Short communication

Separation and quantification of tetraethylene glycol monoheptanoate and diheptanoate by high-performance liquid chromatography

V. Baudrand, Z. Mouloungui*, A. Gaset

Laboratoire de Chimie Agro-industrielle, École Nationale Supérieure de Chimie de Toulouse INPT, 118 Route de Narbonne, 31077 Toulouse, France

Abstract

Tetraethylene glycol mono- and diheptanoate were prepared by direct esterification of heptanoic acid with tetraethylene glycol. The constituents of the reaction were separated by thin layer chromatography with flame ionization detection, but only tetraethyleneglycol diheptanoate could be determined by this method. The main constituents of the reaction mixture were quantified by HPLC using two different mobile phases on a non-bonded silica column. Acetonitrile acidified with 0.3% acetic acid was used to separate and quantify the monoester and tetraethylene glycol, while hexane–isopropanol (80:20, v/v) acidified with 0.3% acetic acid was used to separate and quantify heptanoic acid and the diester. The starting compounds and the products of the reaction could be thus separated and quantified accurately using these two eluents without derivatization of the hydroxylated compounds.

1. Introduction

Tetraethylene glycol monoheptanoate (TEGMH) is a non-ionic surfactant belonging to the family of short hydrophobic alkyl chain wetting agents [1] and is employed in numerous industrial and domestic cleansing formulations [2]. The polyoxyalkylene moiety with a free terminal hydroxyl group is an important structural feature which enhances the properties of these wetting agents.
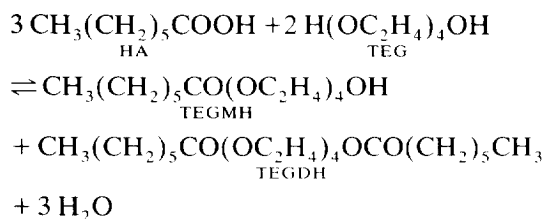
Tetraethylene glycol diheptanoate (TEGDH) is a diester with plastifying properties used as an adhesion control agent in conjunction with polyvinylbutyral (PVB) [3]. It is also employed as an additive in solid photopolymerisable compositions used in the manufacture of optical components in imaging equipment [4].

Analytical data on the separation of commercial polyethoxy fatty acids by HPLC on a silica column have been reported [5], although the main objective of the study was to identify the fatty acids and determine the number of ethylene oxide units in these surfactants.

TEGMH and TEGDH were synthesized from heptanoic acid (HA) and tetraethyleneglycol (TEG) at moderate temperature in the absence of catalyst and solvent [6] (Eq. 1).

---

* Corresponding author.

$$3\,CH_3(CH_2)_5COOH + 2\,H(OC_2H_4)_4OH$$
$$\underset{HA}{} \qquad \underset{TEG}{}$$

$$\rightleftharpoons \underset{TEGMH}{CH_3(CH_2)_5CO(OC_2H_4)_4OH}$$

$$+ \underset{TEGDH}{CH_3(CH_2)_5CO(OC_2H_4)_4OCO(CH_2)_5CH_3}$$

$$+ 3\,H_2O$$

The reaction mixture was analyzed using a combination of TLC–FID and HPLC with silica as stationary phase, which enabled the determination of the content of non-ionic surfactants and starting compounds (HA and TEG) in the reaction mixture.

2. Experimental

2.1. Reagents and analytical set-up

TEG (99%) was supplied by Aldrich (St. Quentin Fallavier, France). HA (99%) was supplied by Fluka (St. Quentin Fallavier, France), and 2,7-difluorescein was purchased from Merck (Nogent-sur-Marne, France). The silica chromagel (60 Å, 60–200 μ) used for the flash-chromatography was obtained from SDS (Peypin, France).

The TLC silica plates (60 F_{254}) were supplied by Merck (Nogent-sur-Marne, France). The detector was a Bioblock Scientific UV lamp (Illkich, France).

An Iatroscan MK-5 was used for TLC–FID, and the samples were applied on S-III chromarods (Iatron Laboratories, Tokyo, Japan) (Flotech, Paris, France). Five 1-μl aliquots (0.25 g reaction mixture in 10 ml ethyl ether) of each sample were applied using a semi-automatic pipette (SES 3202). The chromarods were then placed in a chamber previously saturated with the elution solvent [ethyl ether–pentane–acetic acid (80:20:1, v/v)], removed after 30 min migration and then oven dried (Rod Dryer TK-8; Iatron Laboratories) at 110°C for 5 min. After temperature stabilisation in a desiccator under vacuum, the chromarods were transformed to the flame ionization detector and burned using

the following parameters: hydrogen flow 160 ml/min, air flow 2 ml/min, burn rate 35 s/rod. The results were analyzed using BOREAL software.

The HPLC system was equipped with a Spectra System AS 3000 automatic injector and a Spectra-Physics Analytical P 1500 pump (Spectra-Physics, Paris, France). Samples of 20 μl (0.1 g reaction mixture in 1.4 ml acetonitrile or hexane) were injected. The analytical column was a Nucleosil S 100 5 μm type NC04 (250 × 4.0 mm I.D.) (Bischoff Chromatography) (ICS, Toulouse, France) fitted with a 10 μm Lichrosorb SI 100 precolumn (Bischoff Chromatography). A Car H (30 cm × 7.8 mm I.D.) fitted with a Sarasep precolumn (5 cm × 4.6 mm) (Touzard-Matignon, Grabels, France) eluted with acetonitrile–water (60:40, v/v) acidified with 0.005 M sulfuric acid (flow-rate, 0.4 ml/min; oven temperature, 40°C), and a 5 μm Nucleosil C_{18} column (25 × 4.6 mm I.D.) (Touzard-Matignon) fitted with a precolumn eluted with methanol acidified with 0.3% acetic acid (flow-rate, 0.8 ml/min; oven temperature, 40°C) were also tested. A refractometer (Refractomonitor IV Milton Roy) (LDC, Paris, France) was employed as detector, and the chromatograms were recorded on a Spectra-Physics chromjet integrator.

2.2. Synthesis and purification of TEGMH and TEGDH

TEGMH and TEGDH were synthesized by reacting different amounts of TEG and HA in the absence of solvent in a 100-ml three-necked flask fitted with a cooling system, mechanical stirrer and temperature probe. The reaction mixture was heated to 145°C on a thermostated oil bath for 480 min. The reaction mixture was washed with 0.1 M KOH to extract HA and saturated NaCl to extract TEG. The TEGMH and TEGDH were then separated on a silica column by flash-chromatography using two eluents: ether and ethanol. Analytical grade solvents (SDS; Peypin, France) were employed for the HPLC and TLC–FID.

3. Results and discussion

3.1. Separation by TLC and TLC–FID–Quantification of TEGDH by TLC–FID

In preliminary experiments, the progress of the esterification reaction was followed by TLC. The plates were eluted with ethyl ether–pentane–formic acid (80:20:5, v/v) and the products TEGMH (R_F: 0.10) and TEGDH (R_F: 0.42) along with the starting compounds HA (R_F: 0.50) and TEG (R_F: 0.02) were detected under UV illumination (254 nm) after spraying the plates with 2,7-difluorescein. This produced light yellow spots for the mono- and diester, dark yellow spots for heptanoic acid and orange spots for TEG.

TLC–FID, combining the advantages of TLC and flame ionization detection [7], was carried out using thin silica rods coated with silica (5 μm), and eluted with a mixture of ethyl ether–pentane–acetic acid (80:20:1, v/v). The presence of TEGMH (retention time: 17.90 min) and TEGDH (retention time: 12.11 min) was detected along with HA (retention time: 5.80 min) and TEG (retention time: 23.68 min).

In TLC–FID both TEGMH and TEG were strongly retained on the silica. These highly polar mono- and dihydroxylated compounds interact with silica and are thus strongly bound to the support. TEG with two hydroxyl groups migrated only a little, and tended to contaminate the rods close to the point of sample application. Heptanoic acid is not readily quantified by TLC–FID as it has a low molecular mass and is highly volatile. It tends to evaporate during the drying stage at 110°C and leads to errors in the calculation of mass conservation.

This system was found to be sufficiently linear and reproducible only for accurate quantification of TEGDH. Each point on the calibration curve for the diester corresponds to five separate determinations. The surface areas corresponding to each concentration were calculated to an error of ±3%. The calibration curve for the diester was described by the equation $y = 900.62 + 3379.4\,x$ with a correlation coefficient $r = 0.989$,

which was thus suitable for quantification of the non-ionic surfactant [8].

3.2. Optimization of analytical conditions for HPLC

In view of the limitations of TLC–FID, we turned to HPLC for quantification of TEGMH, TEGDH, TEG and HA. Using a refractometer as detector we obtained linear and reproducible responses for both standards and samples.

Reversed-phase (Nucleosil C_{18}) and high-capacity acid resin (Car H) columns

Various types of columns with different eluents were tested:

(a) A reversed-phase column (5 μm) filled with Nucleosil C_{18} was eluted with methanol acidified with 0.3% acetic acid. TEG (retention time: 4 min) was eluted first followed by HA and TEGMH (retention time: 5 min) and TEGDH (retention time: 6 min). (b) A Car H column filled with a high-capacity cation-exchange resin in the H^+-form has been described for analysis of organic acids and polyols using acetonitrile–water (60:40, v/v) acidified with sulfuric acid (0.005 M) as eluent [9]. Using this system, TEGDH was eluted first (retention time: 8.37 min) followed by HA and TEGMH (retention time: 9.85 min) and TEG (retention time: 20.13 min).

In both cases, TEGMH and HA were eluted together, while the order of elution of TEG and TEGDH was reversed on the two different supports.

Varughèse et al. [10] have investigated the HPLC behavior on reversed-phase supports of a series of non-ionic surfactants bearing different functional and alkyl groups. They found that the chromatographic behavior depended on interactions between the alkyl group of the surfactant and the stationary phase. We assume that the retention of TEGMH and HA on the Car H acid support was influenced by the hydrophobic part of the molecule consisting of the hydrocarbon chain of heptanoic acid.

A drawback of the Car H column was that it

required a perfect match between the eluent and dilution solvent (equal proportions of water and acetonitrile). If these two solvents are not the same, a negative peak was observed just after the TEGMH and HA peak, which tended to interfere with the quantification of these compounds.

Silica column (Nucleosil S100)

The qualitative results obtained with TLC–FID and literature data [5] on HPLC separations using silica columns prompted us to employ a non-coated silica column (Nucleosil S100). Two solvent systems were employed for separation of both starting compounds and products of the esterification reaction:

(a) Hexane–isopropanol (80:20, v/v) acidified with 0.3% acetic acid for separation of HA and TEGDH (Fig. 1). These two compounds were quantified after calibration of the column with external standards. There was a linear relationship between the concentrations injected and the peak heights of HA and the peak areas of TEGDH. The following equations governed the relationships between the peaks and concentrations injected: for HA, $y = 0.26832 + 9.1946 \cdot$

$10^{-2}x$, $r = 0.994$; for TEGDH, $y = 68.685 + 3004.3\ X$, $r = 0.999$. If the dilution solvent differed from the eluent, a negative peak was observed at time t_0 before emergence of HA (Fig. 1). The other compounds (TEG and TEGMH) were too strongly retained for reliable detection and their peak could not be discriminated from baseline noise. To avoid accumulation of polar compounds under these analytical conditions, the column was rinsed with isopropanol after each analysis.

(b) Acetonitrile acidified with 0.3% acetic acid for separation and quantification of TEG and TEGMH (Fig. 2). In this case, HA and TEGDH were eluted first and were not separated. TEG and TEGMH were quantified after calibration using ethylene glycol (EG) as internal standard. Linear relationships were observed between the concentrations injected and the areas of the peaks of TEG, TEGMH and EG. The concentrations of TEG and TEGMH were derived from the following equations: For TEG, $y =$
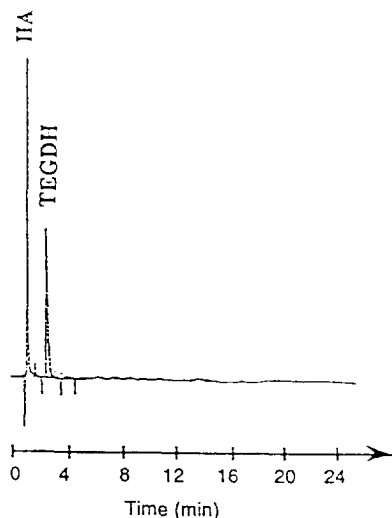
Fig. 1. Chromatogram of HA and TEGDH on a Nucleosil S100 column. Operating conditions: oven temperature, 40°C; duration of analysis, 6 min; retention time (min), HA: 0.8, TEGDH: 3.53.
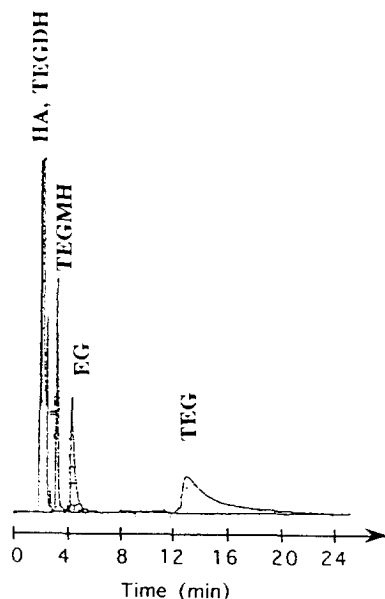
Fig. 2. Chromatogram of TEGMH and TEG on a Nucleosil S 100 column. Operating conditions: eluent, acetonitrile acidified with 0.3% acetic acid; flow-rate, 1.4 ml/min; oven temperature, 40°C; duration of analysis, 25 min; retention time (min), TEGMH: 3.53, TEG: 12.93, EG: 4.27.

$-5623 + 3409.10x$, $r = 0.991$; for TEGMH, $y = -13285 + 2383.70x$, $r = 0.991$; for EG, $y = -2826 + 644.96x$, $r = 0.992$.

3.3. Adaptation of HPLC system to separation of components of esterification medium

Injection system

The esterification reaction between heptanoic acid and tetraethylene glycol depends on the initial ratio of the reactants. A large excess of TEG minimizes formation of TEGDH, whereas an excess of HA maximizes formation of this ester. We thus investigated ratios of HA/TEG ranging from 0.1 to 10. The following conditions were employed: (a) HPLC system with automatic sample loader to gain time and minimize errors on routine analyses, (b) an injection loop which enabled an increase in injection volumes (20–250 μl) and could be adapted to different proportions of reactants and products.

Refractometric detection

The refractometer detected 0.5, 2, 0.15 and 1 μmole respectively of HA, TEG, TEGDH and TEGMH. As in the previous experiments, the refractometric detector could not be employed with a dilution gradient and was highly sensitive to changes in temperature and pressure.

Analysis of media from esterification of HA and TEG

Fig. 3 shows the progress of the esterification reaction for an HA/TEG ratio of 7.52.

The operating conditions with excess HA gave a conversion rate of 100% for TEG and 70% for HA. TEGMH and TEGDH were obtained as a mixture, and the relative proportions of the two depended on the duration of the reaction. TEGMH was obtained in 60% yield after 50 min reaction, while TEGDH was obtained in 95% yield after 480 min.

Analysis of other esterification mixtures

Hexane–isopropanol (80:20, v/v) acidified with 0.3% acetic acid was also employed to
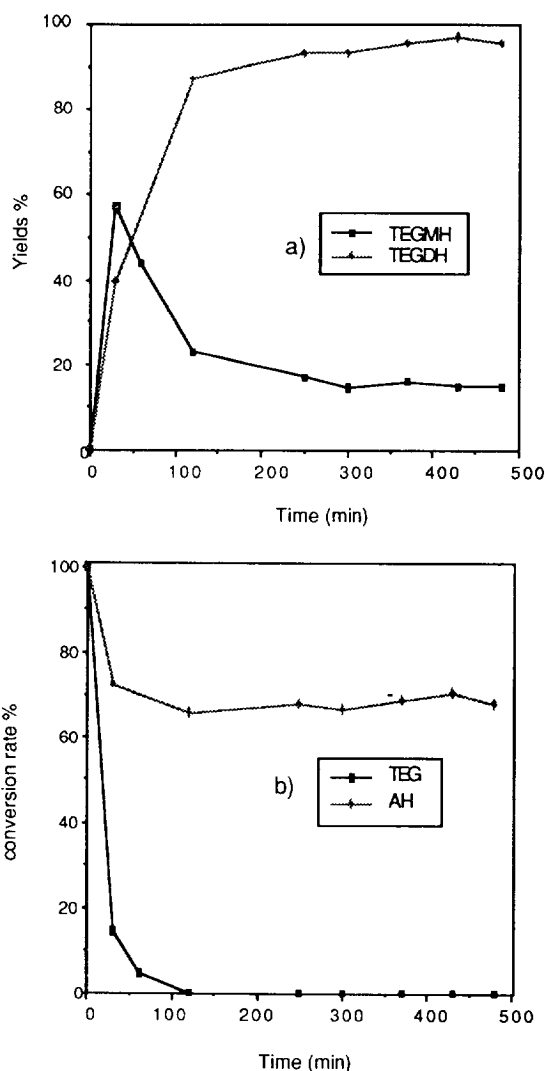

Fig. 3. Reaction progress with HA in excess (HA/TEG: 7.52). (a) Yields of TEGDH and TEGMH, (b) HA and TEG conversion rate. Operating conditions: temperature: 167°C; AH: 6 moles, TEG: 0.79 moles.

separate and quantify compounds from reaction mixtures containing: (a) tetraethyleneglycol monoundecanoate (retention time: 7.17 min), tetraethyleneglycol diundecanoate (retention time: 2.39 min) and undecylenic acid (retention time: 1.86 min); (b) tetraethyleneglycol mono-oleate (retention time: 6.09 min) tetraethyleneglycol dioleate (retention time: 2.05 min), and oleic acid (retention time: 1.77 min).

4. Conclusion

The various components of the reaction mixture from esterification of a fatty acid (HA) with tetraethylene glycol (TEG) were separated by TLC, TLC–FID or HPLC on acidified silica supports. A mobile phase of hydrocarbons (pentane, hexane) mixed with polar solvents such as isopropanol, formic and acetic acid [11], or acetonitrile acidified with acetic acid were found to be suitable for separation of the neutral components, and for separation and quantification of the acid components. Two mobile phases were developed to optimize separation of HA from TEGDH, and TEG from TEGMH by HPLC. The esterification reaction carried out in the absence of solvent could thus be followed both qualitatively and quantitatively by HPLC. The method was adapted for quantification of non-ionic surfactants.

References

[1] J.K. Weil, R.E. Koos, W.M. Linfield and N. Parris. J. Am. Oil Chem. Soc., 139 (1979) 873.

[2] R.A. Reck, in R.W. Johnson and E. Fritz (Editors), Fatty acids in industry, Marcel Dekker, New York, 1989, Ch. 8, p. 177.

[3] T.R. Phillips, Polyvinyl butyral plasticized with tetra-ethyleneglycol di-n-heptanoate, EP 80105092.3 (1980).

[4] W.K. Smothers, Storage stable photopolymerisable composition and element for refractive index imaging, US 4,994,347 (1991).

[5] N. Garti, V.R. Kaufman and A. Aserin, in J. Cross (Editor), Non-ionic surfactant chemical analysis, Marcel Dekker, New York, 1988, Ch. 7, p. 225.

[6] V. Baudrand, Unpublished results.

[7] K.D. Mukherjee, in J. Sherma and B. Fried (Editors), Handbook of Thin-Layer Chromatography, Marcel Dekker, New York, 1991, Ch. 13, p. 339.

[8] H. Pakdel and C. Roy, Biomass, 13 (1987) 155.

[9] Catalogue Touzart-Matignon France (1994), p. 19.

[10] P. Varughèse, M.E. Gangoda and R.K. Gilpin, J. Chromatogr., 499 (1990) 469.

[11] W.W. Christie, Lipid Technol., 5 (1993) 121.