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SEPARATION OF TRITON X-100 AND SIMILAR MIXTURES INTO COMPONENT OLIGOMERS BY HIGH-PRESSURE LIQUID CHROMATOGRAPHY

C. FREEMAN ALLEN and LESLIE I. RICE
Department of Chemistry, Pomona College, Claremont, Calif. 91711 (U.S.A.)
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SUMMARY

A relatively simple liquid chromatographic method for the isolation of individual oligomers of Triton X-100 (Reg. No. 9004-88-0) and similar polyoxyethylene-based surfactants has been developed. The method has been used for separations of mixtures containing between a few milligrams and a few grams of oligomers with up to eighteen oxyethylene subunits on silicic acid type adsorbent using acetic acid–water–ethyl acetate eluents.

INTRODUCTION

A number of biological studies have been carried out using Triton X-100 and other commercial surfactants which are mixed oligomers of polyoxyethylated phenols or alcohols. In some cases it has been of interest to determine whether individual oligomers of these or other surfactants with modified hydrophobic groups might be more effective or display altered activity in these studies. In this paper a procedure is described for separating individual oligomers from such surfactant mixtures (and their tritiated derivatives) by high-pressure liquid chromatography (HPLC).

Triton X-100 is predominantly a mixture of *p-tert.*-octylphenoxy-polyethoxyethanol oligomers (α-[4-(1,1,3,3-tetramethylbutyl)phenyl]-ω-hydroxy poly[oxy-1,2-ethanediyl]) which are abbreviated here as *p-tert.*-OPE$_n$, where n refers to the number of ethylene oxide units attached to the alkylphenol during polymerization. The oligomers are present in approximately Poisson distribution, with $n_{av.}$ of about 9.5. Similar products with other $n_{av.}$ values are commercially available or can be readily prepared by reaction of an appropriate phenol or alcohol with ethylene oxide[1].

Individual oligomers of ^{3}H-labeled Triton X-100, with n up to sixteen, were required for biological studies. Mansfield and Locke[2] have synthesized multi-gram amounts of the first ten members of the *p-tert.*-OPE$_n$ series, but for isolation of small amounts of several oligomers, chromatographic fractionation of Triton preparations offers the multiple advantage of conserving radioactive material and requiring less effort, especially for higher members of the series which are especially tedious to synthesize.

By the use of HPLC on silicic acid adsorbents it has been possible to isolate individual oligomers of Triton with n up to sixteen and more without the use of large columns, and more conveniently than by chromatographic methods previously reported[3–5].

EXPERIMENTAL

Chromatography was carried out in an ALC 201 liquid chromatograph (Waters Ass., Milford, Mass., U.S.A.) fitted with sapphire pistons resistant to corrosive effects of aqueous acetic acid. The adsorbent was Porasil A(60), 37–75 μm (Waters Ass.). Preparative columns ($^3/_8$ in. × 8 ft.) were loaded with up to 4 g of Triton, and analytical columns ($^1/_8$ in. × 2 ft.) were used with up to about 30 mg of Triton.

A linear gradient of ethyl acetate–acetic acid–water (100:32:30) (solution A) against ethyl acetate was used as eluent in the preliminary fractionation of Triton mixtures, and various ratios of ethyl acetate, acetic acid, and water were used for purification of individual *p-tert.*-OPE$_n$ oligomers. Triton concentration in the eluate was followed by absorption at 280 nm using a Model UA-2 ultraviolet (UV) analyzer (Instrument Specialties, Lincoln, Nebr., U.S.A.) with a 2-mm flow cell, or by use of a Techtron 635 Series UV–visible spectrophotometer with an 8 μl flow cell (Varian, Palo Alto, Calif., U.S.A.). A Waters differential refractometer was also used in preparative work, or with compounds which do not absorb adequately in the UV range. The solvent flow-rate was 6 ml/min for the larger diameter column and 0.5 ml/min for the analytical column. The temperature was ambient. Ethyl acetate (99% pure, from Ashland, Santa Fe Springs, Calif., U.S.A.) and acetic acid, reagent grade, were distilled before use. Columns were washed between runs with a 1:1 mixture of acetic acid and water deaerated by boiling and cooled to room temperature before use.

Small-scale reversed-phase separations were carried out on a $^1/_8$-in. × 2-ft. column of Vydac Reverse Phase, 30–44 μm (Applied Science Labs., State College, Pa., U.S.A.).

Thin-layer chromatography (TLC) was carried out on pre-coated silica gel G plates without fluorescent indicator (E. Merck, Darmstadt, G.F.R.) with varying ratios of ethyl acetate, acetic acid and water[6] depending on the polarity of the substrates (140:32:30, 2 cycles, for $n \geqslant 13$; 140:32:30, 1 cycle, for $n = 7$–12; and 140:8:8, for $n \leqslant 7$). The *p-tert.*-OPE$_n$'s were visualized under UV light after spraying with a 0.003% solution of rhodamine 6G in 1 *M* NaOH (ref. 7) or with Dragendorff reagent.

Triton X-100 ($n_{av.} = 9.5$), Triton X-114 ($n_{av.} = 7$–8), and Triton X-165 ($n_{av.} = 16$) (Rohm & Haas, Philadelphia, Pa., U.S.A.) were used, as appropriate, for sources of unlabeled oligomers. Triton X-100 was labeled in the aromatic ring to a specific activity of 472 μCi/mg by the tritium gas exposure method of Wilzbach[8] (New England Nuclear, Boston, Mass., U.S.A.) and used for separation of labeled oligomers.

Authentic *p-tert.*-OPE$_4$ was prepared by condensation of recrystallized *p-tert.*-octylphenol with tetraethylene glycol monochloride as described by Mansfield and Locke[2].

RESULTS

Preliminary fractionation of Triton X-100, using gradient elution, resolved 22 peaks (Fig. 1). TLC of some of the fractions of such an experiment is shown in Fig. 2. TLC was used to detect impurities in individual fractions and to identify the n values of oligomers by comparing their R_F values to that of the synthetic standard oligomer of $n = 4$. Purified fractions obtained from Triton could then be used as standards, since oligomers with increasing n appeared in sequence with decreasing R_F values.

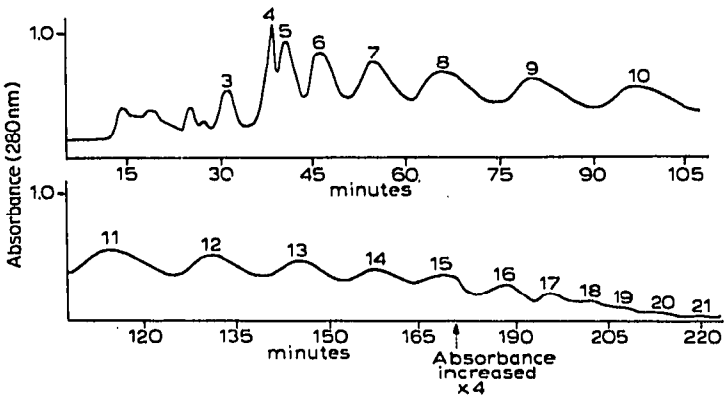

Fig. 1. Liquid chromatography of Triton X-100 (1 g) on 3/8-in. Porasil column. Solvent, linear gradient of ethyl acetate–acetic acid–water (100:32:30) (600 ml) against ethyl acetate (600 ml). Flow-rate, 6 ml/min. Numbers above peaks indicate the n-values of oligomers.

Acetate esters of Triton oligomers are slowly formed via transesterification in the acidic eluents, and prolonged exposure of the oligomers to these solvents should be avoided. The esters have a mobility in the TLC system used similar to the oligomer with three fewer oxyethylene units. (Traces of such esters are present in samples 7, 9, 10, and 11 in Fig. 2).

Fractions from several preliminary chromatograms of 1–4 g of Triton were combined following comparison by TLC and re-chromatographed on the preparative Porasil column to separate the major component from adjacent oligomers. Solvents used were as follows: Ethyl acetate ($n = 3$–5), ethyl acetate–solution A (3:1) ($n =$ 6–8), ethyl acetate–solution A (2:1) ($n = 9$–10), ethyl acetate–solution A (1:1) ($n =$ 11–13), and solution A ($n = 14$–18).

Successive stages in the purification of the $n = 7$ oligomer are shown in Fig. 3. As this figure indicates, a significant amount of material is eluted from the Porasil only after extensive washing of the column with polar eluents. As a result, two cycles of re-chromatography were necessary to bring homogeneity of a given oligomer to $> 97\%$ as judged by relative peak areas and TLC.

The isomeric homogeneity of the oligomers separated by chromatography will reflect the composition of the phenol used in its preparation. Thus, preparation of the pure *p-tert.*-octyl isomer would require starting with pure *p-tert.*-octylphenol. Commercial Tritons contain small amounts of other isomers and perhaps some

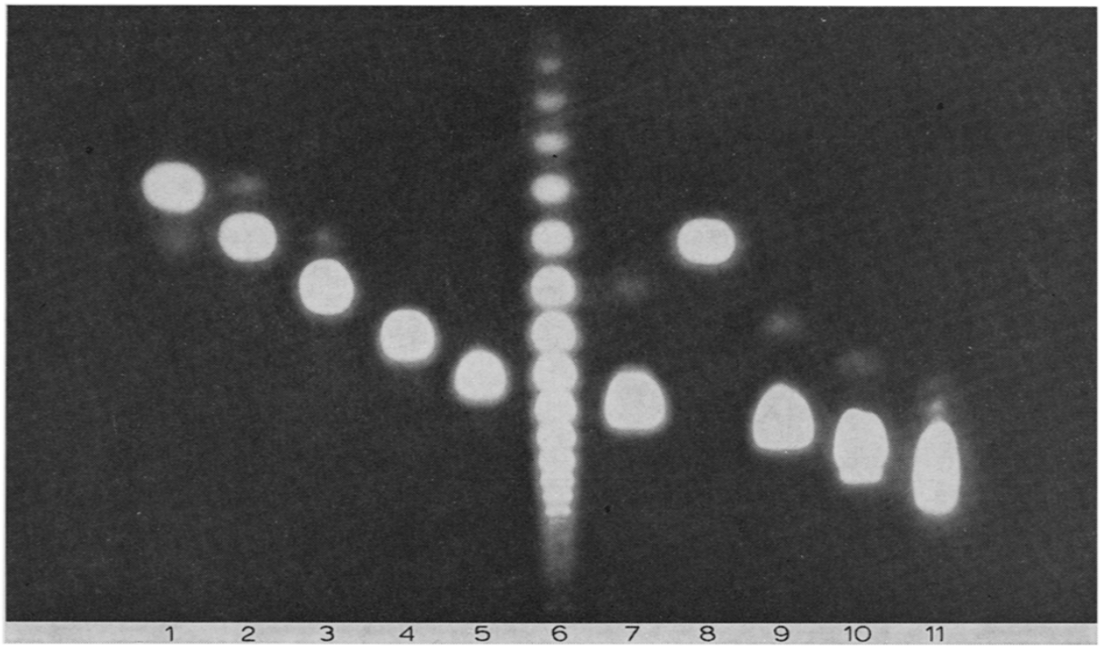

Fig. 2. Thin-layer chromatogram of fractions from a separation of Triton X-100 oligomers as in Fig. 1. Samples: 1–5, n = 7–11 oligomers, respectively; 6, Triton X-100; 7, n = 12 oligomer; 8, n = 8 standard oligomer; 9–11, n = 13–15 oligomers. Solvent, ethyl acetate–acetic acid–water (140:32:30).

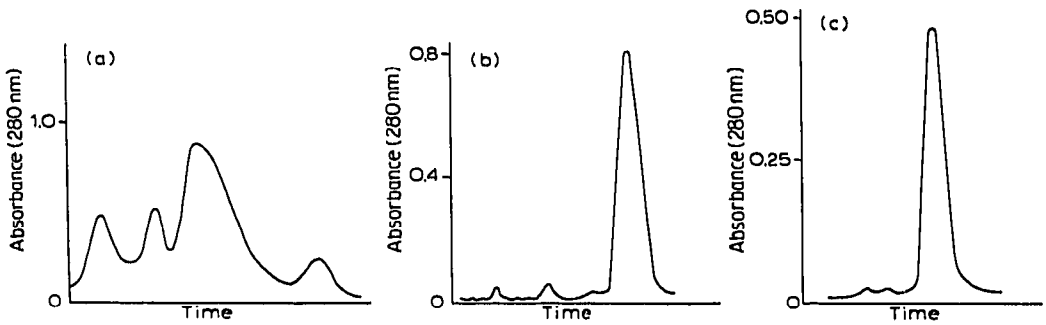

Fig. 3. Purification of n = 7 oligomer by two-stage re-chromatography on Porasil A(60) with ethyl acetate–solution A (2:1). (a) n = 7 oligomer as prepared in Fig. 1; (b) major peak in Fig. 3a; (c) major peak in Fig. 3b.

species with other than eight carbons in the aliphatic residues. Polyethylene glycols may also be present in small amounts.

Reversed-phase chromatography of the purified n = 7 oligomer using 55% methanol as eluent further resolved the isomeric mixture as expected. Three peaks preceding the major component contained in total less than 0.5% of the mixture.

Duplicate elemental analyses of the n = 7 product (Fig. 3c) indicate the composition to be 63.16% C, 9.58% H (calculated values for $C_{28}H_{50}O_8$: 65.30% C,

9.79% H). Nuclear magnetic resonance analysis[9] in $CDCl_3$ solution with a 220 MHz spectrophotometer at 20° indicated a ratio for *tert.*-butyl protons:methyl groups on benzylic carbon:*n* of 9.0:1.97:7.08 (theory, 9:2:7).

The above method has been used on similar mixtures of oligomers prepared in our laboratory from chemically pure alcohols and phenols. This approach is more convenient for obtaining structurally homogeneous oligomers than is separation of individual isomers from a given oligomer derived from impure starting materials.

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