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Technical Note

High-Performance Liquid Chromatographic (HPLC) Analysis of Oligomeric Components of the Spermicide Nonoxynol-9

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INTRODUCTION

Nonoxynol-9 (N-9) is a nonionic surfactant used as the active ingredient in the majority of commercially available spermicides. Recent interest for this compound has arisen because of its *in vitro* toxicity to sexually transmitted pathogens (1–4) and *in vivo* absorption through the vaginal mucosa of rats (5).

The commercially available N-9 is a multicomponent mixture produced by a polymerization reaction between ethylene oxide (9 mol) and nonyl phenol (1 mol). This synthesis produces a complex mixture of oligomers differing in the length of the ethylene oxide (EO) chain (6) (Fig. 1). These oligomers follow a Poisson distribution around the most abundant oligomer, believed to be the one containing nine ethylene oxide residues (EO 9), hence the nomenclature N-9.

Various separation and identification techniques have been developed for the analysis of surfactants of the alkyl phenol polyethoxylate family, mainly in wastewaters and sewage. Techniques such as gas chromatography/mass spectrometry (7), thin-layer chromatography (8), and HPLC (9,10) among others (see references cited in Refs. 9 and 10) have been employed. Gradient elution HPLC of oligomeric mixtures such as ethoxylated octyl phenol has been shown to be an important method for qualitative and quantitative analysis (9,11,12). Qualitative determination of the ethylene oxide distribution for low molecular weight nonoxynols including Nonoxynol-9 by gradient HPLC was also reported. However no specific information regarding N-9 was provided (10).

Gradient HPLC assays for the separation of iodinated N-9 (13) and more recently for [^{14}C]N-9 (5) into their various oligomers were developed in our laboratories.

The objective of this study was to separate N-9 by normal phase gradient elution HPLC and isolate and identify oligomeric components of the surfactant Nonoxynol-9 by NMR and FAB-MS.

MATERIALS AND METHODS

Chemicals. Nonoxynol-9 (Igepal CO-630) was a gift of GAF Corporation (Wayne, NJ). HPLC-grade hexane, 2-propanol, and tetrahydrofuran were purchased from Fisher Scientific (Fairlawn, NJ). All HPLC solvents were filtered through a Millipore nylon-66 0.45-µm filter and degassed before use. Deuterated chloroform (CDCl$_3$) and tetramethysilane (TMS) were purchased from Aldrich Chemical Company (St. Louis, MO).

Instrumentation. The HPLC analyses were performed on a Waters HPLC system (Millipore, Waters Chromatography Division, Millford, MA) consisting of two Waters Model 510 HPLC pumps, a Waters Model 680 automated gradient controller, a Waters Model 440 absorbance detector (280 nm), and a Waters Model SE120 dual-channel recorder. Samples were introduced via a Rheodyne Model 7125 loop injector (Rheodyne, Cotati, CA) equipped with a 100-µl loop onto the HPLC column. The chromatography column used was a Zorbax-NH$_2$, 7 µm, 250 mm × 4.6-mm I.D., which was preceded by a precolumn packed with Zorbax-NH$_2$, 7-µm-particle size packing material.

Nuclear magnetic resonance spectra were recorded using a 300-MHz Varian Model XL300 spectrophotometer (Varian Associates, Palo Alto, CA). Mass spectrum determinations were carried out on a Kratos MS50TA mass spectrometer equipped with a fast atom bombardment source. Spectra were acquired with a Kratos Model 0590 computerized data system.

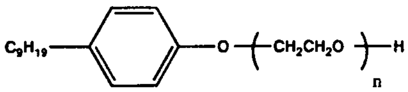
Preparative HPLC Separation and Isolation of Oligomers of N-9. The chromatographic conditions were similar to those published earlier for the analytical separation of N-9 (5) with some modifications. Stock solutions of N-9 (250 mg/ml) were prepared in tetrahydrofuran. A 1.0-ml aliquot was separated on a preparative Zorbax-NH$_2$ 7-µm column, 250 mm × 21.2-mm I.D., using a linear solvent gradient from 98% A–2% B to 50% A–50% B in 90 min, where A = tetrahydrofuran:hexane (20:80, v/v) and B = water:2-propanol (10:90, v/v) delivered at 9.9 ml/min at ambient temperature and detection at 280 nm. The six oligomers eluting between 18 and 22, 32 and 36, 41 and 47, 47 and 51, 57 and 62, and 78 and 80 min were collected, concentrated and reinjected onto an analytical system for further purification.

^{1}H-NMR Spectroscopy. All ^{1}H-NMR spectra of the single oligomers were run in CDCl$_3$ and were referenced to TMS.

FAB-MS Spectroscopy. Glycerol was used as the sample supporting matrix. The generated positive ion mass spectra of the individual N-9 oligomers were continuously acquired.

RESULTS AND DISCUSSION

High-performance liquid chromatography of N-9 using



NONOXYNOL-9 n = 1 - 17

Fig. 1. Nonoxynol-9.

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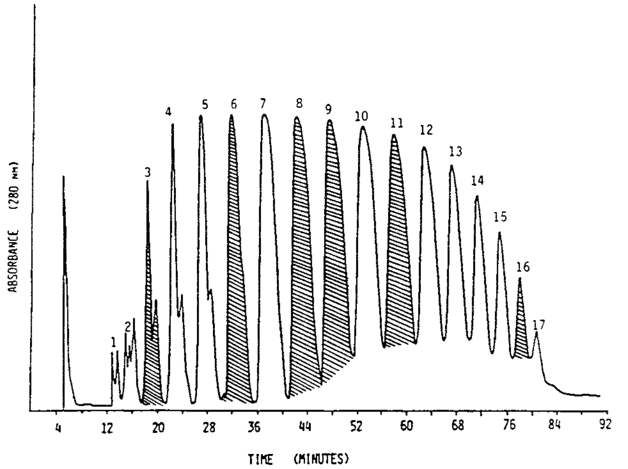

Fig. 2. Preparative normal phase gradient elution HPLC chromatogram of N-9 using a Zorbax-NH_2 (250 × 21.2-mm) column; mobile phase, 90-min linear gradient from 98% A–2% B to 50% A–50% B, where A = tetrahydrofuran–hexane (20:80, v/v) and B = water–2-propanol (10:90, v/v); flow rate, 9.9 ml/min at ambient temperature. Shaded peaks were isolated for further identification.

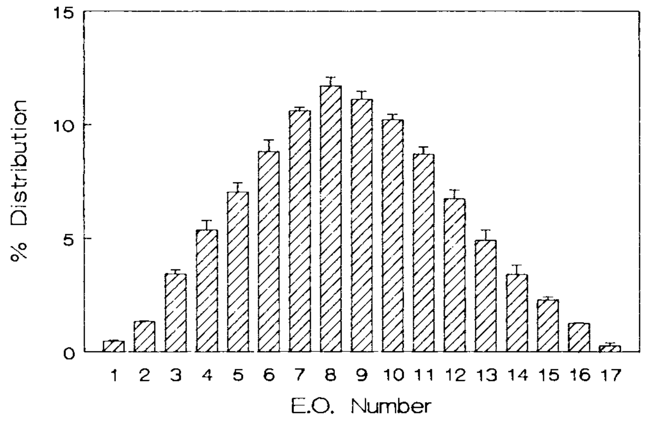

Fig. 3. Nonoxynol-9 oligomer distribution.

gradient elution and UV detection at 280 nm showed that at least 17 oligomers were present in the N-9 mixture (Fig. 2). These followed a Poisson distribution around the most abundant peak eluting at 45.6 min, (11.7%, Table I). As a good separation could be achieved, pure oligomers could be isolated and identified by various spectroscopic techniques. Thus, the most abundant oligomer (45.6 min) and an additional five oligomers of N-9 eluting at 19.6, 34.0, 51.2, 61.6, and 79.2 min were isolated by preparative normal phase HPLC (Fig. 2). The most abundant oligomer was shown to be the oligomer containing eight EO residues (*n* = 8) based on the NMR and FAB-MS studies presented below. It is interesting to note that the EO 8 oligomer was slightly more abundant than the EO 9 for the N-9 sample analyzed. The other oligomers corresponded to the EO 3, EO 6, EO 9, EO 11, and EO 16, which contain 3, 6, 9, 11, and 16 ethylene oxide units/molecule, respectively. The remaining oligomers were assigned from their HPLC elution sequence. The composition of N-9 as a function of ethoxy units is shown in Fig. 3.

Table I. HPLC Distribution of Nonoxynol-9 and FAB-MS Data of Some EO Oligomers

			HPLC	
EO	MW calculated	M + H^+ (FAB-MS)	Retention time (min)	% abundance[a] (*n* = 3)
1	264		14.0	0.47 ± 0.04
2	308		16.4	1.33 ± 0.03
3	352	353	19.6	3.43 ± 0.18
4	396		24.0	5.37 ± 0.42
5	440		28.8	7.04 ± 0.40
6	484	485	34.0	8.81 ± 0.51
7	528		40.0	10.61 ± 0.15
8	572	573	45.6	11.70 ± 0.38
9	616	617	51.2	11.12 ± 0.35
10	660		56.8	10.21 ± 0.23
11	704	705	61.6	8.69 ± 0.32
12	748		66.8	6.73 ± 0.38
13	792		70.4	4.90 ± 0.46
14	836		73.6	3.40 ± 0.41
15	880		77.2	2.27 ± 0.14
16	924	925	79.2	1.25 ± 0.01
17	968		82.4	0.27 ± 0.12

[a] Percentage abundance of EO oligomers = (AUC of oligomer/total AUC) × 100.

The FAB-MS positive ion spectra of all the oligomers showed major ion clusters at M + H^+, M + Na^+, and M + K^+ consistent with the assigned molecular weights as illustrated in Fig. 4 for EO 8 (*n* = 8). The EO 8 gave a mass spectrum with major ions at *m/z* 573 (M + H^+), 595 (M + Na^+), and 611 (M + K^+). Small amounts of the EO 9 (*n* = 9) and EO 10 (*n* = 10) were present as indicated by the lower abundance ions at *m/z* 639 and 683. Analogous spectra were obtained for all the other oligomers.

^1H-NMR spectra were obtained for EO 6, EO 8, and EO 11 oligomers in $CDCl_3$. The ratio of the integral of ethylene oxide protons (δ 3.4–4.4) to that of the aromatic protons (δ 6.7–7.2) gave values of 6, 8, and 11 which were consistent

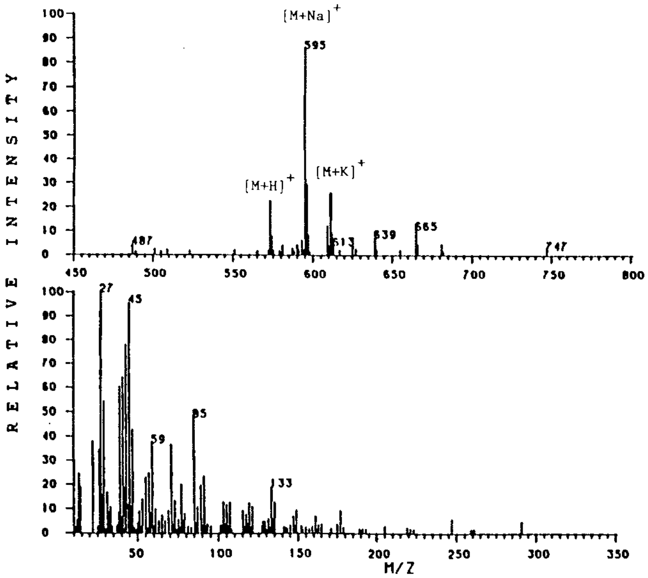

Fig. 4. FAB-MS spectrum of the EO 8 oligomer.

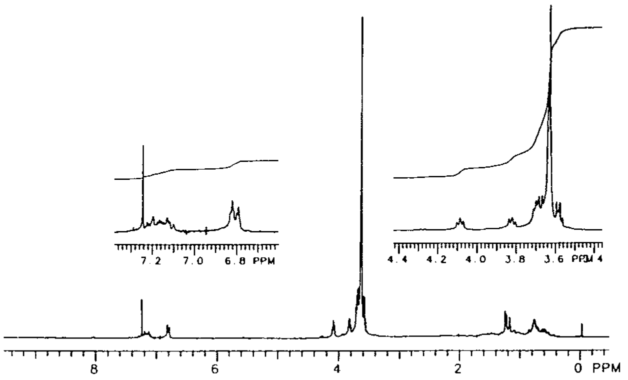

Fig. 5. ^{1}H-NMR spectrum of the EO 8 oligomer in $CDCl_3$.

with EO 6, EO 8, and EO 11 structure, respectively. A typical spectrum is shown in Fig. 5.

Summary

The commercially available Nonoxynol-9 spermicide is a multicomponent mixture of oligomers. When Nonoxynol-9 was separated by normal phase gradient HPLC, 17 components were shown to exist in the commercial mixture. These oligomeric components follow a Poisson distribution around the most abundant oligomer, EO 8 (11.7%). Select oligomers were isolated by preparative HPLC (R_t = 19.6, 34.0, 45.6, 51.2, 61.6, and 79.2 min) and purified by HPLC. These were identified by FAB-MS and NMR to be the oligomers EO 3, EO 6, EO 8, EO 9, EO 11, and EO 16, respectively.

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