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Note

Analytical high-performance liquid chromatography system for separation of components in nonoxynol-9 spermicidal agents[a]

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Extensive use of high-performance liquid chromatography (HPLC) has been made during the past decade to investigate a wide range of ethoxylated non-ionic surfactants[1–8] and anionic surfactants[1,9,10]. HPLC has been particularly valuable for analysing industrial and domestic waste water for levels and distributions of linear alkylbenzenesulphonates (LASs), alkylphenol polyethoxylates (APEOs), and nonylphenol (NP) for environmental control[11–13], for determination of non-ionic surfactants used in tertiary oil recovery[14], and for determining dodecylbenzenesulphonates and ethoxylated alkylphenols in liquid pesticide formulations[15].

Nonoxynol-9 (nonylphenoxypolyethoxyethanol, $CH_3(CH_2)_8C_6H_4(OCH_2CH_2)_nOH$), as commercially produced, is a complex non-ionic surfactant consisting of mixtures of oligomers of polyethoxylated nonylphenol. The average value of n is said to be about 9 (ref. 16), although this value appears to be more coincidental to its name than the fact its oligomers bear chiefly the nonylphenoxy terminal group as compared to the diisobutylphenoxy terminus of octoxynol-9, another widely used nonionic surfactant. In principal, any number of nonoxynol-X mixtures can be produced for each of which X represents the average number of repeating polyethyleneglycol units, which, in tern, affect the viscosity, solubility, polarity, and dispersant properties of the particular surfactant formulation[17]. In particular, nonoxynols-4, -15, and -30 are used as pharmaceutical formulating aids (surfactants)[18]. Our interest in nonoxynol-9 arises over its use as a spermicidal agent, as found in most vaginal contraceptive jellies, creams, aerosol foams and lubricated (coated) condoms sold over-the-counter in many countries including Canada. The purpose of this investigation was to characterize the claimed active spermicidal agent of a new product, a soft polyurethane vaginal contraceptive sponge containing nonoxynol-9, being prepared for introduction to the Canadian market, and to compare its chemical features with those of other commercial nonoxynol-9 raw samples.

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EXPERIMENTAL

Materials

A vaginal sponge, known as Today Vaginal Contraceptive Sponge (VLI Corp., Irvine, CA, U.S.A.) was trimmed of its string loop and soaked in methanol (200 ml, HPLC grade) with occasional agitation for 3 h after which the extract was reduced to a viscous fluid (almost 100% recovery of product claim) in a rotary evaporator under pumping vacuum. For comparison, a sample of the raw nonoxynol-9 (Lot RM-1065-22), as used in manufacture of the Today sponge, was obtained from the VLI Corp. and samples of raw nonoxynol-9 were obtained from Ortho Pharmaceutical (Canada), Don Mills, Ontario, Canada (Lot T1623) and from Rougier, Chambley, Quebec, Canada (Lot 11A30RR). All materials were examined by ^{1}H NMR spectroscopy and by HPLC analysis.

Instrumentation

HPLC analyses of the raw nonoxynol-9 samples, as well as that extracted from the vaginal sponge, were performed with a Chromatography Sciences Company silica S5W 5-μm column (25 cm × 4.6 mm I.D.) employing an ethyl acetate–methanol (1:1, v/v) mobile phase at a flow-rate of 1 ml/min. A Spectra-Physics SP8000B liquid chromatograph was used with a Schoeffel 770 UV–VIS detector at 280 nm.

^{1}H NMR spectra were obtained at 80 MHz and ambient temperature (22°C) from the nonoxynol-9 samples in acetone-d_6 (containing 1% tetramethylsilane internal reference) using a Bruker WP-80 spectrometer.

RESULTS AND DISCUSSION

^{1}H NMR spectral analysis

Acetone-d_6 was found to be the solvent which resulted in the richest ^{1}H NMR spectra (Fig. 1) of the nonoxynol-9 samples, and all samples, including the material extracted from the sponge, gave essentially identical proton spectra. By this analysis, no other component appeared to have been extracted from the urethane sponge. Exchange with ^2H_2O resulted in simplification of the bands near δ 3.6 and suggests that the downfield peaks seen here may arise from the various oligomer hydroxy protons. The integration is supportive of the gross structural features, *i.e.*, on the basis of the low field pattern (δ 7) arising from 4 aromatic protons, the principal band near δ 3.6 accounts surprisingly well for the 36 protons of the archetypal polyethoxy group $(OCH_2CH_2)_n$ where $\bar{n} = 9$, and likewise the integral for the two high-field patterns accounts for the 19 protons of the *para*-nonyl substituent. In dimethyl sulfoxide-d_6 (DMSO-d_6), the sponge extract showed somewhat similar proton spectral features found in acetone-d_6 except for some loss of band character, probably due to increased viscosity of the solution. In ^2H_2O solution (also at ambient temperature), the proton spectrum of the sponge extract suffered serious loss of band character, probably due to the formation of micellar colloids.

HPLC analysis

The chromatograms obtained from analysis of the sponge extract and the three raw nonoxynol-9 samples are shown in Fig. 2. Both the sponge extract and the Rougier

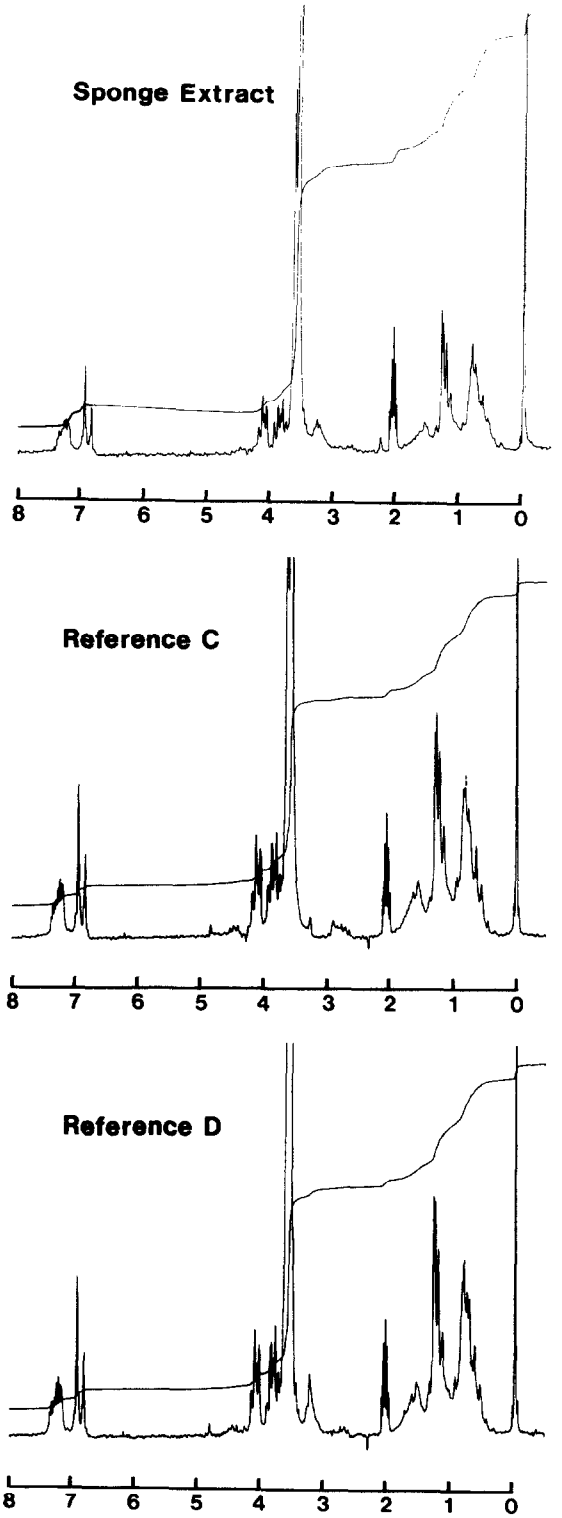

Fig. 1. ^{1}H NMR spectra (80 MHz) of nonoxynol-9 extracted from the sponge (top) and of reference materials C (Rougier, Lot 11A30RR) and D (Ortho, Lot T1623) in acetone, d_6. Chemical shift scale B in ppm.

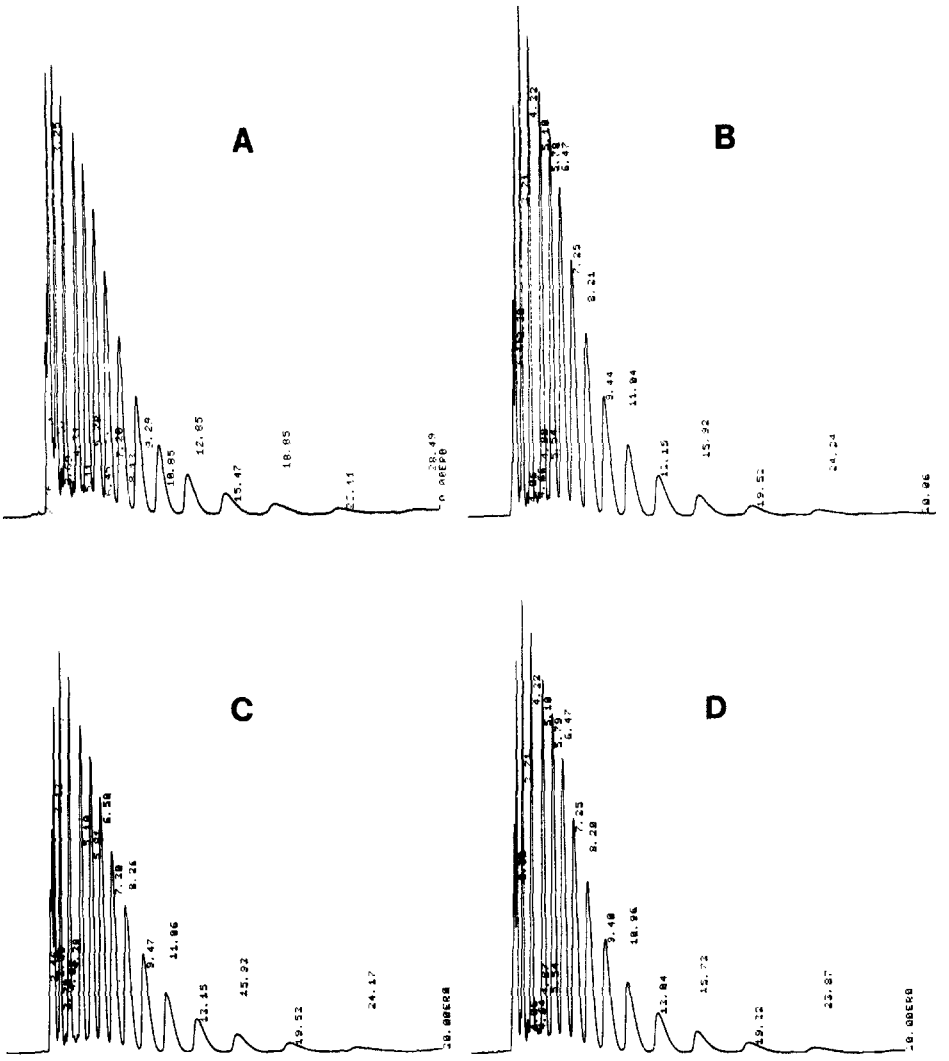

Fig. 2. Chromatograms of nonoxynol-9. (A) Extracted from the Today Vaginal Contraceptive Sponge, (B) raw material (Lot RM-1065-22) used by VLI Corp. for the Today Sponge, (C) Rougier raw material (Lot 11A30RR), and (D) Ortho raw material (Lot T1623). Elution times for individual peaks are given in min.

material showed 17 component peaks, whereas 16 peaks were seen for the raw VLI and the Ortho nonoxynol-9 samples. In each instance, the last component to be eluted was minute (peak 17 for the sponge extract and Rougier material, and peak 16 for the raw VLI and Ortho materials). Interestingly, both the sponge extract and the raw Rougier nonoxynol-9 showed an extra initial peak at the beginning of their chromatograms that was not seen in the chromatograms for the raw VLI and Ortho samples. Apart from these small differences, the four chromatograms showed remarkable similarity in overall profile and retention times, indicative of clean extraction of nonoxynol-9 from

the sponge with no apparent carryover of other components from the urethane material.

The forward phase (normal-phase) non-gradient HPLC system used to obtain the chromatograms shown in Fig. 2, with near baseline separation, constitutes a simpler and faster HPLC procedure for characterizing the oligomeric composition of nonoxynol-9 than reported in the literature to date. While Schreuder and Martijn[15] obtained excellent baseline separation of the oligomers of an ICI (U.K.) ethoxylated nonylphenol (NPEO) with an ethoxylation degree ($\bar{n}$) of 8.5, their procedures required double the time (> 50 min) at the same flow-rate of 1.0 ml/min using an aminopropyl-modified silica column (Hypersil APS, 250 mm × 4.6 mm I.D.) with a linear gradient of propan-2-ol–water (90:10) in a mobile solution of hexane–tetrahydrofuran (70:30) with increased ratio of the former to the latter from 0.05 at time 0 to 0.5 in 60 min. Similarly, Marcomini and Giger[12] separated Marlophen 810, containing NPEO oligomers with an average of 11 and a range of 1–18 ethoxy units, over a 30-min interval using an aminosilica 3-μm column (Hypersil APS, 100 mm × 4 mm I.D.) employing initially a 2-min elution of 100% *n*-hexane–2-propanol mixture (H/IP, 98/2) followed by a 25-min linear elution gradient leading to "50% H/IP (98/2) and 50% H/IP (98/2)" (erroneously reported — a more polar second solvent mix would have to have been used), at a flow-rate of 1.5 ml/min. Using the same normal-phase HPLC system, Marcomini and Giger[12], however, were able to separate 16 components of an NPEO in about 20 min from an unidentified granular laundry detergent in a chromatogram whose peaks were better resolved than those obtained from Marlophen 810, which they had used as an NPEO standard mixture.

In our HPLC analysis of the nonoxynol-9 spermicidal materials, UV detection was effected at 280 nm, very close to the absorption at 277 nm employed by Marcomini and Giger[12] for normal-phase HPLC analyses, because the response factors of NPEO and nonylphenol (NP) were available from the literature[19]. For investigation of more complex detergent systems, Marcomini and Giger[12] used UV at 225 nm to benefit from about a five-fold increase in intensity of absorption by APEOs and NP in the presence of LASs. Schreuder and Martijn[15] also employed UV detection at 225 nm; however, polyethylene glycol (PEG), having no absorbance at 225 nm, was not detected. For HPLC analysis of any of the PEGs, refractometric detection is required as demonstrated by Zeman[2]. We chose, instead, to characterize the oligomers, separated under other conditions, using preparative HPLC, by ^{1}H NMR and MS[20].

In retrospect, simpler, direct forward phase HPLC systems employing silica columns for analysis and separation of APEO preparations were undoubtedly tried, but with little apparent success[21,22]. Subsequent analytical development for such non-ionic surfactants was directed more towards investigation of properties of modified silica columns and gradient elution systems. The better specification and consistancy of manufacture of silica columns in more recent years, together with greater exploration of solvent conditions, now make it possible to employ the simpler, direct approach of non-gradient silica HPLC analyses for these substances.

We believe that the analytical HPLC system reported herein constitutes a convenient and rapid, non-gradient method for assessing pharmaceutical non-oxynol-9 preparations.

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