Quantitative Analysis of Polymerized Fatty Acids using Gel Permeation Chromatography

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ABSTRACT

The acid clay catalyzed dimerization of tall oil fatty acids and oleic acid yields commercial products which are complex mixtures of monomers, dimers, trimers, and higher polymers of various structures. Analyses for monomer, dimer, and trimer concentrations are important to ensure good quality control and reproducible end use performance. Techniques for direct determination of monomer, dimer, and trimer acids by gel permeation chromatography are presented. The components are separated using Bio Beads SX-2 gel. Heptanoic acid is used as an internal standard. The standard deviations for determination of dimer, trimer, and monomer are 1.0, 0.4, and 0.2% respectively. Calibration was accomplished using dimer and trimer fractions isolated from a preparative scale chromatographic system. It is suggested that a measure of higher polymeric acids can be obtained by difference after correction for neutral materials.

INTRODUCTION

The acid clay catalyzed polymerization of unsaturated fatty acids yields a reaction mixture consisting of dimers, trimers, higher oligomers, and monomer. The chemical and physical properties of these mixtures and products derived from them are dependent upon composition; however, analysis of these materials has proven to be difficult. The objective of the work reported here was to develop a rapid, accurate, and precise method for determination of the monomer, dimer, and trimer content of polymerized fatty acid products, particularly those based upon tall oil fatty acids.

Firestone (1) has reviewed the analytical methods used for fatty acid polymers through 1963. Molecular distillation of the methyl esters has been used by a number of workers to determine the monomer and dimer concentrations (2,3). These methods are empirical and tedious. In addition, attainable accuracy is questionable since some polymerization of sample components may take place during analysis under the distillation conditions employed. Frankel, et al., (4) separated dimers and monomers by liquid partition chromatography of the free acids using methanol on silicic acid as the stationary phase and 2% methanol in benzene as the mobile phase. However, this separation is slow, and changes in the mobile phase composition with time make it difficult to reproduce results and limit applicable monitoring techniques. Hase, et al., (5) has described a gas chromatographic determination of monomer and dimer. However, no direct information on trimer or higher oligomers is available by this technique.

The technique of gel permeation chromatography has been applied to polymerized fatty acids and related materials. Bartosiewicz (6) demonstrated reasonably good separation of monomers and dimers with cross-liked polystyrene beads but did not attempt quantitative analysis. Chang (7) used a porous styrene-divinylbenzene gel to determine small amounts of fatty acid dimer in tall oil. Using a modified dextran gel, Sephadex LH20, Hase and Harva (8) showed that the methyl esters of fatty acid monomers could be separated from dimers and higher oligomers, and Inoue et al., (9) achieved resolution up to, and including, tetramer and obtained evidence for the presence of pentamer and higher oligomers. This latter separation requires 24 hr, since low operating pressures must be maintained to avoid collapse of the dextran gel.

EXPERIMENTAL PROCEDURES

Apparatus and Reagents

Liquid chromatograph: Waters Models 301 and 502 instruments equipped with 2 ml liquid sample valves and refractive index detectors were used. Analyses were conducted on a 20 ft x 3/8 in. outside diameter stainless steel column packed with Bio Beads SX-2. Preparative isolation of pure dimer and trimer was carried out on an 8 ft x 1 in. stainless steel column packed with Sephadex LH-20.

Internal standards: Two internal standards were employed in these studies. The first was polystyrene with a mol wt 37,000 and a dispersion factor of less than 1.06 obtained from Pressure Chemical Co., Pittsburgh, Pa. The second was heptanoic acid, Eastman White Label, Cat. no. 821.

Solvents: Tetrahydrofuran (THF) was used for analysis of samples on Bio Beads SX-2. For isolation of standards on the preparative column of Sephadex LH-20, dimethylforamide (DMF) was used. Both were reagent grade materials used without further purification.

Method of Analysis

Samples of 250 ± 10 mg were weighed into 25 ml volumetric flasks. Then 250 ± 10 mg heptanoic acid or 50 ± 1 mg polystyrene was added as the internal standard, and THF was added to dilute to volume. The sample solution was injected, and the chromatogram was recorded. A baseline was drawn across the entire chromatogram, and peak heights were determined for the dimer, trimer, and internal standard peaks. The percentages of dimer and trimer were calculated from the peak height ratios relative to the internal standard, the ratio of weights of internal standard to sample, and the response factor determined from the calibration procedure.

Calibration

Since pure dimer and trimer acids are not commercially available, samples of these components were isolated by preparative scale gel permeation chromatography. The sample to be used for component isolation was dissolved in DMF at a concentration of 500 mg/ml. After adjusting the solvent flow rate at 20 ml/hr, 1 ml sample was injected. Dimer and trimer fractions were collected from the central portions of the peaks to ensure purity of each fraction. The injection-collection procedure was repeated until sufficient material was collected. The combined fractions then were evaporated to near-dryness (1-2 ml) with a rotary evaporator at 55-60 C. The syrup was treated with 25 ml THF and re-evaporated to near-dryness three times to ensure complete removal of DMF which would be detrimental to the Bio Beads SX-2 column. Finally, the syrup was dissolved in THF, transferred to a 50 ml volumetric flask, and diluted to volume. A 2 ml aliquot was dissolved in 25 ml methanol

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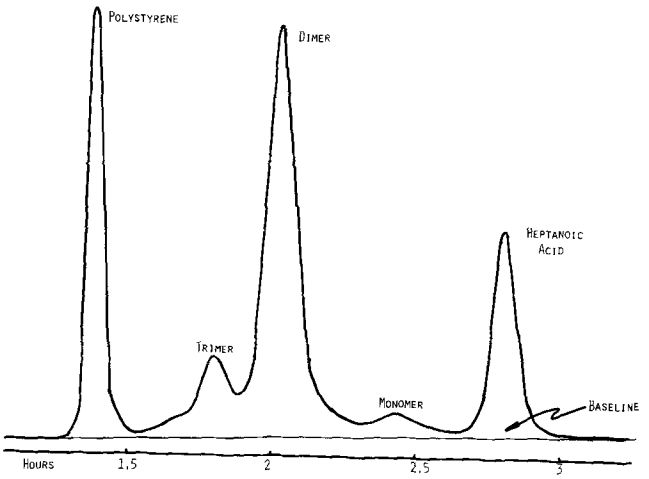

FIG. 1. Gel permeation chromatogram of a typical commercial dimer acid product with added internal standards.

and titrated to a potentiometric end point with 0.05N NaOH delivered from a microburet. The acid concentration was calculated using an equivalent weight of 282, and the average of triplicate titrations was used as the concentration of the standard solutions.

The response factors were determined by mixing portions of isolated dimer and trimer solutions with known wts of innternal standard and diluting to 25 ml with THF. These solutions then were processed by the method given above for sample analysis. Using the 2 ml sample size specified for analysis, detector linearity was demonstrated over the concentration range corresponding from 1-100% for both dimers and trimers.

The monomer response factor was determined from a heads cut obtained by distillation of a crude polymerization product. Gel permeation demonstrated that no polymers were present.

RESULTS AND DISCUSSION

A screening program was carried out to define a column which would provide the resolution required for quantitative analysis of dimer and trimer in a reasonable time. A Bio Beads SX-2 column described above, provided a resolution with a value of 1.05, as defined by Karger (10). This exceeds the value of 1.00 which is considered necessary for quantitative work. The chromatogram obtained from this column is shown in Figure 1. The total chromatographic time required is 3 hr. Since overlapping of chromatograms is possible, four samples can be analyzed on one instrument during a normal working day.

To obtain absolute values for dimer and trimer and to maintain satisfactory quantitative reliability, it was necessary to introduce an internal standard into the procedure. Initial attempts to measure absolute response in terms of peak ht/wt of sample injected were unsuccessful. For

TABLE I

Effects of Solvent Moisture and Temperature on Detector Response

Experimental parameter	Internal standard	
	Polystyrene	Heptanoic acid
H_2O %	% Dimer	
0.01	79.9	78.1
0.25	81.8	78.0
1.00	84.0	---
Temperature		
78 F	79.9	78.1
82 F (estimated)	81.6	78.1
85 F	82.7	78.2

TABLE II

Effect of Process Conditions on Response Factors

Sample	Dimer	Trimer
2 hr reaction (TOFA)[a]		
Crude	2.97	3.29
Residue	2.95	3.28
Overhead	2.98	---
4 hr reaction (TOFA)		
Crude	2.96	3.26
2 hr reaction (Oleic)		
Crude	2.62	---
Hydrogenated	2.37	---

[a]TOFA = tall oil fatty acids.

6 months, the response varied significantly and drifted lower reaching two-thirds of the original value. This behavior was attributed to variations associated with the instrumentation. For example, one set of variations was shown to be due to warping of the source filament in the detector. Response factors also varied between instruments. The use of polystyrene eliminated the variations due to instrumental factors described above. However, due to its high mol wt, polystyrene eluted at the interstitial volume of the column. Since it does not permeate the pores of the gel and is not functionally similar to the sample components being analyzed, polystyrene would not be expected to compensate for noninstrumental variables, e.g., those related to interaction between the solvent and the sample constituents. Therefore, heptanoic acid, which elutes well after the monomer, was investigated as an internal standard.

The effects of solvent, moisture, and temperature on detector response are shown in Table I. These data present the results obtained from a typical product using both internal standards. The normal water content of THF is 0.01%. When additional water was added to the THF, the polystryene failed to compensate for this effect; however, the value obtained using heptanoic acid as the internal standard was not affected by water up to 0.25%. At 1% added water, the heptanoic acid peak became distorted and could not be measured accurately. The temperature data were taken at a time when the laboratory temperature had risen from 78 F to 85 F during successive analyses. Again the polystyrene did not compensate for the temperature change, while heptanoic acid did so satisfactorily. These results agreed with expectations. Water would be expected to influence the degree of solvation of the acids, which would change the peak shape and the peak ht response. A change in temperature would be expected to change the viscosity and affect the solute permeation, which also would change peak shape and peak ht response. Based upon these results, heptanoic acid was chosen as the internal standard for analysis.

A major source of concern was that dimers and trimers

TABLE III

Accuracy of Analysis of Knowns

% Dimer		% Trimer	
Made	Found	Made	Found
89.9	90.3	10.2	10.3
	89.3		10.3
84.6	84.4	15.4	15.5
	84.7		15.0
81.4	81.9	18.6	18.3
	82.0		19.1
73.3	74.8	26.7	26.2
	73.9		26.4

TABLE IV

Analysis of a Commercial Dimer Product

Component	% Component	95% CL[a]
Dimer acids	75.4	± 1.1
Trimer acids	12.9	± 0.4
Monomer acids	2.3	
Neutrals	3.3	
Higher polymeric acids by difference	6.1	
	100.0	

[a]CL = confidence limit.

in samples produced under different processing conditions might have different responses with a refractive index detector. For example, the dimer product produced from tall oil is, in reality, a complex mixture of molecules of different structure; and it is conceivable that distillation might cause a separation within this group of molecules to give dimer fractions with different responses. Therefore, the effects of reaction time, distillation, and source of fatty acids on response factors were determined for both dimers and trimers. The results are shown in Table II. The top row values were determined from a crude product resulting when the bulk of the monomers were stripped. This batch then was carried through a dimer stripping operation where a trimer-rich residue and a dimer overhead resulted. Within these three samples no significant differences for dimer or trimer responses were observed. In an experiment not shown in this table, the dimer-rich overhead from the 2 hr reaction was fractioned in process distillation equipment, and the dimers from the resulting four fractions were found to have the same detector response values shown for the crude product. Therefore, it was concluded that distillation does not have a significant effect on the refractive index detector response. The data in Table II also show that a longer reaction time had no effect on detector response. However, when the feedstock was changed from tall oil fatty acid to oleic acid, there was a change in response factor for the dimer. Post-hydrogenation of the oleic-based dimer, also produced a further change in the response factor.

The analytical method was tested using a series of mixtures in the range of 70-90% dimer and 10-30% trimer prepared from the pure materials isolated from the preparative scale chromatographic system. The difference between the values as made and found are shown in Table III. The average differences of 0.6 for dimer and 0.3 for trimer indicate that the method should be capable of good accuracy. The precision of the method, based upon analysis of 20 samples run in triplicate, was found to be quite satisfactory. The standard deviation found for dimer was 0.98 and for trimer 0.35. This corresponds to a 95% confidence interval 1.1% for dimer and 0.4% for trimer for the average of triplicate determinations.

The monomer is resolved from the dimer and the heptanoic acid internal standard and can be determined from the gel permeation chromatogram. In the range of 1-4%, the standard deviation of the monomer determination was found to be 0.2%. Under the analytical conditions used here for dimer and trimer, the sensitivity of the refractive index detector limits the determination of monomer to a minimum concentration of 1%. It is preferable to use a gas chromatographic method for measurement of monomer contents below 1%.

TABLE V

Long Term Precision of Analysis of a Commercial Dimer Acid Product[a]

Statistical parameter	Dimer	Trimer
Average	79.5%	13.8%
Range	78.1-80.8%	13.4-14.4%
Standard deviation	0.49	0.19
Coefficient of variation	0.6%	1.3%

[a]Sixty analyses for 5 months.

Figure 1 shows the chromatogram of a typical commercial product containing about 75% dimer, 13% trimer, and 2% monomer. Mean values and the 95% confidence limits based upon triplicate determinations are shown in Table IV. The liquid chromatographic measurements of the three major components of this sample accounted for all but 9.4% material present. Neutrals account for another 3.3%. The presence of higher polymers is clearly evident from the shoulder on the ascending slope of the trimer peak. We believe that these higher polymers account for most, if not all, of the 6% not included in the above determinations. This example illustrates the fact that the gel permeation chromatogram presents a good visual accountability of the entire sample.

To define the contribution of the neutral materials to the chromatogram of this sample, the neutral fraction was isolated by alumina chromatography and processed through the gel permeation analytical system. The neutrals eluted as a group slightly earlier than the monomers and are the reason that the monomer peak shows distortion on the ascending slope. Determination of the response factor for the neutrals shows that the detector sensitivity is ca. one-fifth of that for the acids. Therefore, it was concluded that the neutrals do not constitute an interference to the measurement of the acids.

The long term precision of the method is demonstrated by the data in Table V which shows the results obtained for analysis of a commercial sample for 5 months. The low values of the standard deviations obtained over this long time period demonstrate the excellent long term repeatability of the method.

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