

Gas Chromatographic Analysis of Glycols To Determine Biodegradability

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■ The biodegradability of propylene glycol, diethylene glycol, triethylene glycol and trimethylolethane, when challenged by activated or anaerobic sludge microorganisms, was investigated. The disappearance of the substrates was monitored by gas chromatography. Glycols are industrial pollutants also found as hydrolysis products from the corresponding nitrates, which are used as military propellants. Propylene glycol was readily degraded. The initial stage of decomposition of diethylene and triethylene glycol was nonbiological, but trimethylolethane was relatively stable under the conditions tested. A gas chromatographic method was developed for direct injections of the glycols in aqueous solutions having a limit of detection in the low-ppm range. This represents a significant improvement in sensitivity over previously reported methods for aqueous solutions of glycols.

Introduction

Glycols are used extensively in industry, and their biodegradability is of great importance due to the large quantities entering the environment.

Our interest in this area stems from the fact that four nitrate esters, propylene glycol dinitrates (PGDN), diethylene glycol dinitrate (DEGN), triethylene glycol dinitrate (TEGDN), and trimethylolethane trinitrate (TMETN), as military propellants may be found in waste streams from munition plants and loading operations and would therefore be pollutants if discharged into the environment. Initial studies have shown that these esters undergo microbial transformation via successive denitration steps leading to the formation of the corresponding glycols: propylene glycol (PG), diethylene glycol (DEG), triethylene glycol (TEG), and trimethylolethane (TME) (1).

Cox (2) summarized much of the work on the biodegradation of glycols and emphasized the use of indirect methods including biological oxygen demand (BOD), chemical oxygen demand (COD), manometry, and turbidity as indices of biodegradability. Fincher and Payne (3) studied the degradation of PG, DEG, and TEG as sole carbon sources by using turbidity and manometry as evidence for growth. Haines and Alexander (4) investigated the biodegradation of DEG and TEG by using BOD as evidence of decomposition. Kawai et al. (5) reported tetraethylene glycol (10 000 ppm, µg/mL) to be biodegradable; metabolites were identified by gas chromatography/mass spectroscopy (GC/MS) after isolation by column chromatography, chloroform extraction, and derivatization (silylation). Jenkins et al. (6) showed 1000-ppm

solutions of DEG and TEG to be biodegradable by using measurements of total organic carbon (TOC) and turbidity. These results were confirmed by gas chromatography (GC) after extraction and derivatization of the residual glycols. Shumilov et al. (7) used GC to determine the concentration of DEG and TEG between 600 and 2000 ppm in waste waters.

Alternative methods for analysis of glycols have also received attention. Oxidation of glycols to aldehydes followed by derivatization using 3-methylbenzothiazol-2-one hydrazone hydrochloride was developed to analyze for DEG and TEG (8). Ponder (9) employed direct injection of hydrolysates, which contained DEG down to 500 ppm, into a GC equipped with a thermal conductivity detector.

It is the purpose of this work to assess the biodegradability of the four glycols derived from the biological transformation of the corresponding nitrate esters. Detailed study of the biodegradation of glycols at low concentrations in aqueous solutions has not been investigated because of the lack of a suitable analytical method for direct determination of the substrates in the low-ppm range. In this connection, a GC method for the direct analysis of the four glycols of interest has been developed, which allows their determination in the low-ppm range from aqueous media.

PG, DEG, and TEG present minimal toxicological problems (10) and no carcinogenicity hazard. PG is the least toxic of the glycols and is commonly used in the pharmaceutical, cosmetic, and food industries. DEG and TEG are slightly toxic; repeated large doses are needed for the appearance of toxic effects (10). An estimate of the biohazard caused by the release of residual amounts of TME was obtained by determination of its mutagenic properties in the Ames test.

Experimental Section

Media. Basal salts medium consisted of 3.0 g of NH₄H₂PO₄, 1.25 g of K₂HPO₄, 0.75 g of KH₂PO₄, 0.2 g of MgSO₄·7H₂O, 0.01 g of CaCl₂, and 0.01 g of NaCl per liter of distilled water adjusted to pH 7.0. Glucose was added at 1.0 g/L as indicated. The nutrient broth concentration was 4.0 g/L. The viscous nature of PG, DEG, and TEG required the preparation of initial solutions of 1000 ppm by weight, which were diluted to 100 ppm in the culture media for improved accuracy.

Sterile control flasks with the individual glycols in distilled water were filter sterilized by using a 0.2-µm membrane filter, PG and DEG (Baker grade) and TEG (practical grade) were purchased from J. T. Baker Chemical Co., Phillipsburg, NJ. TME (technical grade) was purchased

from Aldrich Chemical Co., Milwaukee, WI.

Culture Conditions. Aerobic batch cultures were incubated in 250-mL Erlenmeyer flasks containing 100 mL of media at 30 °C on an orbital shaker at 225 rpm. Anaerobic (un-aerated) batch cultures were incubated at 37 °C in 250-mL Erlenmeyer flasks filled with media and loosely sealed.

Inocula. Aerobic nutrient broth cultures were inoculated with activated sludge from the Marlborough Easterly sewage treatment plant (Marlborough, MA), and anaerobic broth cultures were inoculated with anaerobic digest from the Nut Island sewage treatment plant (Boston, MA). Individual flasks contained 50 ppm of a glycol. Cell growth was harvested after 2 days. The cell mass was collected at 12 000 rpm on a Sorvall RC-5 centrifuge and washed three times with 0.85% KCl. These cells were used to inoculate corresponding flasks for biodegradation studies.

Lyophilization. Lyophilization was performed on 1000-ppm solutions of the four glycols. PG and DEG were resuspended in ether and TEG and TME in benzene. Residual glycols were derivatized with *N*-(trimethylsilyl)imidazole and injected into a Bendix Model 2500 gas chromatograph equipped with a flame ionization detector and a 183 cm × 0.64 cm stainless steel column packed with 5% OV1 on chromasorb W, 100–200 mesh. Nitrogen carrier gas flowed at 30 mL/min, and the detector and injector were at 225 °C. The column temperature was 100 °C for PG, DEG, and TME and 125 °C for TEG.

Gas Chromatography. Analyses of PG, DEG, and TEG were performed on a Perkin-Elmer Sigma 3B gas chromatograph equipped with a flame ionization detector and a Model 1021A electronic noise filter (Spectrum Scientific Corp.). Nitrogen carrier gas flowed at 25 or 30 mL/min through stainless steel columns, 46 cm × 0.32 cm packed with Poropak Super Q for PG and DEG and 51 cm × 0.32 cm packed with Poropak PS for TEG. Injection, oven, and detector temperatures were 185, 160, and 200 °C for PG, 250, 210, and 250 °C for DEG, and 250, 190, and 250 °C for TEG. Injection volumes were 1 or 2 μL. Analysis of TME was performed on a Bendix Model 2500 gas chromatograph equipped with a flame ionization detector and a glass column (183 cm × 0.64 cm) packed with Tenax GC, 80–100 mesh. Injection port and column were at 270 °C, and the detector was at 300 °C. Injection volumes were 5 μL, and nitrogen carrier gas flowed at 30 mL/min. TME was also analyzed on a Hewlett-Packard 5840 gas chromatograph under similar conditions except the Tenax column was 305 cm long and the carrier gas flowed at 15 mL/min. Detection limits were 3, 1.5, 10, and 3 ppm for PG, DEG, TEG, and TME respectively.

Mass Spectroscopy. Gas chromatographic mass spectral data were obtained on a Finnigan 4000 operating in the EI mode. Mixtures of the four compounds in methanol were separated by gas chromatography with a helium flow at 8 mL/min through an OV-17 (15.2 m × 0.05 cm) SCOT column. Injection port and feed lines were at 225 °C, and the oven temperature was programmed from 60 to 85 °C at 12 °C/min and then 85 to 200 °C at 20 °C/min.

The spectrometer scanned from *m/z* 17 to 160 at 3 ms/amu at 4 s/scan. The ionization energy was 70 eV, the source temperature 220 °C, and the emission current 300 μA.

Separations were also achieved with a Dexil (15.2 m × 0.05 cm) SCOT column with injection and feed lines at 250 °C, the oven programmed from 60 to 260 °C at 8 °C/min and a helium flow rate of 5 mL/min. However, TME and TEG were poorly resolved under these conditions.

Table I. Precision and Accuracy Data

compd	accuracy ^a × 10 ⁻³	std error of estimate
PG ^b	2.41	0.12
DEG ^c	1.07	0.10
TEG ^c	1.37	0.12
TME ^b	-2.96	0.09

^a Slope of regression line of the mean ratio of spiked distilled water vs. spiked salts or nutrient broth.

^b Spiked in 4 g/L nutrient broth. ^c Spiked in basal salts.

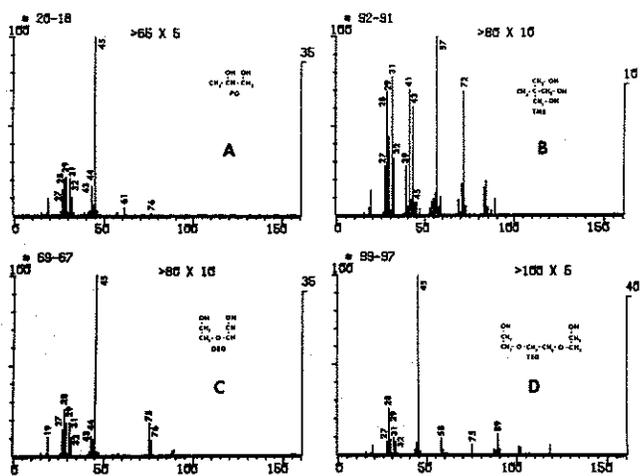


Figure 1. EI mass spectra of (A) PG, (B) TME, (C) DEG, and (D) TEG.

Mutagenicity Testing. A screening test for mutagenicity was performed with TME according to the standard procedures described by Ames (11, 12). Five strains of *Salmonella typhimurium* (TA 98, TA 100, TA 1535, TA 1537, TA 1538) were used to test TME at concentrations ranging from 5 to 5000 μg/plate with and without metabolic activation.

Results

The precision and accuracy data for the method are presented in Table I. Figure 1 illustrates the fragmentation patterns for the four compounds; the major ions are labeled. The most prominent ions are 45, 45, 45 and 57; the most distinguishing ions are 61, 57 or 72, 75, and 58 (>75) for PG, TME, DEG, and TEG, respectively. Only the PG parent ion (*m/z* 76) is present under these conditions. The compounds eluted in the order PG first, then DEG, TME, and TEG, successively. Lyophilization and analysis of 1000-ppm solutions indicated a <1%, 4%, 24%, and 88% recovery of PG, DEG, TEG, and TME, respectively, as determined after derivatization and quantitation by GC. These solutions are 10-fold higher concentrations than those used in biological studies.

At initial concentrations of 100 ppm, PG rapidly disappears from culture flasks under both aerobic and anaerobic conditions, even as the sole carbon source (Figure 2). Some PG disappeared from the sterile controls. After 9 days, 8% and 16% of the PG was lost in sterile anaerobic and aerobic cultures, respectively. In active cultures, PG was not detectable after 2 days in aerobic nutrient broth. In basal salts supplemented with glucose, PG was undetectable after 4 days in aerobic and anaerobic cultures. As the sole carbon source, PG disappeared after 4 days under aerobic and 9 days under anaerobic conditions.

DEG was degraded under both aerobic and anaerobic conditions, but the decomposition appears to be nonbio-

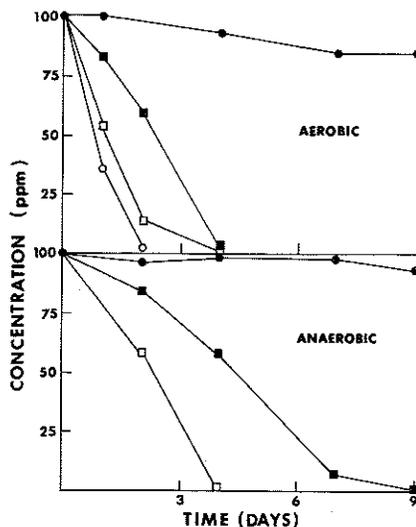


Figure 2. Decomposition of PG under aerobic and anaerobic conditions: filter sterilized distilled water (●), nutrient broth (○), basal salts (■), and basal salts with glucose (□).

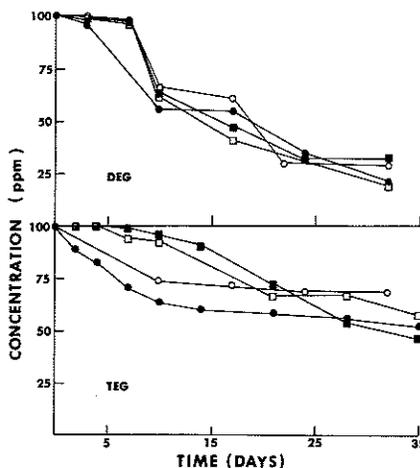


Figure 3. Decomposition of DEG and TEG in filter sterilized distilled water (●), aerobic nutrient broth (○), anaerobic basal salts (■), and anaerobic basal salts with glucose (□).

logical. The disappearance of the substrate in the sterile control (70–80%) was similar to that of the substrate in the inoculated cultures for a period of 32 days (Figure 3). The rate of disappearance was independent of culture media, as similar results were obtained whether nutrient broth, basal salts, or distilled water were used.

The pattern of TEG decomposition was similar to that of DEG (Figure 3), the major difference being the rate of disappearance. TEG was also nonbiologically transformed; after 35 days about 50% of the TEG remained, both in the sterile control and in the inoculated media, whether incubated aerobically or anaerobically.

TME was stable under the conditions tested and showed no evidence of chemical or physical instability during the 34 days. Microbiological results were variable but indicated at best only very slow rates of decomposition.

TME produced no toxic effects in the five *Salmonella* strains, up to 5000 $\mu\text{g}/\text{plate}$, and was negative as a potential mutagen in the Ames test. Toxicity would have been indicated by a decrease in background mutation rate.

Discussion

The low recovery of three of the four glycols by lyophilization illustrates the difficulty in attempting to extract glycols from aqueous solutions by conventional methods.

Jenkins et al. (6) recovered 52% and 62% of DEG and TEG by rotary evaporation followed by chloroform extraction of residual solids, and much lower (<5%) recoveries were obtained by direct extraction with chloroform from solutions saturated with various salts. Similarly, derivatization using silylating agents for GC analysis (13) requires a nonaqueous preparation. Therefore, quantitative extraction of glycols from aqueous solution is extremely difficult.

In this work we successfully detected glycols in aqueous solutions in the low-ppm range. This was accomplished by direct injection into a GC without extraction or concentration steps for sample preparation. Quantitative analysis was successfully performed on samples containing glycols subjected to microbial activity. We have reported on the biodegradability of four glycols.

PG is relatively stable to nonbiological forces at low concentrations in aqueous solutions. Microbial decomposition occurs in rich media and as the sole carbon source. DEG and TEG decompose nonbiologically. No peaks other than the parent peaks were evidence by GC. Therefore, the fragments formed during the decomposition either were amenable to microbial degradation, subject to further nonbiological degradation, or were not detectable by the analytical method used. Cox (2) stressed the potential importance of chemical instability of polyethylene glycols, including factors of heat, peroxide, and acid contaminants formed during their production.

Relative rates of disappearance of the parent compounds indicate a sequence of PG > DEG > TEG > TME from high to low. This agrees with other reports where rates of biodegradation decrease with increasing degree of polymerization (2), but the rate difference may be due to chemical/physical factors affecting rates of depolymerization and not due to microbial factors. Ames testing indicates TME may not pose potential mutagenicity problems.

With the exception of TME, these glycols will undergo degradation under conditions suitable to a biological treatment facility. Degradation occurs through a combination of biological and chemical activities which varies with each glycol.

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