Supercritical Fluid Chromatographic Analysis of the Propoxylated Glycerol Esters of Oleic Acid

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Capillary supercritical fluid chromatographic methods were developed for the separation and quantitation of oligomers of propoxylated glycerols and fatty acid-esterified propoxylated glycerols. Samples containing mole ratios (propylene oxide to glycerol) of 5, 8 and 14 were chromatographed. Separations depended primarily upon the molecular weight or the number of the propylene oxide units. The oligomer distributions in the propoxylated glycerol and the oleic acid-esterified propoxylated glycerol samples were determined.

KEY WORDS: Oleic acid-esterified propoxylated glycerol, supercritical fluid chromatography.

Fatty acid-esterified propoxylated glycerol (EPG) has been synthesized in an effort to develop a heatable, noncaloric fat substitute (1). The characterization of EPGs can be conveniently done by a relatively new chromatographic technique, capillary supercritical fluid chromatography (SFC), to overcome the limitations imposed by gas chromatography (low sample volatility) and high-performance liquid chromatography (HPLC) (insufficient resolution) (2).

Analyses of polyglycerols with HPLC have been reported (3-6). Nozawa and Ohnuma (3) reported an HPLC procedure to analyze ethylene oxide oligomers. The ethoxylates were derivatized with 3,5-dinitrobenzoyl chloride and detected by ultraviolet (UV) absorbance. Allen and Linder (4) have used adsorption chromatography combined with linear gradient elution to separate ethylene oxide oligomers. The ethoxylates were derivatized with phenyl isocyanate and detected by UV. McClure (5) described an HPLC method for analyzing ethylene oxide oligomers with a rotating disk-flame ionization detector (FID). The FID is not a convenient detector for HPLC, and quantitative results can be difficult to achieve. However, without derivatization, propoxylated glycols, as well as propoxylated glycerols, cannot be detected by UV detectors.

SFC has several advantages for the analysis of oligomers. SFC can be used to separate higher molecular weight oligomers at lower temperatures than is possible with gas chromatography (GC). In addition, SFC can be used with most GC and HPLC detectors, including the FID.

Knowles *et al.* (7) reported on the utility of SFC for screening the oligomeric distribution of polyglycols. Geissler (8) developed quantitative SFC methods for mixtures of 2-ethylhexanol and octanol polyethylene oxide oligomers, and Johnson *et al.* (9) studied the relative rate of ethoxylation based on SFC analysis of the ethoxylated alcohols. Kalinoski and Jensen (10) also separated stearyl alcohol ethoxylates and polyethylene glycol oligomers by capillary SFC.

For the analysis of fat- and oil-related samples, Chester and Innis (11) separated derivatized mixtures of polyglycerol fatty acid esters containing 2-4 glycerol units with capillary SFC. In this case, silylation of fatty acid esters enhanced their resolution.

This report describes the analysis of propoxylated glycerol (PG) oligomers and oleic acid-EPGs by capillary SFC. Samples of PGs and oleic acid-containing EPGs with an average of 5, 8 and 14 moles of propylene oxide per mole of glycerol were separated. Estimates of oligomer distribution were obtained from quantitative data based on peak area percent.

EXPERIMENTAL PROCEDURES

Materials. PG and oleic acid-EPG (1) were prepared by Charles Cooper (Arco Chemical Co., Newtown Square, PA). The PGs were synthesized by alkali-catalyzed addition of propylene oxide to glycerol. The polyols were then esterified by heating with oleic acid while removing the by-product H_2O under partial vacuum. During the reaction, some propylene oxide can react with residual moisture to produce a small amount of difunctional propylene glycerol oligomers. Figure 1 outlines the reaction equation and product structures.

The propoxylated glycerols analyzed included polyol 382, polyol 550 and polyol 1000, which contained average mole ratios (propylene oxide to glycerol) of 5, 8 and 14, respectively. The oleic acid-EPGs included EPG-

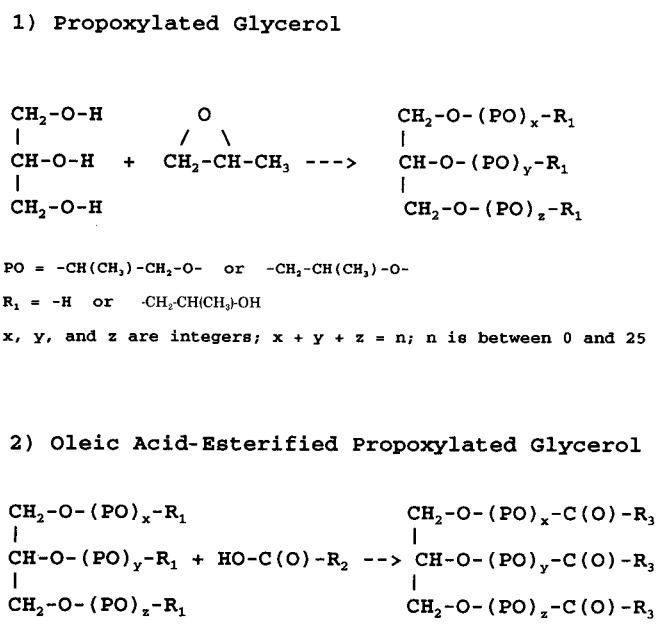

FIG. 1. Structures of propoxylated glycerols and fatty acid-esterified propoxylated glycerols.

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05, EPG-08 and EPG-14 trioleate. They were prepared from polyol 382, 550 and 1000, respectively.

Tridecanoin was used as the internal standard (Nu-Chek-Prep, Inc., Elysian, MN). Methylene chloride was used as the sample solvent (J.T. Baker Chemical Corporation, Phillipsburg, NJ). The concentration of each sample stock solution was approximately 50 mg/mL, and the concentration of the internal standard was approximately 1 mg/mL. Prior to injection, each sample was diluted to approximately 25 mg/mL, and the internal standard was diluted to approximately 0.2 mg/mL.

Instrumental. The supercritical fluid chromatograph used for the analyses was a Lee Scientific Model β501 (Lee Scientific, Inc., Div. Dionex, Salt Lake City, UT) with a Valco A90 injector (Houston, TX) containing a 0.2-μL internal loop operated in a time-split mode. The capillary column was a 20-m SB-methyl-100 (50 μm i.d., d_f = 0.25 μm), except for the EPG-14 oleate sample, which was separated on a 30-m SB-methyl-100 (50 μm i.d., d_f = 0.25 μm) capillary column. The stationary phase for both columns was 100% bonded polymethylsiloxane. The mobile phase was SFE-grade CO_2. (Air Products and Chemicals, Tamaqua, PA). Density programming was

TABLE 1

SFC Pump Programming Parameters[a]

Sample[b,c]	CO_2 density program
Polyol 382	0.2 g/mL, 2.0 min, 0.02 g/mL/min to 0.60 g/mL, followed by an 8.0-min hold.
Polyol 550	0.25 g/mL, 2.0 min, 0.015 g/mL/min to 0.60 g/mL, followed by an 8.0-min hold.
Polyol 1000	0.25 g/mL, 2.0 min, 0.015 g/mL/min to 0.60 g/mL, followed by an 8.0-min hold.
EPG-05 oleate	0.12 g/mL, asymptotic ramp to 0.61 g/mL, 1/2 rise time 15 min, end time 90 min.
EPG-08 oleate	0.15 g/mL, asymptotic ramp to 0.64 g/mL, 1/2 rise time 15 min, end time 90 min.
EPG-14 oleate	0.20 g/mL, 0.0045 g/mL/min to 0.50 g/mL, asymptotic ramp to 0.56 g/mL, 1/2 rise time 20 min, end time 110 min.

[a]SFC, supercritical fluid chromatography; EPG, esterified propoxylated glycerol; FID, flame ionization detector.
[b]Each separation was done at a column temperature of 150°C, except for the EPG-14 oleate sample, which was separated at 180°C.
[c]The FID temperature was 325°C for the Polyol samples, 350°C for the EPG-05 and EPG-08 oleate samples and 400°C for the EPG-14 oleate sample.

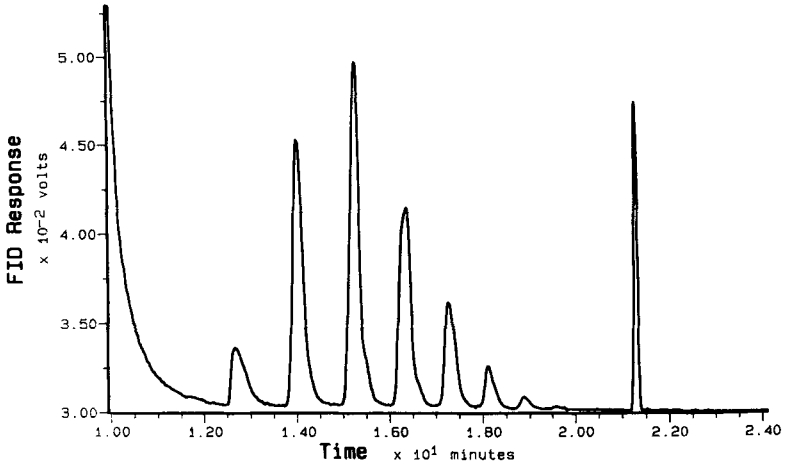

FIG. 2. Supercritical fluid chromatogram of propoxylated glycerol with 5 moles of propylene oxide per mole of glycerol (polyol 382). FID, flame ionization detector.

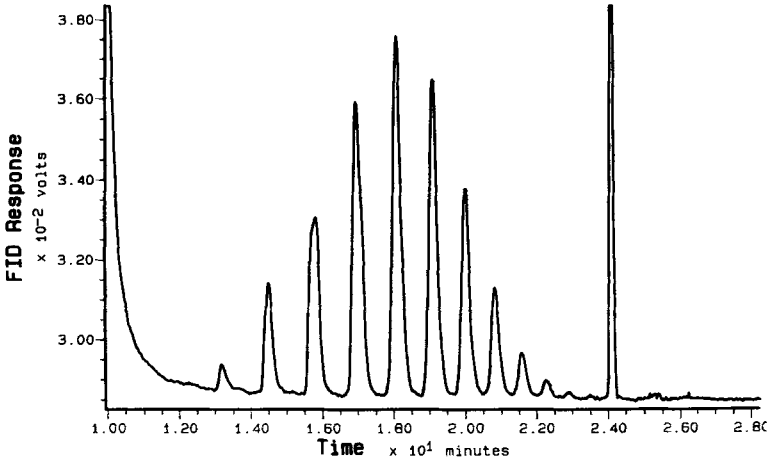

FIG. 3. Supercritical fluid chromatogram of propoxylated glycerol with 8 moles of propylene oxide per mole of glycerol (polyol 550). FID, flame ionization detector.

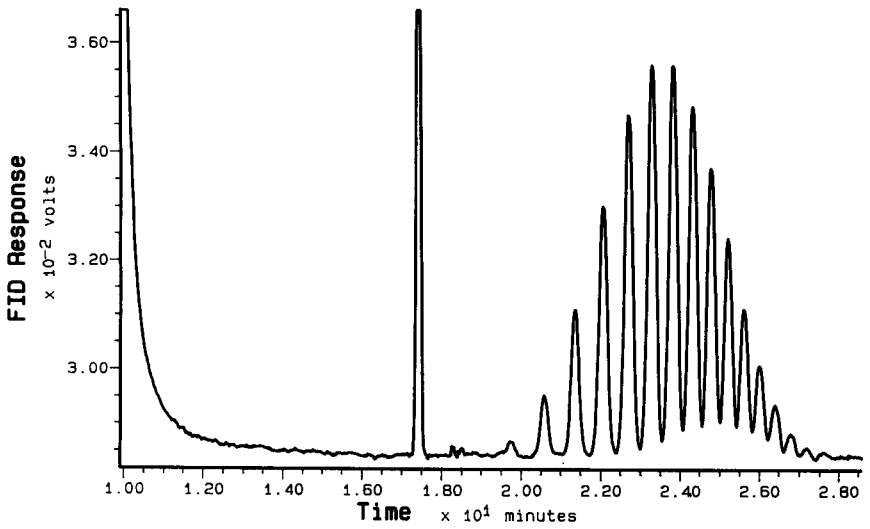

FIG. 4. Supercritical fluid chromatogram of propoxylated glycerol with 14 moles of propylene oxide per mole of glycerol (polyol 1000). FID, flame ionization detector.

TABLE 2

The Propylene Oxide (PO) Oligomeric Distribution in Propoxylated Glycerols

Number of PO units	Polyol 382		Polyol 550		Polyol 1000	
	Percent	RSD[a] (%)	Percent	RSD (%)	Percent	RSD (%)
3	7.5	0.9				
4	25.2	0.3				
5	30.5	0.2	1.42	4.4		
6	21.2	0.4	5.98	0.9		
7	10.5	1.7	12.7	0.8		
8	3.73	2.8	19.2	0.6		
9	1.01	4.1	21.4	0.5		
10			18.0	0.3		
11			11.6	0.5	2.17	1.9
12			5.93	2.6	5.16	0.8
13			2.52	1.3	8.85	0.8
14					12.3	0.8
15					14.2	0.5
16					14.2	0.2
17					12.6	0.7
18					10.1	0.6
19					7.50	1.0
20					5.19	1.3
21					3.35	3.6
22					1.97	5.0
23					1.01	4.2

[a]RSD, relative standard deviation.

employed for each separation (Table 1). The FID temperature was 325–400°C. The chromatographic data were collected and analyzed with a Hyundai 386S PC and Baseline software (Waters Chromatography, Milford, MA). The pump, oven, injector and detector were controlled by an ARC Turbo PC (American Research Corporation, Monterey Park, CA) with software from Lee Scientific, Inc.

RESULTS AND DISCUSSION

GC can be used for the separation and quantitation of low-molecular weight propoxylated glycerols, but not for the separation of the higher-molecular weight propoxylated glycerols or any of the EPGs. The PG oligomer samples (polyol 382 and polyol 550) have been separated by GC in a 10-m polymethylsiloxane megabore column with temperature programming from 0 to 280°C at 10°C/min. SFC separations of the PG oligomers are shown in Figures 2–4. The large sharp peak in Figures 2–4 (eluting at 21.2 min, 25.1 min and a 17.4 min, respectively) is the internal standard (tridecanoin). The SFC separation times and resolution were comparable to the megabore GC separation for the polyol 382 and polyol 550 samples, with the exception of the polyol 1000 sample, which could not be separated with GC. With SFC, a complete separation for the oligomers in the polyol 1000 was achieved (Fig. 4). All of the samples were separated based upon their relative

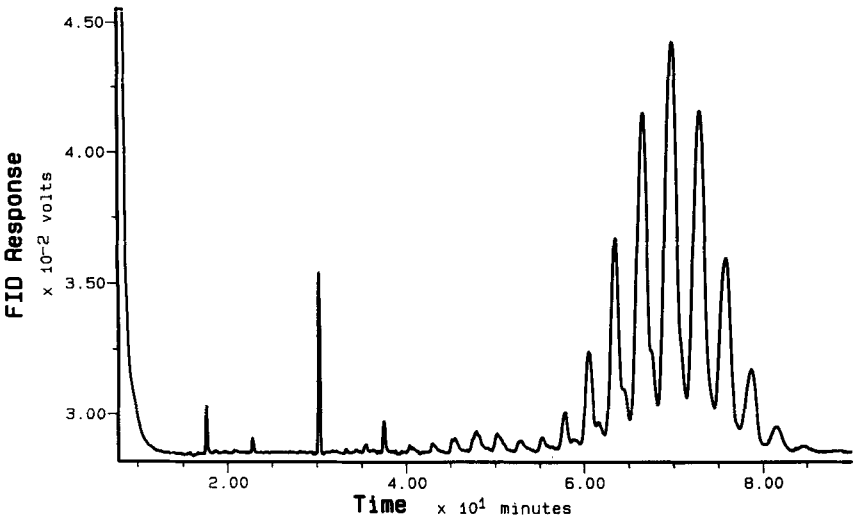

FIG. 5. Supercritical fluid chromatogram of oleic acid-esterified propoxylated glycerol (EPG) with 5 moles of propylene oxide per mole of glycerol (EPG-05). FID, flame ionization detector.

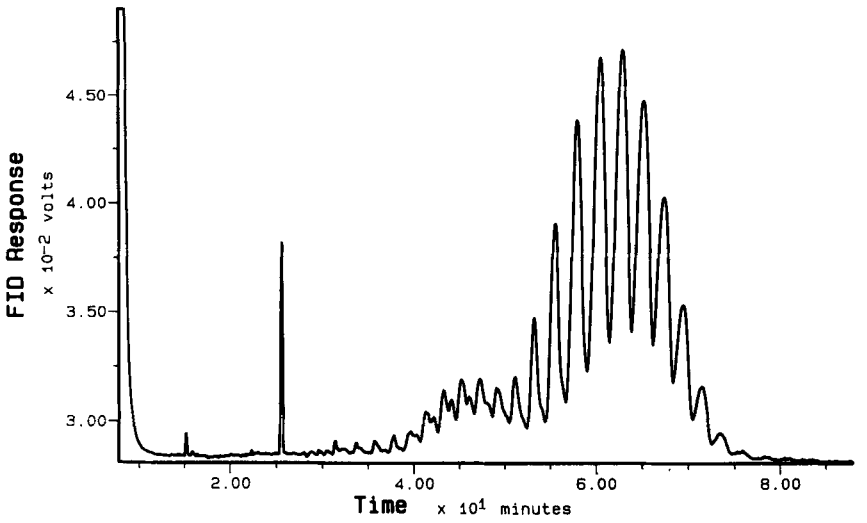

FIG. 6. Supercritical fluid chromatogram of oleic acid-esterified propoxylated glycerol (EPC) with 8 moles of propylene oxide per mole of glycerol (EPG-08). FID, flame ionization detector.

molecular weight. In Figures 2 and 3, several peaks had shoulder peaks, which may indicate structural isomers.

Tentative peak identification and assignment to an oligomer with a specific number of the propylene oxide units was based on GC/mass spectrometry (MS) analysis. Quantitative analysis of the oligomer distribution was based on the peak area percent. Table 2 contains the results for the three polyol samples, which includes the number of propylene oxide units, the peak area percent and the relative standard deviation (RSD) for each of five replicates. Components present at less than 1.0% were not reported.

Figures 5–7 are the separations of the EPG oleates. Two groups of peaks can be discerned from the chromatograms of EPG-05 and EPG-08 oleate (Figs. 5 and 6). The major peaks are the PG triester products (Fig. 5, retention times of 58–85 min; and Fig. 6, retention times of 50–80 min). The other group of peaks tentatively represents the PG di- and monoester products, respectively (Fig. 5, retention times of 35–56 min; and Fig. 6, retention times of 28–48 min). In the EPG-14 oleate chromatogram, two groups of peaks were also apparent; the main trioleate EPGs (Fig. 7, retention times of 79–100 min) and a group of di- and monooleate EPGs Fig. 7., retention times of 60–78 min). The large, sharp, early-eluting peak in each chromatogram is the internal standard (tridecanoin). Any additional conjecture other than this tentative identification will require further evaluation with other techniques, such as SFC/MS or SFC/Fourier transform infrared spectrometry.

Quantitative analysis of the oligomer distributions for the EPG samples was also based on the peak area percent, in conjunction with the internal standard. Tentative-

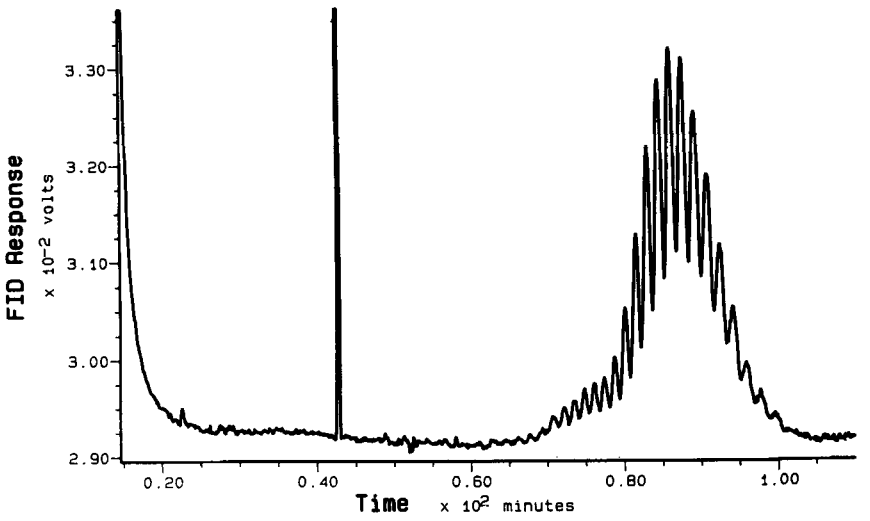

FIG. 7. Supercritical fluid chromatogram of oleic acid-esterified propoxylated glycerol (EPG) with 14 moles of propylene oxide per mole of glycerol (EPG-14). FID, flame ionization detector.

TABLE 3

The Propylene Oxide Oligomeric Distribution in Oleic Acid-Esterified Propoxylated Glycerols (EPG)[a]

Number of PO units	EPG-05		EPG-08		EPG-14	
	Percent	RSD (%)	Percent	RSD (%)	Percent	RSD (%)
1	1.20	2.3				
2	3.71	3.5				
3	8.75	1.6				
4	17.7	0.5				
5	23.8	0.3	3.49	1.5		
6	19.3	0.2	6.49	0.9		
7	9.88	0.5	10.8	0.6		
8	4.01	1.5	14.7	1.5		
9	1.27	5.3	16.2	1.1		
10			14.0	1.7	1.97	8.5
11			9.75	1.4	3.24	2.7
12			5.44	1.4	5.04	0.5
13			2.42	2.7	7.87	3.7
14					10.1	3.1
15					11.6	1.9
16					11.8	1.1
17					10.6	3.0
18					8.88	0.8
19					6.83	2.1
20					4.29	6.5
21					2.90	2.6
22					1.60	9.4

[a]Abbreviations as in Table 2.

ly (based on GC/MS analysis of the polyol 382 and polyol 550 samples), the isomer present in the greatest amount was $Gly(PO)_5(OCOR)_3$ for EPG-05, $Gly(PO)_9(OCOR)_3$ for EPG-08 and $Gly(PO)_{15}(OCOR)_3$ for EPG-14. The oligomer distribution of these EPGs have been summarized in Table 3. The PG tri-, di- and monoesters in each EPG sample were represented by $Gly(PO)_x(OCOR)_3$, $Gly(PO)_x(OCOR)_2(CH_2\text{-}CHCH_3\text{-}OH)$ and $Gly(PO)_x(OCOR)(CH_2\text{-}CHCH_3\text{-}OH)_2$, respectively. As with the analysis of the PGs, five replicates of each sample were analyzed under the same set of experimental conditions.

SFC offers an ability to use much lower temperatures relative to those used in GC for the separation of high-molecular weight analytes. In many instances, an increase in SFC column temperature can improve the separation of higher-molecular weight analytes, although some investigators have used a programmed temperature decrease to enhance SFC separations (12). Hence, EPG-14 was analyzed at a column temperature of 180°C rather than 150°C.

The detector temperature can influence analyte detectability. During method development for the separation of EPG-14 oleate, the detector temperature was initially set at 325°C. However, there were no detectable peaks. An increase in the detector temperature to 350°C still produced no peaks. At 375°C, some early-eluting peaks were

observed. Finally, the detector temperature was set at 400°C. The resulting chromatogram is shown in Figure 7. A similar observation also has been reported by Knowles *et al.* (7).

SFC is an effective technique for the analysis of PG and EPG samples, particularly for the partial separation of EPGs. In some cases the detector temperature can affect analyte detectability.

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