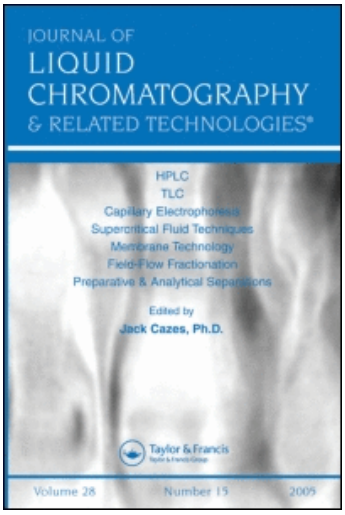

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HPLC Analysis of Oligomeric Ethylene Glyccl Mixtures Via *bis*(2,4-Dinitrophenylation)

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HPLC ANALYSIS OF OLIGOMERIC ETHYLENE GLYCOL MIXTURES VIA BIS(2,4-DINITROPHENYLATION).

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ABSTRACT

Bis(2,4-dinitrophenylation) of oligomeric ethylene glycols of the formula $HO-(CH_2CH_2O)_n-H$ (n=4-16)to the corresponding DNP-$O(CH_2CH_2-O)_n$-DNP (where DNP stands for 2,4-dinitrophenyl) provides chromophoric derivatives, which are separated chromatographically on HPLC column.

The bis(2,4-dinitrophenyl) glycols are stable in presence of triethylamine, but undergo ethanolysis in presence of hydroxide ions. The quantitative removal of the DNP groups allows an integrated scheme to pure glycols from commercially available polyethylene glycol mixtures, by bis(2,4-dinitrophenylation), chromatographic separation, end-group removal, using HPLC of the bis-(2,4-dinitrophenyl) glycols for purity monitoring.

INTRODUCTION

Ethylene glycol oligomers are important starting materials in the synthesis of macrocyclic ethers (1-5). Their special solubility and chain folding properties are extensively studied (6-8). Considerable attention is also given to the use of oligoglycols as liquid polymeric supports in automatic liquid phase peptide synthesis (9).

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Our interest in facile synthetic methods to pure glycols has risen in the course of studies on the synthesis of polymeric crown (10) and pseudocrown ethers (11). This has also led us to develop a highly sensitive and accurate analytical method based on conversion of the glycols to bis(2,4-dinitrophenyl derivatives) (12), coupled with High Performance Liquid Chromatography Analysis (HPLC), which is reported in this paper.

Synthesis of Bis(2,4-Dinitrophenyl) Glycols: Chromophoric Ligands for HPLC Analysis.

The bis(2,4-dinitrophenylation) of glycols converts the water-soluble glycols, to hydrophobic derivatives incorporating high-extinction coefficient chromophores, which can be readily resolved by chromatographic methods using spectroscopic monitoring. The synthesis of bis(2,4-dinitrophenyl) glycols (abbreviated bis-DNP-glycols), the product composition are to be described elsewhere (13). The lower glycols (tri-to-penta glycol) yield exclusively the bis-DNP derivatives after 24 hours reaction. The reactivity of the glycol decreases as chain length increases, and longer reaction times are needed to convert the higher glycols to the bis-DNP derivatives.

High Performance Liquid Chromatography Analysis of Polyethylene Glycol Oligomer Mixtures.

The resolution of a prepared mixture of tetra, penta, hexa, hepta and octa glycols (as the bis-2,4-dinitrophenyl derivatives)

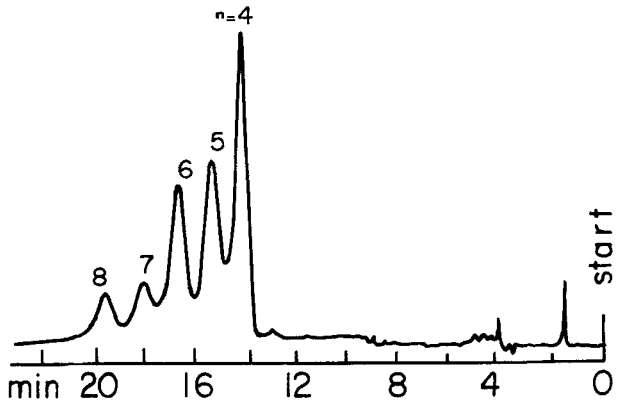


FIGURE 1

HPLC analysis of a prepared mixture of pure bis(2,4-dinitrophenyl) derivatives of tetra to octa glycol.

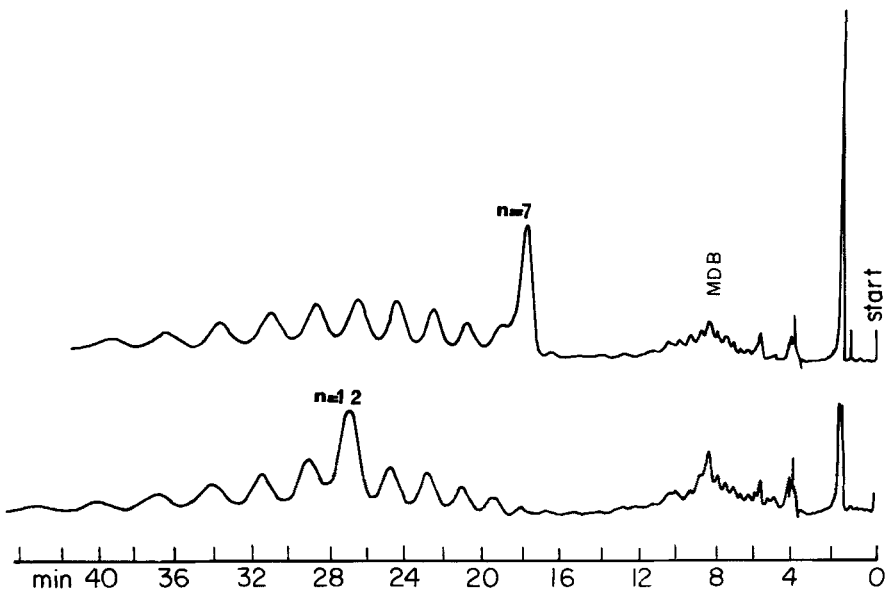


FIGURE 2

HPLC analysis of a mixture of bis(2,4-dinitrophenyl)polyethylene glycol 600, spiked with: (a) bis(2,4-dinitrophenyl)hepta glycol and (b) bis(2,4-dinitrophenyl)octa glycol.

on a nucleosil C_6H_5 (7μm) column is shown in Fig. 1. Next, the chromatogram of the bis(2,4-dinitrophenylated) mixture of polyethylene glycol (PEG)-600 is shown in Fig. 2. Bis(2,4-dinitrophenyl) heptaethylene glycol and bis(2,4-dinitrophenyl) dodecaethylene glycol were added as internal markers.

Under the above conditions, resolution between the bis(2,4-dinitrophenyl) derivatives of ethylene glycols from diglycol to pentadeca glycol is possible. The resolution of crude bis(2,4-dinitrophenylated) mixtures of polyethylene glycols (product of Fluka, Switzerland) are shown in Fig. 3. (Fig. 3A - PEG-200; Fig. 3B - PEG-300; Fig. 3C - PEG-400; Fig. 3D - PEG-600). Each chromatogram contains 2,4-dinitrophenol, 2,4-dinitrofluorobenzene (2,4 DNFB, R_t=6.0min), then a band of closely packed mono (2,4-dinitrophenyl) glycol homologes (abbreviated MDB, for mono-DNP-BAND). Then a band of the bis(2,4-dinitrophenyl) glycols, assigned by numerals identical with the integer n in $HO(CH_2CH_2O-)_{\overline{n}}H$.

The identification of the components in Fig. 3 was through comparison with prepared mixtures of the pure glycols, such as a mixture of tetra to octa glycols shown in Fig. 1. Alternatively, samples were spiked with a known component, as shown in Fig. 2.

HPLC Purity Monitoring in Chromatographic Separation of Bis(2,4-Dinitrophenyl) Oligoethylene Glycol Mixtures.

Bis(2,4-dinitrophenyl) glycols are very sensitive to nucleophilic substitution by ethoxide in the system NaOH/EtOH/$CHCl_3$ [the reaction being completed within 5 minutes at 80°C (13)], and produce a mixture of liberated glycols and 2,4-dinitrophenol. This

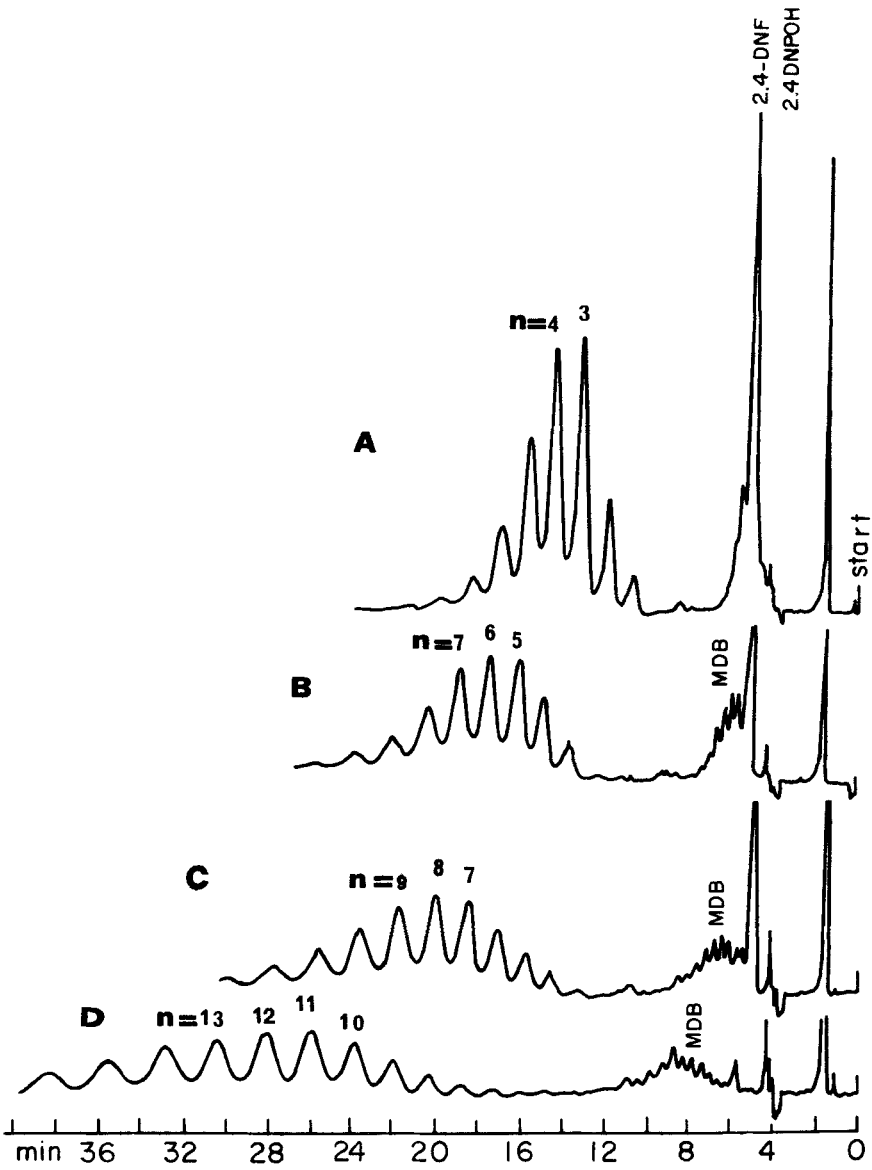


FIGURE 3

HPLC analysis of bis(2,4-dinitrophenyl) derivatives of polyethylene glycol mixtures: (a) PEG-200; (b) PEG-300; (c) PEG-400; (d) PEG-600. Numbers identify with n in $HO-(CH_2CH_2O)_n-H$.

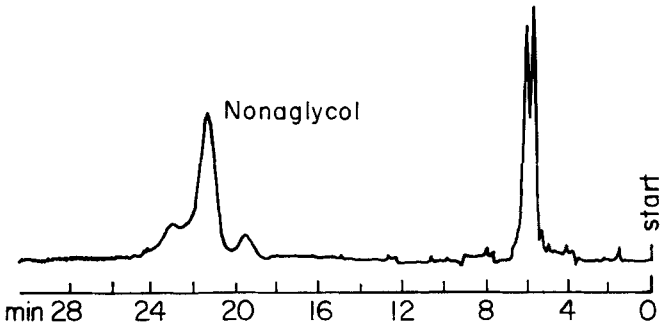


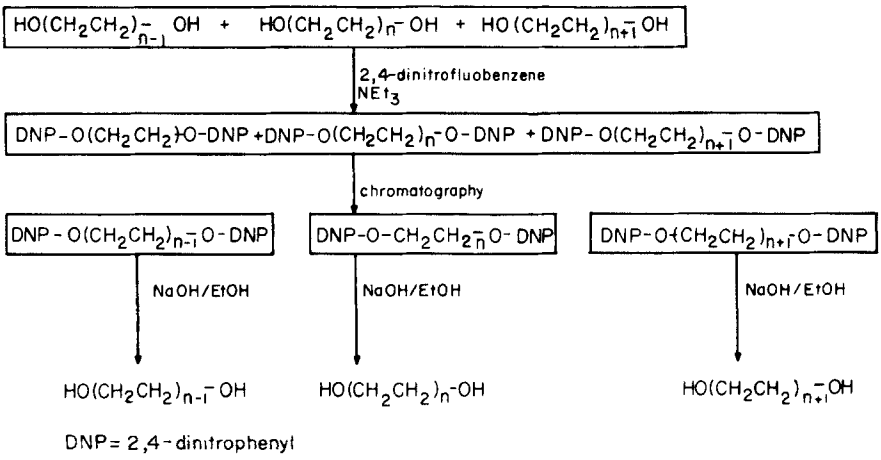
FIGURE 4

HPLC analysis of nonaglycol with octa glycol and decaglycols as impurities: PEG-300, starting material.

property allows the design of an integrated scheme leading from mixtures of polyethylene glycols, via bis-(2,4-dinitrophenylation) chromatographic separation and removal of the 2,4-dinitrophenyl protecting group to pure glycols (see scheme 1). The HPLC method described in this work allows this scheme by ensuring purity monitoring and control of the chromatographic separation procedure. From the composition of the commercial PEG-mixtures (see Fig. 3) it follows that PEG-200 is a source of tri, tetra and penta glycols, PEG-300 and PEG-400 are a source for penta to deca glycols and PEG-600 a source for octa to hexadeca glycols. Fig. 4 shows enriched nonaglycol obtained from PEG-300, and Fig. 5 shows enriched fractions of the Bis-(2,4-dinitrophenyl) derivatives of the higher glycols obtained from PEG-600 using scheme 1. Further chromatographic separation yields pure glycols.

In conclusion, in the present paper we have presented a highly sensitive HPLC method for the resolution and identification of

Scheme no. **1** : pure glycol via bis-(dinitrophenilation)of glycol mixtures, chromatographic separation and deblocking



oligoethylene glycols in synthetic samples or in their commercial admixtures, resulting from anionic polymerization of ethylene oxide. In addition, we have elaborated a method to pure oligo-ethyleneglycols (n=4-16) from commercially available polyethylene glycol mixtures by bis(2,4-dinitrophenylation) chromatographic separation and end-group removal. The HPLC analysis of the bis-DNP glycols enables purity monitoring of the glycols obtained by either route.

Making available high purity glycols will contribute to the synthesis of ultra pure crownethers, and will make evaluation of thermodynamic and biological measurements of various physical properties of crownethers more significant.

Although analysis of higher than hexadeca glycol was not attempted, the method should be applicable to higher glycols.

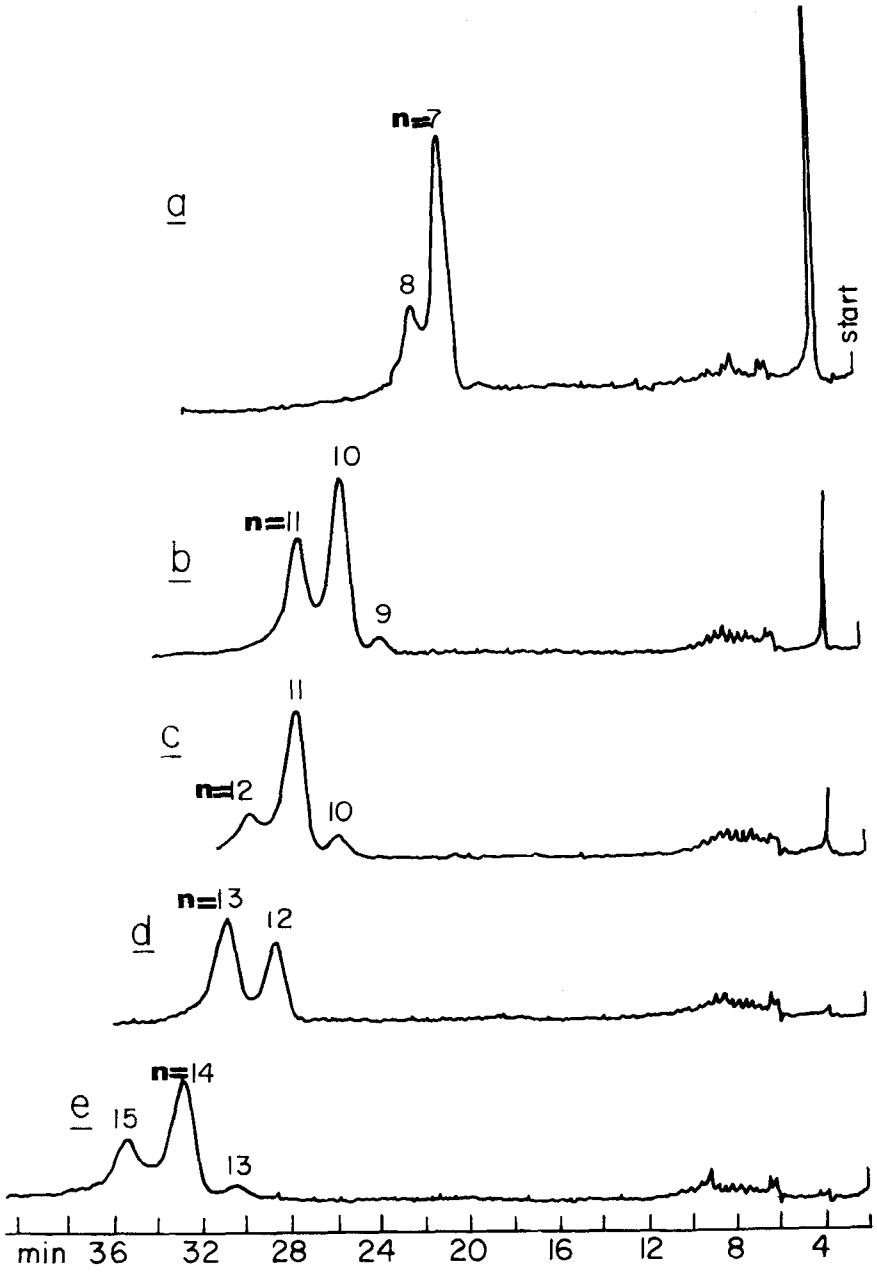


FIGURE 5

HPLC analysis of chromatographically separated bis(2,4-dinitrophenyl derivatives of: (a) hepta, octa; (b) nona, deca, undeca; (c) deca, undeca, dodeca; (d) dodeca, trideca and (e) trideca, tetradeca and pentadeca glycols. PEG-600, as starting material.

EXPERIMENTAL

Preparation of Bis(2,4-Dinitrophenyl) Derivatives.

The glycol (13.0 mmole) is dissolved in 30 ml distilled acetone, and 4.7 ml (66 mmoles) of triethylamine added, followed by 5.0 g (26.4 mmoles) of 2,4-dinitroflurobenzene (A.R). The solution is allowed to stand at room temperature for 24-48 hours in the case of the lower glycols (up to decaglycol), and up to 7 days with the higher glycols.

After evaporating the acetone, the product is taken in 100 ml $CHCl_3$, washed with 2x50 ml in HCl, and then with water to neutral pH. The solution is dried on $MgSO_4$, and the $CHCl_3$ removed by distillation. Chromatographic separation allows isolation of the bis(2,4-dinitrophenyl) derivatives in 45-76% yields (13).

High Performance Liquid Chromatography Analysis.

The HPLC analysis was performed on a Waters Associates Model 244 HPLC using a self-packed Nucleosil (~7 μm) C_6H_5 column of 250 by 4.6 mm, at room temperature. Several solvent elution systems were tried: (a) 20% dioxane in $CHCl_3$; (b) 20% dioxane + 1% isopropanol in $CHCl_3$; (c) 30% H_2O in CH_3OH. The last system was found to be superior and all the results reported here refer to the last system. Flow rate of 1 ml/min and chart speed 0.5 cm/min. Sample concentration in CH_2Cl_2: 10^{-4}M. Sample size: 5-20 μl, UV detector - 254 nm. All the HPLC chromatograms of the crude bis(2,4-dinitrophenyl) glycols contain 2,4-dinitrofluorobenzene (2,4-DNFB) and 2,4-dinitrophenol (2,4-DNPOH). In the case of the 2,4-dinitrophenylated polyethylene glycol mixtures a band corres-

ponding to the mono-2,4-dinitrophenyl derivatives is presented and is designated as MDB.

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