × 4 mm I.D.) packed with LiChrosorb RP-8 (7 μm, 100 Å) and Hypersil MOS WP (10 μm, 300 Å) were used. Mobile phase, acetonitrile–water gradient; detection wavelength, 190 nm.

In our work, the potential of modern reversed-phase chromatography was demonstrated by the separation of PEGs with DPs from 1 to 110. Fig. 1 shows the simultaneous separation of PEGs with MW up to 4800 in one run. The PEGs of the standard mixture were not chemically modified to make them visible at longer wavelengths, *e.g.*, by coupling phenyl or dibenzoyl groups to them[1,8].

The successful separation of so many oligomers and polymers can only be accomplished by maintaining an exact gradient. The most important parameters, such as maximum acetonitrile concentration and temperature, were described by Melander *et al.*[1]. During the gradient elution the acetonitrile concentration in water was increased linearly from 10% to 20% in 15 min, and after a further 95 min up to 35%. The separation was carried out isocratically at this concentration for another 15 min.

Fig. 2a–g shows the wood extracts separated in the same way. In the outer wood layers only (Fig. 2a and g) high-molecular-weight PEGs can be traced. In all other samples only those polymers belonging to PEG 1000 are present. During the 2 weeks of painting with PEG 3000 and during the months of air drying, the large molecules of the PEG 3000 had obviously not diffused further then 5 mm into the wood.

The remainder of the cross-section of the plank seemed to be impregnated evenly with PEG 1000. A "sieve effect" of the wood tissue could not be detected; that would have meant a trend towards the dominance of smaller molecules in the middle of the plank compared with the outermost layers.

It is worth mentioning that the brown wood extracts need not be purified prior to the separation, as the water-soluble substances in archaeological wood such as humic substances, degradation products of carbohydrates and lignin and inorganic infiltrations from the ground do not interfere with the detection at 190 nm. This will facilitate the use of the method for routine analysis in wood conservation science.

From time to time some ghost peaks was observed, as in Fig. 2b after 90 min. We believe, according to Barry[4] and Van der Wal and Snyder[5], that these peaks are due to impurities in the solvents.

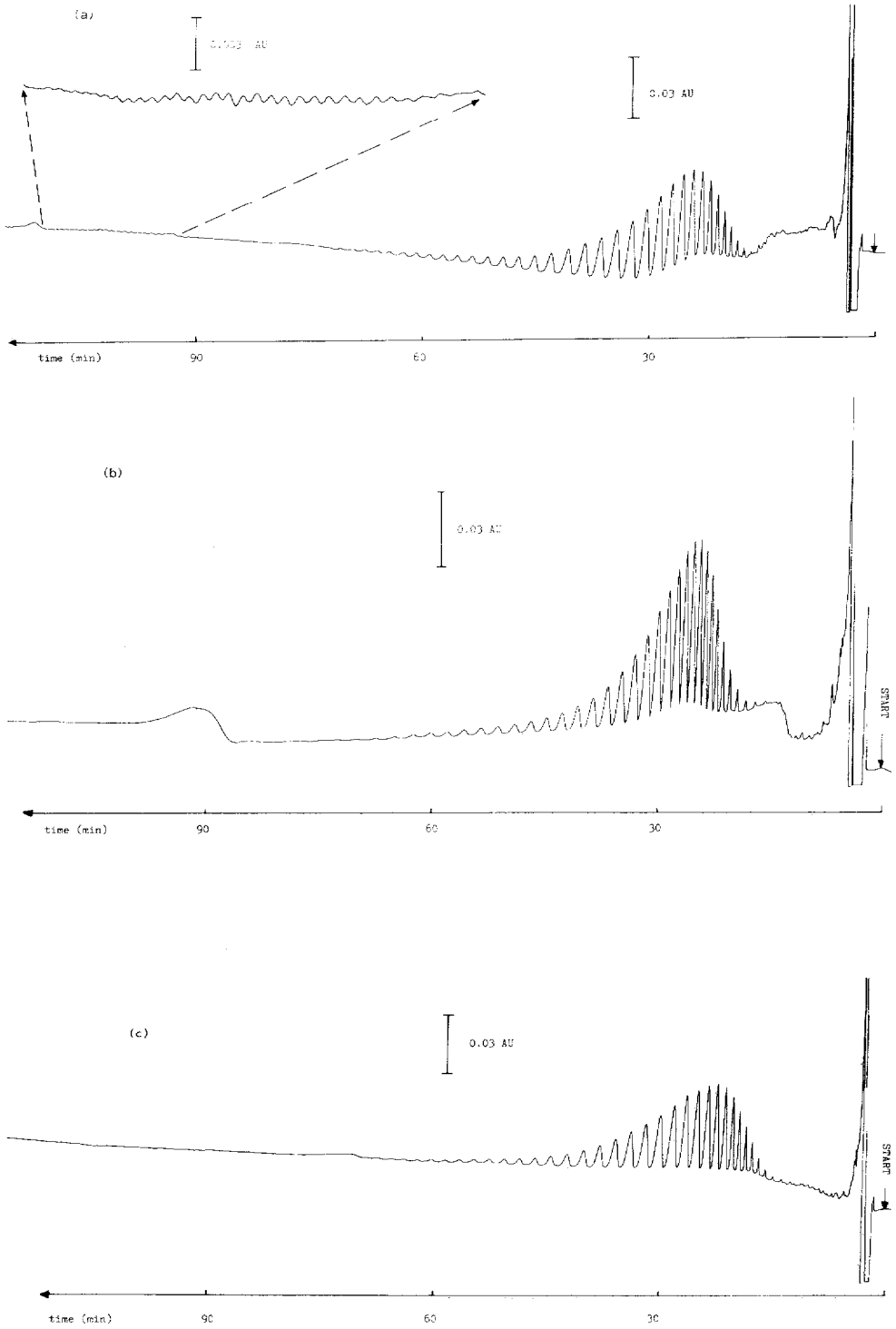
(a)
0.03 AU
time (min)
90
60
30
(b)
0.03 AU
START
time (min)
90
60
30
(c)
0.03 AU
START
time (min)
90
60
30

Fig. 2.

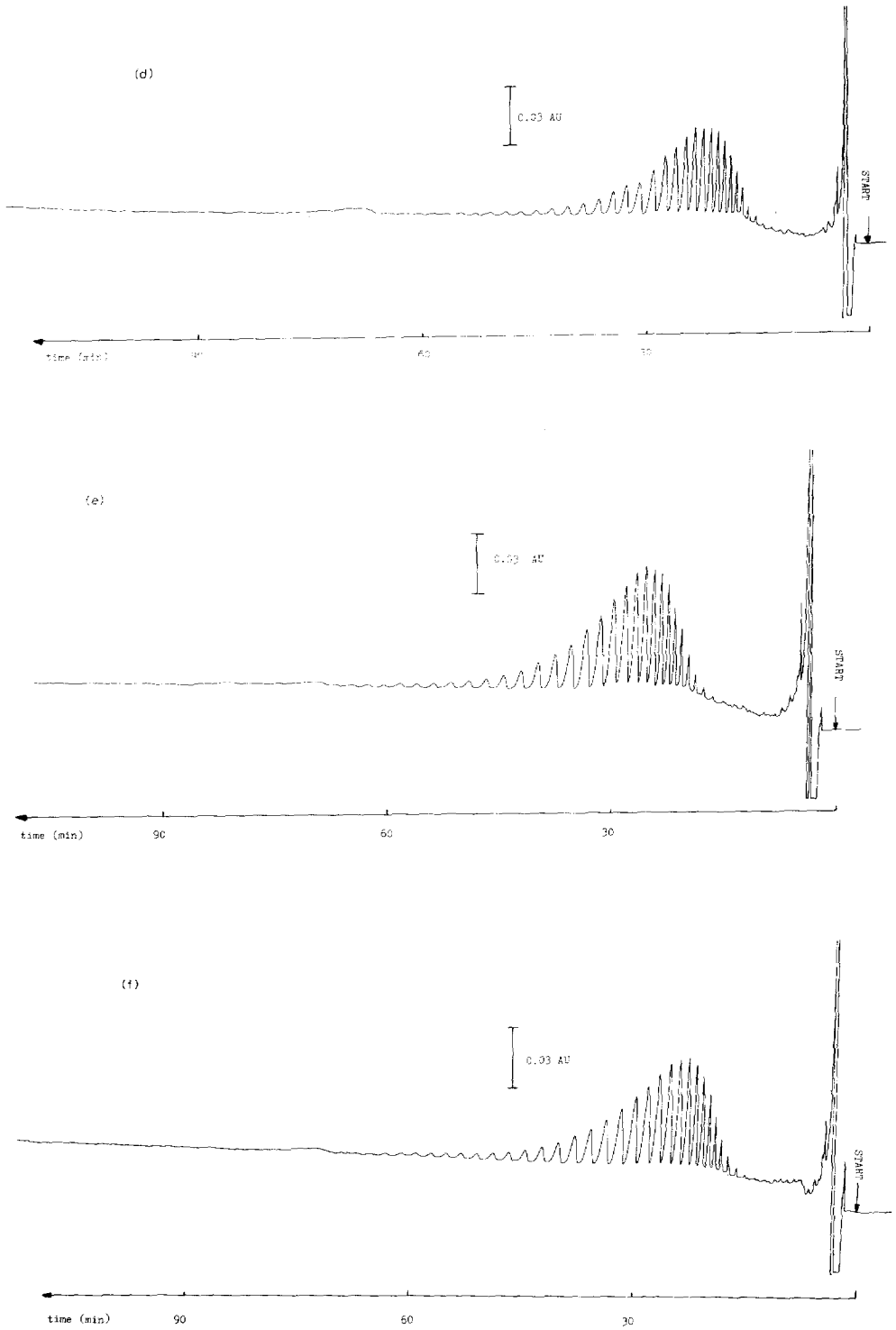

Fig. 2.

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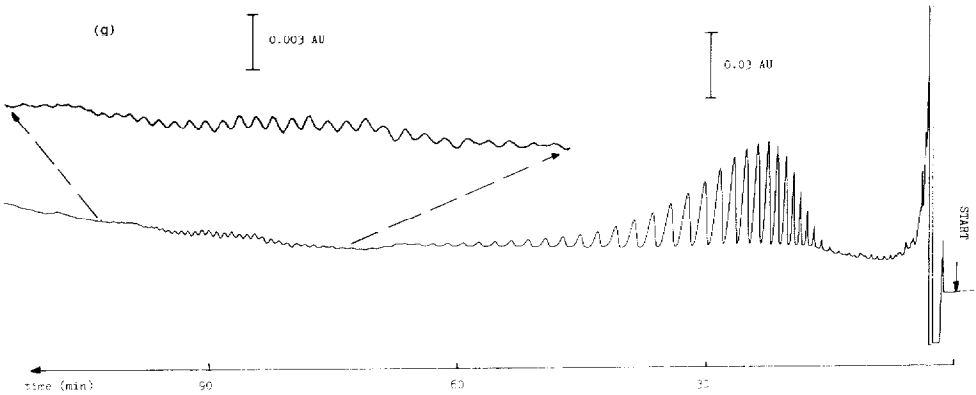

Fig. 2. (a)–(g) Detection and separation of PEGs in aqueous extracts from treated archaeological wood. (a) and (g) represent the PEGs in the outer wood layers, the only region where high-molecular-weight PEGs can be detected. Conditions as in Fig. 1.

CONCLUSIONS

The separation mechanism cannot be explained only by absorption effects. If, for example, two 100 Å columns are connected in series, the low-molecular-weight PEGs up to PEG 1500 can be separated to a substantial extent, but the separation ends at about MW 2000–3000. For PEG 4000 one broad peak is obtained, as is well known from GPC[8,9]. However, a successful separation is accomplished with the combination of columns described in this paper. This leads to the assumption that in our case a separation by molecular size (GPC) takes place together with adsorption effects, in accordance with Klein and Treichel[10] and Engelhardt and Mathes[11].

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