Journal of Chromatography, 298 (1984) 360–365
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,889

Note

Analysis of polyglycerols by high-performance liquid chromatography

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(Received May 9th, 1984)

Polyglycerol esters of fatty acids are used in a variety of food products[1,2] and are permitted for limited use under the Codex Alimentarius Standards[3] of the FAO/WHO and the European Economic Community regulations[4]. The presence of oligomers higher than hexaglycerol is not permitted, however[5]. The composition of polyglycerol esters depends partly on the composition of the polyglycerols used in their manufacture and the analysis of polyglycerols is therefore necessary for monitoring their production. The determination of viscosity, refractive index or hydroxyl value provides information on the rate and degree of polymerization of glycerol[1] but not on the composition of the product. Paper[6] and thin-layer[2,7] chromatographic (TLC) techniques were found not to resolve oligomers higher than hexaglycerol. Gas–liquid chromatographic (GLC) analysis[8,9] involves the preparation of derivatives that decompose at high temperatures.

High-performance liquid chromatography (HPLC) appears to be more suitable, as analysis can be carried out at room temperature without derivatization. Aitzetmüller *et al.*[10] analysed polyglycerols liberated from commercial polyglycerol esters using a silica (LiChrosorb Si 60) column and acetonitrile–water (85:15) as the mobile phase. However, they did not study the separation of cyclic diglycerol and polyglycerols beyond hexaglycerol or quantitation.

In this paper we describe the use of a Carbohydrate Analysis column with acetonitrile–water (83:17) to separate and determine cyclic diglycerol, glycerol and polyglycerol oligomers up to undecaglycerol and demonstrate its utility in monitoring the preparation of polyglycerols.

EXPERIMENTAL

Preparation of polyglycerols

Glycerol (500 g, analytical-reagent grade) was polymerized with stirring in a four necked flask coupled to a Dean and Stark apparatus using sodium hydroxide (1%) as catalyst at 250, 260 and 270°C for 1, 2 and 3 h. A trace amount of magnesium powder was added and nitrogen was bubbled through to prevent development of dark colours or off-odours[1,11]. Water collected was removed. After neutralizing the catalyst with dilute hydrochloric acid the polyglycerol mixture was filtered, dried under vacuum on a steam bath, dissolved in acetonitrile–water (30:70) and analysed by HPLC.

0021-9673/84/$03.00

Samples of cyclic and linear diglycerol and linear triglycerol were obtained from Unilever Research Laboratory (Welwyn, U.K.) and were found to be pure by HPLC as described later and by TLC[2]. Four mixtures of these three components and glycerol were prepared, dissolved in acetonitrile–water (83:17) and analysed by HPLC.

HPLC analysis

HPLC was performed with a Waters Assoc. Model ALC/GPC 244 liquid chromatograph, equipped with a Model 6000 A pump, a U 6K injector, a Model R 401 differential refractometer and a Shimadzu Chromatopak E1A integrator. A Waters Assoc. Carbohydrate Analysis column (30 × 3.9 mm I.D., 10 μm) was used. The polyglycerols, dissolved in acetonitrile–water (83:17), were injected on to the column. The mobile phase was acetonitrile–water (83:17) at a flow-rate of 1.5 ml/min under a pressure of *ca.* 1000 p.s.i. The detector attenuation was × 8 and the recorder chart speed was 1.5 cm/min.

RESULTS AND DISCUSSION

HPLC analysis of prepared mixtures of polyglycerols

Cyclic and linear diglycerol and linear triglycerol gave single peaks with relative retention times (RRTs) of 0.82, 1.09 and 1.22, respectively, with respect to glycerol. As the solvent and the eluent were the same there was no separate solvent peak to interfere with cyclic diglycerol peak. Cyclic diglycerol appeared before glycerol because the former has only two secondary hydroxy groups and no primary hydroxy group for affinity to the stationary phase, unlike the latter. In TLC on silica gel G[2] cyclic diglycerol has a higher R_F value than glycerol for the same reason. In GLC, cyclic diglycerol was eluted after glycerol from JXR and SE-30 columns owing to the higher molecular weight of the former[8,9]. HPLC analysis of four prepared mixtures with different contents of cyclic and linear diglycerol, linear triglycerol and glycerol showed that the peak area percentages correspond to the weight percentages in the mixture (Table I).

HPLC analysis of prepared polyglycerols

The RRTs of polyglycerols higher than triglycerols could not be determined on pure samples as the compounds were not available, but were determined by analysing polyglycerol mixtures obtained under different polymerization conditions (Table II). These polyglycerols were dissolved in acetonitrile–water (30:70) as some of them were not completely soluble in less polar solvent mixtures. Hence cyclic diglycerol, when present in these mixtures, was not well resolved from the solvent. The RRTs of different polyglycerol components present in the mixtures were reproducible. The HPLC trace of a sample obtained by polymerization at the highest temperature (270°C) and for the longest duration (3 h) is shown in Fig. 1. The peaks other than those of cyclic diglycerol and glycerol must be due to homologues of linear polyglycerols from diglycerol to undecaglycerol, as other cyclic and branched polyglycerols are not formed to a significant extent[10,12]. A plot of the logarithm of retention time (t_R) in seconds *vs.* the molecular weight of the expected linear polygly-

TABLE I

HPLC ANALYSIS OF SYNTHETIC MIXTURES OF POLYGLYCEROLS

Component	*RRT**	*Mixture 1*		*Mixture 2*		*Mixture 3*		*Mixture 4*	
		Actual (%, w/w)	*Found (%, w/w)*	*Actual (%, w/w)*	*Found (%, w/w)*	*Actual (%, w/w)*	*Found (%, w/w)*	*Actual (%, w/w)*	*Found (%, w/w)*
Cyclic diglycerol	0.82	18.0	18.1	16.3	16.2	20.2	20.6	20.6	21.5
Glycerol	1.00	28.2	27.6	45.5	45.0	38.7	38.3	27.6	26.9
Linear diglycerol	1.09	38.0	38.7	26.4	26.3	28.6	27.7	41.8	42.2
Linear triglycerol	1.22	15.8	15.6	11.8	12.5	12.5	13.4	10.0	9.4

* Relative retention time with respect to glycerol.

TABLE II

HPLC ANALYSIS OF POLYGLYCEROLS: CHANGES IN COMPOSITION WITH POLYMERIZATION TEMPERATURE AND TIME

Temperature (°C)	*Time (h)*	*Component in polyglycerol mixture (%, w/w)*											
		1 Cyclic diglycerol (0.82)***	*2 Glycerol (1.00)*	*3 Diglycerol (1.09)*	*4 Triglycerol (1.22)*	*5 Tetraglycerol (1.41)*	*6 Pentaglycerol (1.59)*	*7 Hexaglycerol (1.82)*	*8 Heptaglycerol (2.05)*	*9 Octaglycerol (2.35)*	*10 Nonaglycerol (2.69)*	*11 Decaglycerol (3.09)*	*12 Undecaglycerol (3.68)*
250	1	–	73.2	23.6	3.1	0.1							
250	2	–	53.0	37.1	9.2	0.5	0.2						
250	3	–	37.4	38.0	17.3	5.2	1.6	0.5					
260	1	2.0	52.9	32.9	10.0	2.1	0.1						
260	2	6.0	32.9	34.6	16.6	6.6	2.6	0.6	0.1				
260	3	6.6	22.0	31.0	20.3	11.0	5.5	2.5	0.6	0.2			
270	1	6.5	24.1	30.6	19.0	11.0	6.0	1.4	1.2	0.2			
270	2	10.0	12.0	19.0	17.9	14.1	11.4	7.9	6.0	1.6	0.1		
270	3	10.1	7.1	13.0	14.0	14.5	11.7	8.6	7.9	6.3	5.8	0.8	0.2

* Peak number in Fig. 1.

** Retention times relative to glycerol in parentheses.

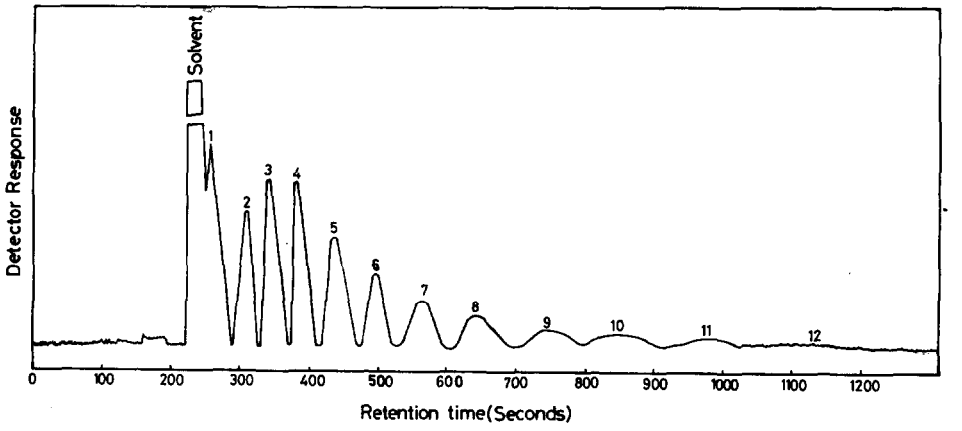

Fig. 1. HPLC separation of polyglycerols obtained by polymerization of glycerol at 270°C for 3 h. Column, Carbohydrate Analysis (30 × 3.9 mm I.D.). Eluent, acetonitrile–water (83:17). Flow-rate, 1.5 ml/min. See Table II for peak identification.

cerol was found to be a straight line (Fig. 2), which by the least-squares method was found to be represented by

$$\log t_R = (7.613 \cdot 10^{-4} \cdot \text{mol. wt.}) + 2.40273$$

The average percentage difference between the experimental and calculated retention times was 1.28. From Fig. 2, it can be concluded that the peaks after triglycerol in Fig. 1 were due to higher homologues of linear polyglycerols.

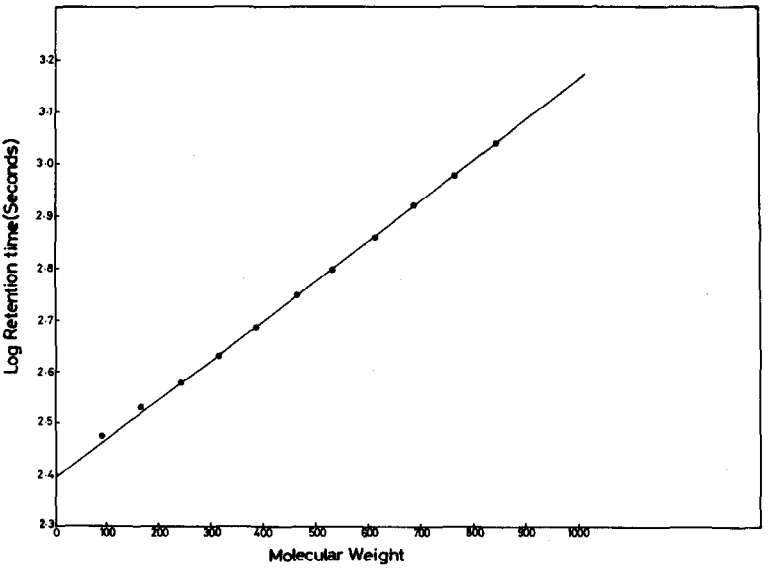

Fig. 2. Plot of molecular weights of polyglycerol linear oligomers *vs.* logarithm of retention times in seconds.

Changes in the composition of polyglycerols due to variations in the temperature and duration of polymerization of glycerol were investigated by HPLC (Table II). Lower temperatures and shorter durations led to higher contents of unreacted glycerol, whereas higher temperatures and longer durations gave higher contents of cyclic diglycerol and oligomers higher than hexaglycerol. Polymerization at 250°C for 3 h was found to give the highest yield of diglycerol with small concentrations of penta- and hexaglycerol and no cyclic diglycerol. HPLC thus appears to be a useful tool for monitoring the production of desired mixtures of polyglycerols and for analysing polyglycerol esters through their polyglycerol moieties.

ACKNOWLEDGEMENT

T.N.K. thanks the Council of Scientific and Industrial Research for the award of a Junior Research Fellowship.

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