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ANALYSIS OF THERMAL POLYMERIC FATTY ACIDS, METHYL ESTERS AND ALCOHOLS ON SEPHADEX LH-20

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SUMMARY

The components of thermal polymeric acids, methyl esters and alcohols were analyzed by gel chromatography on Sephadex LH-20. The conditions for gel chromatography were investigated and adjusted for analysis using a column 2.0 cm in diameter and 230 cm in length. The column temperature was 50°, dimethylformamide was employed as developing solvent at a flow rate of 20 ml/h. The sample size was either 50 or 300 mg. Monomers, dimers, and trimers, and in addition other high molecular weight polymers that could not be confirmed by conventional methods, were separated. Reproducibility of results and recovery were satisfactory.

INTRODUCTION

Thermal polymeric fatty acids, methyl esters and alcohols are industrially unique raw materials for polyamides, polyesters, polyurethanes, etc. Many analytical methods have been studied for investigating the reaction kinetics of polymerization of fatty acids or their derivatives and for examining performance of final products. The determination of molecular weight distribution is especially important and previously several techniques have been reported. COWAN *et al.*[1] determined monomers, dimers and trimers in the thermal polymeric fatty methyl esters by using an alembic pot. PASCHKE *et al.*[2] reported that the micromolecular distribution method lacks disadvantages of the alembic pot distillation method, such as thermal polymerization of methyl eleostearate or the necessity of using a large quantity of sample. FRANKEL *et al.*[3] and EVANS[4] separated monomers, dimers and trimers (plus higher molecular weight polymers) in thermal polymeric fatty acids by elution chromatography on silica gel.

Recently gas chromatographic methods have been investigated[5,6]. However, both microdistillation and chromatographic methods have major disadvantages because the analytical accuracy of the former is poor and polymers having a molecular weight higher than trimers cannot be directly separated by the latter.

Gel chromatography in which separation is based on molecular size has been found widely applied in biochemistry and in synthetic polymer chemistry. In recent

publications gel chromatography has frequently been used in the field of oligomers and small molecules, and its usefulness for analysis of non-volatile substances that cannot be determined by gas chromatography was recognized. Thus this technique appears to be promising for separation of monomers, dimers and trimers in thermal polymeric fatty acids, methyl esters and alcohols. In fact, by using cross-linked polystyrene gel, CHANG[7] and BARTOSIEWICZ[8] analyzed thermal polymeric fatty acids in tall oil and paints, respectively. However, those reports did not refer to the separation of polymers of higher molecular weight than trimers and under gel chromatographic conditions.

The present paper describes the separation and determination of thermal polymeric fatty acids, methyl esters and alcohols by gel chromatography on Sephadex LH-20.

EXPERIMENTAL

Apparatus

Two glass columns (2.0 × 230 cm and 1.5 × 100 cm) equipped with a jacket in order to control the column temperature were used. A differential refractometer (Waters Associates Model R-4) was employed as a detector, and the elution chromatogram was recorded automatically by a recorder. A polyethylene tube (1.8 mm O.D.) was used as a connection between each apparatus.

Reagents and samples

For selecting a developing solvent, analytical grade chloroform, acetone, dioxane, isopropanol, methanol and dimethylformamide (DMF) were used. Sephadex LH-20 gel (Pharmacia Fine Chemicals) was used as column substrate. Commercially available polymeric fatty acids, esters and alcohols were used as samples.

Procedure

Sephadex LH-20 (175 g) was allowed to stand for 24 h in contact with DMF and then was carefully poured into a glass column (2.0 × 230 cm). Furthermore, the gel bed was allowed to settle for 24 h while DMF flowed through the column. The flow rate was controlled by maintaining a reservoir at a certain height. Either 50 or 300 mg of sample were diluted to 1 ml with DMF and carefully applied on top of the gel bed. Each separated component was received in a graduated cylinder, and the developing solvent was removed. Each component was weighed and identified by its infrared

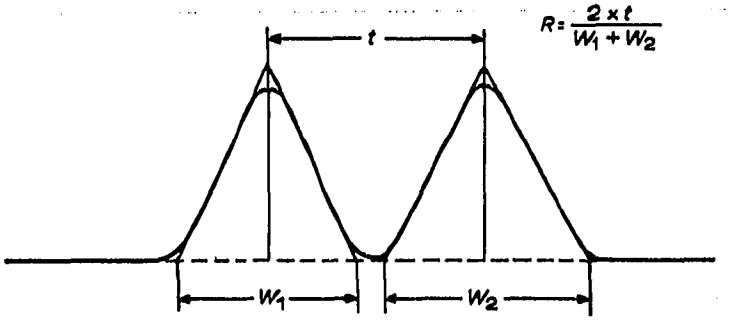

Fig. 1. Peak resolution (R).

spectrum. The molecular weight was determined using a vapor pressure osmometer. Peak resolution, R, was calculated as shown in Fig. 1.

RESULTS AND DISCUSSION

The chromatographic conditions such as developing solvents, sample size, column temperature and flow rate were investigated. In order to shorten the length of the experiment, a glass column, 1.5 × 100 cm, was used because sufficiently good results were obtained to choose the chromatographic conditions. Under these conditions, the R was calculated by using the dimer and trimer peaks.

Developing solvent

Since gel permeability has an important effect upon the saturation and greatly varies when the developing solvent is altered in gel chromatography using Sephadex LH-20, the relationship between resolution and developing solvents was investigated. The results are shown in Table I. From Table I DMF was selected as the developing solvent.

TABLE I

THE RELATIONSHIP BETWEEN SOLVENTS AND PEAK RESOLUTION

Chromatographic conditions: Sample size, 50 mg; column size, 1.5 × 100 cm; flow rate, 38–42 ml/h; temperature, 25–28°.

Solvent	*Solvent regain*[a]	*R values*		
		Polymeric acid	*Polymeric ester*	*Polymeric alcohol*
DMF	2.2	0.48	0.54	0.55
Methanol	1.9	0.43	—	0.43
Isopropanol	1.7	—	0.42	0.30
Chloroform	1.6	—	—	0.29
Dioxane	1.4	0.23	0.39	0.24
Acetone	0.8	0.28	0.41	0.00

[a] Solvent regain, ml/g dry gel.

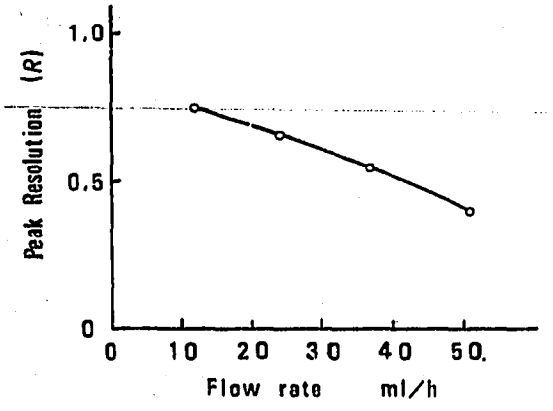

Fig. 2. The relationship between flow rate and peak resolution. Chromatographic conditions: Sample, polymeric alcohol; sample size, 50 mg; column size, 1.5 × 100 cm; temperature: 25–28°.

Flow rate

Using DMF as developing solvent and polymeric alcohol as the sample, the effect of flow rate ranging from 10 to 40 ml/h was investigated, and the peak resolution was plotted as presented in Fig. 2 which shows that the lower the flow rate, the better the separation. However, use of too low a flow rate is time consuming and causes peak broadening. Thus, the flow rate was fixed at 20 ml/h.

Column temperature

The effect of column temperature on the separation was investigated by changing the column temperature from 16 to 50° (Fig. 3). As shown in Fig. 3, the higher the temperature, the better the separation is within the experimental range. It is considered that diffusion of solute into the gel increases with rising temperature and consequently that the equilibrium rate of partition is improved at each theoretical plate. Thus the column temperature was fixed at 50°.

Sample size

The relationship between peak resolution and the sample size ranging from 10 to 100 mg/ml of DMF was studied. Use of sample sizes from 20 to 30 mg gives the best separation, as shown in Fig. 4. However, because of the requirements of the recorder 50 mg of sample were selected. Use of 10 mg of sample gave a poor separation. It is thought that the horizontal diffusion of solute in the column affects separation. For quantitative analysis, a 300-mg sample was selected for convenience.

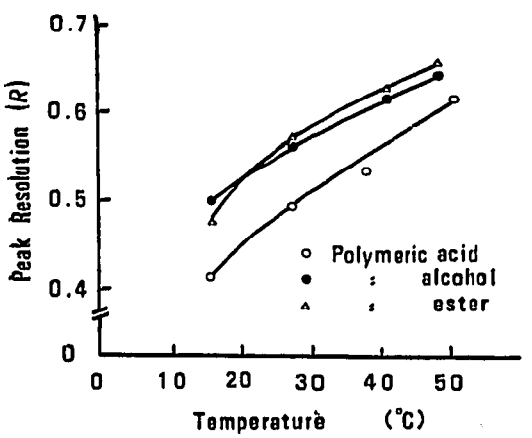

Fig. 3. The relationship between temperature and peak resolution. Chromatographic conditions: Sample size, 50 mg; column size, 1.5 × 100 cm; flow rate, 38–42 ml/h.

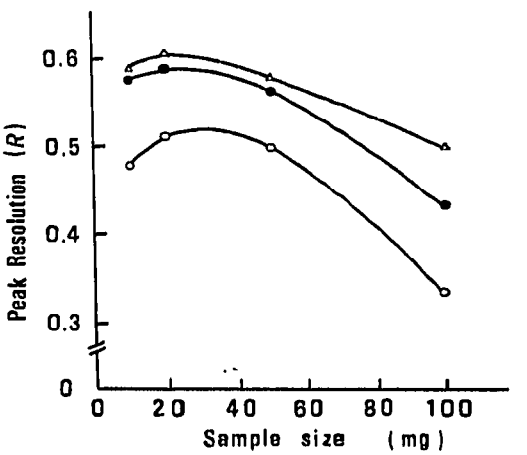

Fig. 4. The relationship between sample size and peak resolution. Chromatographic conditions: Column size, 1.5 × 100 cm; solvent, DMF; flow rate, 38–42 ml/h; temperature, 25–28°.

Gel chromatogram

Gel chromatograms using a glass column (2.0 × 230 cm) under the above-mentioned conditions are shown in Fig. 5. The separation of monomer, dimer and trimer was satisfactory. Also tetramers and higher molecular weight polymers that could not be confirmed by conventional methods could be separated.

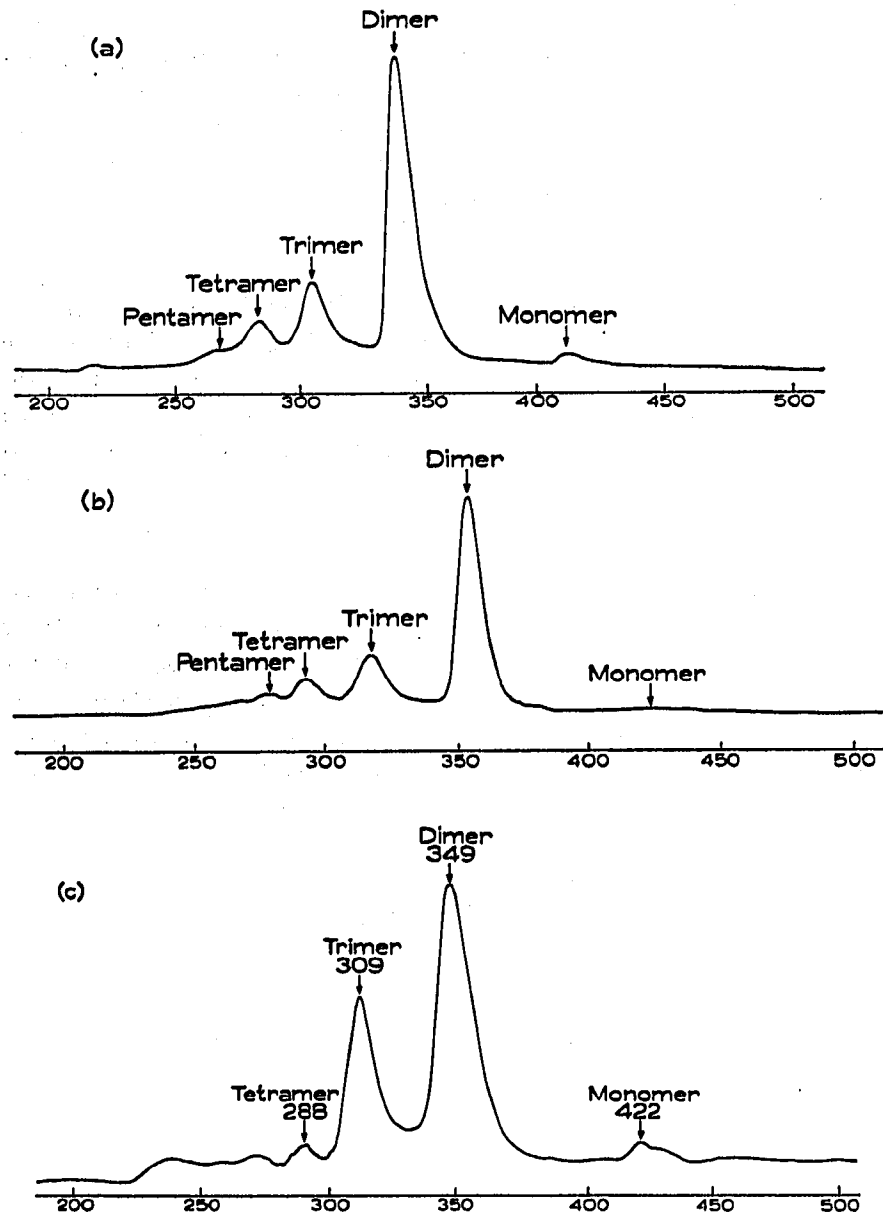

Fig. 5. Gel chromatogram. Chromatographic conditions: Sample size, 50 mg; solvent, DMF; column size, 2.3 × 230 cm; temperature, 48–51°. (a) Thermal polymeric fatty acid. R(dimer–trimer) = 1.47; N(monomer) = 6000; N(dimer) = 4000; N(trimer) = 2590. (b) Thermal polymeric fatty methyl ester. R(dimer–trimer) = 1.64; N(monomer) = 5400; N(dimer) = 2520; N(trimer) = 2140. (c) Thermal polymeric fatty alcohol. R(dimer–trimer) = 1.48; N(monomer) = 6900; N(dimer) = 2500; N(trimer) = 2980. (N = Theoretical plate number).

Reproducibility and recovery

The reproducibility and recovery are summarized in Table II which shows that the recoveries are approx. 97–99 % and that the reproducibility is satisfactory.

Separation mechanisms

Generally it has been observed that Sephadex gel adsorbs solutes more strongly than the cross-linked polystyrene gel[7,9–11]. In order to confirm this, the distribution

TABLE II

SUMMARY OF THE REPRODUCIBILITY AND RECOVERY (wt.%)

Chromatographic conditions: Sample size, 300 mg; solvent, DMF; column size, 2.0 × 230 cm; flow rate, 20 ml/h; temperature, 48–51°.

Component	*Experimental number*				*Average*
	1	*2*	*3*	*4*	
(*A*) *Thermal polymeric fatty acids*					
Monomer	4.1	4.5	5.0	4.6	4.6
Dimer	62.2	62.5	59.8	59.5	61.0
Trimer	17.5	18.6	18.4	18.6	18.3
Tetramer	9.0	8.9	9.4	9.5	9.2
Pentamer	3.1	3.9	3.9	4.1	3.8
Hexamer and higher molecular weight polymers	3.4	2.5	2.5	3.2	2.9
Recovery	99.3	101.1	99.1	99.5	99.8
(*B*) *Thermal polymeric fatty methyl esters*					
Monomer	7.1	7.4	6.1	6.5	6.8
Dimer	56.9	56.2	55.4	56.0	56.1
Trimer	15.6	16.0	17.3	17.2	16.5
Tetramer	7.1	7.7	8.5	8.1	7.9
Pentamer	3.5	3.8	4.2	3.9	3.9
Hexamer and higher molecular weight polymers	8.3	6.8	7.1	7.0	7.3
Recovery	98.5	97.8	98.6	98.7	98.4
(*C*) *Thermal polymeric fatty alcohols*					
Monomer	5.7	5.9	7.1	6.9	6.4
Dimer	51.3	51.5	51.2	52.4	51.6
Trimer	25.8	24.9	26.0	25.1	25.5
Tetramer	5.5	6.2	6.0	6.0	5.9
Pentamer and higher molecular weight polymers	7.7	7.2	7.1	7.6	7.4
Volatile compounds	1.8	1.8	1.8	1.8	1.8
Recovery	97.8	97.5	99.2	99.8	98.6

TABLE III

THE DISTRIBUTION COEFFICIENTS, K_d, OF EACH PEAK

Chromatographic conditions: Sample size, 50 mg; solvent, DMF; column size, 2.0 × 230 cm; flow rate, 20 ml/h; temperature, 48–51°.

Component	*Thermal polymeric components*		
	Acids	*Methyl esters*	*Alcohols*
Monomer	0.55	0.59	0.58
Dimer	0.35	0.36	0.39
Trimer	0.28	0.31	0.29
Tetramer	0.18	0.25	0.23

coefficients of each peak, K_d, were calculated, and the results are summarized in Table III. If strong adsorption occurs, the K_d must be more than 1 or polymeric fatty acids and alcohols must have a K_d larger than polymeric fatty methyl esters. However, the K_d values are less than 1 and almost resemble each other. Consequently, it is suggested that adsorption hardly occurs, and the separation mechanism is based mainly on a molecular sieve effect. Gel chromatograms in Fig. 5 show that the larger the molecule, the poorer the separation because Sephadex LH-20 has comparatively small permeability and decreases the separating ability with higher weight molecules. Thus if a more permeable gel or developing solvent which causes more swelling is employed, the higher molecular weight polymers (penta- or hexamers) should separate as well.

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