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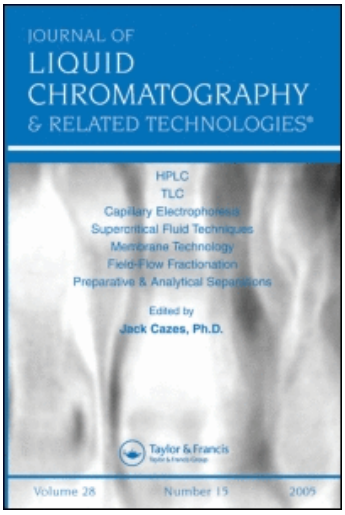

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JOURNAL OF LIQUID CHROMATOGRAPHY, 4(7), 1173-1194 (1981)

ANALYSES OF POLYGLYCEROL ESTERS OF FATTY ACIDS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Polyglycerol esters are important food emulsifiers available commercially as a mixture of various isomers. The need for analyses of these emulsifiers may arise at any stage of their production and use and is of significant importance from the point of view of the health authorities.

Polyglycerol esters of fatty acids can be analyzed by partition HPLC on a 25 cm column packed with 10 μm LiChrosorb Diol. The isomers were eluted gradientally with n-hexane-isopropanol mixture within 60 min. and the components monitored by uv-absorption at 220 nm.

Monoglycerol and polyglycerol monoesters as well as polyglycerol polyesters of oleic acid were separated and evaluated.

INTRODUCTION

Polyglycerol esters of fatty acids, because of their liphophilic and hydrophilic properties are used as food emulsifiers. They are prepared by a two step reaction. Acid or base catalyzed condensation of glycerol to form linear and cyclic polyglycerols, followed by an easy esterification process with fatty acids (1).

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A need for analyses of these emulsifiers may arise at several stages of their production and use, e.g. for production control and the detection of batch-to-batch variations, for comparison of emulsifiers purchased from different suppliers, and for the detection of the type of emulsifiers used in a competitor's food product. Authorities may also require analysis methods to decide whether or not the emulsifiers used in improved food products are compatible with local regulations etc.

These emulsifiers are mixtures of up to hundreds of individuals chemical compounds, differing by degree of esterification of the polyol, by chain length of the fatty acids, by degree of unsaturation, by positional isomerism of the fatty acids esterified with the polyol's hydroxyl groups, and so on.

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Several investigators have reported attempts to analyze and separate commercial polyglycerols and polyglycerol esters of fatty acids. Zajic (2) described a paper chromatography procedure for separation of polyglycerols. Siegel, Bullock and Carter (3) demonstrated a procedure for separation, identification and quantitative estimation of α,α'- and α,β- diglycerol by paper chromatography. Troy and Alrop (4) developed a periodic acid oxidation method for quantitative estimation of several polyglycerols. The use of thin-layer (TLC) (5) and gas-liquid-chromatography (GLC) methods (by derivatization with TMS) (6), also are described for analyses of those polyols. Recently, polyols and polyglycerols were conveniently analyzed by partition HPLC on silica columns without the need of derivatization.

The use of HPLC for separation of monoglycerol mono-, di- and tri-fatty acid esters has been also described by several groups of investigators. Polyglycerol esters of fatty acids have been so far analyzed only in part by an indirect method. TMS-derivatives of short chain polyglycerol esters such as mono-, di- and triglycerols fatty acid esters were injected to gas chromatographs and their patterns were identified.

The following study presents for the first time attempts to analyze a wide range of polyglycerol-poly-fatty acid esters by high performance liquid chromatography technique without the need of any derivatization.

The paper presents chromatograms of monoglycerol mono-, di- and tri-fatty acid esters as well as tri-, hexa-, octa-, decaglycerols monoesters of oleic acid and other polyglycerols poly fatty acid esters.

EXPERIMENTAL

Materials

Glycerol 1-monooleate (puriss) and glycerol 1,3-dioleate (puriss) were obtained from Sigma Chemicals. Glycerol monostearate and mixtures of glycerol mono- and di-stearates were commercially available products from Grindstedt Products and Atlas Europol A.p.S., sunflower oil was commercially available. Polyglycerol esters were synthesized in our laboratory and for comparison were purchased from Capitol City Corp. and Durkee Corp. The eluents were n-hexane (uvasol) and isopropanol (A.R.) purchased from E. Merck, Darmstadt, West Germany.

Procedure technique

The analyses were performed on Spectra Physics model SP-8000 HPLC chromatograph equipped with SP770 variable wavelength UV-detector at 220 nm. The separations were achieved on 25 cm x 4.6 mm ID steel columns prepacked with 10 µm LiChrosorb Diol purchased from Alltech Associates, Inc.

A gradient elution with n-hexane and isopropanol was carried out as described in table 1 at a flow rate of 1 ml/min and a pressure of about 150 psi, and the chromatographs were analyzed using SP-8000

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0.0	0.0	100
1.0	1.0	99
22.0	1.0	99
24.0	3.0	97
58.0	3.0	97
60.0	0.0	100

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data system built in the instrument to achieve peak areas and retention times. The samples were dissolved in isopropanol (up to 15% w/w) and 10 μl of solution were injected by automatic loop injector.

RESULTS AND DISCUSSION

Figure 1 shows the chromatograms of some standard authentic solutions of glycerol esters of mono-, di- and trioleates as well as glycerol mono-, di-, and tristeareates.

The separation is not the best for monoglycerol esters but we have chosen to use those conditions since they were found suitable and essential for the separation of the polyglycerol esters and the comparison is inevitable.

it is possible to achieve separation between mono-, di- and tri-fatty acid esters of glycerol. Figure 1B is the standard pure 99% glycerol monooleate in which peak 9 is composed of two components: glycerol α-monooleate eluting first and glycerol β-monooleate (7), being only an impurity and more polar, elutes second as a shoulder peak.

When 90% distilled monoglyceride of unsaturated fatty acids (Hercules 90-15) is injected (Figure 1C) the peak corresponding

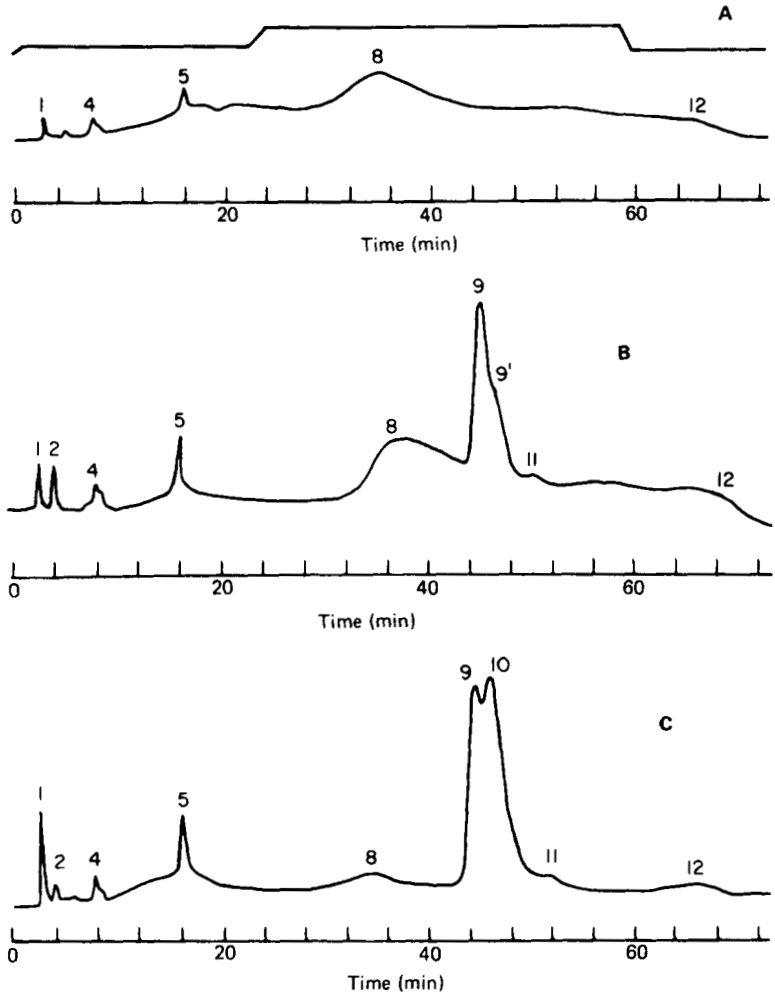

Figure 1A, B, & C (continued)

to the mono isomer is again composed of two components which seems to be glycerol monooleate and glycerol monolinoleate (according to the manufacture those are the two main components in the product).

Figure 1D shows the chromatogram of the commercially so called glycerol monostearate, (90% mono glycerol of mainly stearic and

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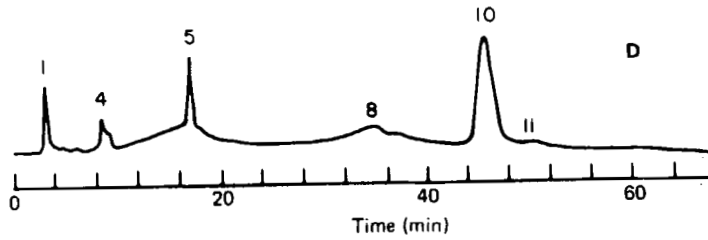

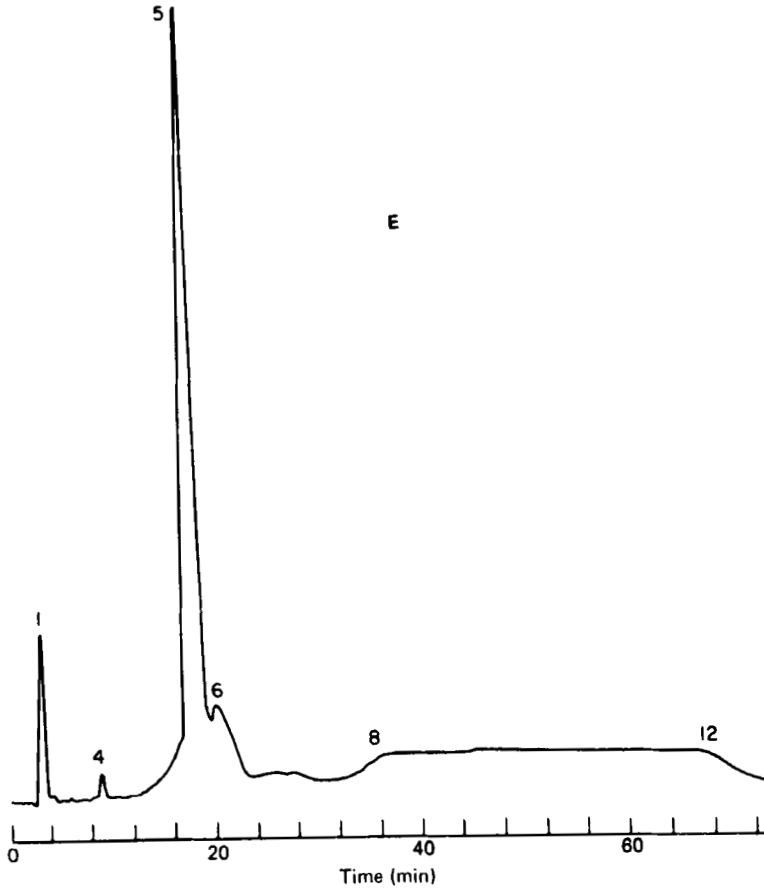

Figure 1D & E

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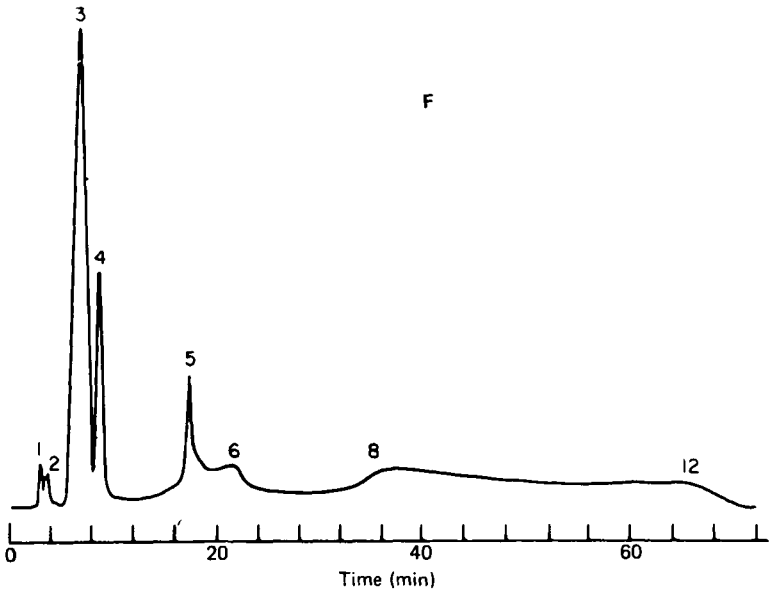

Figure 1: HPLC chromatograms of same standard monoglycerol mono-, di and trioleate and stearates

A: Pure isopropanol
B: Standard monoglycerol monooleate from Sigma Chemicals (99.5% pure)
C: Standard "monoglycerol monooleate" from Hercules Corp. (90% distilled monoglycerides of unsaturated fatty acids, mainly oleic and linoleic and 1.0% di- and triglycerides)
D: Standard "monoglycerol monostearate" from Hercules Corp. (90% distilled monoglycerides of saturated fatty acids mainly stearic and palmitic and 10% di- and triglycerides)
E: Standard monoglycerol dioleate from Sigma Chemicals (99.5% pure, contains mainly 1,3-dioleate and traces of 1,2-dioleates)
F: Sunflower oil (commercial grade) containing monoglycerol tri- fatty acid esters of unsaturated fatty acids mainly oleic 22% and linoleic 66%

Peaks 3-4: glycerol triester
Peaks 5-7: glycerol diester
Peaks 9-11: glycerol monoester

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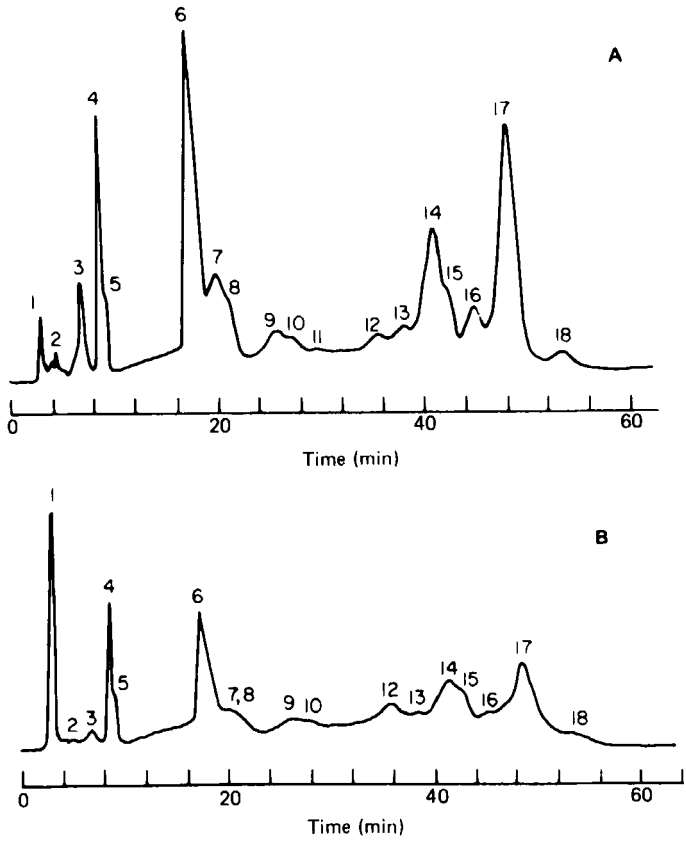

Figure 2A & B (continued)

palmitic acids from Hercules). It can be clearly seen that under those conditions the column is not capable of separating glycerol esters of stearic, palmitic or oleic acids.

Glycerol 1,2- and 1,3-dioleates are eluted as peaks 5-6 as it can be seen from injection of standard solution of pure glycerol dioleate (Figure 1E). The separation between these two isomers is not perfect. The 1,2-glycerol dioleate (peaks 6 or 7) is more polar and elutes shortly after the 1,3-dioleate (which is the main component in the mixture).

Triglycerides of unsaturated fatty acids were eluted shortly after their injection (peaks 3 and 4 of Figure 1F). Several oils

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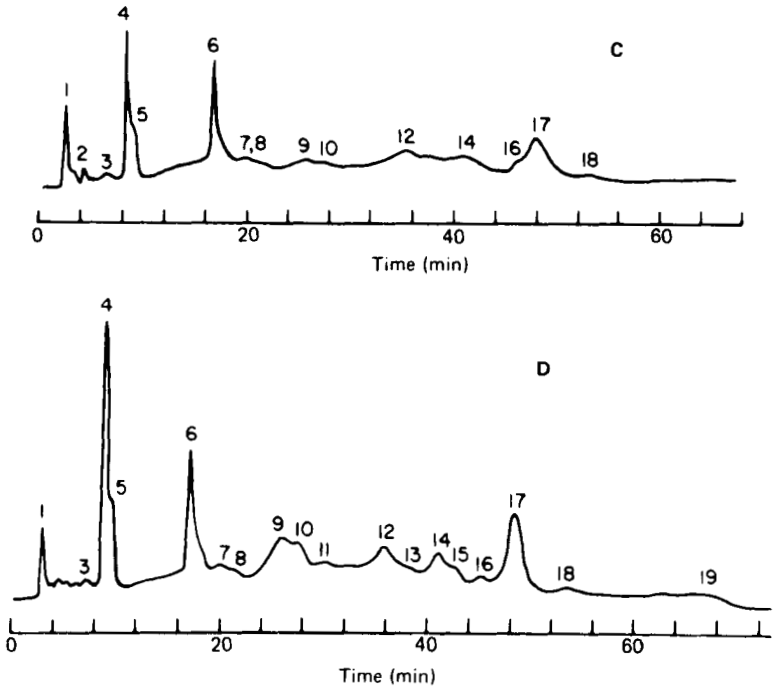

Figure 2: HPLC chromatograms of some crude commercial polyglycerol monooleates (from Capitol City Corp.)

A: Triglycerol monooleate
B: Hexaglycerol monooleate
C: Octaglycerol monooleate
D: Decaglycerol monooleate

Peaks 3-5: triglycerol tri- and polyoleates
Peaks 6-11: diester isomers
Peaks 12-18: monooleate isomers of mono and polyglycerols

such as sunflower, linseed and soybean were also tested by this method to confirm the retention times for the triglycerides esters.

Peaks 1,2,5,6,8,12 are most probably artifacts or impurities and some of them appear usually even when injecting pure isopropanol (Figure 1A) and thus are attributed to the use of gradient.

Figure 2 demonstrates the chromatograms obtained after injecting a series of several polyglycerol monoleates. It is important to realize that the materials are not pure and a compound commercially

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named, for example triglycerol monooleate (3.G.1.0), might as well contain a wide range of several polyglycerols from one to ten. The degree of polymerization is given by the producer according to the number of moles of water formed and distilled out during the polymerization process of the glycerol and also based on other physical properties of the polyglycerol obtained (such as refractive index, viscosity, density, hydroxyl value etc.) (1). Thus the triglycerol is an average number of glycerols in such a polymer. The same idea stands for the number of the oleic acids present in such a polymer. Emulsifier containing one mole equivalent of oleic acid per chain of polyglycerol is claimed by the manufacturer to be polyglycerol monooleate despite the fact that several positions on the polyglycerol chain can be esterified and thus the compound can be, as well, a mixture of polyglycerol mono-, di-, tri-, tetraoleate. (See Figure 4).

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The absence of free fatty acid is assured by allowing the reaction to proceed up to a point where the acid value of the product is zero, indicating absence of free fatty acid in the system. Free polyglycerols are also not present in the crude product due to its separation by simple decantation of the two immiscible phases (when still hot).

When the crude product named "triglycerol monooleate" (3.G.1.0) was injected to the HPLC column a complex chromatogram, containing a large number of peaks, was recorded (Figure 2). The three main peaks in Figure 2A correspond according to their retention time to glycerol monooleate (peak #17), glycerol dioleate (peak #6) and glycerol trioleate (peak #4). In addition several other significantly big peaks are present in the above chromatogram. At 7 min. (peak #3) another trioleate isomer with more apolar structure is eluted. The main dioleate peak is accompanied by additional smaller peaks at 20 min. (peak #7) and its tailing at 21 min. (peak #8). Those are interpreted as additional polyglycerol dioleate isomers being more polar than the main dioleate one. At 25 to 30 min. (peaks #9, 10, 11) appear additional small peaks most probably corresponding

to other dioleate structures. The monooleate pattern is very complex containing a series of less polar structures and more polar isomers (the main apolar isomer elutes after 41 min., peak #14). It is worth noticing that while for monoglycerol monooleate there are only two possible positions to be esterified (see Figure 3) and thus two isomers can be obtained; one can account for two isomers of monoglycerol dioleate and only one isomer of monoglycerol trioleate (see Figure 3). The situation with triglycerol oleates is more complex. There are - isomers of 3.G.1.0, - isomers of 3.G.2.0, - isomers of 3.G.3.0 and isomers of 3.G.4.0. Considering all these possible isomers one can imagine a large variety of isomers eluting in every set of peaks as can be seen from the chromatogram. Since

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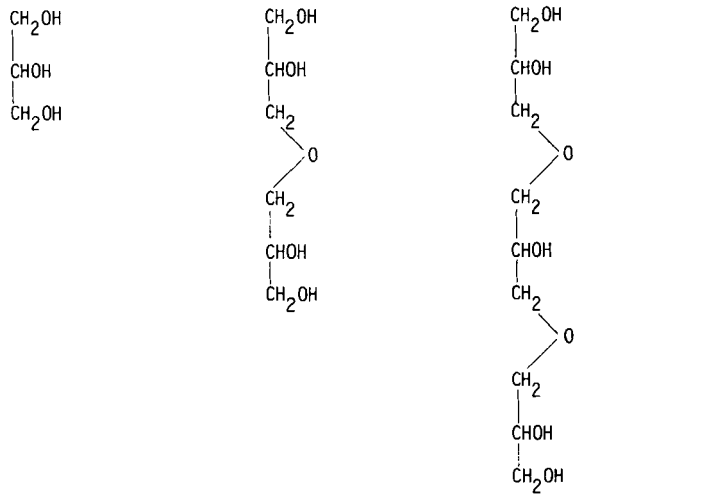

Figure 3: Illustration of several possible isomers obtained from mono-, di- and tri linear glycerol esters.

the crude product contains longer polyol chains such as tetra-, penta- etc. it will be reasonable to consider additional sites for esterification and thus one would expect the appearance of a large number of peaks corresponding to a variety of mono-, di- and tri-oleates. It can be concluded that each chromatogram is divided in three main parts corresponding to mono-, di- and tri-esters. Each group contains several subpeaks being the less polar structures of the above groups.

In order to identify the compounds eluted in the first set of peaks (peaks #3-5) a semi-quantitative separation has been accomplished. The eluted compounds were further analyzed using mass-spectrometer equipped with chemical ionization detector. The analysis confirmed that triglycerol trioleate as well as diglycerol trioleates and monoglycerol trioleates and most probably tetraoleates are present in this fraction. It was impossible to detect higher molecular weight materials but those are most probably persent. In the second set of peaks it was clear that isomers of triglycerol dioleates (MW=768) are present. The third set of peaks corresponds well to triglycerol monooleates.

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When the crude mixture of 3.G.1.0 was analyzed by the mass-spectra technique mass fractions of MW = 356 (1.G.1.0) as well as MW = 430 (2.G.1.0) and MW = 504 (3.G.1.0) were found. In addition fractions corresponding to 3.G.2.0 and 3.G.3.0 were identified. Higher molecular weight fractions are detectable but hard to interpret.

By examining the chromatogram of hexaglycerol monooleate (6.G.1.0) (Figure 2B) in comparison to triglycerol monooleate (Figure 2A) it can be seen that the main three peaks in each chromatogram appear after the same retention times (peaks marked as 4, 6 and 17). These findings indicate again that the column does not differentiate between the chain length of the polyol. Close examination of the peak areas in chromatograms 2A and 2B shows that the monooleate peak area (#17) is significantly smaller than the dioleate peak area (#6) and the trioleate peaks (#4-5) in Figure 2B in comparison to the same ratios in Figure 2A (see also table 2). Furthermore

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TABLE 2

Product Composition of Tri-, Hexa-, Octa- and Decaglycerol Monooleates from the HPLC Chromatogram.

Type of emulsifier	Trioleate peaks		Dioleate peaks			Monooleate peaks						mono #17 / di #6	mono #17 / tri #4-5
	3	4,5	6	7,8	9-11	12	13	14,15	16	17	18		
3.G.1.0	4.7	8.2	36.0	4.8	3.5	0.6	0.9	13.4	2.5	23.7	1.3	0.66	2.9
6.G.1.0	1.8	12.3	4.0	1.7	0.83	8.9	---	13.3	0.4	20.7	---	0.52	1.68
8.G.1.0	1.4	17.7	45.4	2.2	1.6	8.4	---	4.0	---	17.2	---	0.38	0.97
10.G.1.0	1.0	24.2	28.6	4.6	13.7	6.6	---	6.0	0.9	18.6	1.5	0.65	0.76

(*) All the calculations are averaged from at least four different injections. The response for each compound at 220 nm is, of course, not similar and thus the numbers have only qualitative meaning.

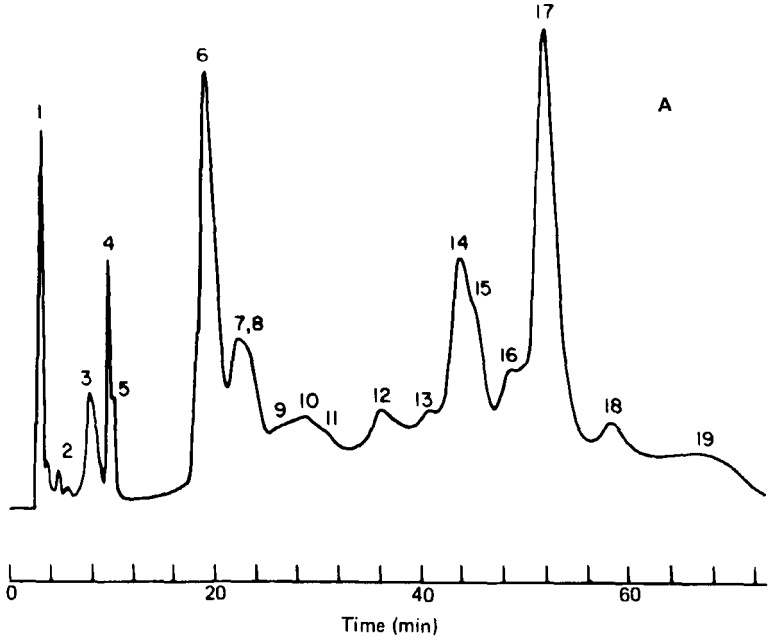

Figure 4: HPLC chromatograms of several triglycerol polyoleates

A: Triglycerol monooleate (3.G.1.0)
B: Triglycerol dioleate (3.G.2.0)
C: Triglycerol trioleate (3.G.3.0)
D: Triglycerol tetraoleate (3.G.4.0)

Peaks 3-5: triglycerol tri- and polyoleates
Peaks 6-11: diesters isomers
Peaks 12-18: monooleate isomers of mono- and polyglycerols

the nonpolar portion of the group of peaks (#12-16) corresponding to the monooleate increased significantly in comparison to the polar portion of the monooleate (peaks #17). The same phenomenon exists (when comparing Figure 2A to 2B) for the group of peaks corresponding to the dioleates (compare peaks #6 to 9 and 10). Thus the more polar compounds (peaks #9, 10) are consumed in comparison to the less polar compounds (peak #6) (see Table 2).

All peaks in octaglycerol monooleate and decaglycerol monooleate are less sharp and tend to tail due to the big number of possible

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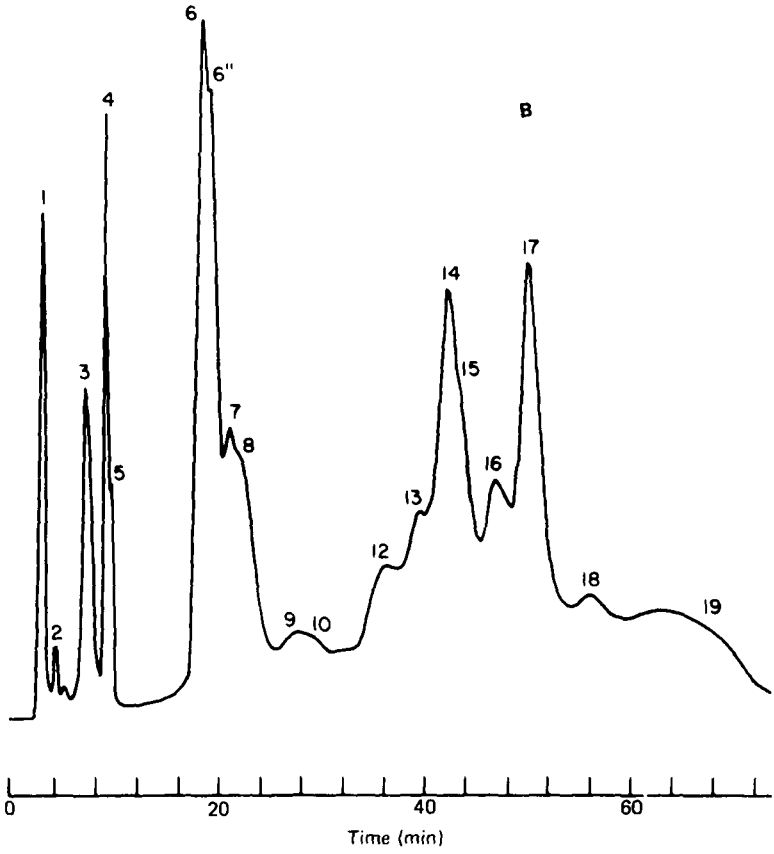

Figure 4B (continued)

isomers existing in the crude product. From Figure 2C and 2D one can see that the monooleate peaks are less pronounced than the dioleate since the oleic acid tends statistically to esterify the short polyols twice and more rather than being esterified once by the long chain polyol.

Figure 4(A-D) and Table 3 illustrates the separation and the product composition of various triglycerol oleic acid esters with increasing amounts of esterified oleic acid in each polyol. Figure 4A shows the following intercomposition; 60.1% mono-,

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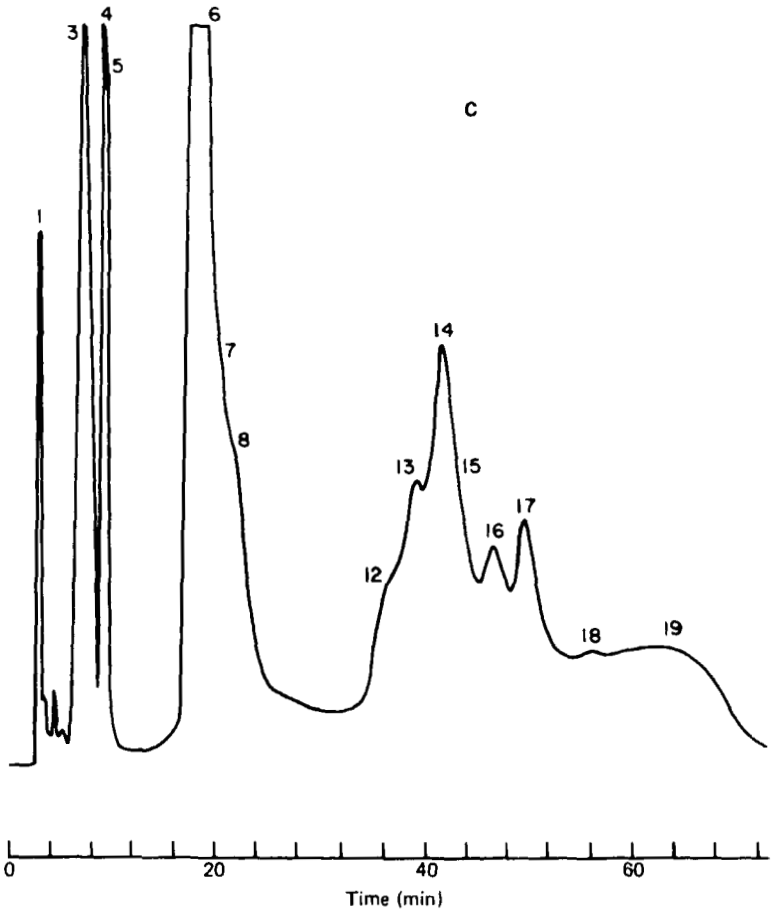

Figure 4C

36% di-, and 7.3% trioleates. The mono isomers are distributed among less polar isomers (peaks #12, 13, 14, 15; 24.8%) and the main polar isomer (peak #17; 29.8%). When 3.G.2.0 is examined it can be clearly seen that the same series of peaks exist exactly at the same retention times but the area proportions have been changed. The monooleates are only 52.2% while the dioleate increased and are 39.1% of the total composition. The

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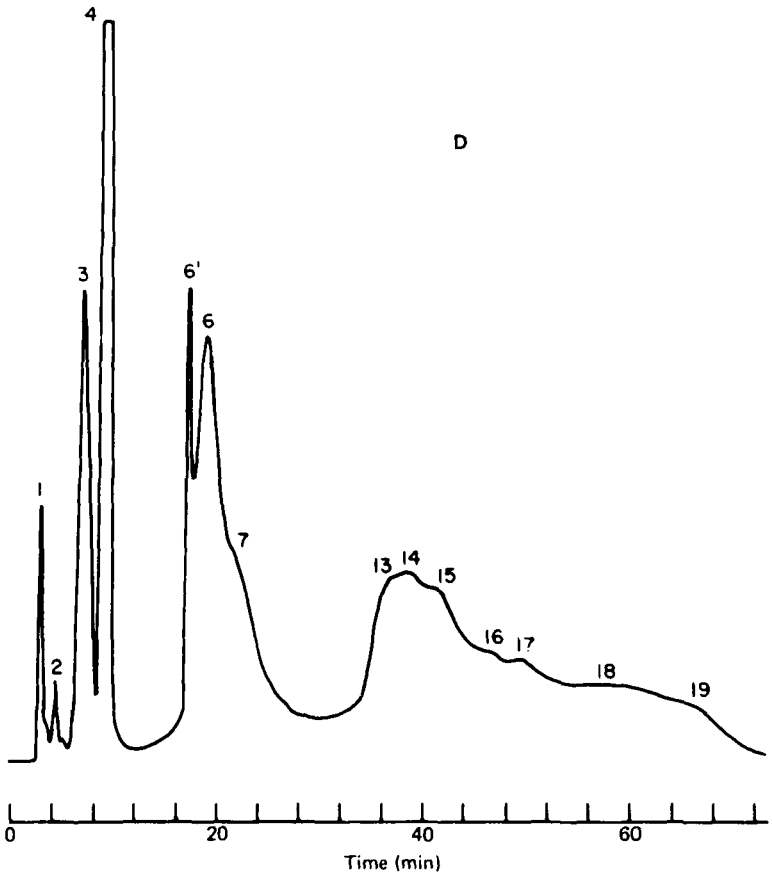

Figure 4D

trioleates are 8.6%. The internal composition in the monooleates also changed and the proportion of the less polar isomers is more pronounced (see peaks #15 and 17 for comparison). The same phenomena is evidently noticed for the dioleates and trioleates peaks.

Increasing the number of the oleic acids in each compound (see 3.G.3.0 and 3.G.4.0) caused a deformation in the chromatogram. The monooleate peaks were significantly reduced and the

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TABLE 3

Product Composition of Several Triglycerol Mono-, Di-, Tri- and Tetraoleates Crude Mixtures.

Type of emulsifier	Trioleate isomers		Dioleate isomers			Monooleate isomers						mono #17 / di #6-11	mono-polar #17 / mono-apolar #15
	3	4,5	6	7,8	9-11	12	13	14,15	16	17	18		
3.G.1.0	3.3	4.0	30	4.0	2.6	3.4	2.0	16.8	2.4	30	1.3	0.82	1.35
3.G.2.0	4.3	4.3	33.4	4.1	1.5	5.3	5.5	18.5	5.7	16.7	0.4	0.43	0.57
3.G.3.0	9.8	12.8	(	40.0	)	(	9.5)	17.4	3.0	7.2	0.1	0.18	0.27
3.G.4.0	10.8	27.5	(	40.8	)	(		20.6	)	0.3	---	0.007	0.01

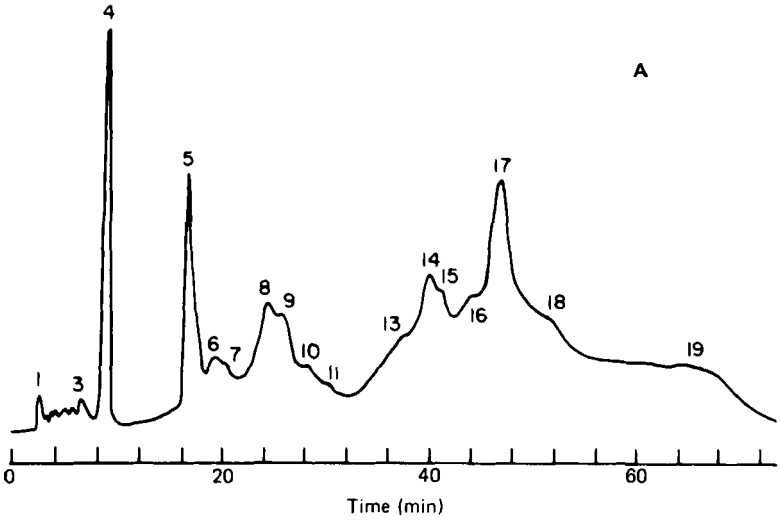

Figure 5: HPLC chromatograms of several decaglycerol polyoleates

A: Decaglycerol monooleate (10.G.1.0)
B: Decaglycerol dioleate (10.G.3.0)
C: Decaglycerol decaoleate (10.G.10.0)

Peaks 3-4: decaglycerol tri to decaoleates
Peaks 5-11: diesters isomers
Peaks 12-18: monooleate isomers of the polyglycerols (decaglycerol)

(continued)

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di-, and trioleates increased dramatically. The phenomena is demonstrated clearly when comparing the product composition of 3.G.3.0 to 3.G.1.0 (see Table 3). In the crude triglycerol tetraoleate (3.G.4.0) one can imagine three isomers of 3.G.1.0; six isomers of 3.G.2.0; six isomers of 3.G.3.0 and two isomers of 3.G.4.0 and thus the chromatogram is very complex and deformed. The main polar monooleate (peak #17) almost disappeared while the nonpolar monooleate structures are dominent (peaks #14 15). The deformation in the chromatogram and the wide unresolved peaks may also indicate existing of many isomers of longer and shorter chains eluting at the same time.

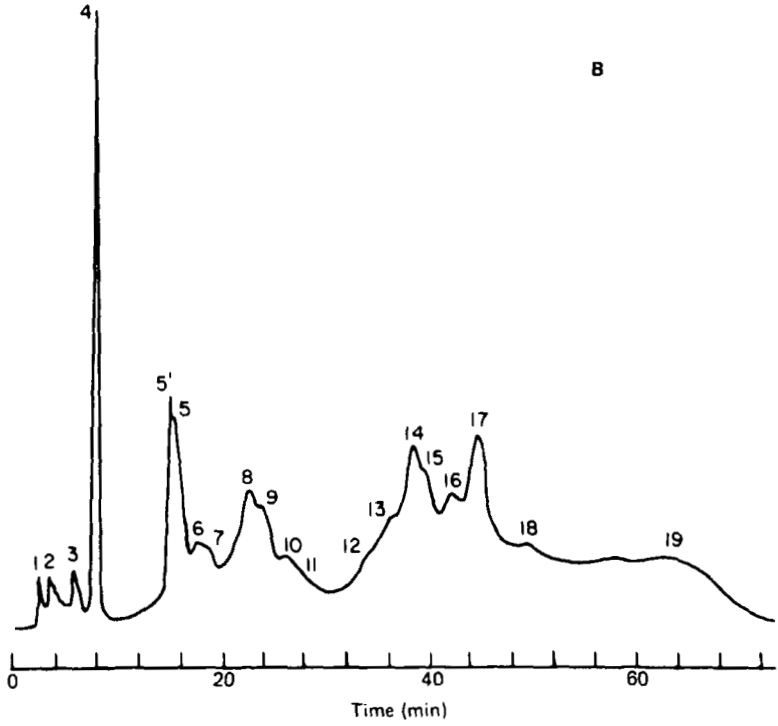

Figure 5B

Injection of the decaglycerol esters of mono-, di- and decaoleates is illustrated in Figure 5(A-C). The same type of separation is seen for these compounds in spite of the fact that when comparing 10.G.1.0 to 10.G.10.0 the chromatograms are more deformed and the peak proportions have changed.

For long polymeric glycerol esterified by many oleic acids (10.G.10.0) it is hard to obtain a reasonable chromatogram from which product composition can be calculated with any accuracy. Thus those chromatogram have only quantitative value.

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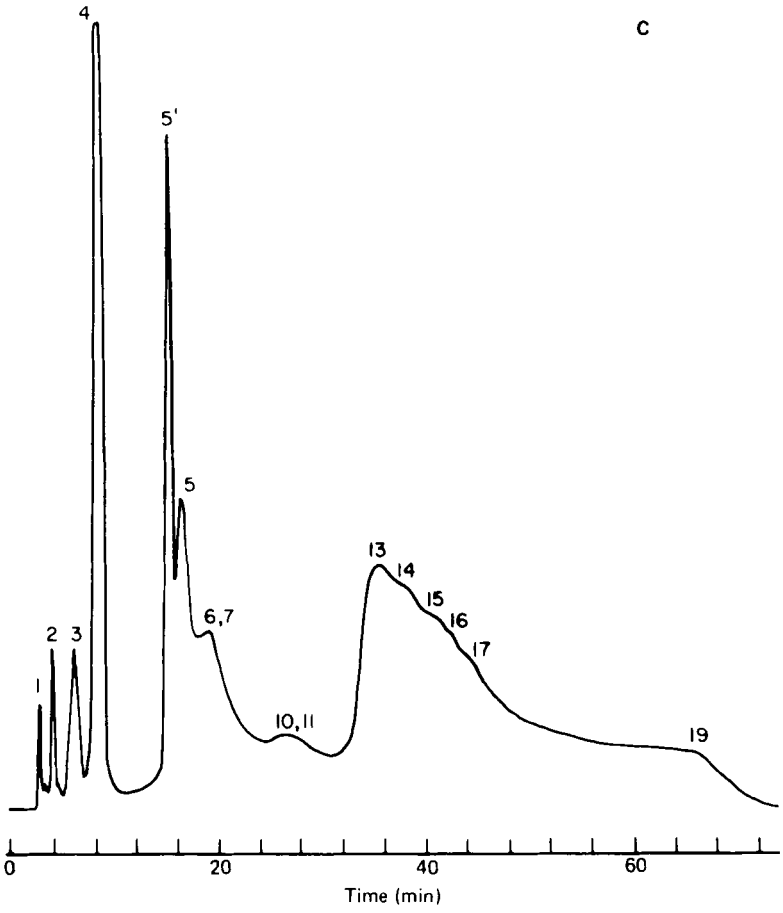

Figure 5C

CONCLUSIONS

Polyglycerol esters of fatty acids are an important group of emulsifiers with a very complex product composition. Many attempts have been made to analyze quantitatively those mixtures with only minor success. Our attempts to separate the crude

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product by HPLC showed that some differentiation can be made with regard to the number of fatty acids which esterify the glycerol. For short chain polyols separation can be achieved and the complexity of the chromatogram can be resolved to some extent. The chromatogram of long chain polyols in which a large variety of isomers exist in the product, is deformed and the analysis is ambiguous.

The study in this respect is still in progress and we hope to bring additional results after accomplishing synthetic work to obtain relatively pure standards for comparison.

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