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ANALYSIS OF NONIONIC SURFACTANTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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General Considerations

"Surfactant", a convenient contraction of "surface active agent" which was originated in one of the industry's leading laboratories and is now widely accepted, suggests that this large group of substances has become well known enough to require a simple yet distinctive tag. "Surfactant" connotes organic molecules or an unformulated compound having surface active properties, when dissolving in water or another solvent, orient themselves at the interface between the liquid and a solid, liquid, or gaseous phase and modify the properties of the interface. The modifications may be accompanied by frothing or foaming and by formation of colloids, emulsions, suspensions, dispersions, aerosols, or foams.

Surfactants are not only important as the active constituents of cleansing agents (soaps, detergents, etc.) but are also vital in the stabilization of emulsions (e.g. foods, cosmetics, pharmaceuticals), as mold release agents in plastics industry, in fabric softening, in contraceptive pastes, in flotation, for biocidal activity, in oil well drilling, as well as in a host of many other applications.

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Most surfactants have a common molecular structure consisting of a long nonpolar chain, frequently a hydrocarbon chain, sometimes a fluorocarbon or silicone based polymer, and hydrophilic end which is sufficiently "hydrophilic" to confer some water solubility on the compound as a whole.

Surfactants are, in most cases, industrial products prepared via a series of chemical reactions, with almost no product separation and purification. Thus most of the commercial end products are complex mixtures of a variety of components.

Today's usage of surfactants takes full advantage of the many different properties of nonionic, cationic, anionic, and ampholytic compounds and results in commercial products that are, to various degrees, complex mixtures. An added complication is that most of the surfactants contain unreacted chemicals present in them. Also, the initial hydrophobe may be an impure material, often a mixture of isomers or a natural product. In addition, the nonionic surfactants involving ethylene or propylene oxides consist of hydrophilic portions of the molecules, with varying lengths. All in all, then, analytical efforts are needed to evaluate surfactants since they consist of mixtures.

To the industrial chemist the over-all problem of surfactant analysis is to evaluate the performance of the product and its physical properties such as solubility, melting or boiling points, density, refractive index, etc.

To the analytical chemist the full analysis of surfactant is tedious and requires the full complement of analytical tools which today's technology provides. To satisfy demands of research, the analyst must act fast and surely. Not only must he classify the nature of the surfactants, but he must always be fairly certain of its structure, not to mention its quantitative make-up.

Many investigators have attempted to find suitable tools of analysis [1-3], including methods of classification and separation of any unknown type of surfactant and their quantitative determination. This sometimes can be done on the surfactant mixture wihtout attendant separation, but the best data are generally obtained from the isolated component.

Most quantitative analysis approaches include: a) gravimetric procedures, b) colorimetric methods and c) volumetric methods. In addition to these important classes of quantitative analysis most authors would include wet-reaction methods such as those based on saponification or hydrolysis followed by: 1) Instrumental methods, which include infrared, mass spectrometry, NMR, ultraviolet, conductometric titration and polarographic analysis and 2) Chromatographic methods including all modes of chromatography and ion exchange[3].

Surfactants, being mostly long chain molecules with both a hydrophilic and lipophilic nature, are non volatile materials and these are difficult to elute by gas-liquid-chromatography means.

Several authors describe, thus, methods based on saponification or hydrolysis of the surfactant and a separate analysis to each part of the molecule. The main disadvantage of the above methods lies in the fact that the internal structure composition of the product cannot be evaluated. The internal structure of any given surfactant, mainly of those applied in the food industry, is of significant interest since the health authorities are restricting their use and require as full an analysis as can be achieved of the product. High performance liquid chromatography is an ideal tool for accurate evaluation of the product distribution of surfactants.

No review articles have been published on the use of HPLC

as a method for analysis of surfactants to date.

Nadeau and Waszeciate[4] in their review on separational methods for analysis of nonionic surfactants discussed many procedures to analyze nonionic surfactants including the use of gel-permeation chromatography. They predicted an increased use of the technique in the future "because of its rapidity and a great producibility and its ability to separate cleanly molecular species based on size...". Cross[5] in his review on analytical chemistry of anionic surfactants examined the use of TLC, paper chromatography, and Gas chromatography, but does not mention any additional options to evaluate the product composition.

A close look at the literature published recently reveals that several investigators have discovered the potential of the HPLC technique to separate and to analyze surfactants and a few studies already have been published in this area.

We felt that a review of the subject could help analytical chemists to evaluate the potential of this method and use it as an analytical tool.

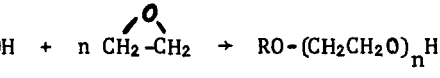
Nonionic Surfactants

The term nonionic surfactants refers chiefly to polyoxyethylene and polypropylene derivatives, but other surfactants are also included in this category, such as an hydroxyhexitol derivative, sugar esters, fatty alkanolamides and fatty amine oxides[3].

The nonionic surfactants are usually prepared by:

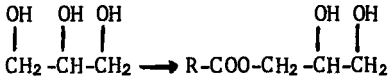
1: The addition of ethylene oxide to compounds containing one or more active hydrogen atom such as alkylphenols, fatty alcohols,

fatty acids, fatty mercaptans, fatty amines, fatty amides and polyols. For example:



$$ROH + n\ CH_2\text{-}CH_2 \rightarrow RO\text{-}(CH_2CH_2O)_nH$$

3: The esterification or transesterification of fatty acids by polyols. For example:

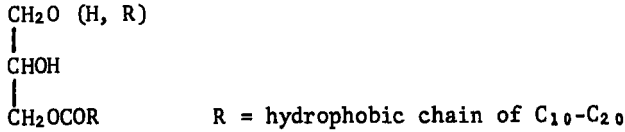


$$RCOOH + CH_2(OH)\text{-}CH(OH)\text{-}CH_2 \longrightarrow R\text{-}COO\text{-}CH_2\text{-}CH(OH)\text{-}CH_2$$

In any of these reactions the product is a mixture of a variety of components. In most cases the product specifications are related to the acid value, hydroxyl number, esterification value, etc., which are only indicative of the average but not the full product composition. Several attempts have been made to analyze the products by various chromatographic methods including paper chromatography[6,7], thin layer chromatography[8-14] and gas-liquid chromatography[6,15-29]. Only a few attempts have been made to analyze nonionic surfactants by the HPLC method.

The present review divides the nonionic surfactants into several groups according to the most common classification:

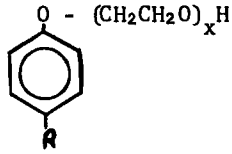
1. Mono-diglycerides of fatty acid esters having the following general structure:



CH_2O (H, R)
|
CHOH
|
CH_2OCOR R = hydrophobic chain of C_{10}-C_{20}

2. Polyol surfactants containing the residue of polyhydroxy/ compounds as the hydrophilic moiety ester combination with hydrophobic groups derived from fatty acids.

3. Polyoxyethylene alkylphenols having the following general structure:



4. Polyoxyethylene alcohols and polyoxyethylene esters of fatty acids with the general structure of:

$$RO-(CH_2CH_2O)_xH$$

$$RCOO-(CH_2CH_2O)_xH$$

5. Alkylamides and Polyoxyethylene alkylamides:

$$RCONHCH_2CH_2OH \quad \text{and} \quad RCON(CH_2CH_2OH)_2$$

$$RCONH-(CH_2CH_2O)_xH \quad \text{and} \quad RCON\begin{matrix}(CH_2CH_2O)_xH \\ (CH_2CH_2O)_yH\end{matrix}$$

1. Mono-Diglycerides of Esters of Fatty Acids

In a series of studies published almost at the same time in 1975 by several authors [30,31,32], an effort was made to use HPLC techniques for separation and detection of tri-, di- and mono-glycerides of fatty acids and sterols in order to accomplish full analysis of these compounds in various food products. Table I summarizes, in part, the results from these studies, and others,

TABLE I
HPLC Analysis of Mono-Diglyceride Esters of Fatty Acids

		Column	Detector	Eluent	Figure	Author (ref)
1.	Mono- and diglyceride of fatty acids	MicroPak SH-10 (10μ)	FID	EtOH:H_2O gradient	1	Kiuchi (1975) (30)
2.	Monoglycerides of oleic acid	SI (Silica)	Moving wire (FID)	Mixture of solvents	-	Aitzetmüller (1975) (31)
3.	Mono-diglycerides	Zorbax-Sil	RI	Ether:Hexane	-	Sinsel (1975) (32)
4.	Mono-diglycerides	Lichrosorb SI-60 (5 or 10μ)	Moving wire (FID)	Mixture of solvents	2,3,4	Aitzetmüller (1977) (33)
5.	Mono-diglycerides	Lichrosorb Diol 10μ	UV	Isooctane: Isopropanol	5,6	Riisom (1978) (34)
6.	Mono-diglycerides	Partisil PXS 10/25 PAC	Infrared (5.72 μm)	Mixture of solvents	-	Payne-Wahl (35)
7.	Mono-, di-olein	Lichrosorb Diol (10μ)	UV	Hexane (gradient)	-	Garti (1981) (36)
8.	Monomyristin, palmin, stearin, arachidin	Lichrosorb -RP-18 (10μ)	UV	Acetonitrile	7	Sudraud (1981) (37)
9.	Mono-diglyceride stearin olein	μ-Porasil (10μ)	UV	Isooctane: i-PrOH (gradient)	-	Brüschweiler (1977) (43)
10.	Mono-diglycerides	Zorbax-SIL	Moving wire (FID)	acetone:hexane (gradient)	-	Nakamura (57)

the methods and conditions for optimum separation obtained by them.

Kiuchi[30] has used both UV, refractive index and flame ionization detector in order to separate total lipids from soybean oils in various soybean foods. Figure 1[30] demonstrates separation of lipids, triglycerides, diglycerides, sterols, free fatty acids and monoglycerides of fatty acids using two 5000 psi pumps, a solvent flow programmer and a stainless steel MicroPak SH-10 column.

Aitzetmüller[31] has demonstrated in his studies the usefulness of moving wire detectors and other transport flame ionization detectors for the liquid chromatography of fats and monoglycerides of fatty acids. By using the moving wire detector and a sequence of solvents, Aitzetmüller[33] was able to keep the analysis time of partial glycerides as short as 30 minutes. A complex mixture of solvents of increasing polarity was employed. The solvent mixture was carbon tetrachloride and isooctane (34:36) (Solvent I); chloroform,dioxane and hexane (40:11:49) (Solvent II) and chloroform,methanol and isopropylether (34:36:30) (Solvent III). A remarkable separation of the mono- and diglyceride of fatty acid esters was achieved by this technique[33] (figs. 1-3). A Lichrosorb Si-60 column (5 or 10 μm) was used. Tripalmitin was eluted after 5 min., followed by 1,3-dipalmitin and 1,2-dipalmitin. The monopalmitin was eluted last after less than 15 min. (fig. 2).

Figure 3 [33] illustrates a typical chromatogram of three different commercially available partial glyceride food emulsifiers using the same technique. Several mixtures of partial glycerides of oleic and stearic acids were also tested (see Figure 4) together with some so-called commercial "monoglycerides".

In a more recent study by Riisom and Hoffmeyer[34], a new

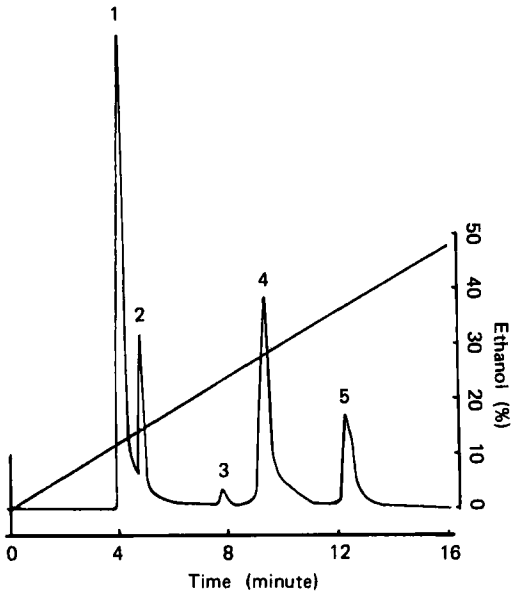


Figure 1: Separation of glycerol esters of fatty acids using microPak SH-10 column: 1 - triglycerides; 2 - diglycerides; 3 - sterols; 4 - free fatty acids; 5 - monoglycerides.

HPLC method was described for the separation of mono-, di- and triglycerides of fatty acids. A 25 cm column packed with 10 μm Lichrosorb Diol was used. The monoglycerides were eluted isocratically with isooctane-isopropanol (95:5) and the detection was accomplished by UV-absorption detection at 213 nm (see Table I, line 5).

The applicability of the method for the quantitative determination of these compounds using an internal standard, in any mixture, has been demonstrated (see Figures 5 and 6, Table II). The method shows excellent reproducibility and accuracy with standard deviations of less than 1%. The method is applicable

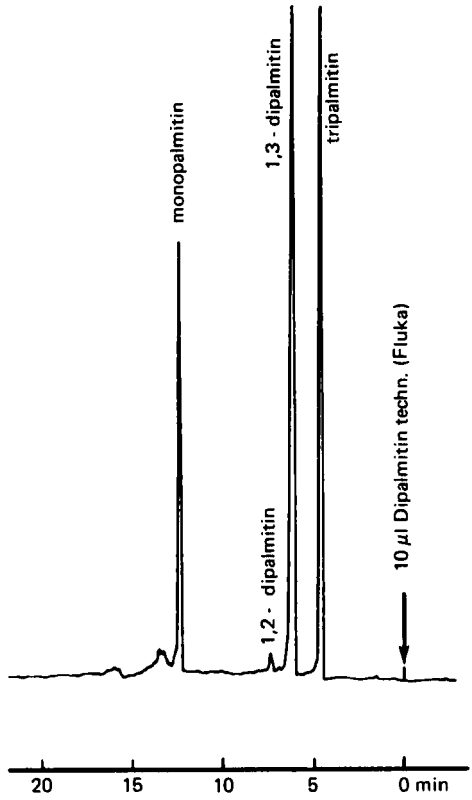


Figure 2: Chromatogram of technical glycerol dipalmitate.

to other types of emulsifiers; for instance, acetylated monoglyceride and propylene glycol esters of fatty acids.

Recent papers published on the separation of mono- and diglycerides of fatty acids concentrate mainly on finding new detection methods (infrared detector at 5.72 µm, see ref. 35) new and better columns (Partisil PXS 10/25 PAC)[35], Lichrosorb Diol[34,36], Lichrosorb RP-18 [37]. Sudraud[37] was able to conduct a full analysis of a commercial distilled monoglyceride on a

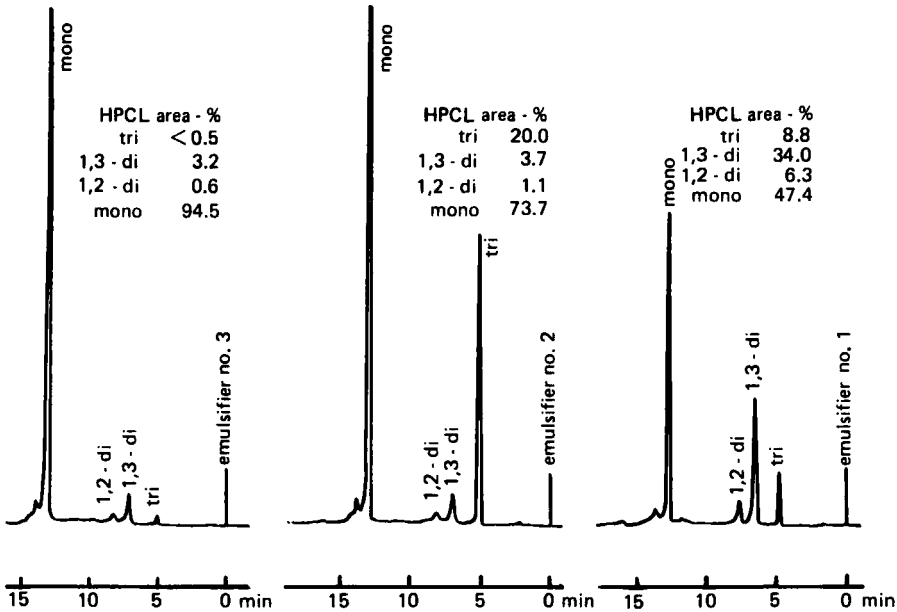


Figure 3: Typical chromatograms of three different commercially available partial glyceride food emulsifiers.

Lichrosorb RP-18 column with acetonitrile as a mobile phase as shown in Figure 7. A reasonable separation of monomyristin from monopalmitin, monostearin and monoarchidin was achieved.

2. Polyol Surfactants

The polyol surfactants are materials containing a residue of a polyhydroxy compound as the hydrophilic portion of the molecule in ester combination with hydrophobic groups derived from fatty acids. Polyol starting materials include straight-chain polyhydroxy compounds with two to six hydroxyl groups per chain, pentaerythritol, polyglycerols, carbohydrates and higher polyol fatty acid esters[3].

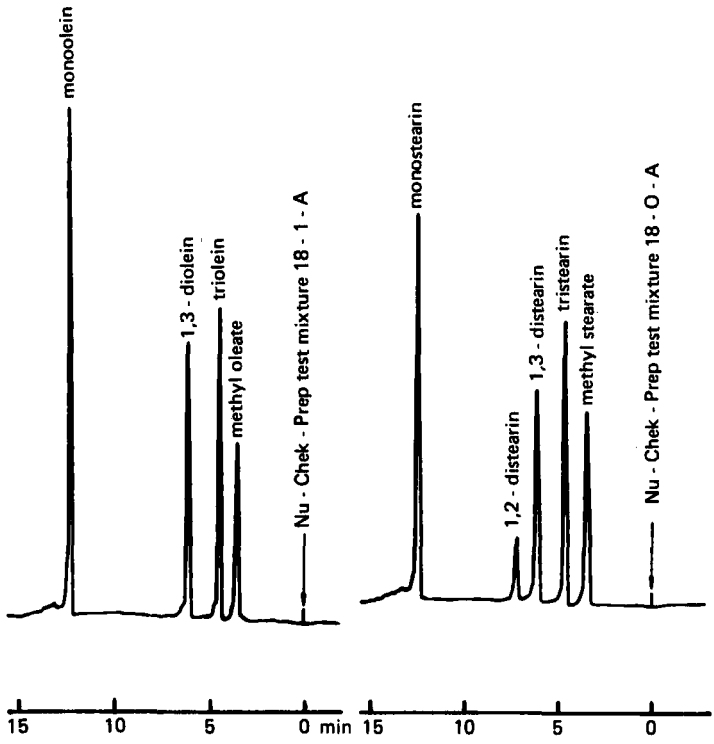


Figure 4: Typical chromatograms of several glycerol esters of stearic and oleic acid test mixtures.

Most polyol surfactants, particularly commercial materials, are complicated mixtures. The complexity of composition is due to a distribution of degree and position of esterification, to the mixed composition of starting fatty acids, to the varying extent of ether linkages, and to the presence of different oxyalkylene chain lengths[38].

The considerable variation in physical properties of polyol surfactants makes it possible to use them in a variety of end-products. They find applications in food, pharmaceutical, cosmetic, and industrial products. The polyol surfactants are usually

TABLE II
Molar Extinction Coefficients of
Fatty Acid Esters, and Internal Standard

Compound	ε at 213 nm[a,b]
Glycerol 1-monopalmitate	66
Glycerol 2-monopalmitate	63
Glycerol 1-monostearate	64
Glycerol 1,2-dipalmitate	118
Glycerol 1,3-dipalmitate	117
Glycerol tristearate	180
Di-n-propyltartrate	295

[a] Molar extinction coefficient.

[b] Accurate to ±5%.

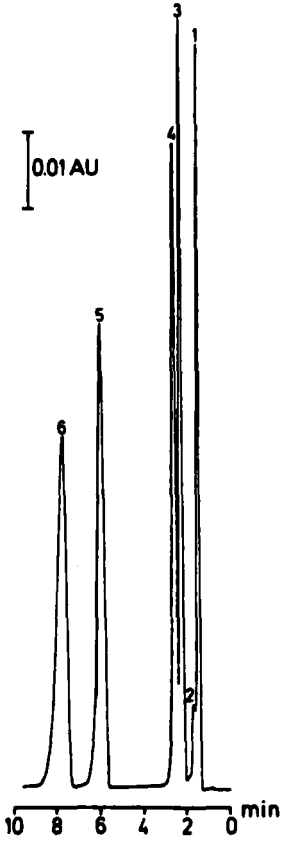


Figure 5: HPLC chromatogram of test mixtures of glycerol esters: 1 - glycerol tristearate; 2 - artefact peak due to loop injection; 3 - glycerol 1,3-dipalmitate; 4 - glycerol 1,2-palmitate; 5 - di-n-propyltartarate (initial standard); 6 - glycerol 1-monostearate.

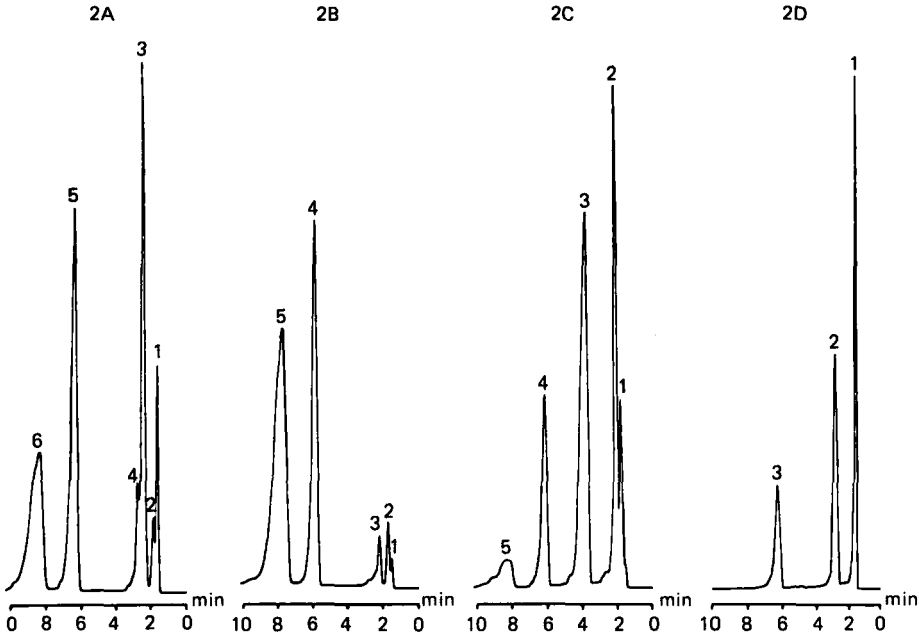


Figure 6: HPLC chromatograms of nonionic emulsifiers. 2A. Mono-diglyceride: 1 - triacylglycerols; 2 - artefact peak; 3 + 4 - diacylglycerol; 5 - internal standard; 6 - monoacylglycerols. 2B. Distilled monoglyceride: 1 - triacyglycerols; 2 - artefact peak; 3 - diacylglycerol; 4 - internal standard; 5 - monoacylglycerols. 2C. Acetic acid esters of distilled monoglycerides: 1 - triacylglycerol; 2 - diacetic acid esters of monoacylglycerols; 3 - monoacetic acid esters of monoacylglycerols; 4 - internal standard; 5 - monoacylglycerol. 2D. Propylene glycol esters of fatty acids: 1 - diacylpropyleneglycols; 2 - monoacylpropyleneglycols; 3 - internal standard.

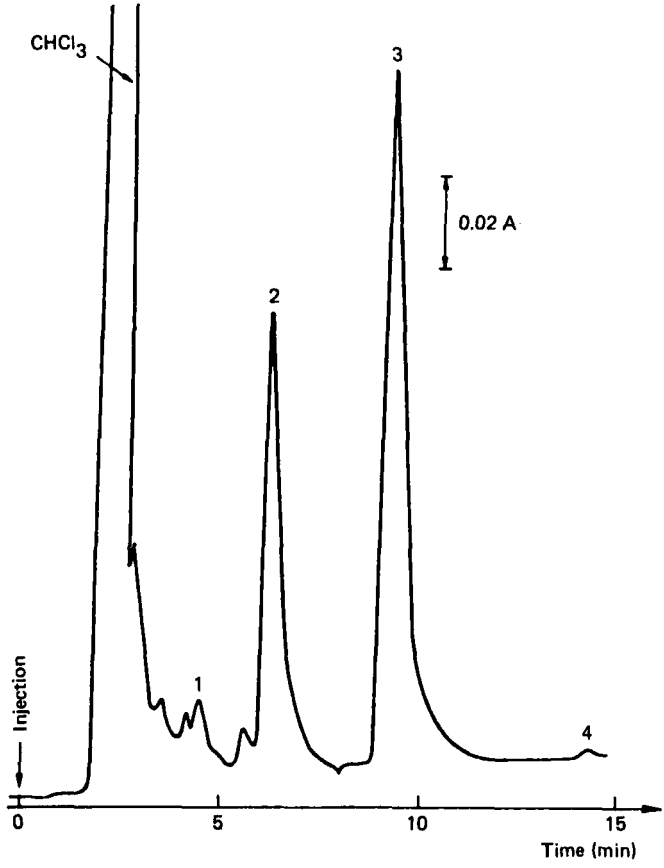


Figure 7: Separation of saturated distilled monoglycerides by reversed-phase chromatography. Column: 15 x 0.47 cm I.D. Stationary phase: LiChrosorb RP-18, 10 µm. Mobile phase: Acetonitrile; flow-rate 0.9 ml/min. UV detection at 204 nm. Peaks: 1 - monomyristine; 2 - monopalmitine; 3 - monostearine; 4 - monoarachidine.

TABLE III
HPLC Analysis of Polyol Surfactants

Nature of Surfactant	Column	Detector	Eluent	Figure	Author (ref)
Propylene glycol esters	SI (silica)	Moving wire (FID)	Mixture of solvents	8d	Aitzetmüller (31)
Polyglycerol esters	SI (silica)	Moving wire (FID)	Mixture of solvents	8b	Aitzetmüller (31)
Polyglycerol esters	SI (silica)	Moving wire (FID)	Mixture of solvents	-	Aitzetmüller (39)
	Lichrosorb Diol (10μ)	UV	Hexane-i-PrOH (gradient)	9,10	Garti (36)
	μ-Porasil (10μ)	UV	Isooctane:i-PrOH (gradient)	-	Brüschweiler (43)
Sorbitan esters	SI (silica)	Moving wire (FID)	Mixture of solvents	8g	Aitzetmüller (31)
	Silica-Amine Modifier	Moving wire (FID)	Acetonitrile	-	Aitzetmüller (39)
	μ-Porasil (10μ)	UV	Isooctane:i-PrOH (gradient)	-	Brüschweiler (43)
Sugar esters	Fluoroether-Sil-x-1	UV, RI	n-Chloropropane: MeOH	12	Cormier (40)
	Lichrosorb-RP-18	UV, RI	Methanol, H_2O, i-PrOH	13,14,15	Kaufman (41)
	Fluoroether-Sil-x-1	RI	n-Chloropropane: MeOH	-	Brich (42)
	μ-Porasil (10μ)	UV	Isoocatne:i-PrOH (gradient)	-	Brüschweiler (43)

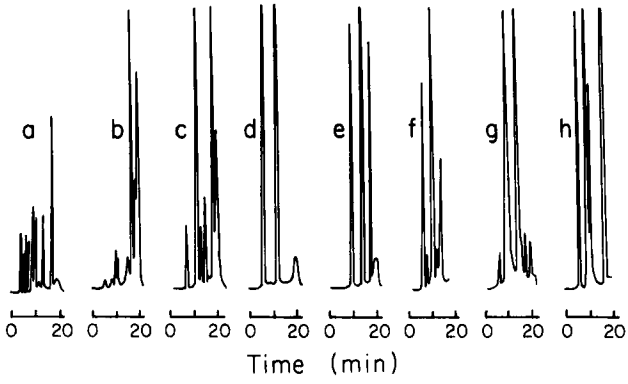


Figure 8: Fingerprint Liquid Chromatograms of a series of food emulsifiers. a - test mixture containing all the commercial emulsifiers together; b - polyglycerol esters of palmitic and stearic acid; c - citric acid esters of monoglycerides; d - propylene glycol esters of saturated fatty acids; e - monoglyceride acetates of fatty acids; f - DATA, diacetyl tartaric esters of monoglyceride; g - sorbitan esters of saturated fatty acids; h - monodiglycerides.

divided into subgroups according to their hydrophilic functional groups, e.g. glycol esters, glycerol esters, polyglycerol esters, hexitol esters, anhydrohoxital esters, sugar esters, etc.

Close examination of the methods described in the literature reveals that most workers used wet techniques, TLC and other chromatographic methods to evaluate the product composition of these materials. Only few studies have been dedicated to the separation of polyol surfactants by HPLC.

The need for a better detection method of these compounds, which have low UV absorbance, led Aitzetmüller[31] to use a moving wire detector to separate some polyol surfactants (see Table III). A fingerprint chromatogram of a variety of food emulsifiers including propylene glycol mono- and diesters of fatty acids have been achieved by Aitzetmüller[31], who has used a silica gel

column and a gradient elution. Figure 8d shows that under these conditions the diester of fatty acids with propylene glycol is eluted first followed by the monoester isomer. Glycerol esters of fatty acids have been described separately in the previous Section, in Table I and will not be further discussed here.

Polyglycerol esters provide an especially difficult task for the analytical chemist since they are usually a very complex mixture of isomers. These emulsifiers are mixtures of up to hundreds of individual 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. The existence of polyglycerol esters in a mixture has been shown as several unresolved peaks (fig. 8b) in chromatography.

An indirect method for the detection of polyglycerol esters has been developed by Aitzetmüller[39]. After an hydrolysis of the esters, the polyglycerols have been injected in HPLC columns in order to estimate the variation in the degree of polymerization of each polyglycerol. The method is not satisfactory since there is no consideration as to the existence of several isomers of fatty acids on each polyol. Most polyol surfactants such as propylene glycol esters, sorbitan esters and sugar ester have been analyzed by this method[39]. Typical analyses of the glycerol oligomer content of a number of polyglycerol containing emulsifiers are presented in Table IV. Since calibration standards for the individual oligomers were not available, the results are expressed as area-percent of the RI detector signal.

In a recent study [36] an attempt to analyze the polyglycerol esters without pretreatment has been made on a wide range of polyglycerol-poly-fatty acid esters. The analyses were performed on a Specta Physics model SP-8000 HPLC chromatograph equipped with

TABLE IV
Polyglycerol in Commercially Available Emulsifiers (by HPLC-RI Area % of the Total Polyol Fraction Obtained by Saponification of the Emulsifier).[39]

	Area %					
Emulsifier	Mono-	Di-*	Tri-	Tetra-	Penta-	Hexa-
A	29.0	30.3	17.6	11.0	7.8	
B	6.3	48.1	20.1	11.9	6.7	3.2
C	85.3	9.7	2.2	1.3	0.4	
D	60.3	19.4	9.5	5.9	3.7	

* including partly separated isomer

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.

A gradient elution of n-hexane and isopropanol was carried out. Figure 9 demonstrates the chromatograms obtained after injecting a series of several polyglycerol monooleates. It is important to realize that the materials are not pure as the commercial name might indicate. For example, triglycerol monooleate (3.G.1.0), might as well contain a wide range of several polyglycerols from one to ten. Thus the triglycerol is an average number of the oleic acids present in such a polymer.

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 (fig. 9). The three main peaks in Figure 9A correspond, according to their retention time, to glycerol monooleate (peak 17), glycerol dioleate (peak 6) and glycerol trioleate (peak 4). At 7 min. (peak 3) another trioleate isomer with more apolar structure is eluted. The main dioleate peak is accompanied by additional smaller peaks (peaks 7 and 8). Those are interpreted as additional polyglycerol dioleate isomers being more polar than the main dioleate one. 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).

By examining the chromatogram of hexaglycerol monooleate (6.G.1.0) (fig. 9B) in comparison to triglycerol monooleate, (fig. 9A) it can be seen that the main three peaks in each chromatogram appear at the same retention times (peaks 4, 6 and 17). These findings indicate again that the column does not differentiate between the chain length of the polyol.

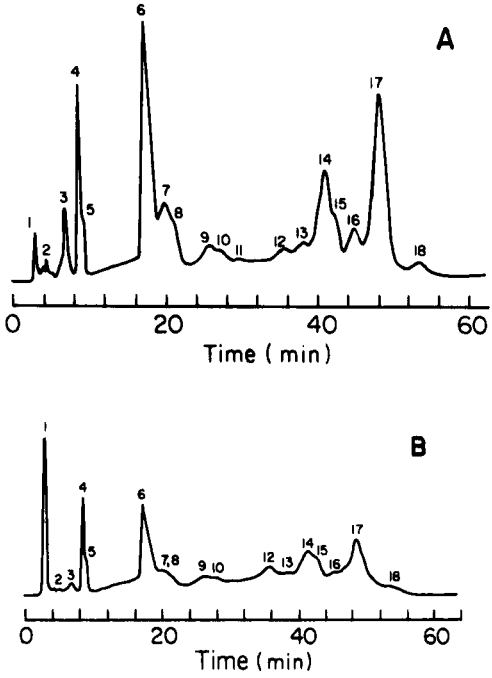


Figure 9: HPLC chromatograms of some crude commercial polyglycerol monooleates (from Capitol City Corp.) A: Triglycerol monooleate; B: Hexaglycerol monooleate; C: Octaglycerol monooleate; D: Decaglycerol monooleate; Peaks 3-5: triglycerol tri- and polyoleates; Peaks 6-11: diester isomers; Peaks 12-18: monooleate isomers of mono and polyglycerols.

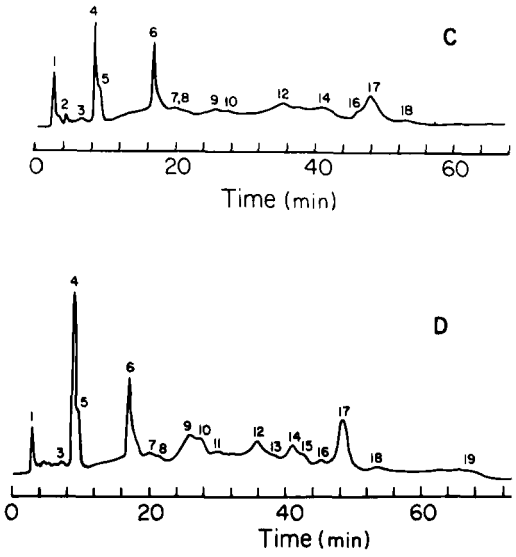


Figure 9: continued

All peaks in octaglycerol monooleate (Figure 9C) and decaglycerol monooleate (fig. 9D) are less sharp and tend to tail, due to the large number of possible isomers existing in the crude product. Figure 10 (A-D) illustrates the separation and the product composition of various triglycerol oleic acid esters with increasing amounts of esterified oleic acid in each polyol. Figure 10A shows the following intercomposition: 60.1% mono-, 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 is clearly seen that the same series of peaks exist exactly at the same retention times but the area proportions have been changed. The monooleates make up 52.2% while the amount of dioleate increased and are 39.1% of the total composition. The trioleates make up 8.6%. The internal composition in the monooleate structures are dominant (peaks 14, 15). The unsymmetrical peaks and the large number of unresolved peaks may also indicate

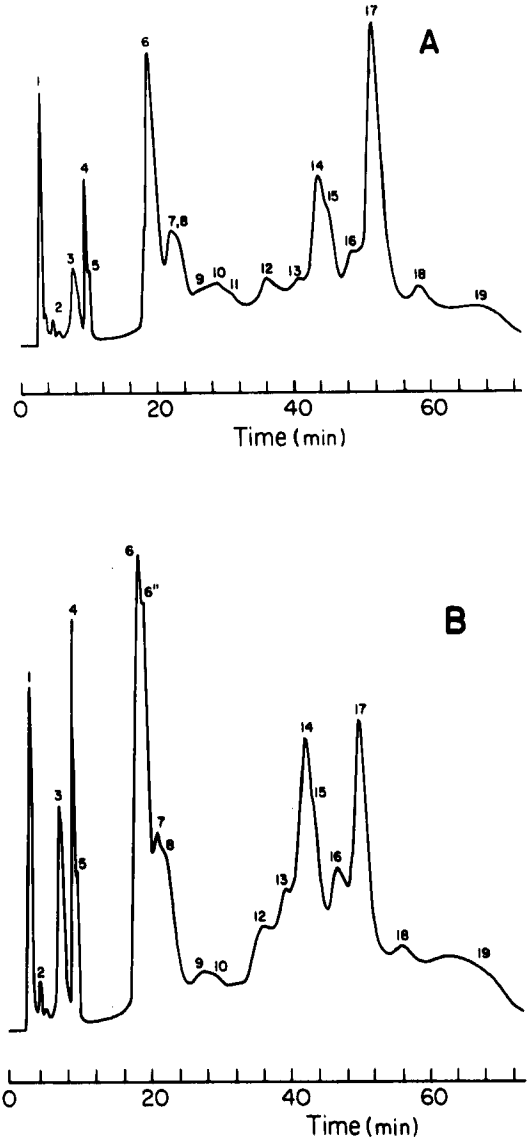


Figure 10: HPLC chromatograms of several triglycerol polyoleates. A: Triglycerol monooleate (3.G.1.0.); B: Triglycerol dioleate (3.G.2.0); C: Triglycerol trioleate (3.G.3.0); D: Triglycerol tetraoleate (3.G.4.0); Peaks 3-5: triglycerol tri and polyoleates; Peaks 6-11: diesters isomers; Peaks 12-18: monooleate isomers of mono- and polyglycerols.

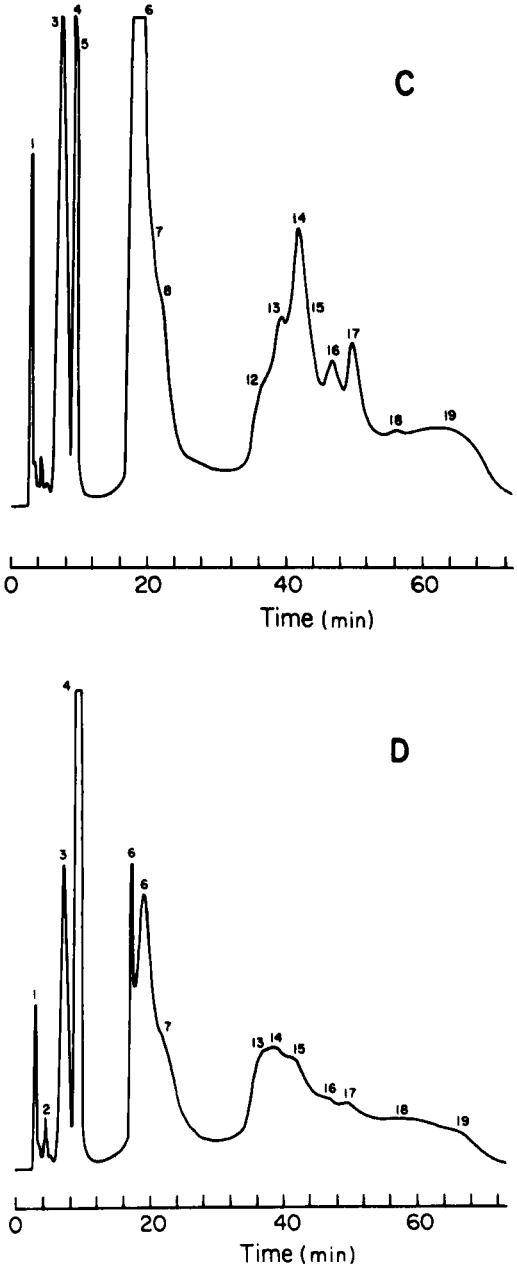


Figure 10: continued

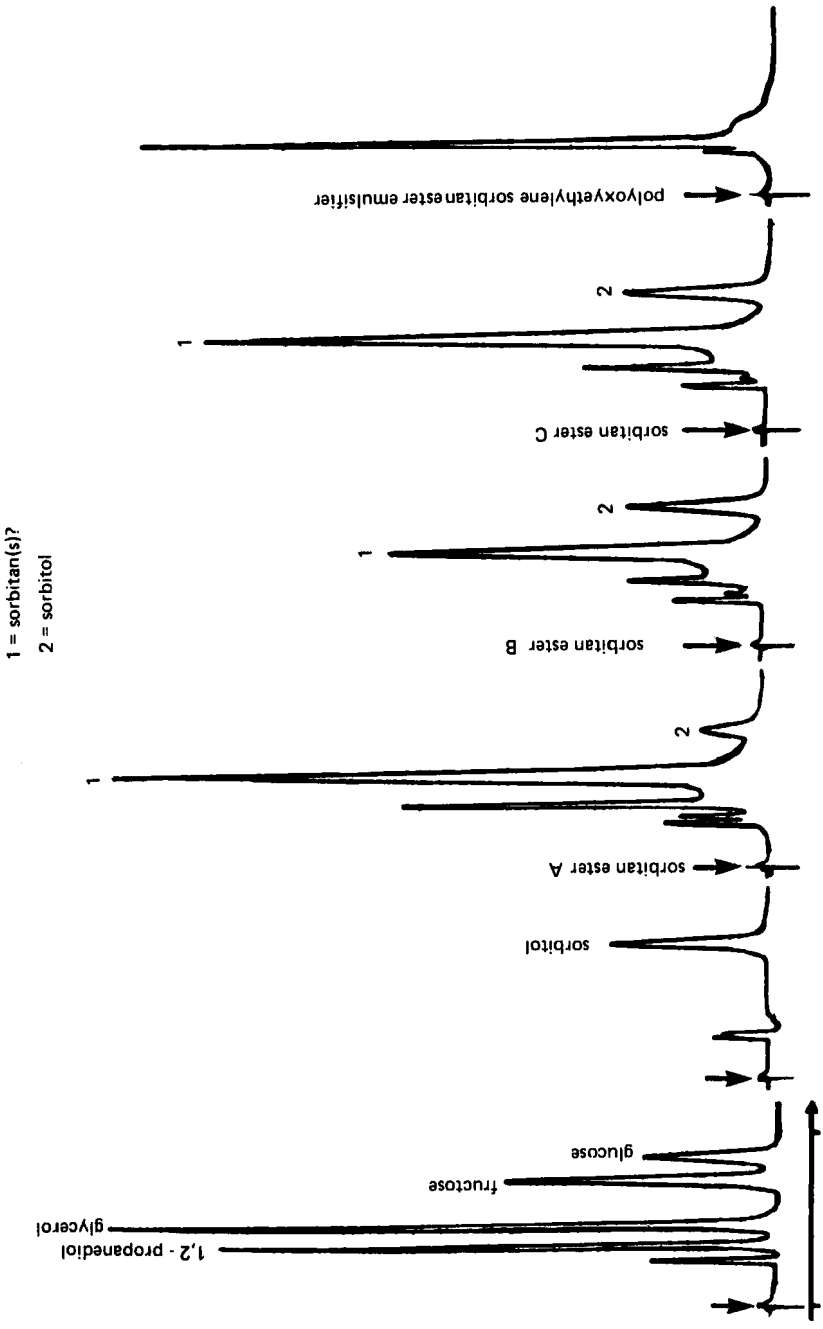


Figure 11: Polyhydric alcohols from sorbitan esters after saponification. A silica column impregnated with amine modifier I.

the existence of many isomers of longer and shorter chains which elute at the same time.

Direct esterification of erythritol, manitol or sorbitol with fatty acids leads to mixtures of mono-, di-, tri- and tetra-esters. The most common and commercially available emulsifiers are the sorbitan esters of fatty acids. Unresolved peaks of the sorbitan esters of saturated fatty acids were shown in Figure 8g[31]. No other attempts have been made by other investigators to analyze commercially available sorbitan esters.

Full saponification of these esters yielded sorbitol, sorbitan and isosorbide allowing their detection by amine modifier 1 column using mixture of acetonitrile and water (fig. 11)[39].

Reaction of carbohydrates, mainly sugar, with fatty acyl chlorides, fatty acids or methyl esters of fatty acids, form a sucrose-ester of fatty acids which are potentially an important class of emulsifiers and detergents.

An analytical method based on high pressure liquid chromatography has been developed in order to separate and identify these sucrose fatty acid esters[40]. The monoesters and diesters of sucrose stearate have been separated and quantitated. A complex mixture of sucrose tallowate has also been analyzed. The determinations were made using a one meter column packed with Fluoro-ether-Sil-x-1, with a solvent consisting of 91% n-chloropropane, and 9% anhydrous methanol. The peaks of positional isomers are then measured for each type of ester.

Figure 12 illustrates the chromatogram of commercial sucrose mono- and distearate samples. The numbering of the peaks refers to the numbered spots on the corresponding TLC analysis made on the collected fractions. The shape of the chromatogram and the

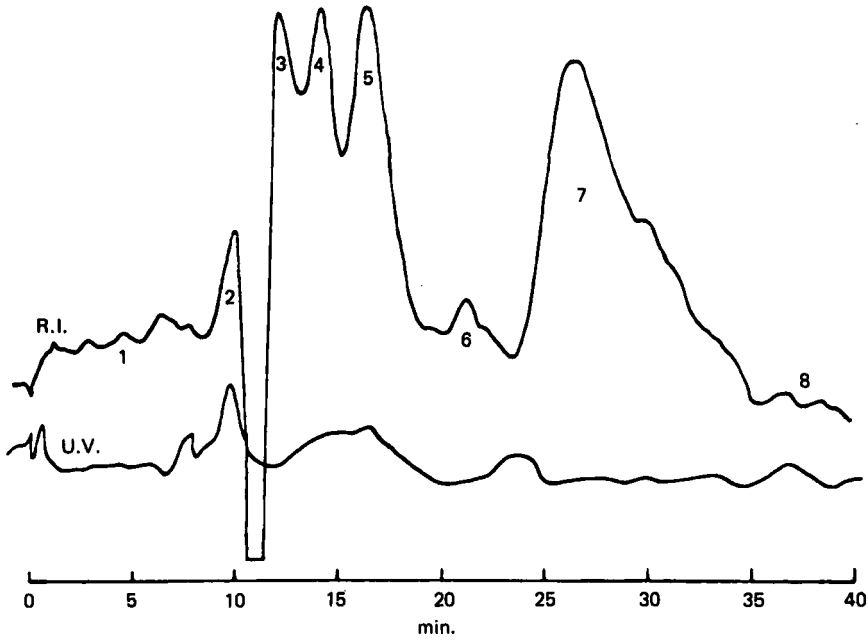


Figure 12: Separation of commercial sucrose monoesters using Fluoroether Sil-x-1 column and mixture of chloropropane and methanol as eluent.

fact that the peaks are hardly resolved emphasizes the complexity of such analysis. Even though it can be observed that the compounds are eluted in order of increasing polarity, it was found that peaks 6 and 7 correspond to the monoesters. The diester appears as peaks 4 and 5. Peak 3 corresponds to triesters. The situation is much more complex when other commercial materials are examined.

Improved conditions and better column selection allowed better separation of sucrose esters of fatty acids. The work was carried out on Lichrosorb RP-18 [41], using UV (220 nm) and RI detectors and a (95:5) methanol-isopropanol mobile phase (40°C, isocratically). Figure 13 demonstrates the separation of commercial sucrose esters containing 70% and 30% monoester respectively

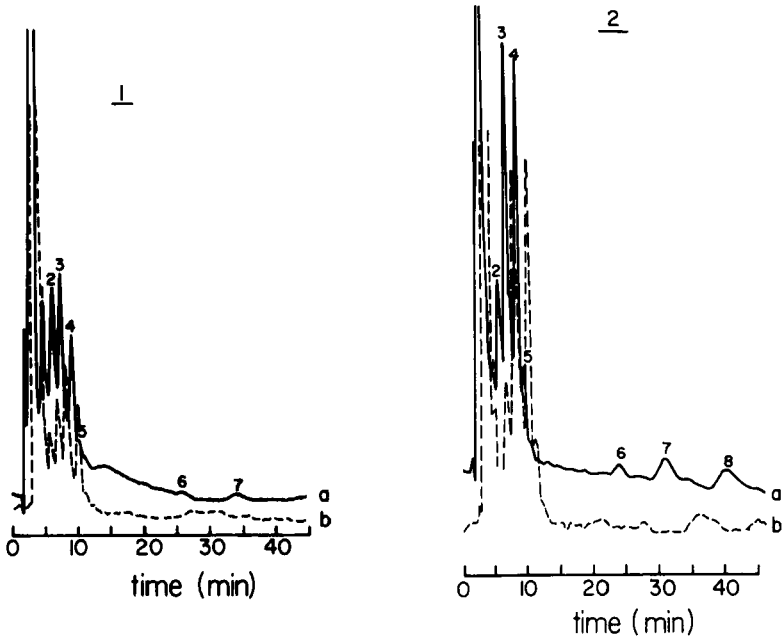


Figure 13: A typical chromatogram of commercial sucrose esters, F160 (70% monoester) (Figure 13.1) and F50 (30% monoester) (Figure 13.2); methanol and isopropanol 95:5 (v/v) 40°C. a - UV detector at 220 nm; b - RI detector. Peak 1 methylpalmitate; Peak 2 methylstearate and sucrose monopalmitate; Peak 3 sucrose monostearate; Peaks 4,5 sucrose diesters; Peak 6-8 sucrose polyesters.

(according to producer claims). The first materials to be eluted are methyl palmitate and stearate which are the reactants in the transesterification reaction. Sucrose mono-palmitate and mono-stearate appear together as one peak. Sucrose diester (peak 4-5) and polyesters (peaks 6-8) elute last.

When a different mobile phase (methanol: H_2O 95:5) was used (55°C, isocratically) better separation of sucrose esters was obtained (see Figure 14). Sucrose stearate or palmitate prepared

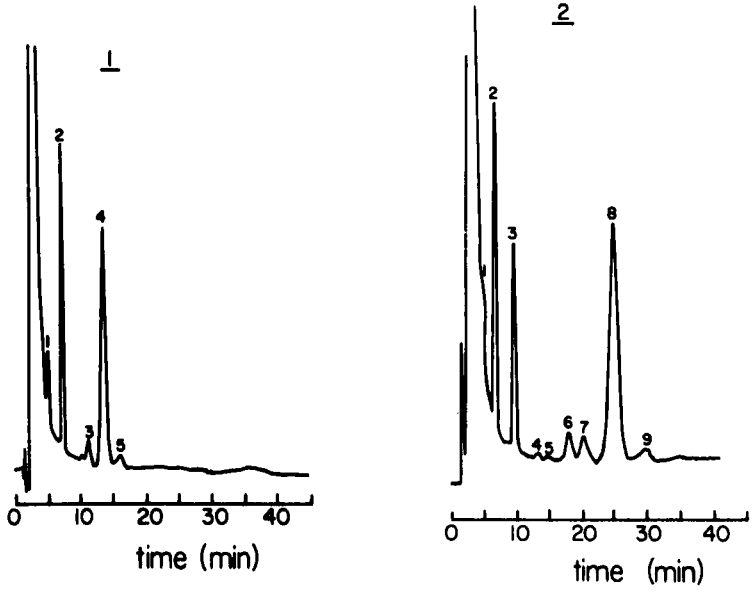


Figure 14: HPLC chromatogram of crude sucrose esters of saturated fatty acid (in DMF) UV detector; methanol and water (95:5); figure 14.1 - sucrose palmitate; Figure 14.2 - sucrose monostearate; Figure 14.3 - sucrose monopalmitate and monostearate mixture.
Figure 14.1: Peak 2 methylpalmitate; Peak 3-5 sucrose monopalmitate isomers; Figure 14.2: Peak 2 methylpalmitate; Peak 3 methylstearate; Peaks 4-5 sucrose monopalmitate and 6-7 sucrose monostearate isomers; Figure 14.3: Peak 3 methylpalmitate; Peak 4 methylstearate; Peaks 5-7 sucrose monopalmitate and 8-10 sucrose monostearate isomers; Peak 11 sucrose dipalmitate; Peak 12 sucrose distearate.

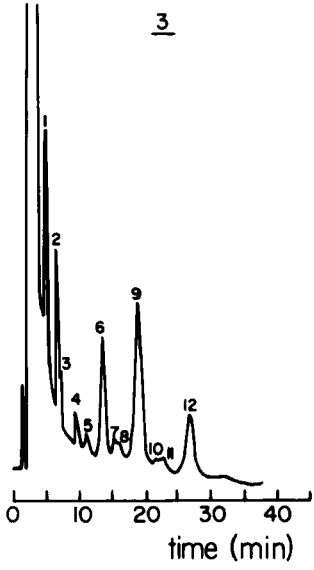


Figure 14: continued

from pure methyl stearate or pure methyl palmitate were eluted significantly earlier and better separations were obtained (see Figure 15).

Determination of sucrose polyesters such as sucrose hexa-, hepta- and octastearates and palmitates were also achieved[42]. Fluoroether-Sil-x-1 column was used with a RI detector. The mobile phase was n-chloropropane-methanol (91:9). A typical composition of sucrose polyester (SPE) as obtained by the base-catalyzed transesterification of sucrose and fatty acid methyl esters, is summarized in Table V. Although the isomers are not fully resolved, a good indication of the proportions of each isomer can be derived.

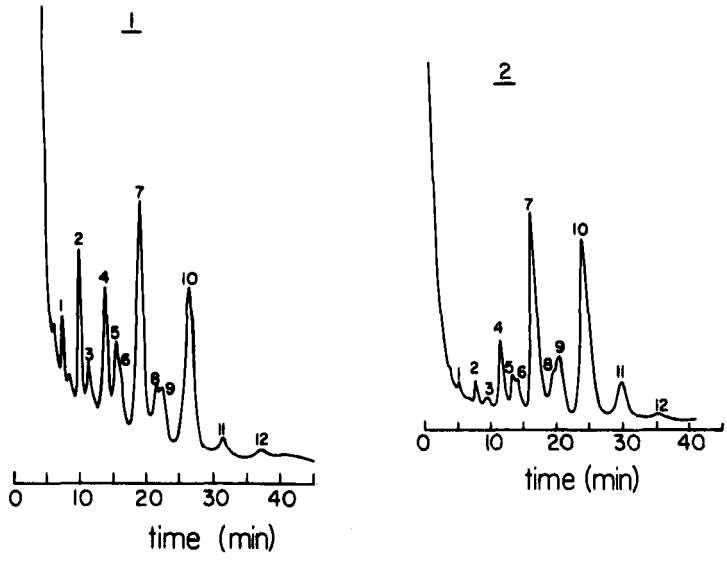


Figure 15: HPLC chromatogram of commercial sucrose esters F160 (Figure 15.1) and F50 (Figure 15.2). UV detector at 220 nm. Methanol and H_2O 95:5 (v/v); 55°C; Peak 1 methylpalmitate; Peak 2 methylstearate; Peaks 3-5 sucrose monopalmitate; Peaks 6-8 sucrose monostearate; Peak 9 sucrose dipalmitate; Peak 10 sucrose distearate; Peaks 11-12 sucrose polyesters.

3. Polyoxyethylene derivatives of fatty acids and alcohols

Ethoxylation of fatty acids with ethylene oxide in the presence of a variety of basic catalysts yields a mixture of polyethylene glycol monoester, polyethylene glycol diesters and polyethylene glycols. The composition of these three components has been shown to be close to the theoretical molar proportions of 1:2:1 and the equilibrium constant is expressed as:

$$K = \frac{(\text{monoester})^2}{(\text{diester})\ (\text{polyethyleneglycol})}$$

TABLE V

Typical Composition of Sucrose Polyester (SPE)

Percent of sucrose in SPE:	14.7%
Percent of TFA[a] in SPE:	91.5%

SPE distribution (by TLC):

% SE_8 =	54%
% SE_7 =	41%
% SE_6 =	5%
	100%

Ester Fatty Acid Distribution (by gas chromatography):

C_{18}	10.4%
$C_{18(1=)}$	59.0%
$C_{18(2=)}$	17.6%
$C_{18(3=)}$	12.3%
C_{18}	0.5%
	99.8%

Molecular Weights (as oleates):

SE_8	2458
SE_7	2193
SE_6	1929

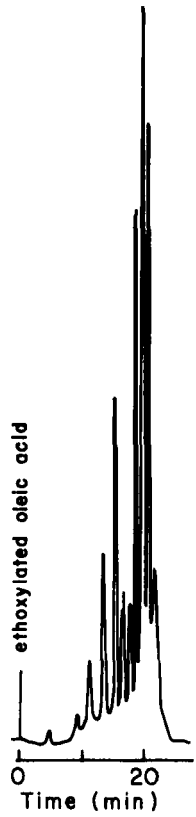


Figure 16: Fingerprints of gradient elution chromatogram of technical ethoxylated oleic acid.

TABLE VI
HPLC Analysis of Polyoxyethylene Derivatives of Fatty Acids and Alcohols

Type of Surfactant	Column	Detector	Eluent	Figure	Author (ref)
Ethoxylated fatty acids	SI (silica)	Moving wire (FID)	Mixture of solvents	16	Aitzetmüller (31)
Lauric acid ethoxylated	Zorbax SIL	Moving wire	acetone:hexane (gradient)	17	Nakamura (44)
Ethoxylated fatty acids	μ-Porasil (10μ)	UV	isooctane:i-PrOH (gradient)	-	Brüschweiler (43)
Ethoxylated fatty acids	only abstract available no details				Przondo (1975) (47)
Ethoxylated fatty acids	Merckosorb SI 60 (5μ)	UV	acetone	-	Nagami (1975) (48)
Ethoxylated fatty acids	Styrene-divinyl benzene (mol.sieve)	IR	MeOH:$CHCl_3$	-	Cassidy (1976) (56)
Ethoxylated fatty alcohols	SI (silica)	Moving wire (FID)	Mixture of solvents	18	Aitzetmüller (31)
Ethoxylated lauryl alcohol	Bondapak C_{18} Corasil	RI	acetonitrile: H_2O	19	Turner (1976) (45)
Dodecyl alcohol ethoxylated 5-25 EO	Zorbax SIL	Moving wire (FID)	acetone:hexane (gradient)	20-24	Nakamura (44) (derivatized)
Ethoxylated fatty alcohols	Lichrosorb RP-2 (5μ)	UV	acetonitrile: H_2O	25	Nozawa (46) (derivatized)
Ethoxylated fatty alcohols	μ-Porasil (10μ)	UV	isooctane:i-PrOH (gradient)	-	Brüschweiler (43) (derivatized)

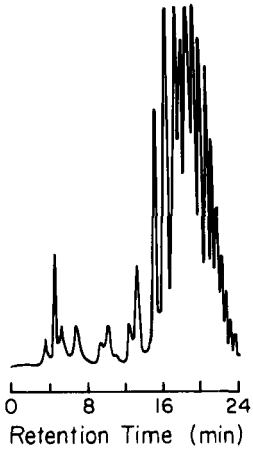


Figure 17: Fingerprints of commercial lauric acid ethoxylated (10 EO units). Zorbax sil column; moving wire detector and acetone-hexane mixture as eluent.

The product composition of these surfactants has been analyzed in the past by wet chemistry. The chromatography methods and mainly the use of HPLC were shown to be more accurate and give better insight into the nature of the product.

a. Polyoxyethylene fatty acids - Aitzetmüller[31] was the first to utilize HPLC analysis on a commercial product of fatty and ethoxylated acid. Figure 16 demonstrates the obtained chromatogram. There was no identification of the isomers. Only the fingerprints of the commercial material were shown in comparison to the ethoxylated fatty alcohols.

Qualitative analysis of polyoxyethylene type of surfactants including fatty acids were carried out by Nakamura[44] (see Table VI). The samples were acetylated prior to chromatography in order to decrease the adsorption on the column. The molar distribution of ethylene oxide (EO) adducts in the range of about 30 was

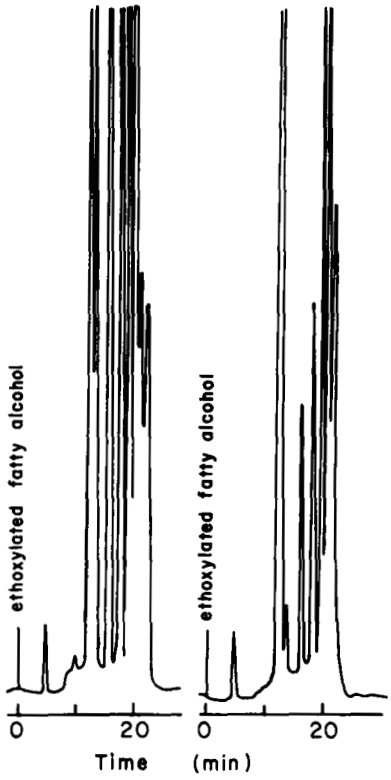


Figure 18: Fingerprint chromatogram of ethoxylated commercial fatty alcohols, using silica column, moving wire detector and complex mixture of solvents.

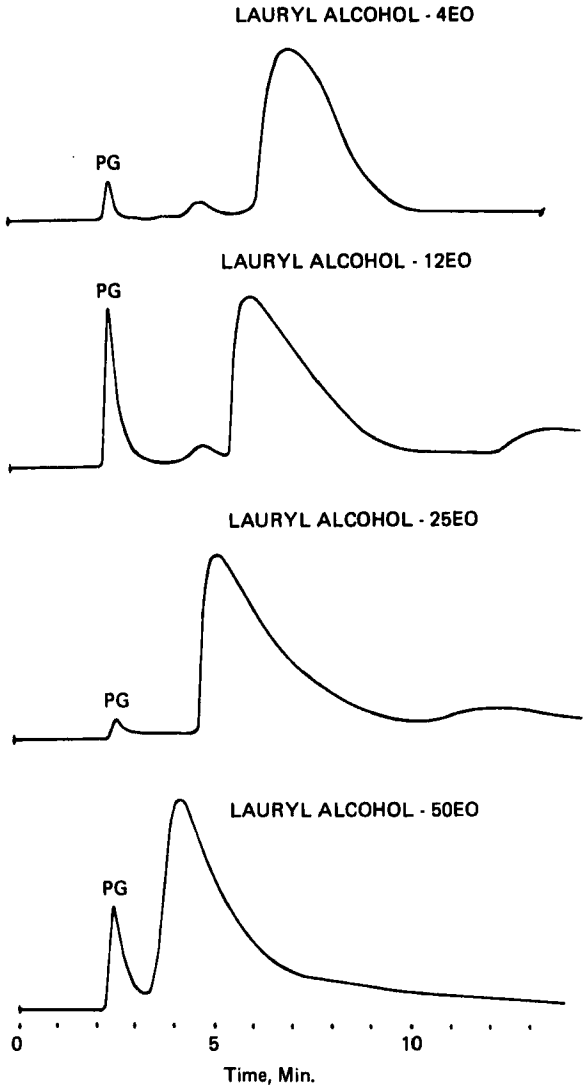


Figure 19: Lauric alcohol ethoxylated with 4,12,25 and 50 ethylene oxide units as eluted using Bondapak C_{18}/corasil column and acetonitrile water mixture as eluent.

established by the analysis of HPLC. The retention time was directly proportional to the EO mole number. Several mobile phases were tested (Zorbax-Sil column) including n-hexane, methanol, benzene and 2-propanol and their effect on the retention times and the detector response were examined. In a typical example, commercial lauric acid with 10 EO units was analyzed. There was no identification of the peaks (see fig. 17)[44].

b. Polyoxyethylene fatty alcohols - More detailed work was carried out on polyoxyethylene fatty alcohols. The fingerprints (fig. 18) of two commercial ethoxylated fatty alcohols, of a low to medium degree of ethoxylation were eluted[31]. The aim of that work was to show the possibility of using the transport-flame ionization detector (moving wire) to analyze food emulsifiers including ethoxylated fatty alcohols.

A method employing high pressure liquid chromatography has been developed for the quantitative determination of ethoxylated fatty alcohols and alkylphenols. This technique overcomes some of the problems encountered in the Aitzetmüller method. The polyethylene glycols were separated from the ethoxylated product and other materials using a 65/35 acetonitrile/water mobile phase and a Bondapak C_{18}/Corasil-reverse phase column system[45]. The molecular weight of the polyethylene glycols of each sample was approximated using TLC technique prior to the HPLC analysis. The precision of this method for determination of polyethylene glycols is 4% relative, or better, and the recovery of added polyethylene glycols is quantitative. Figure 19 represents the separation of four lauryl alcohols ethoxylated with 4, 12, 25, and 50 EO units. Turner[45] managed to find the response factors for his fatty alcohol ethoxylated materials according to their carbowax molecular weight.

An important work was carried out by Nakamura[44] to analyze the ethoxylated fatty alcohols. Pattern analysis of EO adducts

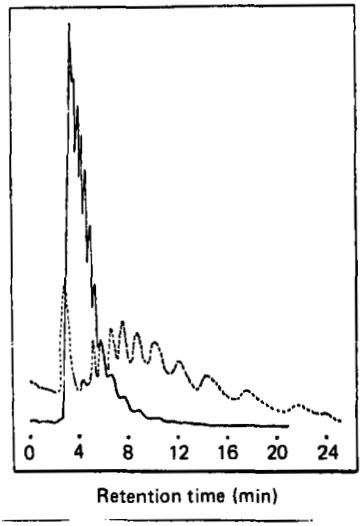


Figure 20: Chromatograms of commercial dodecyl alcohol 15 EO adducts and their acetates (Mobile phase; Acetone) ——— : Acetylized sample, -----: Sample only.

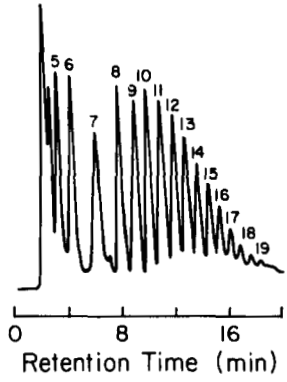


Figure 21: Chromatogram of commercial dodecyl alcohol 10 EO adducts.

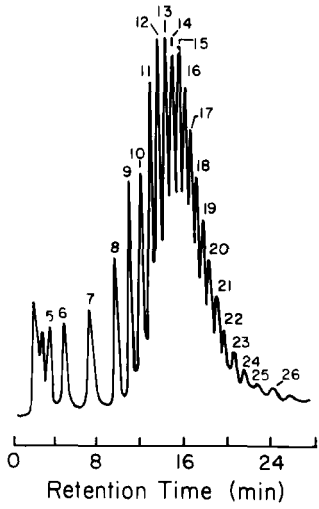


Figure 22: Chromatogram of commercial dodecyl alcohol 15 EO adducts mobile phase: Gradient from acetone/ n-hexane (25/75) to acetone at 5% acetone/min.

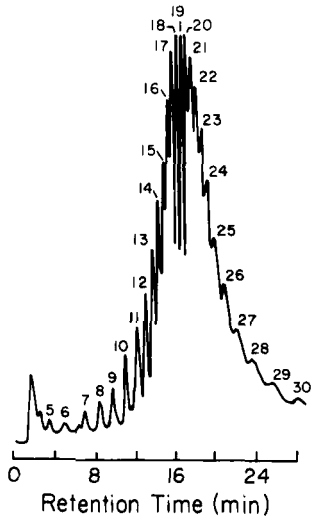


Figure 23: Commercial dodecyl alcohol 25 EO adducts, mobile phase same as in Figure 22.

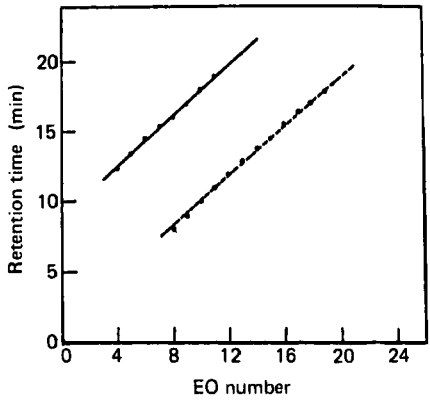


Figure 24: Relationship between EO number and retention time. _____ : Mixture of nondistributed EO adducts, 4∿11 (Mobile phase; Gradient from n-hexane to acetone at 5% acetone/min). ---: In Fig. 22.

and polyethyleneglycols were carried out under the following conditions: Zorbax-Sil column: 2.1 mm i.d. x 25 cm, mobile phase: mixture of acetone and n-hexane (gradient rate, 5% acetone/min), temperature 30°C, pressure 1500 psi and moving wire detector (flame ionization). The samples were acetylated prior to the chromatography in order to decrease the adsorption on column. According to the author the retention time was directly proportional to an EO mole number and therefore partial identification of the product composition could be done. The author used a GLC technique in order to determine the alkyl group composition of the peaks appearing in the HPLC chromatogram. Figures 20-24 demonstrate the Nakamura's findings for three commercial ethoxylated fatty alcohols surfactants. Figure 20 shows the difference between the separations achieved with acetylated and nonacetylated surfactants. Figures 21-23 show the complexity of the chromatogram of dodecylalcohols of 10, 15, and 25 EO adducts. The relationship between EO number and the retention times id demonstrated in Figure 24 indicating that there is a straight correlation between these two parameters.

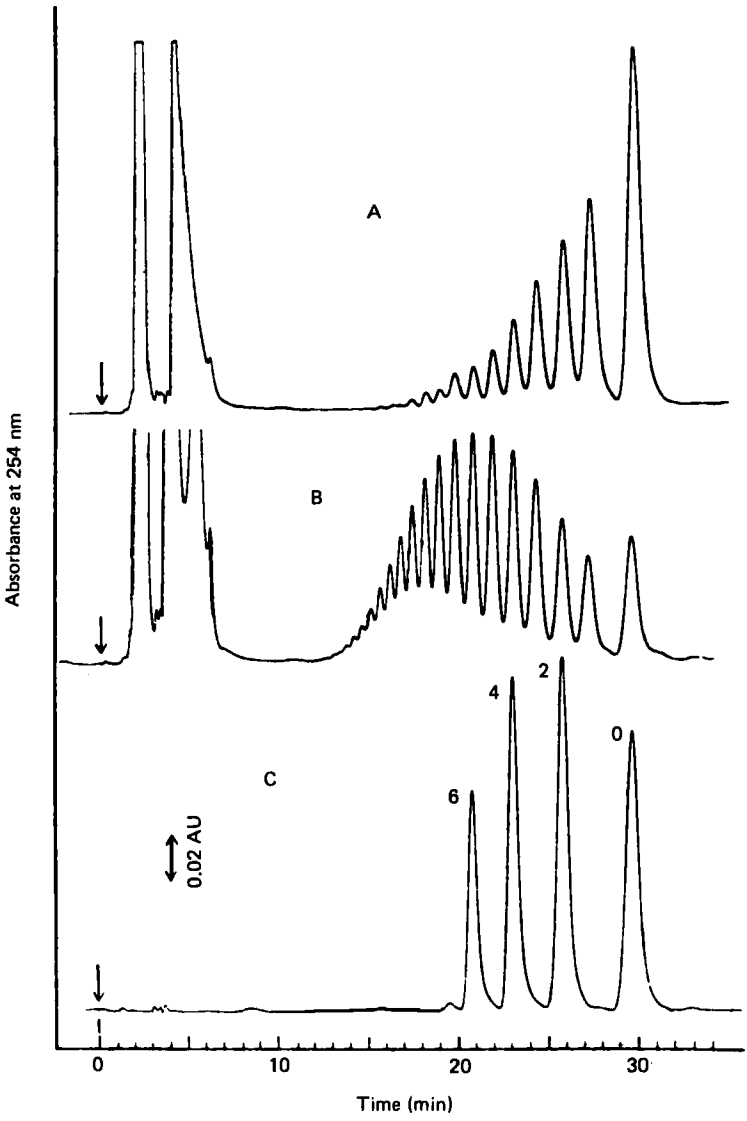


Figure 25: Chromatograms of esterification products of polyoxyethylene monododecyl ethers with 3,5-dinitrobenzoyl chloride samples. (A) $\bar{n}$ of the condensates was 2, the peaks were identified as materials of 0 and 1-10 ethylene oxide units from right to left; pre-peaks on the left were due to excess 3,5-dinitrobenzoyl chloride. (B) $\bar{n}$ of the condensates was 7, the peaks were identified as materials of 0 and 1-16 ethylene oxide units from right to left; pre-peaks on the left were due to excess 3,5-dinitrobenzoyl chloride, (C) Standard sample prepared by mixing homogeneous esters having polyoxyethylene length 0,2,4 and 6.

The above methods are not convenient for routine work because of the lengthy chromatographic steps and the complexity of the procedures. Nozawa [46] suggests the use of a UV detector after derivatization with 3,5-dinitrobenzoyl chloride for better and easier separation and detection of the polyoxyethylenated fatty alcohols. Monododecyl ethers ethoxylated were prepared by standard methods under based catalyzed conditions. The arithemetic average value of the ethylene oxide units were measured by determining the number of moles of the hydroxyl group per gram by an acetylation method. The columns were 250 x 4.0 min ID stainless stell tubes, packed with Lichrosorb RP-2, 5 μ size. An acetonitrile:water (6:4) mixture was used as the mobile phase at a flow rate of 0.8 ml/min. Figure 25 demonstrates a typical chromatogram of polyoxyethylene monododecyl ethers derivatized with 3,5-dinitrobenzoyl chloride samples. As indicated in the figure most peaks are identified.

4. Alkyl phenols ethoxylated

The polyoxyethylene alkylphenols have held an important position in the field of nonionic surfactants since the mid 1940's. Millions of pounds per year of these materials have been used in household products such as liquid dishwashing formulations and hard-surface cleaners since 1950.

The alkylphenols are generally oxyethylated in an autoclave at high temperatures and ethylene oxide pressures of 20 to 60 psi with 0.1 to 0.5% NaOH as catalyst. The first mole of ethylene oxide adds easily even at atmospheric pressure. The second and additional moles of EO will be added more difficultly. The final product is usually a mixture of several polyoxyethyleneated compounds[43].

TABLE VII

HPLC Analysis of Alkylphenol Ethoxylated

	Column	Detector	Eluent	Figure	Author (ref)
Nonylphenol ethoxylated	Zorbax-SIL	Moving wire	Acetone: hexane	26-27	Nakamura (1975) (44)
Octylphenol ethoxylated	Bondapak C_{18} Corasil	RI	Acetonitrile: water	-	Turner (1976) (45)
Nonylphenol ethoxylated	Merckosorb SI 60 (5μ)	UV	Acetone	28-29	Nagami (1977) (48)
Nonylphenol ethoxylated	Bondapak NH_2 (10μ)	UV	THF:hexane (gradient)	30	Maeden (1978) (49)
Octylphenol ethoxylated	Bondapak C_{18} Corasil	UV	Methanol:water (gradient)	-	Otsuki (1979) (50)
Alkylphenol ethoxylated	μ-Porasil (10μ)	UV	Isooctane: i-PrOH (gradient)	-	Brüschweiler (1977) (43)
Nonylphenol ethoxylated	various modified silica	UV	CCl_4:isooctane (mixture)	-	Huber (1972) (54)
p-tert-octylphenol ethoxylated	Porasil A	UV	AcOH:H_2O:EtOAc	-	Freeman (1975) (55)

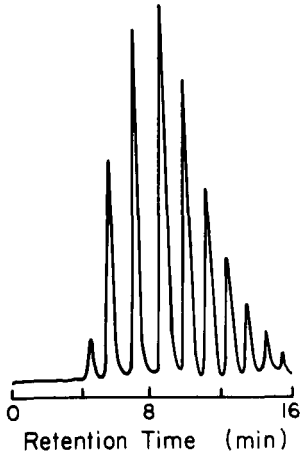


Figure 26: Chromatogram of commercial nonylphenol 5 EO adducts, mobile phase as in Figure 22.

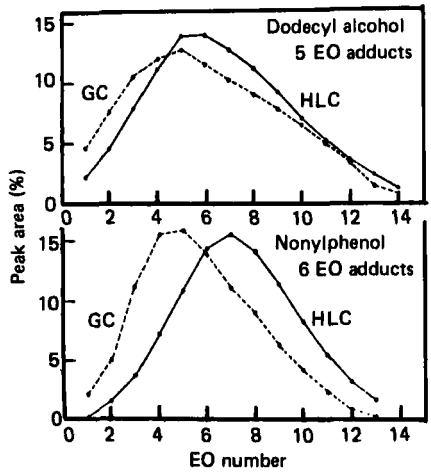


Figure 27: Comparison of EO distribution. Conditions - HLC: Mobile phase, gradient from acetone/n-hexane (10/90) to acetone at 5% acetone/min. GC conditions - Column: 2% SE-52 on AW-DMCS chromosorb G (60/80) 3 mm i.d. x 100 cm, glass column. Temp: 160→300°C (prog. rate, 4°C/min) Detector: FID, 330°C; Carrier gas: He, 60 ml/min.

Several attempts have been made since 1975 to separate and analyze these materials using HPLC (Table VII). Nakamura[44] acetylated his samples prior to their analysis in order to decrease adsorption to column. He used Zorbax-SIL column and a moving wire detector. A commercial nonylphenol ethoxylated with 5 EO units was injected using a gradient of acetone:n-hexane (25:75 or 10:90) while increasing the acetone concentration at 5% per minute. Figure 26 presents a typical chromatogram of such separation. Nakamura compared his method with existing GLC methods and has correlated the peak area of various EO nonylphenol adducts as eluted by GLC and HPLC, versus the number of EO distribution in order to demonstrate the validity of the separation (see Figure 27). The retention times were directly proportional to the EO mole number.

Turner[45] managed to analyze several octylphenol EO adducts (10 EO, 25 EO, 35 EO and 60 EO) using 60:40 V/V acetonitrile/water as mobile phase, a refractive index detector and BondaPak C_{18}/Corasil reverse phase column. The average molecular weight of the isooctyl derivatives was determined as described earlier in this review using TLC techniques.

When Merckosorb (10 mm x 500 mm) 5 μ SI 60 was used as stationary phase[48] and acetone as the eluent, good separation of the nonylphenol ethoxylated derivatives has been obtained (Figure 28). Figure 29 and Table VIII show the separation and the retention times measured for nonylphenols. With increasing EO units (n) it can be seen that the non-polar derivatives are eluted at relatively higher retention times and the peaks are broader. Nagami[48] has also plotted the HLB (Hydrophile-Lypophile Balance) values of the surfactants versus their retention times and showed a direct correlation between the two parameters. The identification of the lipophilic groups by infrared spectroscopy and the chromatographic determination of HLB permitted the

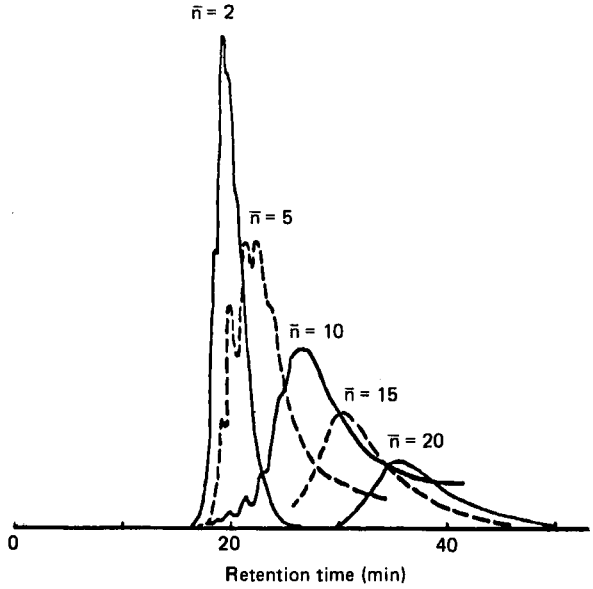


Figure 28: Chromatograms of polyoxyethylene monononylphenyl-ethers.

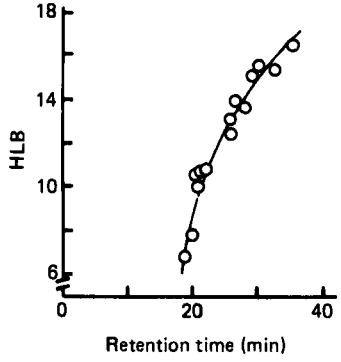


Figure 29: Relationship between HLB and retention time.

TABLE VIII

Chromatography of POE Type Surfactants

	$\bar{n}$	HLB	Retention time (min.)
Nonylphenyl ether	2	6.8	19.0
	2.6	7.8	20.0
	5	10.7	21.5
	10	13.9	26.5
	11.8	14.5	29.0
	15	15.5	30.5
	20	16.4	35.5
Oleyl ether	5.3	10.0	21.0
	10	12.4	26.0
	11.7	13.6	28.0
	20	15.3	33.0
Octylphenyl ether	7.8	13.1	26.0
Lauryl ether	3.9	10.5	20.5
Cetyl ether	5.5	10.5	22.0

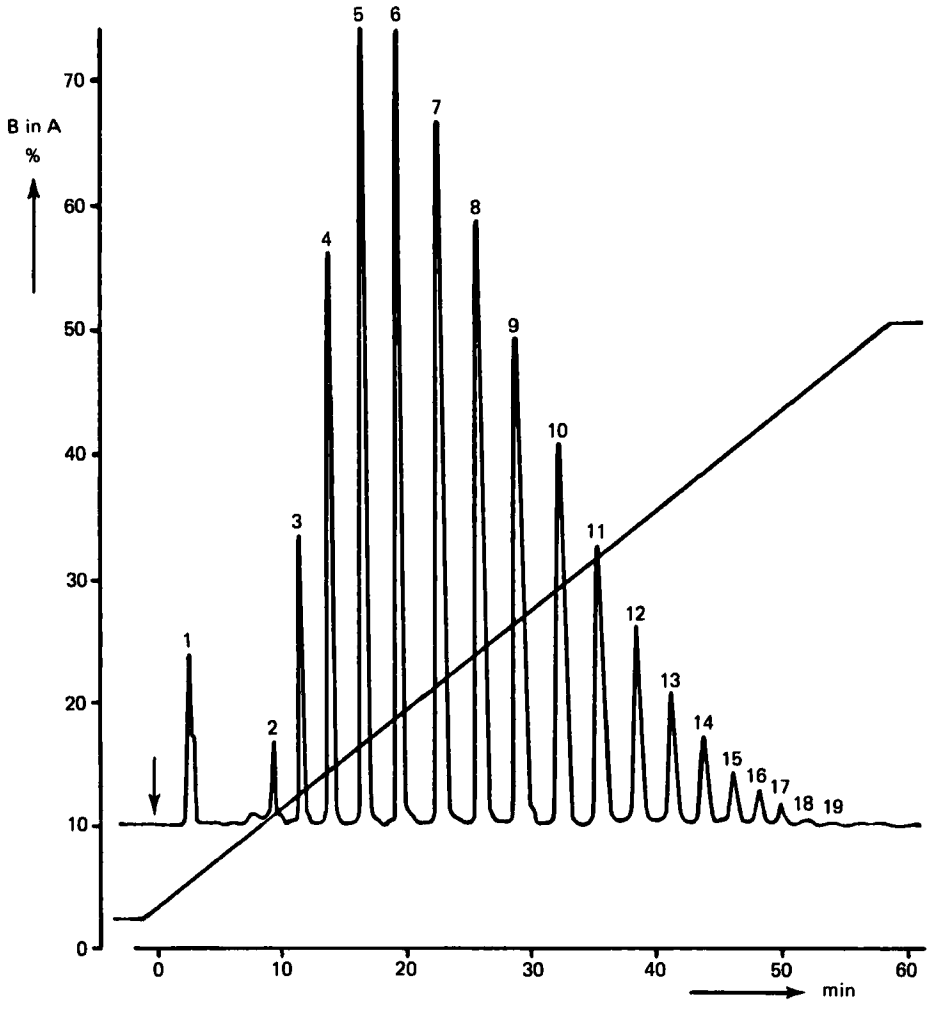


Figure 30: HPLC chromatogram of an ethoxylated octylphenol on a μBondapak NH_2 column. Peaks: 2 - oligomer with 2 EO units; 3 - 3 EO units; etc. Conditions: solvent gradient from 2% to 50% of solvent B in A in 60 min; solvent A is 20% THF in n-hexane; Solvent B is 10% water in isopropanol; sample size, 200 μg in 20 μℓ of solvent A; flow-rate, 1 ml/min; UV detection at 280 nm, 0.5 a.u.f.s.

qualitative analysis of these compounds. Table 8 compares the retention times and the HLB's to the number of EO units of the nonylphenyl adducts to other alkyl phenols and alkyl fatty alcohol adducts (ethers).

Gradient elution high performance liquid chromatography on chemically modified silica was found to be an excellent method for both qualitative and quantitative analysis of alkylphenol ethoxylated. The column used by Van der Maeden[49], Figure 30, TableVII,was a 30 cm x 3.9 mm I.D. semipolar μ-BondaPak-NH_2 column, with a UV detector at 280 nm, and a solvent gradient from 2% to 50% of solvent B (water-isopropanol 10:90) in solvent A (THF-n-hexane 20:30) in 60 min. flow-rate of 1 ml/min was used. A full assignment of the various isomers is given.

Polyoxyethylene alkylphenyl ether nonionic surfactants in water were adsorbed on an octadecyltrichlorosilane bonded glass bead in a packed column and eluted by gradient elution from water to 100% methanol. The elution order is octylphenyl, nonyphenyl and dodecylphenyl ethers. The recovery was more than 96% at 1 mg/L level and 71% at the 50 mg/L level. Field desorption mass spectrometry was used to confirm these compounds in the fraction collected, and to determine the degree of polymerization of polyethylene oxide[50].

The above methods have been widely used in the literature to detect small quantities of surfactants in final products.

5. Fatty alkanolamides

The condensation of fatty acids or esters with alkanolamine yields nonionic surfactants of wide utility and economic importance. Diethanolamine condensates, especially of lauric acid are

TABLE IX

HPLC Analysis of Alkanolamides

Type of surfactant	Column	Detector	Eluent	Figure	Author (ref)
Fatty amides	μ-Porasil	UV	Isooctane-i-PrOH (gradient)	-	Brüschweiler (43) (derivatized)
Monoethanolamides (C_{10}-C_{12})	Poly(styrene-divinylbenzene) gel (10-15μ)	UV	MeOH:water	31	Nakae (1978) (51)
Diethanolamides (C_{10}-C_{12})	"	UV	MeOH:water	32	Nakae (1978) (51)
Fatty acid mono-isopropanol amides (C_{12}, C_{14}, C_{16})	Octadecyl silica (TSK, Gel, 5μ)	UV	MeOH:water (pH 2.2 H_3PO_4)	33	Nakamura (1980) (52)
N-acylated sarcosinates	"	UV	"	34	Nakamura (1980) (52)
Commercial fatty acid	"	UV	"	35-36	Nakamura (1980) (52)
Fatty acids monoethanolamides	"	UV	MeOH:water	39	Nakamura (1981) (53)

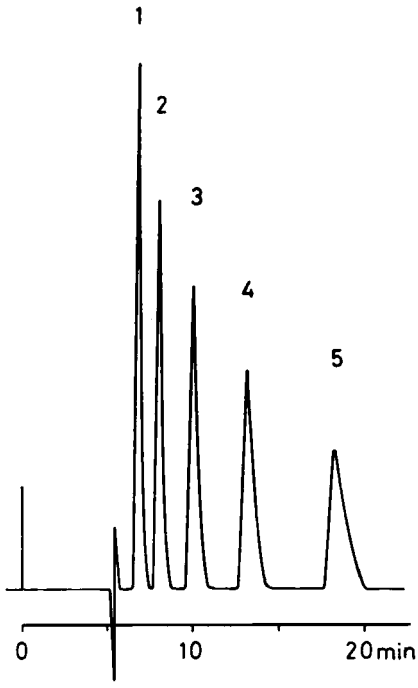


Figure 31: Chromatogram of fatty acid monoethanolamides. Column: Hitachi Gel 3011, 500 x 4 mm I.D. Mobile phase: water-methanol (3:97), Flow rate: 1.1 ml/min. Pressure: 40 kg/cm^2. Column temperature: 30^0. Detector: UV (215 nm), 0.5 a.u.f.s. Peaks: 1 - capric acid monoethanolamide; 2 - lauric acid monoethanolamide; 3 - myristic acid monoethanolamide; 4 - palmitic acid monoethanolamide; 5 - stearic acid monoethanolamide.

the main products available in the detergents market.

The reaction between a carboxylic acid (or ester) and a mono- or diethanolamine is complicated by the competing reactivity of the several functional groups present. Consequently, the composition of the products can vary considerably depending on the mole ratios and reaction conditions employed. Whereas derivatives from the reaction of equimolar quantities of dietanolamine and a fatty acid are water insoluble (high in ester-amide) it was noted that the use of 2 moles of diethanolamine led to water soluble materials with surfactant properties. Therefore it is very important to find suitable easy methods for evaluation of the commercial product distribution. Unfortunately only few studies were carried out concerning the fatty alkanolamides.

Brüschweiler (1977) used μ-Porasil column and UV detector to analyze the fatty amides[43] (see Table IX). While elution was claimed, no chromatograms were given in this article.

Homologous series of fatty acid mono- and diethanolamides with C_{10}-C_{18} alkyl groups were separated by high-performance liquid chromatography, employing a porous micro-spherical poly-(styrene-divinylbenzene) gel as the stationary phase[51]. The recommended conditions for the analysis were as follows: column, 500 mm, 4 mm I.D.; mobile phase, water-methanol (3:97); and column temperature 30°C. The logarithm of the capacity factors for each homologous series was directly proportional to their alkyl chain lengths. The reciprocal of the column temperature was also proportional to the capacity factor of each component.

The chromatograms of fatty acid monoethanolamides are presented in Figure 31 and Figure 32. It can be seen that easy separation is achieved, according to the hydrophobic chain length of the surfactants. Capric acid monoethanolamide is

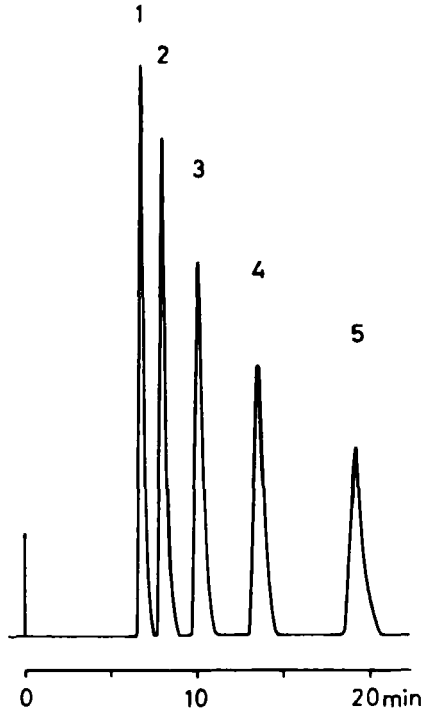


Figure 32: Chromatogram of fatty acid diethanolamides. Conditions as in Fig. 31. Peaks: 1 - capric acid diethanolamide; 2 - lauric acid diethanolamide; 3 - myristic acid diethanolamide; 4 - palmitic acid diethanolamide; 5 - stearic acid diethanolamide.

TABLE X

Capacity Factors of Fatty Acid Alkanolamides

Temperature 30° except where indicated otherwise

Compound	Mobile phase												
	CH_3OH	H_2O-CH_3OH (3:97)				H_2O-CH_3OH	0.05 M	0.1 M	0.5 M	0.5 M	0.5 M	0.5 M	0.1 M
		30°	40°	50°	60°	(5:95)	$HClO_4$*	$HClO_4$*	$HClO_4$*	$NaClO_4$*	HCl*	LiCl*	NH_4Cl*
Fatty acid monoethanolamides													
C_{10}	0.46	0.60	0.50	0.44	0.35	0.69	0.36	0.36	0.47	0.61	0.44	0.68	0.57
C_{12}	0.64	0.89	0.74	0.65	0.52	1.09	0.52	0.51	0.70	0.92	0.64	0.95	0.78
C_{14}	0.90	1.36	1.11	0.95	0.75	1.76	0.76	0.75	1.07	1.42	0.95	1.42	1.10
C_{16}	1.30	2.11	1.69	1.40	1.12	2.93	1.11	1.11	1.66	2.23	1.42	2.11	1.58
C_{18}	1.87	3.31	2.59	2.08	1.65	4.90	1.63	1.65	2.64	3.51	2.14	3.23	2.30
Fatty acid diethanolamides													
C_{10}	0.57	0.61	0.51	0.44	0.36	0.71	0.40	0.41	0.50	0.63	0.51	0.71	0.58
C_{12}	0.75	0.92	0.75	0.65	0.53	1.12	0.57	0.58	0.75	0.92	0.74	1.01	0.81
C_{14}	1.02	1.41	1.14	0.96	0.79	1.84	0.84	0.86	1.16	1.42	1.09	1.49	1.15
C_{16}	1.46	2.23	1.75	1.43	1.16	3.08	1.25	1.29	1.85	2.24	1.66	2.25	1.66
C_{18}	2.10	3.56	2.73	2.16	1.72	5.27	1.88	1.94	2.78	3.57	2.56	3.51	2.46

* In methanol

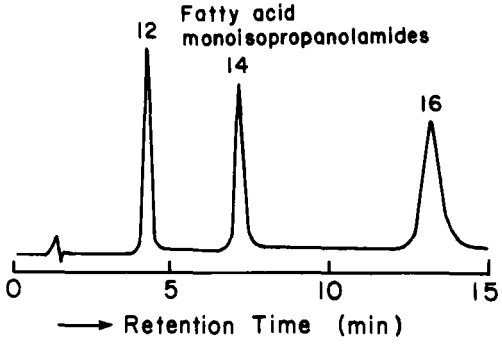


Figure 33: HPLC chromatogram of fatty acid monoisopropanolamides. (Numbers of each peak are lypophilic groups; i.e., 12 = lauroyl) Conditions: Eluent: Methanol - water (85-15), pH = 2.2, Column temp. 50°C, Detector UV (210 nm).

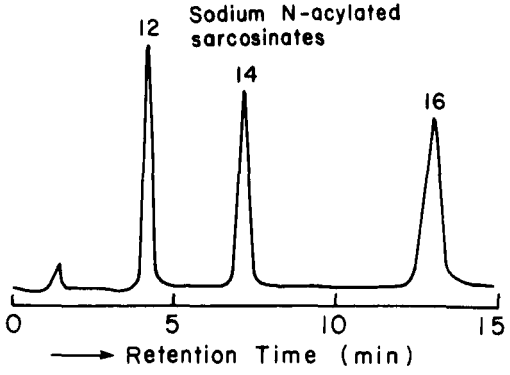


Figure 34: HPLC chromatogram of sodium n-acylated sarcosinates at same conditions as in figure 33.

eluted first, followed by lauric acid monoethanolamide and myristic, palmatic and stearic monoethanolamides.

Table X represents the capacity factors of several fatty acid alkanolamides as calculated by Nakae[51] at several elution conditions (various solvent mixtures and increasing column temperatures). The higher the hydrophobicity of the surfactant, the larger is the capacity factor of the compound indicating slower elution from the gel permeation column.

Separation of homologous series of standard and sodium-N-acylated sarcosinated and fatty acid monoisopropanol-amides with C_{12}, C_{14} and C_{16} alkyl groups were studied by Nakamura[52] using high speed liquid chromatography employing octadecylsilica (TSK-Gel; LS-410, 5 μ column). The recommended conditions for the separation were as follows: column size, 4 mm I.D. x 25 cm, eluent water-methanol (15:35) adjusted to pH=2.2 with phosphoric acid, and a UV detector, (figs. 33-34). By using the same eluent conditions nonionic surfactants such as fatty acid mono- and diethanolamides could be easily separated to their corresponding homologous series. Typical separation of coconut and hydrogenated tallow fatty acid derivatives is shown in Figures 35-36.

The capacity factor (k') values of homologous series of sodium N-acylated sarcosinates were drastically affected with the eluent pH; the k' values increased by lowering the eluent pH. However, fatty acid and monoisopropanolamides were not affected by the eluent pH. The logarithms of k' were directly proportional to the alkyl chain length of the standard surfactants, (figs. 37,38).

Recently Nakamura[53] has shown that the use of octadecylsilica column allows the separation of a variety of nonionic,

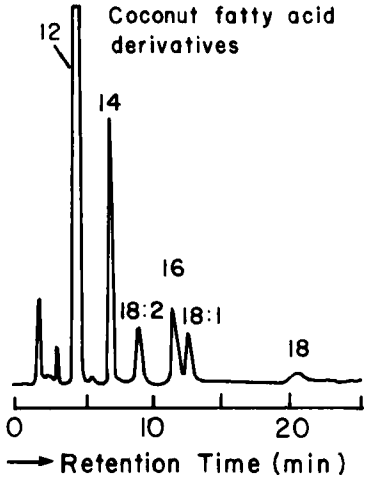


Figure 35: HPLC chromatogram of coconut fatty acid monoethanolamide at same conditions as in figure 33.

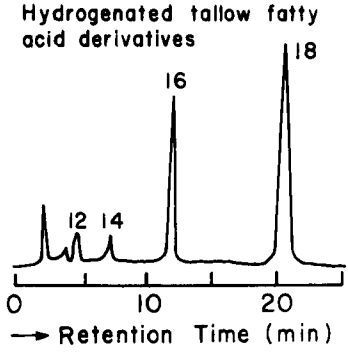


Figure 36: HPLC chromatogram of hydrogenated tallow fatty acid monoethanolamide at same conditions as in figure 33.

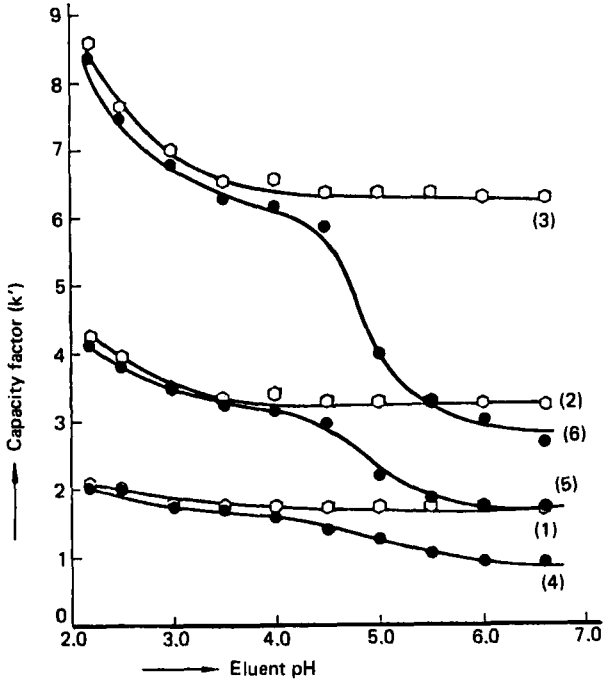


Figure 37: Effect of eluent pH on capacity factor (k'). Eluent:Methanol-water (85-15). (1) lauroyl-; (2) mirystoyl-; (3) palmitoyl-monoisopropanolamides; (4) sodium N-lauroyl-; sodium N-mirystoyl; (6) sodium N-palmitoyl sarcosinate.

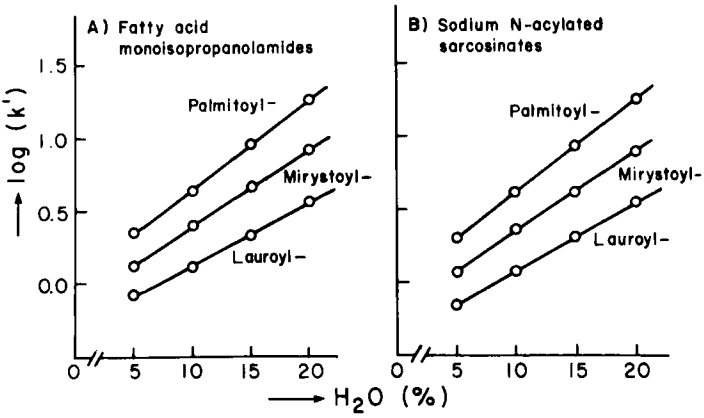


Figure 38: Effect of water content on capacity factor (k'). Eluent pH were adjusted to 2.2 with phosphoric acid.

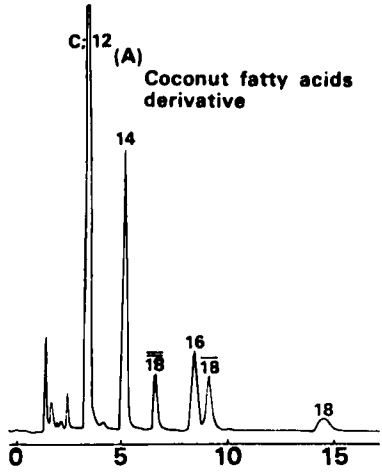


Figure 39: HPLC chromatogram of commercial fatty acid monoethanolamide; from coconut fatty acids.

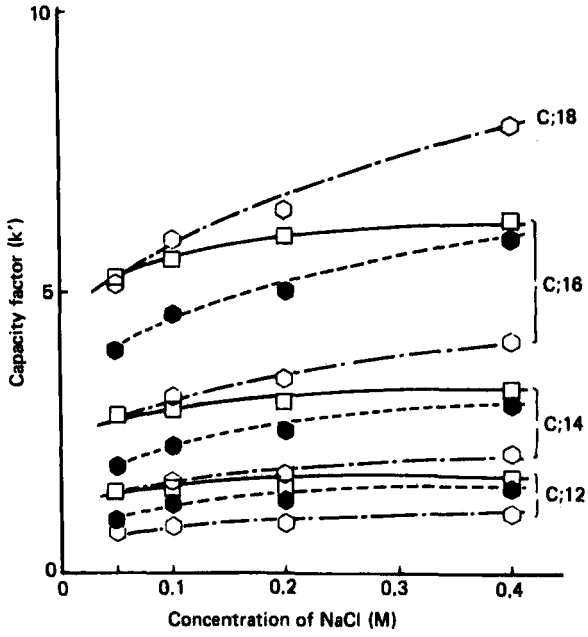


Figure 40: Effect of concentration of NaCL on separation of homologs.
(● - alkylbenzyldimethylammonium chlorides,
○ - sodium alkylsulfates and ◘ - fatty acid monoisopropanolamides. C-12 means alkyl chain length in lipophilic group is 12).

cationic and anionic surfactants when small amounts of NaCl were present in the eluent mixture of MeOH-water. The analytical results of several commercial surfactants were excellent and in good accordance with those obtained by gas chromatography. Excellent separation of fatty acid monoethanol amides (Figure 39) was shown.

Nakamura has demonstrated both the effect of the concentration of the concentration of NaCl, and the effect of water content in the mobile phase on separation of homologs (Figures 40, 41).

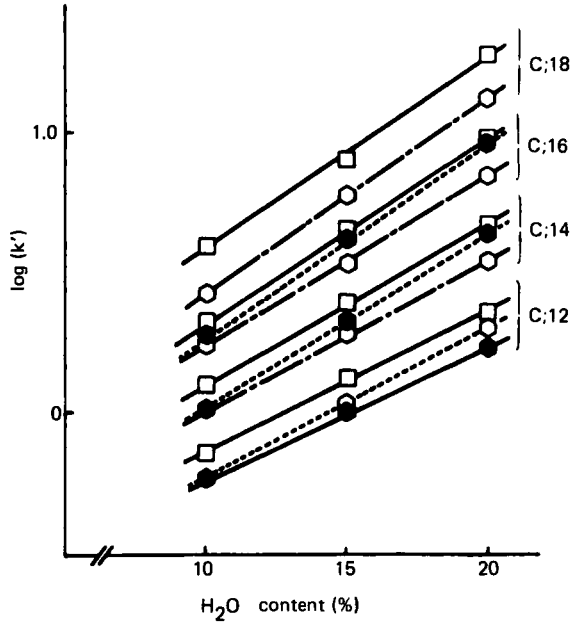


Figure 41: Effect of water content in mobile phase on separation of homologs. (NaCl concentration 0.4 M/L, and symbols are the same as in fig. 40.).

Summary

High-performance liquid chromatography is gaining an increasing role in the interaction between scientists and technologists, in producing units. Many of the food emulsifiers are evaluated by this technique both from quality control and product distribution purposes.

The major drawback of the method in emulsifier analysis is in the detection of the separated components, since most emulsifiers are based on fatty acids or other fatty components which normally do not contain chromophores. A universal detector, in which solvent energy from a column was coated on a moving wire and then

dried off before pressing through a flame ionization detector for quantification of any substances remaining was marketed for several years. This did not appear to be a commercial success and is no longer available, possibly because of low sensitivity. Today UV detectors are the most common devices for monitoring solutes absorbing in the 220-230 nm range. For surfactants containing only isolated double bonds and carbonyl groups, some variable wavelength detectors have been developed and are commercially available. Some analysts managed to convert specific emulsifiers to derivatives that contain aromatic groups which can be monitored by UV detectors. Refractive index detectors were also used for such compounds. These detectors are sensitive but can only be used with an eluting solvent of constant composition, thus limiting the range of applications.

The columns used are of two types: adsorption and reversed phase. The former is usually based on a silica gel matrix. The latter is frequently made up of octadecyl grafted to silica gel.

The eluents used for the separation of nonionic emulsifiers are mostly combinations of water with several selected polar organic solvents such as ethanol, acetonitrile, methanol etc. In some cases nonpolar solvents were also used.

The lack of pure standard compounds and the complexity of each emulsifier are the cause for the lack of reliable and quantitative studies of the nonionic emulsifiers. The development of variable wavelength detectors, the use of high purity solvents and the availability of a large variety of types of columns will most probably help the analysts to obtain better separations of these emulsifiers in the near future.

Many investigators have tried to analyze the product composition of a given emulsifier in two stages: Splitting the

hydrophilic portion of the surfactant from its hydrophobic part and injecting each into GLC or HPLC instruments. The present report does not intend to review studies carried out in the analysis of the hydrophilic or hydrophobic moieties of the molecule even if it was accomplished using HPLC techniques. We do not see the advantage of the HPLC technique in such cases.

The review article presented here is only a small part of the studies carried out on other surfactants, namely anionic, cationic and amphoteric. These compounds are a subject for a separate report.

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