

Quantitative Proton NMR Determination of Linoleic Acid Mono- and Diesters of Polyethyleneglycols via Reaction With Trichloroacetyl Isocyanate

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A method has been developed for qualitative identification and quantitative determination of linoleic acid mono- and diesters of polyethyleneglycols with various molecular masses. The method is based on proton nuclear magnetic resonance (NMR) spectroscopy analysis of the product obtained after in situ reaction of the ester mixture with trichloroacetyl isocyanate.

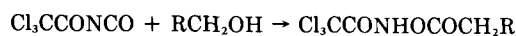
The fatty acid esters of polyethyleneglycols (PEG) are widely used in the pharmaceutical, cosmetic and other industries (1). It is well known, however, that regardless of the method used, the reaction product in most cases represents a mixture of mono- and diesters as well as PEG itself (2).

There are several methods described in the literature for quantitative determination of the components in such mixtures, based on chemical analysis (3), countercurrent extraction (4) or column chromatography (5). All are time-consuming and/or not very accurate. A HPLC-based method proposed more recently (6) deals with esters of PEG of relatively low molecular mass (up to ca. 200).

NMR spectroscopy is a rapid and convenient method for quantitative analysis of mixtures of organic compounds provided that distinct signals for the different species can be observed in the spectrum (7). In the case of mixtures of linoleic acid monoesters (ME) and diesters (DE) of polyethyleneglycols, the proton signals of ME and DE are of similar position and multiplicity, which makes the direct quantitative analysis practically impossible. Moreover, the signal of the OH-proton in ME is rarely observed, due to its fast exchange and/or the relatively high mean molecular mass of the polyethyleneglycols used.

There are various methods described in the literature for chemical transformation of hydroxyl compounds into derivatives with more easily NMR-identifiable groups: acetylation and benzylation (8), dichloroacetylation (9), trifluoroacetylation and subsequent ^{19}F NMR investigation (10). In addition to the signal for the acyl substituent, the esterification usually causes characteristic low-field shifts of the α -proton signals.

Trichloroacetyl isocyanate (Cl_3CCONCO , TAI) was reported to be an effective acylating agent which reacts quantitatively with hydroxylic compounds (11). The dominant reaction is 1,2- addition leading to formation of trichloroacetyl carbamate (O-TAC):



The reaction is usually fast and can be conveniently carried out in situ, i.e. in the NMR tube. The OH-proton is transformed into an imide NH-proton observable at 8–11 ppm which easily can be determined quantitatively by integration. In addition, low-field shifts for the signals of protons in proximity to the OH-group are observed.

In this work, the derivatization with trichloroacetyl isocyanate (TAI) followed by ^1H NMR spectral analysis was used for qualitative identification and quantitative determination in mixtures of linoleic acid mono- and diesters of polyethyleneglycols with various molecular mass.

EXPERIMENTAL PROCEDURE AND DATA

Materials used. Polyethyleneglycols, PEG with mean molecular mass 200, 400, 600, 1000, 1500, 2000 (Fluka); linoleic acid 99% by GLC (12); trichloroacetyl isocyanate, TAI, A.R. (Fluka); deuteriochloroform (Fluka), and tetramethylsilane, TMS (Fluka). PEG 2000 monolinoleate ($\text{ME}_{2000}^{\text{Li}}$) was prepared by treatment of pure linoleic acid with PEG 2000, according to the method of Rankoff et al. (13). PEG 1500 monolinoleate ($\text{ME}_{1500}^{\text{Li}}$), PEG 1000 monolinoleate ($\text{ME}_{1000}^{\text{Li}}$), PEG 600 monolinoleate ($\text{ME}_{600}^{\text{Li}}$), PEG 400 monolinoleate ($\text{ME}_{400}^{\text{Li}}$) and PEG 200 monolinoleate ($\text{ME}_{200}^{\text{Li}}$) were prepared in a similar manner. PEG 2000 dilinoleate ($\text{DE}_{2000}^{\text{Li}}$), PEG 1500 dilinoleate ($\text{DE}_{1500}^{\text{Li}}$), PEG 1000 dilinoleate ($\text{DE}_{1000}^{\text{Li}}$), PEG 600 dilinoleate ($\text{DE}_{600}^{\text{Li}}$), PEG 400 dilinoleate ($\text{DE}_{400}^{\text{Li}}$) and PEG 200 dilinoleate ($\text{DE}_{200}^{\text{Li}}$) were isolated by preparative TLC of mixtures of ME and DE of the corresponding PEG (14).

Analysis and procedures. The ^1H NMR spectra were taken on a pulsed FT spectrometer, Bruker WM-250, at 250.13 MHz and ambient temperature, using 5-mm tubes. Typical measurement conditions were: pulse width ca. 30° ; digital resolution 0.4 Hz/point; memory size 16K. Deuteriochloroform was used for solvent and tetramethylsilane for internal standard. The following analytical procedure was used: The solution of the sample (15–20 mg) in 0.4 ml solvent was placed in a clean, dry NMR tube and a normal proton spectrum was taken (Fig. 1). A few drops (a small excess) of TAI were added to the NMR tube and the NMR measurement was repeated (Fig. 2).

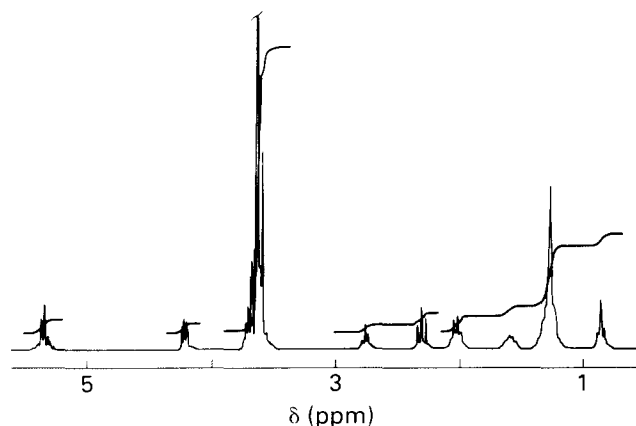


FIG. 1. 250.13 MHz ^1H NMR spectrum of mixture of linoleic acid mono- and diesters of polyethyleneglycol with mean molecular mass of 1000 in CDCl_3 .

NMR DETERMINATION OF LINOLEIC ACID ESTERS

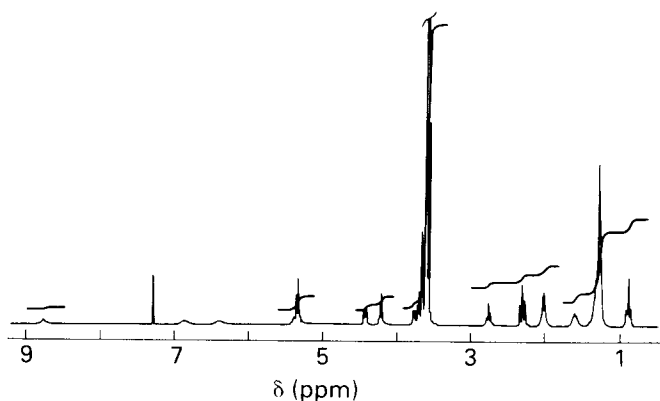


FIG. 2. 250.13 MHz ^1H NMR spectrum of mixture of linoleic acid mono- and diesters of polyethyleneglycol with mean molecular mass of 1000 after addition of TAI.

The quantitative analysis is most conveniently based on the integral intensities of the signals for the CH_2OTAC -protons in ME-OTAC at 4.43 ppm and for the COOCH_2 -protons of ME and DE at 4.22 ppm. The percentage of ME in the sample is calculated using the formula:

$$\text{ME (\%)} = 2I_x \cdot 100 / I_x + I_y$$

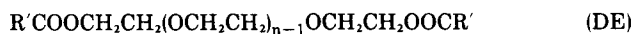
where I_x is the integral intensity of the signal at 4.43 ppm and I_y is the integral intensity of the signal at 4.22 ppm. In order to obtain accurate results, the following conditions are required:

- The sample should be practically free of PEG. This can be achieved easily by washing the product several times with a saturated (3) or diluted (13) aqueous solution of sodium chloride.
- The solution concentration should not exceed ca. 30 mg sample/0.4 ml solvent. Otherwise, the solution may be overheated upon addition of TAI.
- It is desirable to minimize the amount of moisture in the sample, solvent and reagent because it may cause secondary reactions and/or crystallization of trichloroacetamide in the NMR tube.

Mixtures of linoleic acid monoesters and diesters of polyethyleneglycols have been prepared according to the following procedure: In a three-necked flask fitted with a stirrer, nitrogen blowing device and vacuum line are placed 0.1 mole of the corresponding polyethyleneglycol (20 g PEG 200, 40 g PEG 400, 60 g PEG 600, 100 g PEG 1000, 150 g PEG 1500 and 200 g PEG 2000), 0.1 mole (28 g) of pure linoleic acid and 1 wt% of *p*-toluene sulfonic acid as catalyst. The mixture is heated to 140 C and stirred for two hr under vacuum and nitrogen. As soon as the reaction is completed the mixture is cooled to 90–100 C and washed five times with aqueous solution of sodium chloride under heating in order to remove the free PEG. [In the case of the esters of PEG 200, 400 and 600 the washing is done with saturated sodium chloride solution (3); with 15% sodium chloride solution for the esters of PEG 1000; with 12% and 10% sodium chloride solution, respectively, for the esters of PEG 1500 and PEG 2000 (13).] The upper layer containing mono- and diesters of the corresponding PEG is dried under vacuum and filtered to separate the solid sodium chloride.

RESULTS AND DISCUSSION

The samples studied were mixtures of linoleic acid mono- and diesters of polyethyleneglycols with mean molecular mass of 200, 400, 600, 1000, 1500 and 2000:

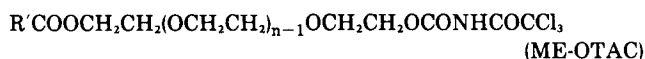
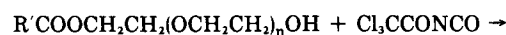


where $\text{R}' = \text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7$.

In the proton NMR spectra of samples containing varying amounts of ME and DE measured in deuteriochloroform without addition of TAI (Fig. 1) one can observe a broad multiplet signal for the polyethylene unit (OCH_2CH_2) at 3.65 ppm. The COOCH_2 protons in ME and DE are observed as a quartet at 4.22 ppm due to the deshielding effect of the COO group. As already mentioned above, the signal of the OH proton of ME is not observed. The linoleic acid protons are assigned as follows: methyl group, triplet at 0.89 ppm; 2-methylene, triplet at 2.32 ppm; 3-methylene, broad quintet at 1.62 ppm; 8- and 14-methylene, quartet at 2.04 ppm; 11-methylene, triplet at 2.77 ppm; 4-, 5-, 6-, 7-, 15-, 16- and 17-methylene, broad multiplet at 1.31 ppm; olefinic protons, multiplet at 5.34 ppm.

It is clear that the direct determination of ME and DE is practically impossible due to the overlap of signals at 4.22 ppm for the COOCH_2 group in both compounds. This fact, as well as the overlap of the signals at 3.65 ppm, excludes the possible determination of the mean molecular mass of the polyethyleneglycols by comparison of the intensities of the signals of the linoleic acid and glycol moieties.

All these problems were surmounted by addition of TAI. The latter reacts with ME according to the following scheme:



The reaction proceeds rapidly and is complete at the time when the proton NMR spectrum is remeasured (Fig. 2). In addition to the signals already observed, the spectrum now contains a well defined multiplet at 4.43 ppm due to CH_2OTAC protons; another multiplet for the β -methylene with respect to the OTAC group at 3.78 ppm (these two multiplets are parts of an AA'BB' spin system), and a singlet at 8.76 ppm due to the CONHCO proton. All these signals can be used for quantitative determination of the amount of ME in the sample under investigation. The presence of moisture in the sample leads to additional broad singlets at 6.34 ppm and 6.83 ppm, caused by the trichloroacetamide formed by the reaction of TAI with water. The latter signals do not present a problem for the interpretation of the spectra and the quantitative analysis.

The results obtained for the model mixture with known ratio of linoleic acid monoesters and diesters of polyethyleneglycols with various molecular mass obtained according to (13,14) are given in Table 1.

The results for the composition of the samples investigated prepared by esterification of linoleic acid with

TABLE 1

Composition of Linoleic Acid Monoesters (ME) and Diesters (DE) with Polyethyleneglycols in Model Mixtures

Trademark of PEG	Introduced:	ME:DE ratio (wt)			
		99:1	95:5	70:30	5:95
		Found			
200	99.1 ^a ± 0.1 ^b	95.4 ^a ± 0.1 ^b	70.5 ^a ± 0.1 ^b	5.1 ^a ± 0.2 ^b	
	0.9 ± 0.1	4.6 ± 0.1	29.5 ± 0.1	94.9 ± 0.2	
400	99.1 ± 0.1	95.4 ± 0.1	70.3 ± 0.2	5.0 ± 0.1	
	0.9 ± 0.1	4.6 ± 0.1	29.7 ± 0.2	95.0 ± 0.1	
600	99.3 ± 0.3	94.8 ± 0.2	70.3 ± 0.1	5.1 ± 0.1	
	0.7 ± 0.3	5.2 ± 0.2	29.7 ± 0.1	94.9 ± 0.1	
1000	99.2 ± 0.2	95.4 ± 0.2	70.1 ± 0.2	5.2 ± 0.1	
	0.8 ± 0.2	4.6 ± 0.2	29.9 ± 0.2	94.8 ± 0.1	
1500	99.3 ± 0.3	95.1 ± 0.3	70.4 ± 0.2	5.0 ± 0.1	
	0.7 ± 0.3	4.9 ± 0.3	29.6 ± 0.2	95.0 ± 0.1	
2000	99.2 ± 0.2	95.1 ± 0.1	70.4 ± 0.2	5.4 ± 0.2	
	0.8 ± 0.2	4.9 ± 0.1	29.6 ± 0.2	94.6 ± 0.2	

^aMean value of triplicate analysis.^bStandard deviation.

the corresponding polyethyleneglycols (Analysis and Procedures) are presented in Table 2.

TABLE 2

Amounts of Linoleic Acid Monoesters (ME) and Diesters (DE) With Polyethyleneglycols in Esterification Products

Trademark of PEG	ME % (wt)	DE %(wt)
200	60.0 ^a	40.0 ^a
400	62.5	37.5
600	64.5	35.5
1000	74.3	25.7
1500	81.0	19.0
2000	89.2	10.8

^aMean value of triplicate analysis.

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