PREPARATION OF [^{131}I] IODINATED NONOXYNOL-9

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SUMMARY

A novel method for the radioiodination [^{131}I] of the multicomponent non-ionic surfactant nonoxynol-9 (N-9) is described. The extent of incorporation and distribution of the ^{131}I-label into the aromatic moiety of various ethoxymer units of N-9 was determined utilizing a high pressure liquid chromatographic (HPLC) method.

Key Words: Nonoxynol-9, Nonylphenoxy (polyethoxy) ethanol, ^{131}I-iodinated, Thallic trifluoroacetate, HPLC, Ethoxymers.

INTRODUCTION

Today, the vast majority of commercially available vaginal spermicides rely on non-ionic surface active agents as their active ingredient.

Nonoxynol-9 [nonylphenoxy (polyethoxy) ethanol] (N-9) is the most widely used non-ionic surfactant in vaginal spermicides. In the past, these surfactants have been used in various pharmaceutical forms on the premise that they are not absorbed through the vaginal mucosa. Recently, however, speculation to the contrary has developed. Using commercially available ^{14}C-labeled N-9 (^{14}C-polyethylene glycol moiety) it has been suggested that considerable absorption occurs through the vaginal wall into the systemic circulation of barren (1,2) and pregnant rats (3).

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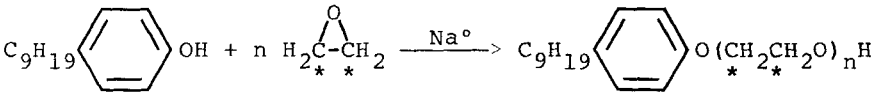
Commercially available ^{14}C-labeled N-9 is prepared by reacting one equivalent of nonyl phenol with nine equivalents of ethylene oxide [1,2-^{14}C] in the presence of a catalytic amount of sodium (4) as shown in equation 1.



*^{14}C-labeled Nonoxynol-9; avg. n=9

Equation 1

The radiochemical purity of this ^{14}C-labeled product was not previously determined since this material is not one distinct compound but is a mixture of ethoxymer species with different molecular weights (4), (i.e., variable n), and an efficient methodology for their separation was not available. Furthermore, animal studies utilizing N-9 labeled with ^{14}C in the manner described above may suffer from the fact that the relative abundance or distribution of the ethoxymer species with various molecular weights existing in commercially available N-9 (prepared in kilogram quantities), could possibly be different from the distribution of radioactivity present in a custom made ^{14}C-labeled sample of N-9 (prepared in milligram quantities).

The above potential radiochemical problems with [^{14}C] N-9, and the need to perform absorption and tissue distribution studies as a function of time by external scintigraphy (5), prompted us to develop a method for labeling the aromatic moiety of the commercially produced N-9 with a gamma emitter (^{131}I). Additionally, high pressure liquid chromatography (HPLC) methodology is reported for the separation of the different components (variable number of ethylene oxide units) in N-9 and [^{131}I] N-9. HPLC data are also presented that establish that the partition coefficient and solubility characteristics, as reflected by chromatographic mobility in a

reverse phase system, for N-9 and [^{131}I] N-9 are very similar.

EXPERIMENTAL

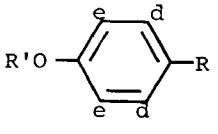
Synthesis of Cold Iodinated Nonoxynol-9

In a 250 ml Erlenmeyer flask a mixture of nonoxynol-9 (Ortho Pharmaceutical Company, Raritan, New Jersey) (12 g, 18 mmol), thallic trifluoroacetate (TTFA) (12 g, 22 mmol) and trifluoroacetic acid (TFA) (25 ml) was stirred for 30 minutes at room temperature until all the solid dissolved. To the resulting brown solution, KI (20 g, 170 mmol) in H_2O (75 ml) was added; the color turned blue then dissappeared and a yellow precipitate was formed. The reaction mixture was stirred for another 20 minutes, sodium metabisulfite (2.5 g) was added, the mixture was basified with NaOH (pH ~ 8.5), filtered and the filtrate was extracted into chloroform (4 portions of 50 ml). The chloroform layer was washed three times with water (50 ml), dried with $MgSO_4$ overnight, filtered and the solvent was evaporated to give the yellow iodinated liquid product (12 g).

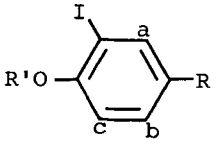
The UV spectrum of the iodinated product taken in EtOH showed a bathochromic shift accompanied with an increase in absorptivity.

UV spectrum of N-9, λ_{max}^{EtOH} 283 (ε=356), 275 (ε=437), nm; UV spectrum of iodinated N-9, λ_{max}^{EtOH} 290 (ε=541), 283 (ε=746), 275 (Sh) (ε=600), nm.

The nmr spectrum of nonoxynol-9 taken in $CDCl_3$ showed in the aromatic region an AB quartet characteristic of <u>para</u> substitution in a benzene ring. Upon iodination, the spectrum changed into two doublets ($H_{b,c}$) and one singlet (H_a) corresponding to the proton adjacent to the iodinated carbon.



Nonoxynol-9



Iodinated Nonoxynol-9

nmr spectrum of N-9 δ ($CDCl_3$): 6.90 (d, J=8Hz, $2H_d$), 7.26 (d, J=8Hz, $2H_e$).

nmr spectrum of iodinated N-9 δ($CDCl_3$): 6.90 (d, J=8Hz,$1H_b$) 7.26 (d, J=8Hz, $1H_c$), 7.70 (s, $1H_a$).

Synthesis of [^{131}I] Iodinated Nonoxynol-9

In a 50 ml Erlenmeyer flask a mixture of nonoxynol-9 (0.60 g, 0.9 mmol), TTFA (0.65 g, 1.1 mmol) and TFA (2 ml) was stirred for 30 minutes at room temperature until all the solid dissolved. To the brown solution $Na^{131}I/H_2O$ (0.25 ml, 1 mCi) was added followed by the addition of cold KI/H_2O (1.0 g, 8.5 mmol/10 ml): the color turned blue then dissappeared and a yellow solid precipitated. The reaction mixture was stirred for another 20 minutes, sodium metabisulfite (0.2 g) was added, the mixture was basified with NaOH (20 ml, 10% aq. solution), filtered and the filtrate was extracted into chloroform (3 portions of 15 ml). The chloroform layer was then washed thoroughly with water (several portions of 15 ml) until no radioactivity could be detected in the washings, dried over $MgSO_4$ overnight, filtered, and the solvent was evaporated by a stream of nitrogen yielding the yellow iodinated product as a viscous liquid. Yield, 0.5 g; radiochemical yield, 400 μCi (~40%). An autoradiogram (TLC, silica gel) of the product against $K^{131}I$ (developed in a mixture of benzene:ethanol 3:1) was taken and it was shown that the product (Rf = 0.62) was not contaminated with $K^{131}I$ (Rf = 0.25).

The radiochemical yield in a typical reaction run was found to be about 40% leading to a product with a specific activity of 800 μCi/g. Further chemical characterization of the [^{131}I] iodinated N-9 was obtained by HPLC (see details below). The data obtained indicated that the distribution of radioactivity in the iodinated N-9 sample (Fig. 2) was similar to the ethoxymer distribution of the non-labeled N-9 (Fig. 1). This showed that the

radionuclide [^{131}I] was introduced into the nonyl phenol moiety of each of the ethoxymer species constituting commercially available N-9.

HPLC Separation of Ethoxymer Units in N-9

The method utilized was basically similar to that developed by Rothman (6) for the determination of the ethoxymer distribution in Triton-X.

All HPLC work was performed at ambient temperature using a mini-pump (The Munhall Co., Ohio, U.S.A.), a Beckman Model 153 Analytical U.V. Detector at 280 nm and a DuPont Zorbax-NH_2 6 µm (25 cm x 4.6 mm) analytical column and an in-house packed LiChrosorb NH_2 10-µm guard (5 cm x 4.6 mm) in series. Injection volumes were 25 µl. The chromatography of N-9 and iodinated N-9 was accomplished by linear gradient using an LKB 11300 Ultro-grad Gradient Mixer. Time of linear gradient = 30 minutes; gradient limits: 100% A to 50% A - 50% B; where A is isooctane - methylene chloride - methanol (95:5:3), and B is isooctane-methylene chloride - methanol (60:40:7.5). All flow rates were at 2 ml/min.

Peak areas were calculated by multiplying the height of the peak by half the width at the base and the mole percent was determined by comparing these peak areas to the total area under the chromatogram; thus obtaining the relative abundance of each ethoxymer unit.

Determination of the chromatographic mobility (retention time) of the ethoxymer units in N-9 was based on the assumption that the peak with the greatest area corresponded to the species containing nine ethylene oxide units. This assumption was made by comparing a chromatogram of N-9 with a series of chromatograms of nonylphenoxy-(polyethoxy) ethanols in which the most abundant species contained 5, 6, 7 and 8 ethylene oxide units (Igepal-CO series from GAF Corporation, New Jersey). A typical HPLC chromatogram of N-9 is shown in Figure 1.

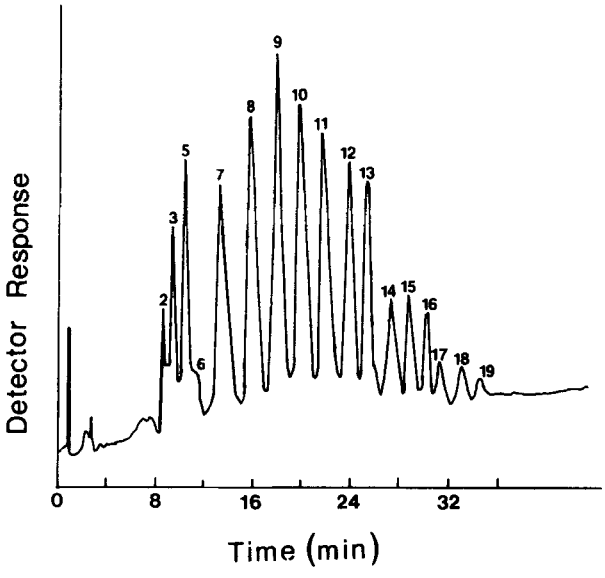


Fig. 1. HPLC chromatogram of N-9; 30-min linear gradient, 100% A to 50%A - 50%B where A=isooctane - methylene chloride - methanol (95:5:3); B=isooctane - methylene chloride - methanol (60:40:7.5); UV detector at 280 nm. (numbers 1-19 denote the number of ethylene oxide units in each molecular species of N-9)

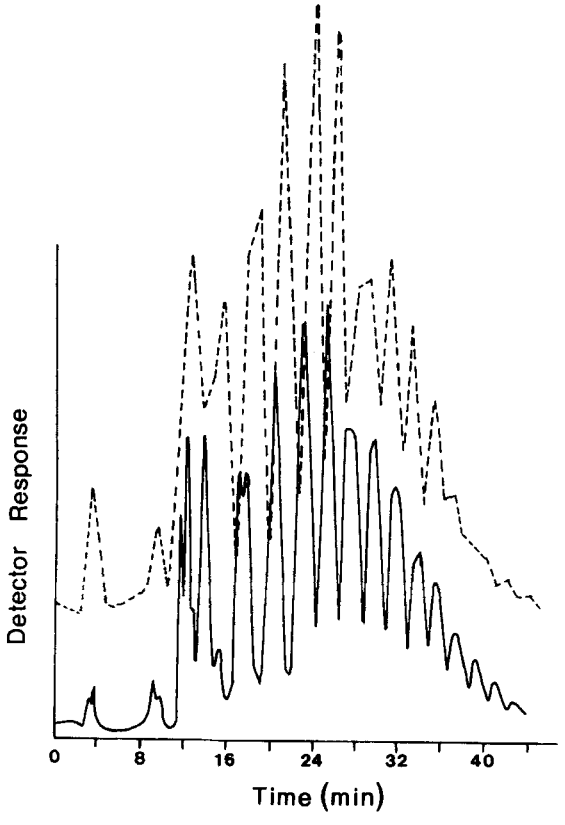


Fig. 2. Radiomonitored HPLC chromatogram of a mixture of N-9 and ^{131}I-iodinated N-9 (same experimental conditions as in Fig. 1); (a) ———————— UV detector (at 280 nm) (b) ---------- γ Detector (counts per 50 sec.).

Determination of Radioactivity Distribution in the Various Ethoxymer Units of [^{131}I] Iodinated N-9 by HPLC.

HPLC solutions were prepared by mixing N-9 (150 mg) and [^{131}I] iodinated N-9 (40 μCi) in 10 ml of methylene chloride. Injection volumes were 25 μl. The HPLC conditions described above for the separation of the various ethoxymer units were employed. Sixty one-minute fractions of the effluent were collected and counted for radioactivity in a gamma counter (Packard Model 9012 Auto-Gamma Scintillation Spectrometer). A radiochromatogram (Figure 2b) was constructed and superimposed on the HPLC UV response obtained for the same injection sample (Fig. 2a).

Assessment of Partition Characteristics of N-9 and Iodinated N-9 by Reverse-Phase HPLC.

A reverse-phase HPLC system was used with a Waters μ-Bondapak C_{18} column (30 cm x 3.9 mm), at 2.0 ml/min and ambient temperature using 80/20 MeCN/H_2O as the mobile phase. Detection was accomplished with a UV detector at 280 nm. Analysis of the composition of the iodinated N-9 using the above reverse phase system at both 254 and 280 nm indicated the presence of about 20% unreacted nonoxynol-9. As expected the iodinated nonoxynol-9 eluted after nonoxynol-9, however, only a small difference in retention times between the two peaks was observed (retention times for N-9 and iodinated N-9 in MeCN/H_2O were 3.4 and 4.4 minutes respectively). The capacity factors (k') for N-9 and iodinated N-9 were calculated and found to be 3.3 and 4.5 respectively. These capacity factors were taken to be a valid indication of the partition coefficients of these compounds (7).

RESULTS AND DISCUSSION

Commercially available nonoxynol-9 (N-9) is not a single compound but a mixture of several molecular species that result in the course of reacting ethylene oxide with nonyl phenol as shown by equation 1 and figure 1. Recently, Rothman (6) has shown, using HPLC techniques,

that Triton-X [octylphenoxy (polyethoxy) ethanol], which is very similar in composition to N-9, is a mixture of ethoxymer species with different molecular weights that follows a Poisson distribution.

The presence of aliphatic and aromatic ether linkages and a primary alcohol function in N-9 provide some challenge to the iodination of its aromatic ring. Thus, in our hands, attempts to iodinate N-9 by reacting it with chloramine T and potassium iodide (KI), as well as with iodine monochloride (ICl) and NaI, led to poor or no incorporation of the radionuclide. Apparently, heating under strongly acidic conditions leads to facile cleavage of the ethereal linkage(s) in N-9.

The iodination of N-9 was accomplished using thallic trifluoroacetate (TTFA) and KI. Data from reverse-phase HPLC indicated that the product consisted of 80% monoiodinated N-9 and 20% unreacted starting material. This reaction utilizes <u>in situ</u> formation of aryl-thallium difluoroacetate and is known to proceed in high yields in aqueous solutions at room temeprature (8) according to the following equations:

a) $ArH + \underset{(TTFA)}{Tl(OCOCF_3)_3} \xrightarrow{CF_3COOH} ArTl(OCOCF_3)_2 + CF_3COOH$

b) $ArTl(OCOCF_3)_2 + 2KI \longrightarrow ArI + 2K^+ \ ^-OCOCF_3 + TlI$

Using the synthetic approach described above, [^{131}I]-iodinated N-9 was synthesized in 40% radiochemical yield.

The identity of the iodinated N-9 was supported by a bathochromic shift in its UV spectrum and the appearance of two doublets and one singlet in the aromatic region of the nmr spectrum (see experimental section for details). The nmr spectrum tends to indicate that the iodination is predominatly <u>ortho</u> to the alkoxide substituent. This is not surprising since thallation usually occurs in the <u>ortho</u> or <u>para</u>

positions when conducted at room temperature, and in the meta position when reflux conditions are used (8). Furthermore, when the [^{131}I] iodinated N-9 was mixed with non-radioactive N-9 and subjected to radiomonitored HPLC it was shown that the distribution of the radioactivity was similar to the ethoxymer distribution of N-9 as recorded by UV detection, (See Fig. 2). This indicates that the radionuclide [^{131}I] was introduced into the nonyl phenol moiety of each of the ethoxymer species constituting commercially available N-9.

A plot of mole percent versus the number of ethylene oxide units (n, equation 1) shows that the molecular species with highest abundance in N-9 is that containing nine (n=9) ethylene oxide units (Fig. 3).

Likewise, a histogram (Fig. 4) of the radioactivity distribution within the synthesized iodinated N-9 showed that the highest incorporation of the radionuclide [^{131}I] was achieved in the ethoxymer species containing nine ethylene oxide units.

In contrast to the above, ^{14}C-labeled samples of N-9 synthesized from ^{14}C-ethylene oxide and nonyl phenol (equation 1) in milligram quantities may not exhibit the highest incorporation of ^{14}C-label into the species of highest abundance (n=9) found in commercial N-9.

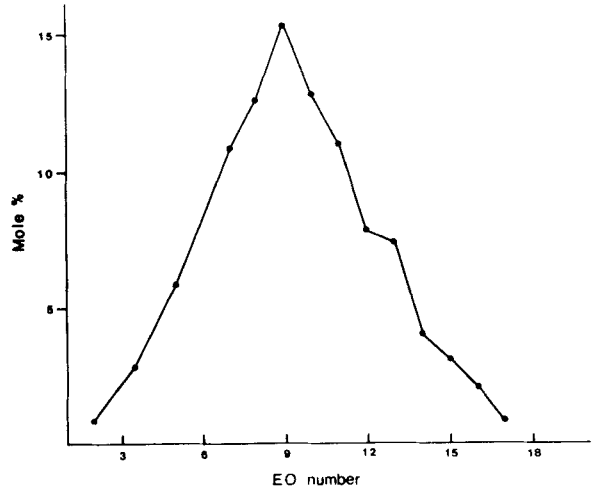


Fig. 3. HPLC distribution of ethoxymer units (EO) in N-9.

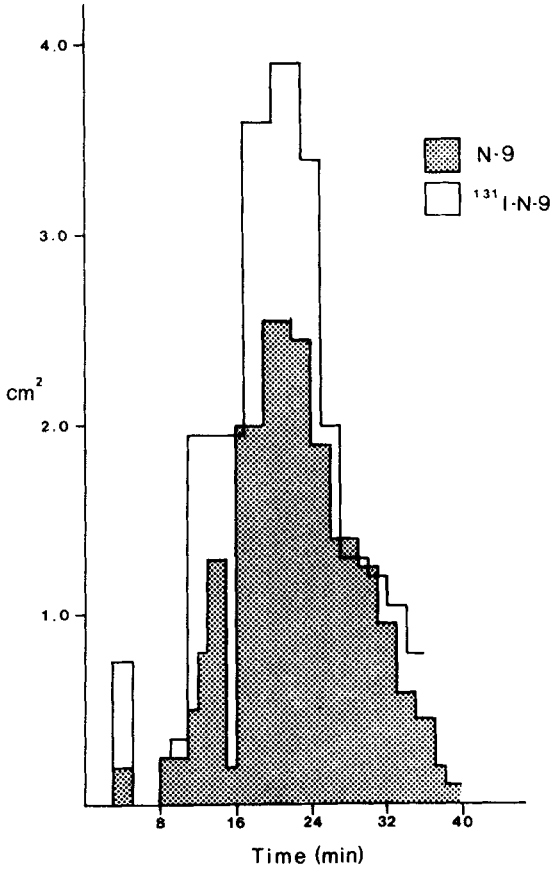


Fig. 4. A histogram of peak areas (see Fig. 1) as a function of chromatographic retention times; N-9 by UV detector at 280 nm and ^{131}I-N-9 by γ detector.

The similarity in the solubility and partition coefficient between N-9 and iodinated N-9, as gauged by their close retention times in reversed-phase HPLC in two chromatographic elution systems, suggest that iodinated N-9 would be a good model compound for studying, by external scintigraphic techniques, the in vivo disposition of N-9 from vaginal administration. This is particularly important since preliminary data from our laboratories (9) indicate that no deiodination of [^{131}I] iodinated N-9 occurs in vivo 24 h after vaginal administration of the compound in rats as evidenced by the fact that no iodine-131 uptake was observed in the thyroid.

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