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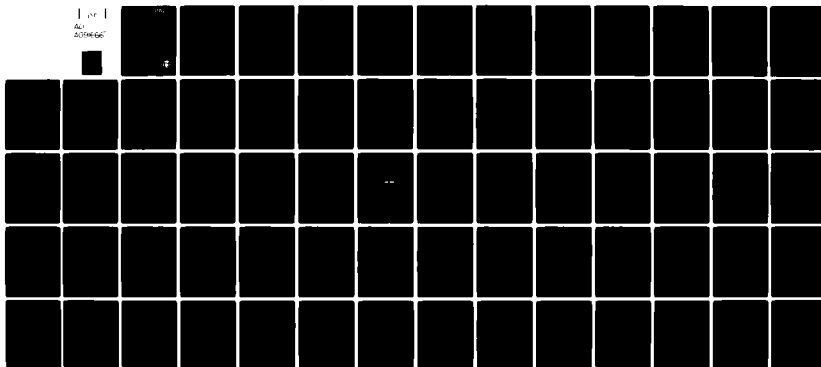   EVALUATION OF METHODS FOR THE ANALYSIS OF SMALL MOLECULAR WEIGH--ETC(U)
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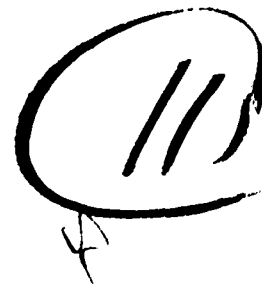UNCLASSIFIED USAMBRDL-TR-8011   NL

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TECHNICAL REPORT 8011

EVALUATION OF METHODS FOR THE ANALYSIS
OF SMALL MOLECULAR WEIGHT END-PRODUCTS
OF WASTEWATER OZONATION

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Acetic Acid	Glyoxal	Oxalic Acid
Dimethylglyoxal	Glyoxylic Acid	Ozonation
Formaldehyde	Methylglyoxal	Wastewater Analysis
Formic Acid		

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The treatment of hospital laboratory wastewaters by ozonation produces a number of organic products: methanol and acetone are converted to formaldehyde, formic acid, and acetic acid and phenols are converted to oxalic acid, glyoxylic acid, and glyoxal. Methods for detecting these residual organic compounds at levels of < 5 mg/L in laboratory wastewater were evaluated and compared:.

Acetic and Formic Acids - A method of analysis that provides for isolation of formic and acetic acids from water as lipophilic tetra-n-butylammonium hydroxide salts, treatment with benzyl bromide to form benzyl esters, and detection of

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20.   ABSTRACT (Continued)

benzyl esters by FID-GC was tested. In a modified procedure, pentafluoro-
benzyl bromide was used instead of benzyl bromide; results showed no
advantage over the benzyl bromide method, which easily detected acetic
and formic acid levels as low as 0.25 mg/L. Recovery was quantitative,
with a precision of 1 to 4% of the mean.

Oxalic Acid - Attempts to quantitatively derivatize 0- to 10-mg/L
solutions of oxalic acid with pentafluorobenzyl bromide were unsuccessful.

Glyoxals - A method for FID-GC detection of glyoxals in aqueous
solution was modified by heating the reaction mixture at 80°C for 30
minutes, by using methylene chloride as a solvent, and by choosing a
more polar GC column. Although the method gave reasonable precision
for methylglyoxal and dimethylglyoxal at about 2-mg/L levels, the
precision for glyoxal was not acceptable.

Formaldehyde - Formaldehyde was readily determined by colorimetry for
concentrations up to 8 mg/L and by fluorimetry for levels of 0.05 to 2 mg/L.

Glyoxylic Acid - At least 0.1 mg/L could be detected by a resorcinol
procedure, with a precision of 7% relative standard deviation.

ACKNOWLEDGMENT

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I. INTRODUCTION

As part of the mission of the U.S. Army Medical Bioengineering Research and Development Laboratory (USAMBRDL) to develop water quality criteria for reuse of Army wastewaters, an in-house capability to determine concentrations of organic compounds in treated wastewaters is required.

One of the candidate methods for treating hospital laboratory wastewaters is ozonation,[1] which was shown by Chian and Kuo[2] to convert the major components, methanol and acetone, to formaldehyde, formic acid, and acetic acid. Similarly, ozonation of phenols by Gould and Weber[3] was shown to produce oxalic acid, glyoxylic acid, and glyoxal. Since phenols may also be present in hospital laboratory wastewaters, their ozonation products were considered in the analytical requirements list. Figure 1 shows the structures of the compounds suspected as the major products from ozonation of hospital laboratory wastewaters. In general, ozonated organic compounds are converted to more oxidized forms such as aldehydes, ketones, and acids before loss of organic carbon as carbon dioxide. One form of evidence for this is the ratio of chemical oxygen demand to total organic carbon (COD/TOC), which has been shown by McCarthy and co-workers[1] to decrease during the ozonation of synthetic laboratory wastewater.

Because the level of residual TOC in highly ozonated wastewaters would be expected to lie at or below 5 mg/L, relatively sensitive methods are required for the detection of residual organic compounds. For example, a 1-mg/L residual TOC concentration due to oxalic acid would be equivalent to 3.75 mg/L of oxalic acid.

II. OBJECTIVE

The objective of these studies was to evaluate analytical methods capable of detecting levels of < 5 mg/L. The sources of methods were: (a) Army contractors (Dr. P.K. Kuo and Dr. E.S.K. Chian, University of Illinois, Champaign/Urbana, Illinois), (b) chemical literature, and (c) methods modified during the present studies from those of sources (a) and (b). By documenting the successful methods found from these studies, it is hoped that this report will be useful to researchers as a manual of analytical methods in future ozonation studies.

III. ACETIC AND FORMIC ACIDS

A. Literature Review

Acetic and formic acids have been determined by direct aqueous injection onto a gas-liquid chromatography column packed with 15% SP-1220/1% H_3PO_4 on 100/120 mesh Chromosorb WAW, with thermal conductivity detection.[4] Acetic acid has been determined by the same method, using a graphitized carbon packing (Carbopack B/3% Carbowax 20 M/0.5% H_3PO_4)[4] or Chromosorb 101

$H_2C=O$ $HCOOH$ CH_3COOH $HOOC-COOH$

Formaldehyde   Formic   Acetic   Oxalic
                 Acid   Acid   Acid

$$HC=O \qquad CH_3-C=O \qquad CH_3-C=O \qquad COOH$$
$$| \qquad\qquad | \qquad\qquad | \qquad\qquad |$$
$$HC=O \qquad\quad HC=O \qquad CH_3-C=O \qquad HC=O$$

Glyoxal   Methyl   Dimethyl   Glyoxylic
             Glyoxal   Glyoxal   Acid
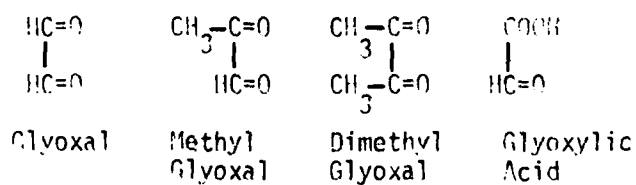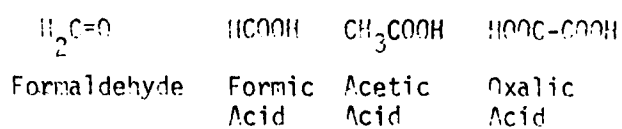
Figure 1.   Structures of suspected small molecular
               weight end-products in ozonated hospital
               laboratory wastewater.

coated with 3% FFAP liquid phase.[5] The sensitivity of thermal conductivity (TC) detectors is generally less than that of flame ionization detectors (FID), so that detectability by TC would not be expected to match the levels reached by FID detectors. In the case of aqueous formic acid, however, the FID response would be expected to be minimal, because there is only one C-H bond. By judicious selection of derivatives of these acids, the FID response per mole of acid can be increased, and better separation of the two compounds can be achieved.

Benzyl esters of formic, acetic, and higher homologous fatty acids have been used by Bethge and Lindstrom[6] for quantification of these acids. They isolated the acids from water by first forming lipophilic tetra-n-butyl-ammonium hydroxide (TBAOH) salts of the acids and then removing the water by rotary evaporation. The remaining salts were dissolved in acetone and treated with benzyl bromide to form the benzyl esters. The detection limit was related to the volume of the sample aliquot taken for analysis; starting with a 10-mL aliquot, they were able to detect acids present at the 5×10^{-5} M level in the original sample. This would be equivalent to about 3 mg/L of acetic acid. Davis[7] used 18-crown-6 (crown ether) as a catalyst with the potassium salts of acetic and higher homologous acids to form pentafluoro-benzyl esters with pentafluorobenzyl bromide (PFB-Br) in an organic solvent. However, no procedure was given for extracting the acetic acid from a water solution. The pentafluorobenzyl esters were detected with a highly sensitive electron capture detector. The lower limit of sensitivity was 10 ng because the unreacted pentafluorobenzyl bromide peak and artifact peaks made work at lower attenuations impossible. In addition, Davis[7] did not attempt to form the pentafluorobenzyl ester of formic acid.

Thus, the literature indicated that a good method existed for the isolation of formic and acetic acids from water in a form that would react in an organic solvent to displace bromide from benzyl bromide. By replacing benzyl bromide with pentafluorobenzyl bromide, the resulting derivative would be susceptible to detection by gas chromatography (GC) detectors more sensitive and specific than FID, namely electron capture or electrolytic conductivity detectors. In addition, there appeared to be a chance that oxalic acid could be determined simultaneously with the acetic and formic acids.

B. Materials and Methods

1. Standard Acetic and Formic Acids

Reagent grade formic acid and glacial acetic acid were diluted to approximately 1-g/L concentrations in glass-distilled water. These stock solutions were titrated to pH 7 (formic) or pH 7.5 (acetic) with standard sodium hydroxide on a Metrohm Herisau Dosimat-pH meter combination. The sodium hydroxide was standardized against reagent grade potassium acid phthalate. Appropriate volumes of the two stock solutions were diluted to 100 mL with glass-distilled water to give a combined 100-mg/L standard of the two acids. This standard was then diluted as required for standard curves.

2. Benzyl Formate and Acetate

The following procedure is essentially that presented by Bethge and Lindstrom.[6] To 10 mL of sample or standard were added 100 µL of approximately 500-mg/L propionic acid in water as an internal standard. The mixture of acids was passed through a 12-mm diameter x 100-mm length column of 20-50 mesh AG-50W x 8 ion exchange resin (Bio-Rad Laboratories) in the hydrogen form, followed by two 5-mL water washes. The solution passing through the column was collected in a small beaker and titrated to pH 8 with 0.03 N tetra-n-butylammonium hydroxide. The resulting solution was evaporated under aspirator vacuum on a rotary evaporator with a 50°C water bath. A special flask designed by Junk et al.[8] was used for the evaporations. This flask was made by joining a tapered centrifuge tube to the bottom of a 100-mL round-bottom flask. To form the tetra-n-butylammonium salt derivatives of the acids in the residue, 0.5 mL of redistilled acetone and an appropriate excess of 10% (v/v) benzyl bromide in acetone were added to the residue in the rotary evaporation flask. The flask was stoppered and allowed to stand at least 15 minutes at room temperature.

The benzyl esters formed in the reaction were determined by injecting 1 µL of the solution onto the gas chromatograph. The GC conditions were:

GC:	Hewlett-Packard 5830A, FID detector
Column:	6 ft x 1/8 in O.D. stainless steel, packed with 3% butane-1,4-diol succinate on 80/100 mesh Chromosorb WHP (Supelco, Inc.)
Carrier:	Helium, 25 cc/minute
Column temperature:	110°C isothermal
FID temperature:	190°C
Injection port temperature:	160°C

3. Pentafluorobenzyl (PFB) Formate and Acetate

To 10 mL of sample or standard were added 100 µL of approximately 500-mg/L n-butyric acid as an internal standard. This solution was passed through a 12-mm diameter x 100-mm length column of 20-50 mesh AG-50W x 8 ion exchange resin (Bio-Rad Laboratories) in the hydrogen form, followed by two 5-mL portions of water washes. The solution passing through the column was collected in a small beaker and titrated to pH 8 with 0.03 N tetra-n-butyl-ammonium hydroxide. The resulting solution was evaporated under aspirator vacuum at 50°C in the special flasks described previously. The lipophilic tetra-n-butylammonium salts in the residue were dissolved in 0.5 mL of redistilled acetone added directly to the evaporation flask. An appropriate excess of 10% PFB-Br in acetone was added to the flask, which was then stoppered and allowed to stand at least 20 minutes before a 1-µL sample was taken for GC analysis.

The GC conditions were:

GC: Hewlett-Packard 5830A, equipped with a Tracor Model 700 Hall electrolytic conductivity detector

Transfer line temperature: 250°C

Detector resin column: 65% IRN-77 pump side
35% IRN-150 cell side

Detector solvent: 50:50 1-propanol:water (v/v) at 0.8 cc/minute flow

Detector mode: Quartz tube, in halogen mode with hydrogen reactor gas at 50 cc/minute, 850°C oven temperature

Column: 6 ft x 1/8 in O.D. stainless steel, packed with 3% OV-225 on 100/120 mesh Gas Chrom Q (Applied Science Laboratories, Inc.)

Carrier: Helium, ultra high purity, at 43 cc/minute

Column temperature: 80°C (4 minutes), 8°C/minute to 175°C (1 minute)

Injection port temperature: 150°C

4. Ozonated Laboratory Wastewater

Table 1 shows the composition of the synthetic reverse osmosis permeate of laboratory wastewater (RO-Lab) from an Army hospital. This wastewater formulation was also used by McCarthy et al.[1] The wastewater was ozonated

Table 1. Synthetic Laboratory Reverse Osmosis Permeate Formulation

Chemical	Concentration[a] (mg/L except where noted)
Diethyl ether	1.8
Methanol	289.1
Urea	0.9
Glycerol	2.1
Ethanol	3.3
Formaldehyde (37% sol.)	3.5 µL/L
o-Phenylphenol	0.4
o-Benzyl-p-chlorophenol	0.3
2,4-Xylenol	0.2
2-Propanol	0.1
Acetone	32.8

a. Tap water was used for all batches of this synthetic wastewater.

for 8 hours in the Life Systems Modified Torricelli Ozone Contactor (LMTOC), designed by Life Systems, Inc., Cleveland, Ohio, under Army contract No. DAMD 17-76-C-6041.[9]

The following conditions were used in the ozonation:

Gas flow:	Air at 20 standard cubic ft/hr
O_3 dose:	1.5 weight %
UV light:	On
pH control:	None
Sample period:	Hourly
Temperature:	Ambient

This formulation and the ozonation protocol were used whenever a source of ozonated wastewater was required for testing analytical methods, at various periods in fall 1977 and spring 1978. No preservative was added to the samples, which were stored at 4°C until analyzed. Hourly samples were collected in glass bottles with Teflon-lined caps, whereas samples for spiking tests were collected in plastic containers or glass jars.

C. Results and Discussion

    1. Benzyl Formate and Acetate

    Figure 2 shows the separation of these esters from the benzyl bromide and internal standard (benzyl propionate) peaks on the 3% butane-1,4-diol succinate column. The only problem seen was the presence of an artifact peak following the internal standard (I.S.) peak, ascribed to benzyl alcohol by Bethge and Lindstrom.[6] However, the GC integrator was able to reproducibly split the peak areas, as shown by the least-squares regression of relative peak area (area standard peak/area I.S. peak) versus concentration (Figure 3). For these standard curves, 36 µL of 10% (v/v) benzyl bromide in acetone was added to the standards and blanks. This value was chosen from a titration of a spiked (see below) sample of RO-Lab wastewater after passage through the H+ form ion exchange resin, with equation (1) used to calculate the required amount of reagent.

$$V = 36 \text{ µL} = (0.673 \text{ mL TBAOH}) (0.03 \text{ N TBAOH}) \times \frac{171.04 \text{ mg/mmole}}{1.438 \text{ mg/µL}} \qquad (1)$$
$$\times 10 \times 1.5$$

where

    factor 1.438 mg/µL = the density of benzyl bromide
    171.04 mg/mmole = the molecular weight of benzyl bromide
    factor 10 = the correction for use of 10% reagent benzyl bromide
    factor 1.5 = the 1.5-fold molar excess chosen for the ratio of
                 benzyl bromide/titrated acids
    0.673 mL TBAOH = volume of TBAOH reagent required for titration of acids
                      in the sample.

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Peak (1) = Benzyl Br
     (2) = Benzyl formate
     (3) = Benzyl acetate
     (4) = Benzyl propionate
           internal standard
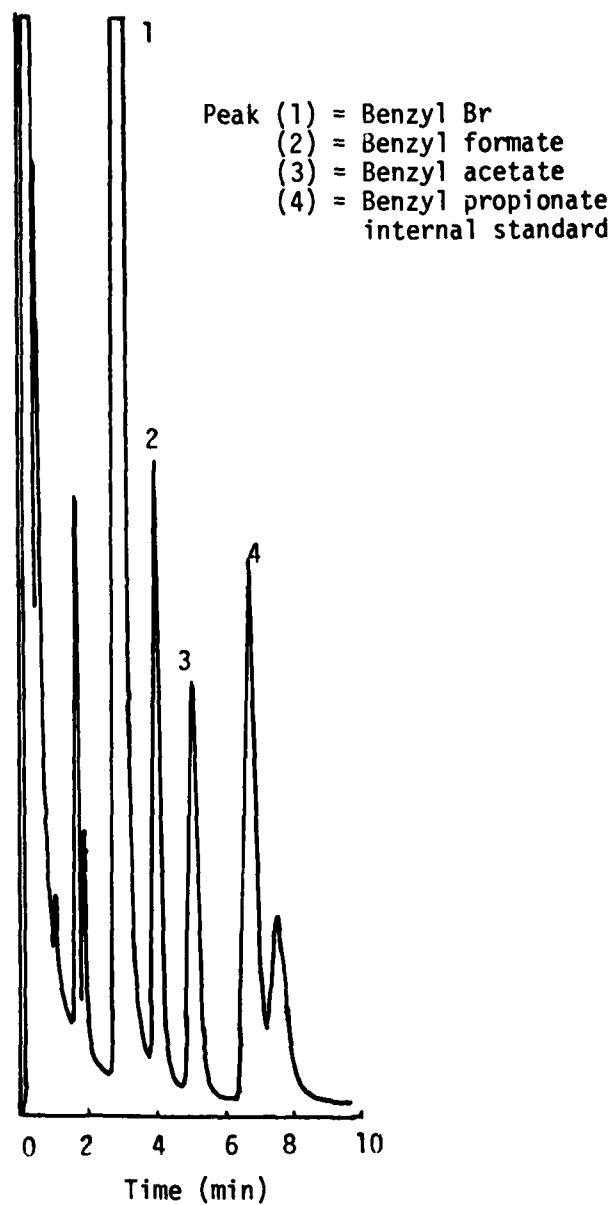
Figure 2.   FID-GC of a 2.5-mg/L standard of acetic
            and formic acids, treated with benzyl bromide.

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Because the RO-Lab waste was made up in tap water, the titrated acids included carbonic acid from bicarbonate in the tap water as well as formic and acetic acids added as spikes.

The standard curves in Figure 3 showed sample correlation coefficients (r^2) of 0.99981 (formic) and 0.99945 (acetic) for the range of 1 to 10 mg/L; hence, there was excellent linearity of FID response relative to the internal standard.

Addition of 4.35 mg/L of formic acid and 4.87 mg/L of acetic acid to the RO-Lab wastewater provided a sample with a mid-range level of acids for recovery and precision tests. Analysis of one spiked sample with only 8 µL of 10% benzyl bromide (calculated to be 1.5 molar excess for a 10-mg/L combined acid standard plus internal standard in distilled water) showed approximate concentrations of 0.7 mg/L (formic) and 4.8 mg/L (acetic). Hence, the carbonic acid in these samples was probably competing effectively with the formic acid for the benzyl bromide, but the acetic acid was not affected. When 36 µL were added, as required by the titration calculation of equation (1), the recoveries of both acids were comparable. Table 2 shows the data collected from seven replicate aliquots from a single spiked RO-Lab waste. The coefficients of variation were 4.34 and 1.35% for formic and acetic acids, respectively, at the 4- to 5-mg/L level of these acids.

Table 2. Benzyl Method of Recovery of Formic and Acetic Acids
from RO-Lab Wastewater

	Concentration Found (mg/L)	
Spiked Sample No.	Formic Acid	Acetic Acid
1	4.04	5.22
2	4.56	5.39
3	4.53	5.42
4	4.32	5.38
5	4.32	5.29
6	4.43	5.33
7	4.18	5.27
Mean value ($\bar{x}$)	4.34	5.33
Standard deviation (s)	0.187	0.072
Coefficient of variation ($s/\bar{x} \times 100$)	4.3%	1.4%
mg/L acid added (z)[a]	4.35	4.87
Average % recovery ($\bar{x}/z \times 100$)	100%	109%

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a. No formic or acetic acids were present in the unspiked wastewater.
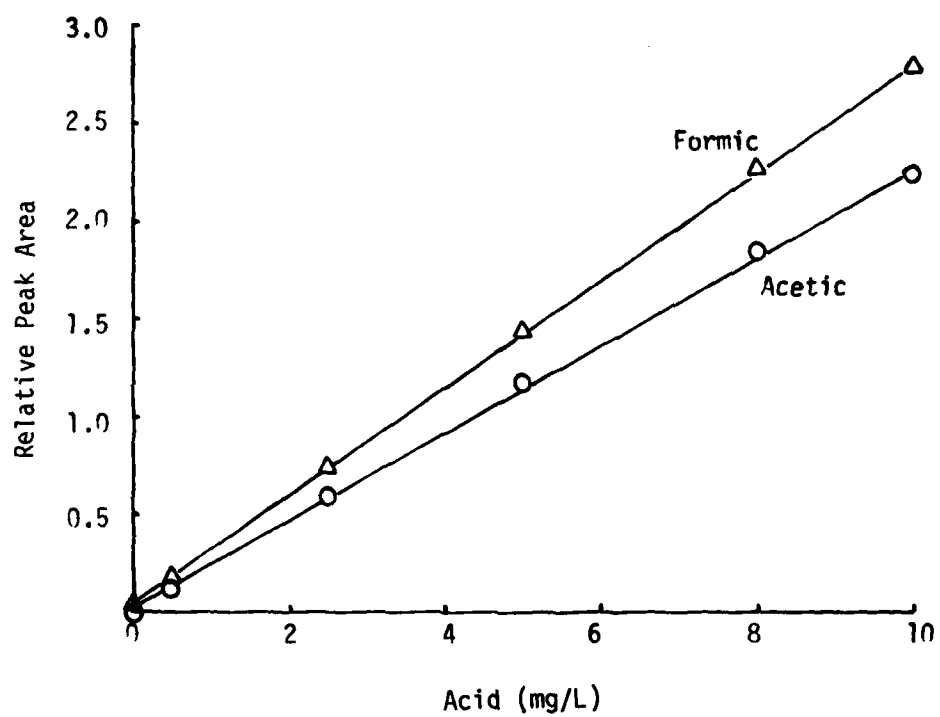
Figure 3. Standard curves for benzyl bromide derivatization of formic and acetic acids.

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As a test to see whether the derivatization reaction conditions (at least 15 minutes, room temperature) were adequate, the data in Table 3 were collected from a spiked RO-Lab sample. It appeared that the peak area ratio was constant by 15 minutes and did not change significantly for periods up to an hour.

Table 3. Kinetic Study of Benzyl Formate and Benzyl Acetate Derivatization Reaction at Room Temperature

Reaction Time (min)	Peak Areas			Relative Peak Areas[a]	
	Formate	Acetate	Propionate	Formate	Acetate
0	0	0	0	0	0
15	124,900	123,000	100,600	1.242	1.223
30	127,600	124,500	102,300	1.247	1.217
45	130,500	126,200	102,900	1.268	1.226
60	137,900	132,200	107,600	1.282	1.233

a. Area formate or acetate/area propionate.

In order to find a detection limit for this procedure, standards of 0, 0.1, 0.25, and 0.5 mg/L formic and acetic acids were run. The data were analyzed by peak height at an attenuation of 2^4 on the Hewlett-Packard recorder. Figure 4 indicates that 0.25 mg/L of these acids could be easily distinguished from the blank responses.

2. Pentafluorobenzyl (PFB) Formate and Acetate

Because the volatilities of the PFB esters were close to the volatility of PFB-Br and the PFB esters were more volatile than the benzyl esters, a lower column temperature and different liquid phase was chosen for the PFB esters. Figure 5 shows the separation of esters from the PFB-Br and side-reaction peaks as detected by the halogen-specific Hall electrolytic conductivity detector. GC/MS studies indicated that the large peak just preceding the PFB-Br peak was pentafluorobenzyl alcohol. The peak between the PFB-acetate and PFB-butyrate was not identified, but may have been an ether of structure PFB-O-PFB, as suggested by Davis.[7]

Because the electronic integration system was not able to reliably skim the acetate and formate peaks from the PFB-Br peak, these peaks were manually skimmed and their peak heights were measured relative to the internal standard peak height. Figure 6 shows the resulting calibration curve for distilled water standards permitted to react with 10 µL of 10% PFB-Br (1.5 molar excess for a 10-mg/L standard with internal standard). Fairly good correlation coefficients (r^2) of 0.99807 and 0.99176 were obtained for the least-squares regressions of formic and acetic acids, respectively.

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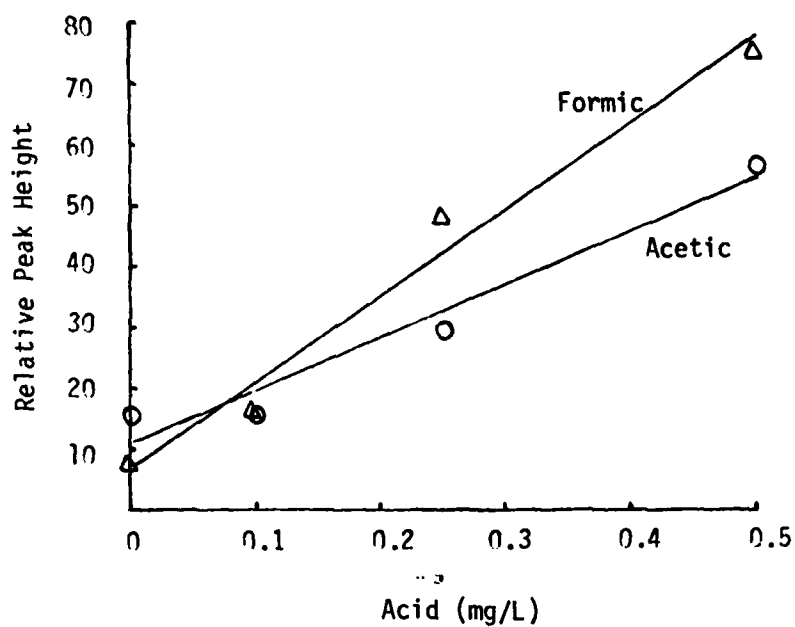
Figure 4.   Low-level standard curves for benzyl bromide
derivatization of formic and acetic acids.

Peak (1) = PFB-Br
(2) = PFB-formate
(3) = PFB-acetate
(4) = PFB-butyrate
internal standard

Time (min)

Figure 5. Hall detector-GC of a 2.5-mg/L standard
of acetic and formic acids.

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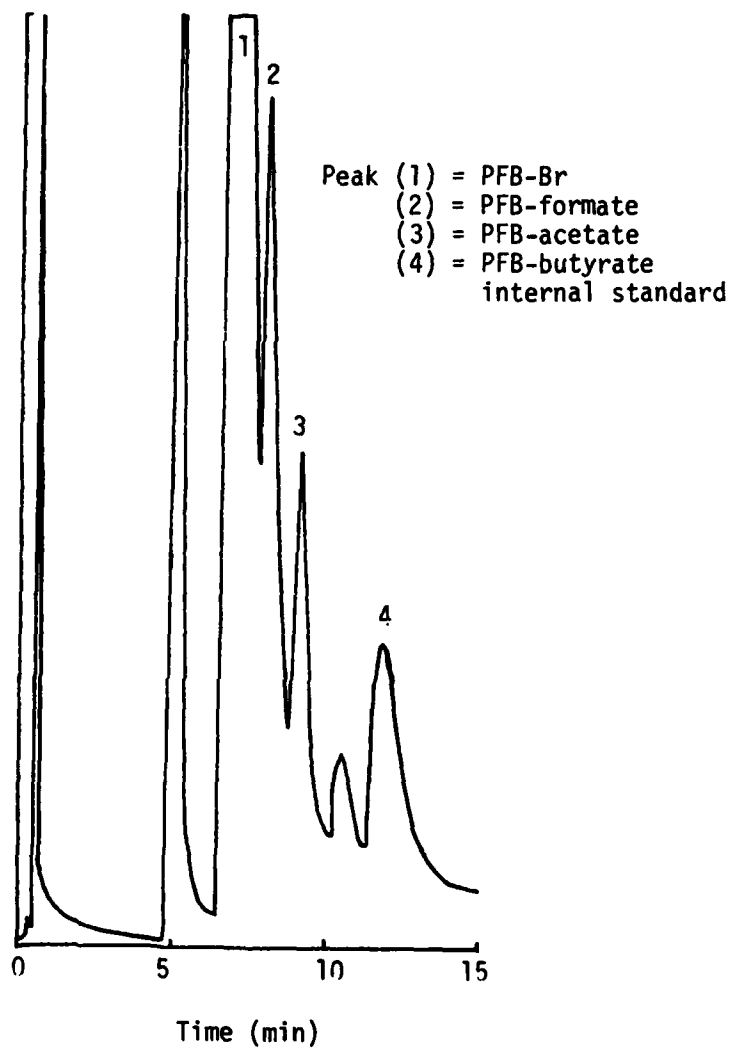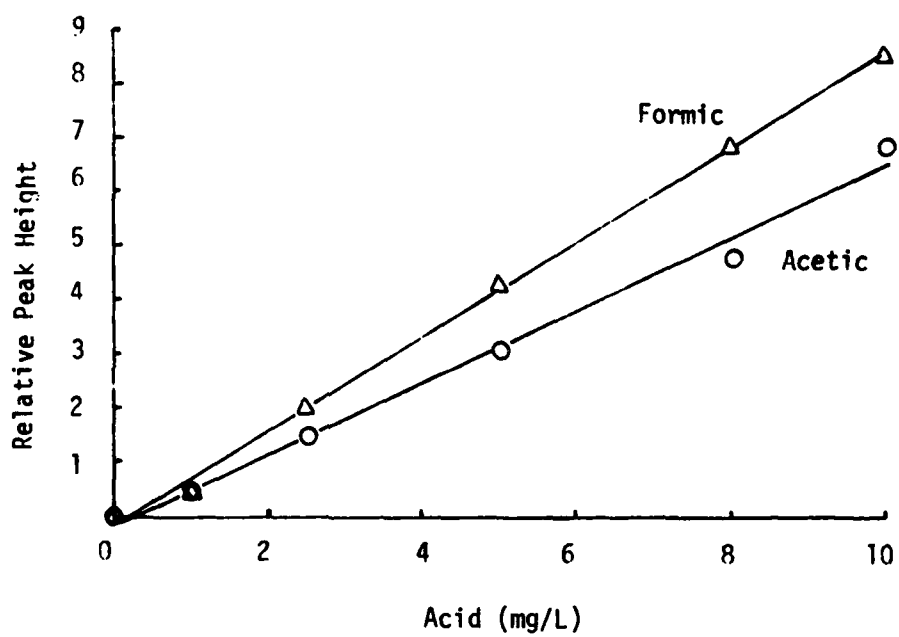
Figure 6. Standard curves for pentafluorobenzyl bromide
derivatization of formic and acetic acids.

Titration of a spiked RO-Lab wastewater demonstrated a requirement for 45 µL of 10% PFB-Br for a 1.5 molar ratio to titrated acids. Figure 7 shows a chromatogram from such a sample. The PFB alcohol and other artifact peaks were very large, probably because of the PFB-Br reaction with carbonic acid (derived by ion exchange) from the bicarbonate in the tap water used to make up the wastewater. Carbonic acid would react with TBAOH in the titration of the acids to form $(TBA)_2CO_3$, which would then react with PFB-Br to form $(PFB)_2CO_3$ and this would possibly hydrolyze to form the alcohol (PFBOH) and HCO_3^- in the presence of moisture. The relative (to internal standard) peak heights were 2.33 and 3.10 for formate and acetate, respectively, in Figure 7. By comparison, a spiked sample with only 10 µL PFB-Br used to make the acid derivatives is shown in Figure 8. It can be seen that the PFB-Br peak was almost entirely converted to PFB alcohol, and the relative peak heights for formate and acetate were 1.35 and 3.41, respectively. The formate peak, but not the acetate peak, was reduced relative to butyrate; thus, the reaction to form the alcohol competed with the reaction to form PFB-formate but not with that to form PFB-acetate. This is also apparent from the data in Table 4, which show relative peak heights for three different volumes of 10% PFB-Br added to prepare the acid derivatives in a separate test from that shown in Figure 8.

Table 4. Relative Response of Formate and Acetate Peaks to Various Amounts of PFB-Br Used to Derivatize a Spiked RO-Lab Wastewater[a]

Volume of 10% PFB-Br Used (µL)	Relative Peak Height	
	PFB-Formate	PFB-Acetate
10	0.83	2.40
20	2.50	2.43
30	1.97	2.47

a. Spike added: 4.35 mg/L formic acid, 4.87 mg/L acetic acid added to raw RO-Lab wastewater containing no acetic or formic acids.

An attempt was made to see whether the interference from bicarbonate in the sample could be eliminated by stripping a spiked RO-Lab wastewater sample with nitrogen gas for 5 minutes after acidification with the ion exchange column. This treatment reduced the TBAOH titer by about 18% and resulted in a chromatogram similar to that shown in Figure 9, with 10 µL of PFB-Br. Comparison with Figure 8 shows that the PFB alcohol was still present, but the formate peak had increased relative to the internal standard peak. Relative peak height ratios of 2.10 (formate) and 2.67 (acetate) were recorded for this sample, in approximate agreement with the values of Table 4 for 20 and 30 µL PFB-Br used. Consequently, the competitive interference from bicarbonate could be reduced by stripping out the carbon dioxide in equilibrium with the carbonic acid of the sample before TBAOH titration, or more PFB-Br could be
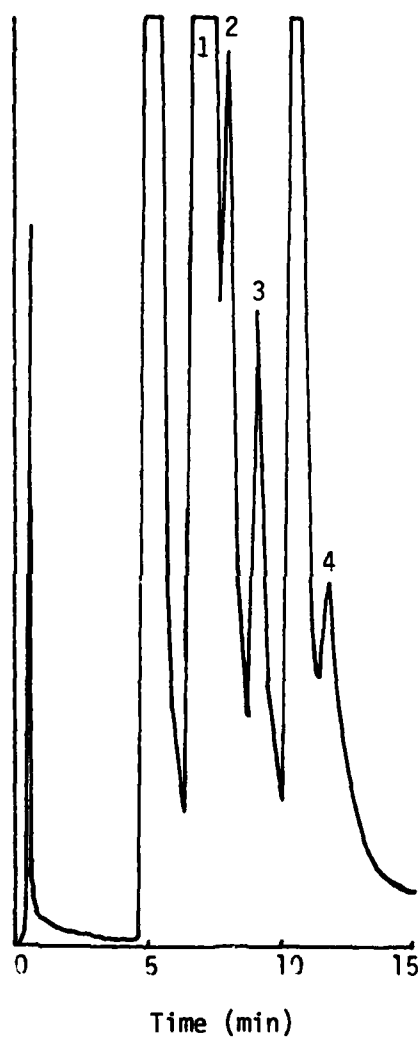
Figure 7. Hall detector-GC of a RO-Lab wastewater
sample spiked with 4.35 mg/L of formic
acid and 4.87 mg/L of acetic acid and
derivatized with 45 μL of 10% (v/v) PFB-Br
in acetone. Peak identification is the
same as in Figure 5.

Figure 8. Hall detector-GC of a RO-Lab wastewater sample spiked with 4.35 mg/L of formic acid and 4.87 mg/L acetic acid and derivatized with 10 µL of 10% (v/v) PFB-Br in acetone. Peak identification is the same as in Figure 5.

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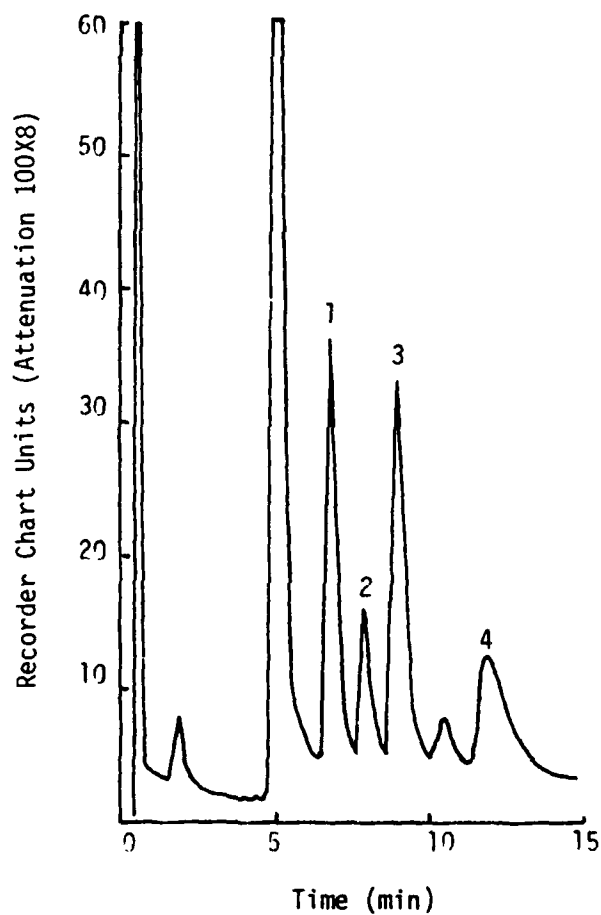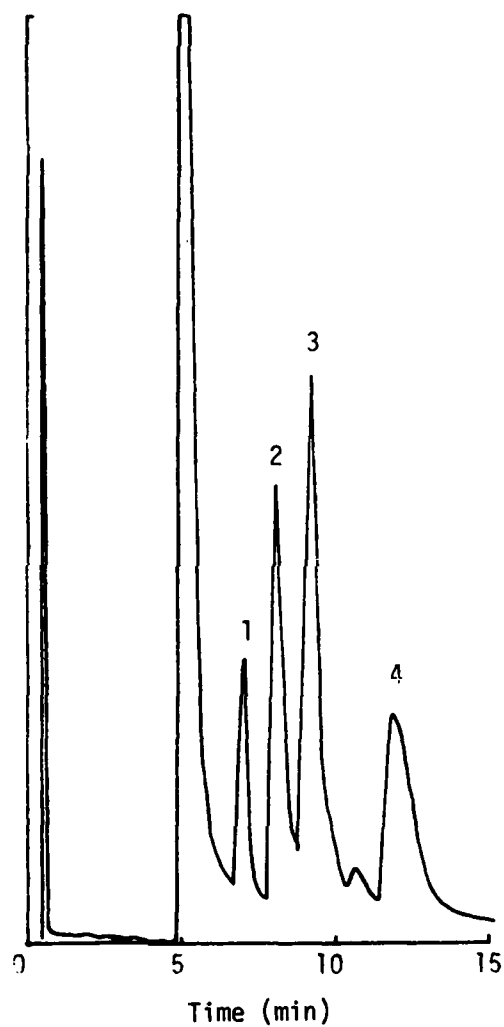
Figure 9. Hall detector-GC of a RO-Lab wastewater
sample treated as in Figure 8 and using
N_2 gas stripping to remove CO_2 after
acidification by ion exchange. Peak
identification is the same as in Figure 5.

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used, in accord with equation (1) to cover the extra acids present from tap water. In the latter case, the chromatograms (see Figure 7) were difficult to quantify accurately because of the large by-product peaks. Recovery data (Table 5), for example, showed relatively poor precision for acetic acid and poor recoveries (accuracy) for both acids, compared to the benzyl bromide procedure (Table 2). The data in Table 5 were collected from derivatization using 45 µL of 10% (v/v) PFB-Br, in accord with equation (1).

Table 5. PFB Method of Recovery of Formic and Acetic Acids from RO-Lab Wastewater

Spiked Sample No.	Concentration Found (mg/L)	
	Formic Acid	Acetic Acid
1	2.86	4.91
2	3.06	5.29
3	3.06	4.40
4	3.02	5.50
5	3.60	7.97
6	3.28	5.88
Mean value ($\bar{x}$)	3.15	5.66
Standard deviation (s)	0.26	1.24
Coefficient of variation (s/$\bar{x}$ x 100)	8.2%	21.9%
mg/L acid added (z)[a]	4.35	4.87
Average % recovery ($\bar{x}$/z x 100)	72%	116%

a. No formic or acetic acids were present in the unspiked wastewater.

Figure 10 shows the results of a low-level standard curve run with distilled water standards and 2 µL of 10% (v/v) PFB-Br added to derivatize the acids. A Hall detector range of 30 with attenuation of 1 to 4 was used to detect these levels. From the figure, it was apparent that 0.25-mg/L levels could be detected.

The PFB-Br procedure was tested unsuccessfully several times with oxalic acid in the combined standard. This lack of success may have been due to the poor nucleophilicity expected of the oxalate anion, because of mutual electron withdrawing by one COO^- group from the other. A dipentafluorobenzyl oxalate diester was prepared with PFB-Br and tetra-n-butylammonium oxalate as shown by infrared, NMR, and mass spectra (Appendix A). However, the retention time of this diester was very close to that of the high-boiling reaction by-product eluting just ahead of PFB-butyrate, making quantification difficult. The oxalate diester peak never showed a proportional area increase as a function of oxalic acid concentration, as in the case of acetic and formic acids in the procedure outlined previously. Appendix A shows the mass spectrum of the PFB-oxalate, as well as GC/MS data for PFB-acetate and PFB-formate.

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Figure 10. Low-level standard curves for PFB-Br derivatization
of formic and acetic acids.

3. Test of Benzyl Acetate and Formate Method with Ozonated RO-Lab
   Wastewater

Because there appeared to be no difference in sensitivity of the benzyl
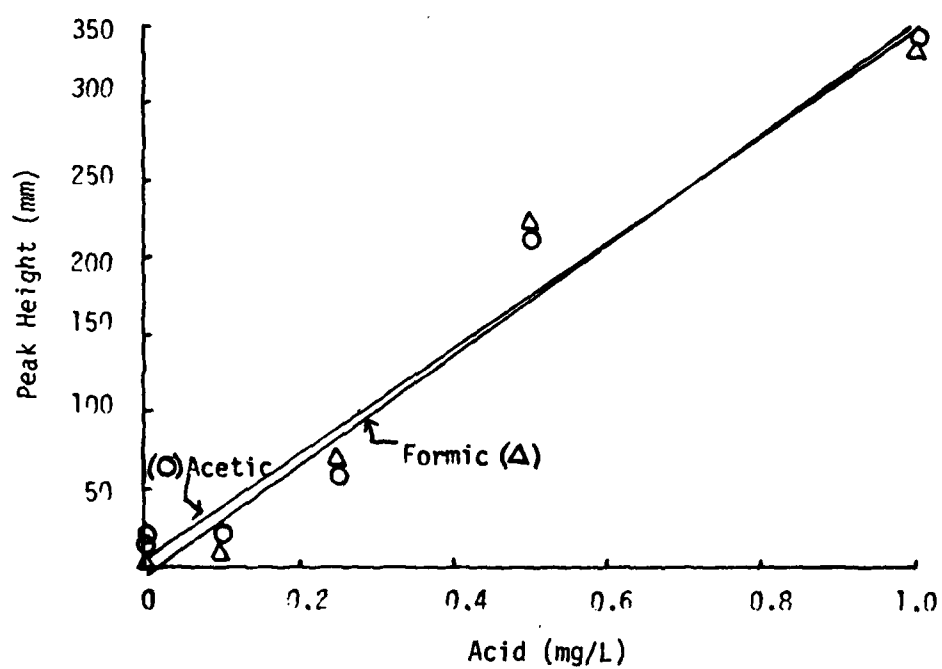and pentafluorobenzyl methods, and because the benzyl method was much easier
to quantify, the benzyl procedure was used to measure the concentrations of
acetic and formic acids in an ozonated RO-Lab wastewater. Figure 11 shows the
changes in formic and acetic acids and TOC as a function of time. As expected
from the high initial methanol concentration, the formic acid concentration
increased to a maximum early in the ozonation. Acetic acid, primarily from
acetone breakdown, did not reach a peak until later in the run. The only
point which did not appear to fall on a smooth curve was the 180-minute point
for both acids. No explanation is currently available for this decrease in
concentration from the values on either side of 180 minutes. The samples from
60 through 300 minutes were diluted 1:10 for this analysis, and this may not
have been the optimal dilution for all of these samples. The final
concentrations of 1.48 mg/L of formic acid and 0.5 mg/L of acetic acid
accounted for 0.4 and 0.2 mg/L of a final TOC, which could only be measured as
< 5 mg/L on a Dohrman DC-50 carbon analyzer.

IV. OXALIC ACID

A. Literature Review

Oxalic acid has been determined fluorimetrically by a rather complex
procedure involving extraction with tri-n-butyl phosphate, co-precipitation
with calcium sulfate, reduction to glyoxylic acid, then reaction with
resorcinol to form a fluorescent complex.[10] A linear fluorescence curve was
found between 0 and 5 mg/L oxalic acid.

Oxalic acid has also been determined by GC after derivatization to its
bis-trimethylsilyl (TMS) ester, dimethyl ester, or diisopropyl ester. Rumsey
and Noller[11] used hydrogen chloride and methanol to form the methyl ester in
the range of about 45 to 630 mg/L. Mee and Stanley[12] used the same reaction
in the range of 2 to 25 µg of oxalic acid (derivatized) injected onto the GC
column. This would be a concentration of 2,000 to 25,000 mg/L in the injected
sample, for a 1-µL injection. More recently, Chian and Kuo[2] used a
small-scale diazomethane procedure similar to that of Schlenk and
Gellerman[13] to esterify oxalic acid, which was concentrated from a water
sample by rotary evaporation, in the range of about 2 to 30 mg/L. Gould and
Weber[3] reacted dried residues of wastewater samples with 2-propanol and
sulfuric acid, then extracted with chloroform, and analyzed the extract on the
GC for diisopropyl oxalate.

For trimethylsilylation, Butts and Rainey[14] made the ammonium salt of
oxalic acid and then reacted it with bis-(trimethylsilyl) trifluoroacetamide
(BSTFA) in dimethylformamide. No quantification was attempted. Von Nicolai
and Zilliken[15] concentrated oxalic acid from urine by rotary evaporation and
then permitted the acid to react with a mixture of pyridine-BSTFA-trimethyl-
chlorosilane (TMCS) to form the trimethylsilyl (TMS) ester. Quantification by
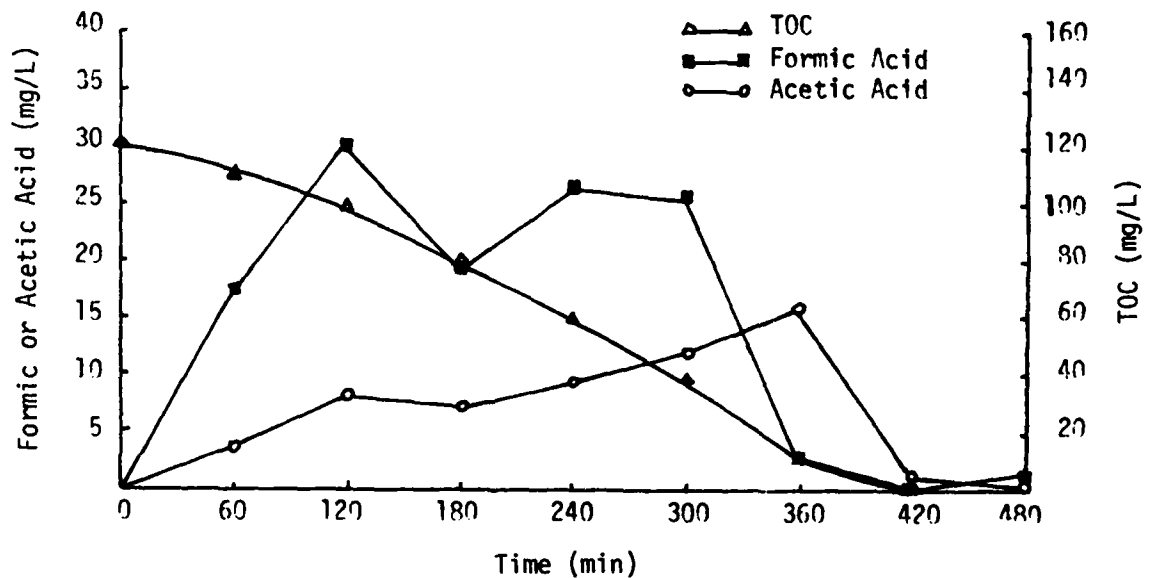
Figure 11. Ozonation of synthetic RO-Lab wastewater, with analyses of formic and acetic acids by the benzyl bromide method of Bethge and Lindstrom.[6]

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GC was attempted in the range of 2 to 16 µg of oxalic acid injected as its derivative in a 1-µL injection or 2,000- to 16,000-mg/L solution concentration.

B. Materials and Methods

1. Trimethylsilylation of Oxalic Acid

The derivatizing reagent of Von Nicolai and Zilliken[15] was used with the ammonium salt of oxalic acid as proposed by Butts and Rainey.[16] Because of the volatility of oxalic acid (melting point 101°C, sublimation beginning at 100°C), it was expected that losses of the acid might be experienced during rotary evaporation of the water; this would not be the case with the ammonium salt.

A 10-mL sample or standard was passed through a 12-mm diameter x 100-mm length column of AG-50W x 8 resin (Bio-Rad Laboratories), 20-50 mesh, in the NH_4^+ form. The column was then washed with two 5-mL portions of distilled water. All solutions were collected from the column in a 50-mL round-bottom flask, and this solution was evaporated under aspirator vacuum on a rotary evaporator with a 56°C water bath. The dry flask containing the oxalate was purged with nitrogen gas to remove moisture and then 200 µL of 5:3:0.1 (v/v/v) pyridine:BSTFA:TMCS reagent was added. The flask was stoppered and heated for 2 minutes at 80°C and then cooled. A 20-µL volume of 10 µL/10 mL dodecane in pyridine was added as an internal standard, and 2 µL was injected onto the GC.

The GC conditions were:

GC:	Hewlett-Packard 5830A, FID detector
Column:	6 ft x 2 mm inner diameter glass, packed with 10% OV-1 on 80/100 mesh Chromosorb WHP (Alltech Associates)
Carrier:	Nitrogen at 24 cc/minute
Column temperature:	150°C (6 minutes), 4°C/minute to 125°C (2 minutes)
FID temperature:	220°C
Injection port temperature:	200°C

All silylation reagents were silylation grade (Pierce Chemical Co.). Oxalic acid was dried by placing ACS grade oxalic acid dihydrate in an oven at 98° to 99°C for 2 hours and then storing over P_2O_5 in a desiccator. Purity by pH titration with standard sodium hydroxide was found to be 100.2%.

2. Methylation of Oxalic Acid

The procedure evaluated in this study was recommended by Chian and Kuo,[2] and is derived from that proposed by Schlenk and Gellerman.[13] However, Chian and Kuo used a butanediol succinate (polyester) GC column packing, whereas the procedure reported below used an OV-225 (cyanopropyl phenyl silicone) column packing. In addition, ether was used as a solvent instead of methylene chloride for the derivatization.

A 20-mL aliquot of sample or standard was passed through a 12-mm diameter x 100-mm length column of 20-50 mesh AG-50W x 8 ion exchange resin (Bio-Rad Laboratories) in the hydrogen form, followed by 5 mL of distilled water. The effluent from the resin was evaporated at 30°C on a rotary evaporator under water aspirator vacuum in a 50-mL round-bottom flask. Five mL of laboratory grade ether, which had been redistilled in glass before use, were added, and the exit tube of the diazomethane generator (Figure 12) was dipped into the ether to bubble in CH_2N_2 gas (performed in a fume hood) until the solution turned yellow, indicating an excess of CH_2N_2. Diazomethane was generated from a mixture of 2 mL of potassium hydroxide (60 g/100 mL H_2O) plus approximately 0.3 g of Diazald (Aldrich Chemical Co., N-methyl-N-nitroso-p-toluenesulfonamide) in the second tube. The first tube contained ether to scrub the helium sweep gas. Rubber stoppers and plastic and glass tubing were used for flexible connections between the helium source and between the tubes.

The ether solution was poured into a 10-mL Kuderna-Danish (Kontes No. K-570050) concentrator tube, the round-bottom flask was washed with 2 to 3 mL of ether, and the washings were added to the concentrator tube (final volume 6 to 8 mL).

An evaporative concentrator microreflux column (Kontes No. K-569251) was placed on the tube and heated in a Kuderna-Danish tube heater block maintained at 70°C, with bumping protection provided by capillary tubes that bubbled nitrogen gas into the tubes. The solutions were concentrated to a minimum of 0.5 mL (caution was taken not to allow further evaporation). Finally, 30 μL of 10 μL/10 mL hexadecane in methylene chloride was added as an internal standard, and 2 μL were injected onto the gas chromatograph with conditions as follows:

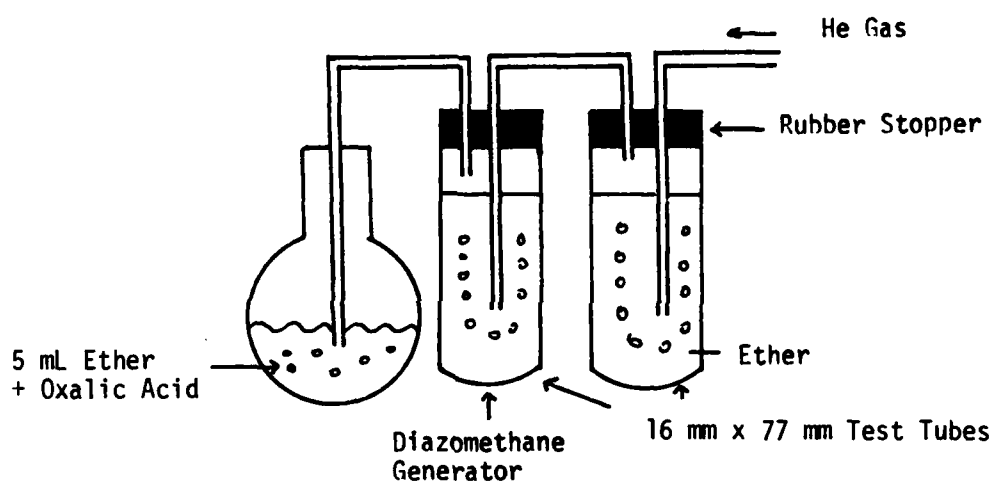|	
GC:	Hewlett-Packard 5830A, FID detector
Column:	6 ft x 1/8 in O.D. stainless steel, packed with 3% OV-225 on 100/120 mesh Gas Chrom Q (Applied Science Laboratories, Inc.)
Carrier:	Nitrogen at 30 cc/minute
Column temperature:	90°C (4 minutes), 15°C/minute to 150°C (3 minutes)
FID temperature:	200°C
Injection port temperature:	150°C

Figure 12. Diazomethane generator.

C. Results and Discussion

1. (TMS)$_2$ Oxalate

Figure 13 shows a typical gas chromatogram of (TMS)$_2$ oxalate, from analysis of a 2.0-mg/L standard of oxalic acid. The peak was well separated from the reagent peaks. Figure 14 shows a calibration curve prepared from distilled water standards. Since the line of best fit for the data did not appear to intersect the x-axis at zero, a constant loss of material in the procedure was indicated. To check the FID response, a 20-mg/L oxalic acid sample was derivatized and the resulting (TMS)$_2$ oxalate was diluted serially with derivatizing reagent and injected onto the GC. Figure 15 shows that the response curve still did not intersect at zero; thus, an irreversible column adsorption or breakdown of the (TMS)$_2$ oxalate was suspected. The test was repeated after the column had been conditioned with Silyl-8 (Pierce Chemical Co.) silylating reagent at 175°C (300 μL), but the results were the same. Silylation of a 6-mg/L standard with 1/4-strength reagent mixture (diluted with pyridine) gave an area ratio of 1.321 relative to dodecane. After the mixture stood overnight, the ratio dropped to 0.205. However, injection of 3 μL of Silyl-8 at 180°C onto the GC column, followed by another injection of the standard, showed an increase in the ratio to 0.871. A repeated (3 μL) GC column conditioning with Silyl-8 before sample injection gave a ratio of 1.089. Finally, preconditioning with 12 μL of Silyl-8 showed an area ratio of 1.528 and 1.476 for duplicate injections of the standard. Further tests indicated that injection of TMS reagent after an injection of 10-mg/L standard oxalate (peak area = 282,700 units) would result in a "ghost" peak of TMS oxalate (area = 42,910 units), so that column adsorption of (TMS)$_2$ oxalate was reversible or the column breakdown of the ester was reversible by injection of excess reagent. It was apparent that at low levels this method could lead to very confusing results, and further evaluation was not attempted.

2. Dimethyl Oxalate

Because of suspected breakdown[16] of dimethyl oxalate on the 1,4-butanediol succinate polyester column used by Chian and Kuo,[2] a 3% OV-225, polar silicone column was chosen to determine this ester for these studies.

Although methylene chloride was suggested by Chian[17] for use as a solvent for the diazomethane reaction, the solubility of oxalic acid in this solvent was found to be low. Consequently, ether was tried, because this solvent readily dissolved the oxalic acid, and Schlenk and Gellerman[18] had used ether (with 10% methanol) as a solvent for diazomethane's reaction with fatty acids.
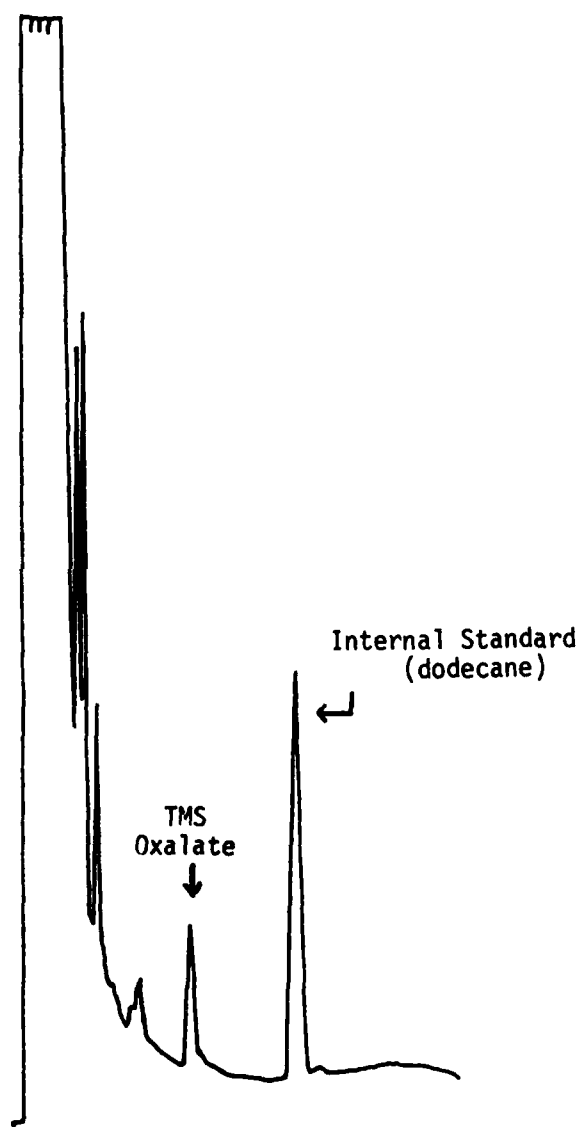
31

Figure 13. Gas chromatogram of TMS oxalate
reaction mixture (2 mg/L oxalic
acid standard).

32
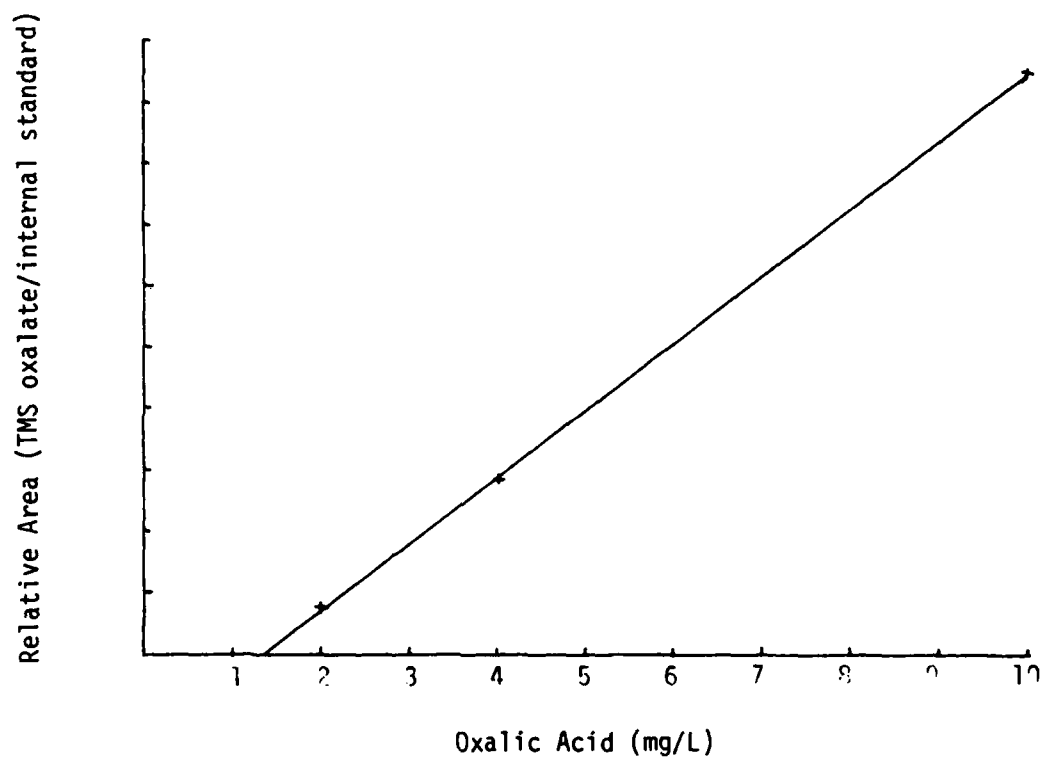
Figure 14. Standard curve for oxalic acid by the TMS method.

33
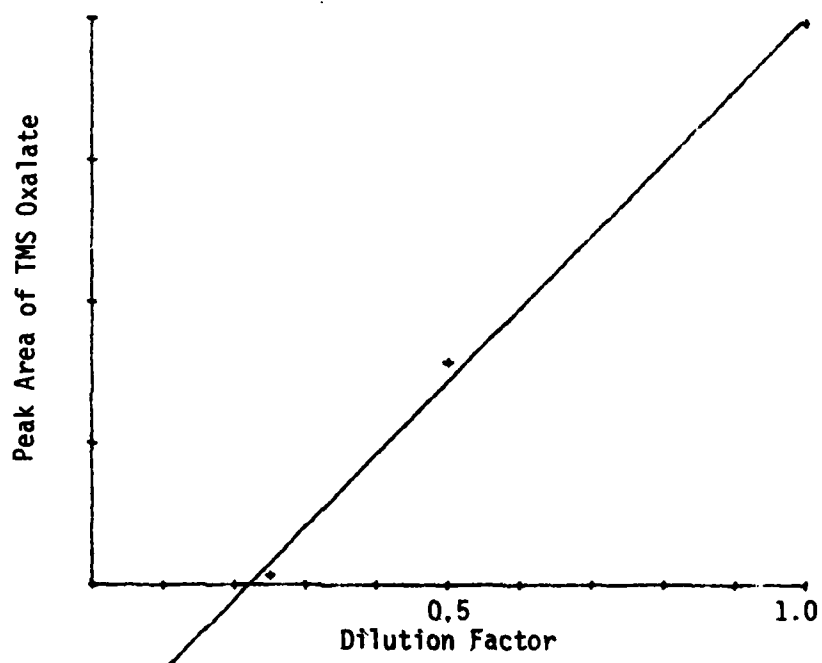
Figure 15. Serial dilution of TMS oxalate reaction mixture (20 mg/L oxalic acid standard) with pyridine-TMCS-BSTFA reagent.

Figure 16 shows the separation of dimethyl oxalate from the solvent peak and the hexadecane internal standard under the GC conditions described previously. Early work at low levels, with rotary evaporation temperatures of 50°C or more, showed chromatograms without the oxalate peak. Figure 17 presents a typical curve for 50°C rotary evaporated distilled water standards, plotted without the blank value, to show that the data (least-squares regression) extrapolated to 66 µg of oxalic acid on the x-axis for a relative area of zero. This indicated a loss of 66 µg from all the standards. When oxalic acid standards in ether were converted to derivatives and analyzed on the GC without the rotary evaporation step, the least-squares regression curve extrapolated to a value of 5 µg on the x-axis, which was much closer to the expected value of zero (Figure 17). Hence, most of the loss must have occurred during rotary evaporation at 50°C. Table 6 indicates that the results were low or erratic at 40° or 50°C, whereas the evaporation gave higher and more consistent results at 30°C.

Table 6. Relative Peak Areas from Analysis of 20 mL of 9.83 mg/L Oxalic Acid Standard, at 30° to 50°C Rotary Evaporation Bath Temperatures

Temperature (°C)	Relative Peak Area of Dimethyl Oxalate
30	1.118
30	1.115
40	0.766
40	1.111
50	0.726
50	0.798

Even at 30°C, the rotary evaporation still caused small losses of oxalic acid (Figure 18). The two extrapolated curves in Figure 18 show 1.3 mg/L on the x-axis, so that a lower limit of detection would have to be somewhere above 1.3 mg/L. A low-level standard curve (Figure 19) indicated that about 2 mg/L was the lower limit of detection.

Recovery of oxalic acid from synthetic RO-Lab wastewater that had been ozonated for 4 hours is given in Table 7. The recovery at the level tested (9.9 mg/L) was 97%, and the coefficient of variation was 7%.

Table 7. Dimethyl Oxalate Method of Recovery of Oxalic Acid
from Ozonated RO-Lab Wastewater

Spiked Sample No.	Concentration Found (mg/L)
1	10.00
2	9.68
3	9.68
4	10.59
5	8.32
6	9.55
7	9.62
Mean value ($\bar{x}$)	9.63
Standard deviation (s)	0.68
Coefficient of variation (s/$\bar{x}$ x 100)	7%
mg/L acid added (z)[a]	9.9
Average % recovery ($\bar{x}$/z x 100)	97%

a. Oxalic acid in unspiked wastewater = 0 mg/L by analysis.

Analysis of samples from an 8-hour ozonation of RO-Lab wastewater, taken at 60, 180, 300, and 420 minutes, showed no oxalic acid at these times during the ozonation; hence, the concentration was less than 2 mg/L in this wastewater at the intervals sampled. Two large peaks at retention times greater than that of dimethyl oxalate were seen. Qualitative standards of malonic, succinic, maleic, and fumaric acids were run with diazomethane, but these did not match the unknown peak retention times.
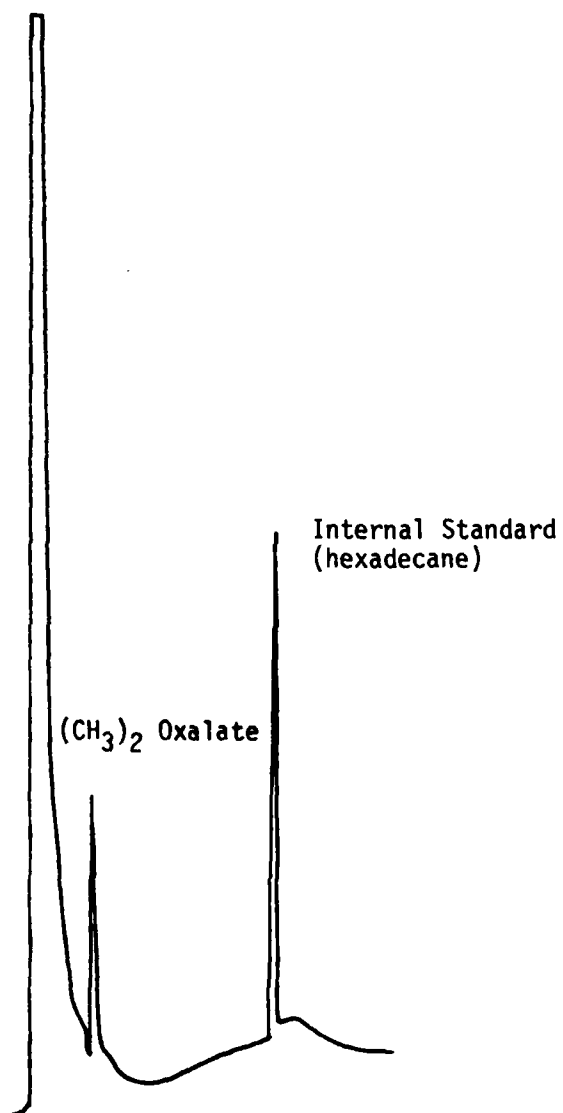
Figure 16. Gas chromatogram of reaction mixture from a 5-mg/L oxalic standard with diazomethane.

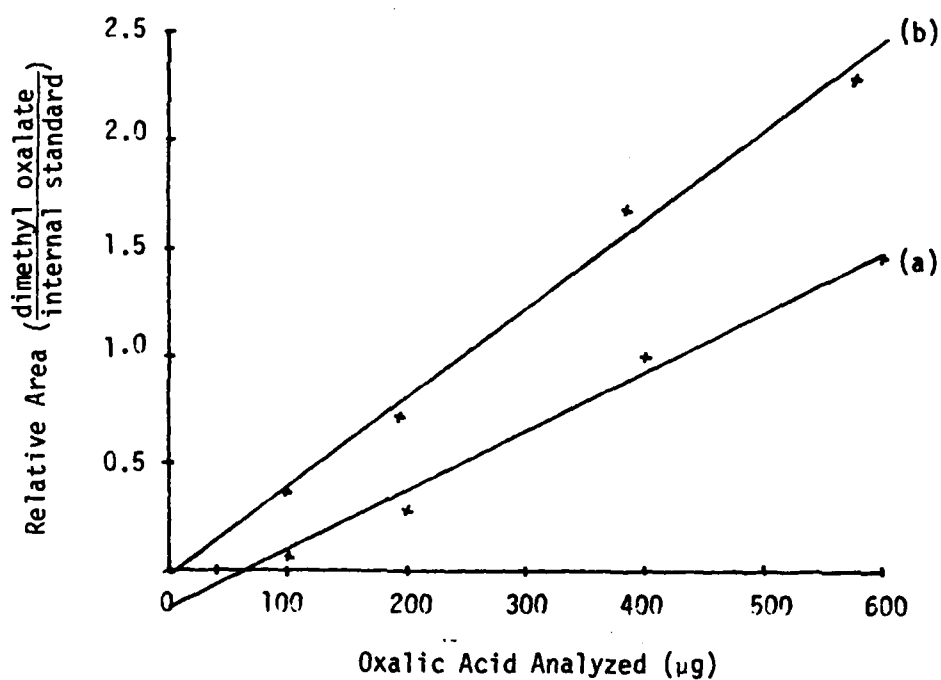Figure 17. Standard curves for dimethyl oxalate: (a) from aqueous standards using rotary evaporation at 50°C, then methylation in ether for GC; (b) from standards in ether, methylated for GC.

38
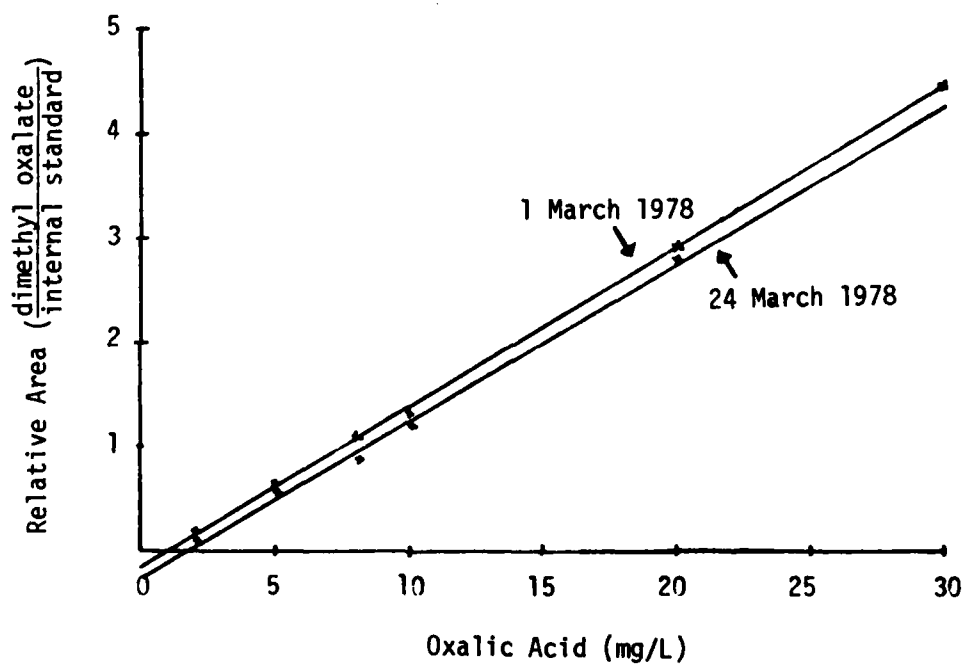
Figure 18. Standard curves for oxalic acid with the 30°C rotary
evaporation method, 1 March and 24 March 1978.
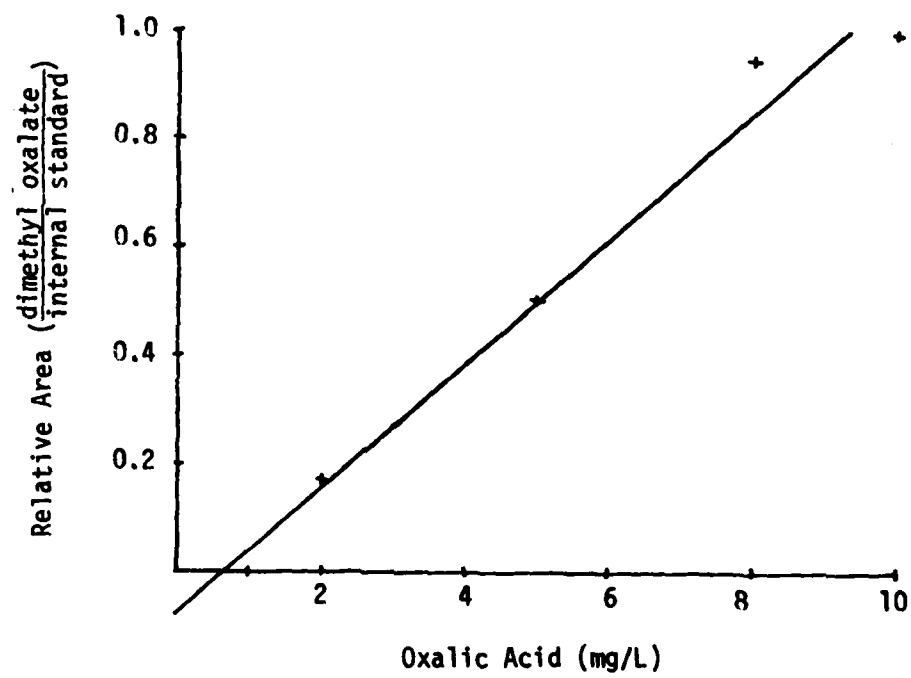
Figure 19. Low-level standard curve for oxalic acid.

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V. GLYOXALS

A. Literature Review

Glyoxal, OHC-CHO, has been determined colorimetrically by the formation of bis(2,4-dinitrophenylhydrazone) (DNP). Gould and Weber[9] measured the loss in color of a standard solution of 2,4-DNP as a measure of the amount of glyoxal reacted (molar extinction coefficient = 13,200 L/mole·cm at 365 nm for DNP). Johnson et al.[18] used a mildly basic solution of diethanolamine in pyridine to develop the color of the phenylhydrazone formed. This method was used by Gilbert[19] to follow the production of glyoxal during ozonation of organic compounds at 1-mmole/L initial concentrations.

Gas chromatographic methods have been developed to determine glyoxal, methylglyoxal (pyruvaldehyde), and dimethylglyoxal (2,3-butanedione) in a single chromatogram, using the reaction of glyoxals with ortho-phenylene-diamine to form quinoxalines (equation 2):

$$
\begin{array}{c} R \\ | \\ C{=}O \\ | \\ C{=}O \\ | \\ R \end{array}
\; + \;
\text{(1,2-diaminobenzene)}
\; \longrightarrow \;
\text{(2,3-R-substituted quinoxaline)}
\qquad (2)
$$
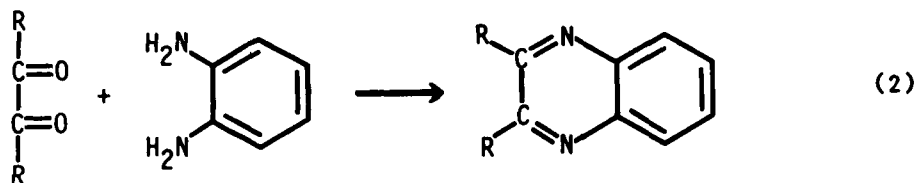
R = H (in glyoxal and methylglyoxal) or CH_3 (in methyl- and dimethylglyoxal)

Nojima et al.[20] used 4-chloro-o-phenylenediamine to detect glyoxals in air pollution studies, with electron capture (^{63}Ni) gas chromatography for quantification. Chian and Kuo[21] developed a method for glyoxals in aqueous solution by FID-GC detection. They reacted a 10-mL water sample with 1 mL of 0.185 M methanolic-o-phenylenediamine solution and 1 mL of 10% aqueous sodium bisulfite for 30 minutes at room temperature. Benzene was added to extract the quinoxalines and the extract was evaporated to 0.5 mL in a micro Kuderna-Danish evaporator and analyzed on a 3% OV-17 GC column. Yields of quinoxaline (Q), methylquinoxaline (MQ), and dimethylquinoxaline (DMQ) (from glyoxal, methylglyoxal, and dimethylglyoxal, respectively) were 81%, 32%, and 33%.

Extraction efficiencies were 27%, 85%, and 83%, whereas the solvent evaporation efficiencies were 95%, 98%, and 94%, respectively. Detection limits were reported as 0.08, 0.05, and 0.2 mg/L. Precision estimates were 2%, 4.3%, and 2.5% (relative standard deviation) at the 10-mg/L level.

B. Materials and Methods

The method of Chian and Kuo[21] was modified by heating the reaction mixture at 80°C for 30 minutes in sealed (Teflon-lined caps) test tubes instead of shaking 30 minutes at room temperature. In addition, methylene chloride was used as a solvent instead of benzene, and a more polar (OV-225 instead of OV-17) GC column was chosen.

Ten mL of combined glyoxal standard or sample were pipetted into a 30-mL test tube. One mL each of 0.185 M o-phenylenediamine (Aldrich Chemical Co., recrystallized from ether after treatment with Norit carbon and filtration) in methanol and 10% (wt/vol) aqueous sodium bisulfite were added, and the tubes were capped and shaken to mix the solutions. The mixture was heated in an 80°C water bath for 30 minutes, cooled, and shaken with 5 mL of methylene chloride for 1 minute. Four mL of the lower phase was withdrawn with a pipette and evaporated to 0.5 mL in a 10-mL micro Kuderna-Danish concentrator tube with reflux column and nitrogen gas ebullator. The heating block temperature was set at 80°C. A 1/1,000 (v/v) solution of hexadecane in hexane was added to each concentrate as an internal standard, and 2 µL of the concentrate was injected onto the GC with the following conditions:

GC:	Hewlett-Packard 5830A, FID detector
Column:	6 ft x 1/8 in O.D. stainless steel, packed with 3% OV-225 on 100/120 mesh Gas Chrom Q (Applied Science Laboratories, Inc.)
Carrier:	Helium at 30 cc/minute
Column temperature:	130°C (2 minutes), 10°C/minute to 200°C (2 minutes)
FID temperature:	220°C
Injection port temperature:	200°C

Glyoxal was purchased as a 40% aqueous solution (Pfaltz and Bauer Chemical Co.), which was found to have a density of 1.26 g/mL. Dimethyl-glyoxal (2,3-butanedione) was obtained as a 99% pure liquid (density = 0.9809 g/mL) from Aldrich Chemical Co. Methylglyoxal (density = 1.045 g/mL) was obtained as a 99% pure liquid from Pfaltz and Bauer. A stock standard was made up with 250 µL of 40% glyoxal solution, 100 µL of methylglyoxal, and 100 µL of dimethylglyoxal diluted to 100 mL with water. The concentrations of this stock solution were calculated to be:

1,260 mg/L glyoxal
1,000 µL/L methylglyoxal = 1,045 mg/L
1,000 µL/L dimethylglyoxal = 981 mg/L

The concentrations of methyl- and dimethylglyoxal in this standard were considered as 1,000 mg/L to within 5% error, based on the densities of the pure liquids, and subsequent concentrations are reported as mg/L in this report.

For recovery studies, 500 mL of RO—Lab wastewater were spiked with 10 mL of 1/10 diluted stock glyoxal combined standard to give concentrations of 2.47 mg/L of glyoxal and 1.96 mg/L of methylglyoxal and dimethylglyoxal.

C. Results and Discussion

Substitution of OV-225 for OV-17 as the liquid phase for gas chromatography did not change the order of elution of quinoxalines, but did shift the peak due to unreacted o-phenylenediamine from its position between quinoxaline (Q) and methylquinoxaline (MQ), as reported by Chian and Kuo,[21] to a position after the dimethylquinoxaline (DMQ) peak. This peak was generally very large and tailed significantly, but caused no problem because it was at the end of the chromatogram (Figure 20).

A standard curve for low levels of glyoxals (0.25 to 6.3 mg/L range) is shown in Figure 21. The curves were linear and showed that the sensitivity followed the order glyoxal < methylglyoxal < dimethylglyoxal. The lower limit of detection was no greater than 0.25 mg/L in these tests; no attempts were made to try lower concentrations. The data in Figure 21 were obtained with methylene chloride as an extractant for the quinoxalines. When pentane was used, the relative areas for Q, MQ, and DMQ were reduced by about 86%, 45%, and 22%, respectively. The amount of o-phenylenediamine extracted also decreased when pentane replaced methylene chloride. Chian and Kuo[21] reported an extraction efficiency of only 27% for Q with benzene, which resembles the reduced recovery seen with pentane. Hence, methylene chloride was a much better solvent for quinoxaline extraction, and the extra amount of o-phenylenediamine extracted did not interfere with determination of the quinoxalines on the OV-225 column.

Table 8 gives the recoveries of glyoxals added to synthetic RO—Lab permeate wastewater. At approximately 2-mg/L concentrations, the recoveries averaged 79 to 88%. The variation in glyoxal recovery was much higher than that of methyl- or dimethylglyoxal. A test with distilled water standards showed better precision for glyoxal (11% coefficient of variation, for four replicates at 3.15 mg/L) and approximately the same precision values as those in Table 8 for the other glyoxals. The low recoveries for all the glyoxals and poor precision for glyoxal were probably due to the low level of the spike, which was only about 10 times the estimated limit of detection.

The effect of temperature on the derivatization reaction was not studied here. Chian and Kuo[21] indicated that heating was not necessary in tests with 10 mL of 1,000 ppm of glyoxals. An 80°C temperature was used here to ensure a complete reaction even for low levels of glyoxals.

The results of an 8-hour ozonation of RO—Lab wastewater are presented in Table 9. Glyoxal concentration was less than 0.25 mg/L in all samples. Methylglyoxal increased to about 3 mg/L after 360 minutes and disappeared by 420 minutes. Dimethylglyoxal showed 0.4 mg/L in the 60-minute sample and undetectable concentrations thereafter. Hence, of the three glyoxals, only methylglyoxal was a significant reaction product in the ozonation of this wastewater.

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Table 8. Recoveries of Glyoxals from RO-Lab Wastewater

Spiked Sample No.	Concentration Found (mg/L)		
	Glyoxal	Methylglyoxal	Dimethylglyoxal
1	1.19	1.46	1.70
2	1.02	1.55	1.83
3	3.17	1.73	1.72
4	1.53	1.50	1.81
5	1.82	1.74	1.81
6	2.37	1.62	1.54
7	2.64	1.68	1.72
Mean value ($\bar{x}$)	1.96	1.61	1.73
Standard deviation (s)	0.79	0.11	0.10
Coefficient of variation (s/$\bar{x}$ x 100)	40.0%	6.9%	5.8%
mg/L added (z)[a]	2.47	1.96	1.96
Average % recoveries ($\bar{x}$/z x 100)	79%	82%	88%

a. No glyoxals were detected in the unspiked wastewater.

Table 9. Production of Glyoxals During Ozonation of RO-Lab Wastewater

O_3 Time (min)	mg/L		
	Glyoxal	Methylglyoxal	Dimethylglyoxal
0	<0.25	<0.25	<0.25
60	<0.25	0.59	0.41
120	<0.25	1.05	<0.25
180	<0.25	1.79	<0.25
240	<0.25	2.45	<0.25
300	<0.25	3.18	<0.25
360	<0.25	3.26	<0.25
420	<0.25	<0.25	<0.25
480	<0.25	<0.25	<0.25

Peak (1) = Internal standard
              (hexadecane)
     (2) = Quinoxaline
     (3) = Methyl quinoxaline
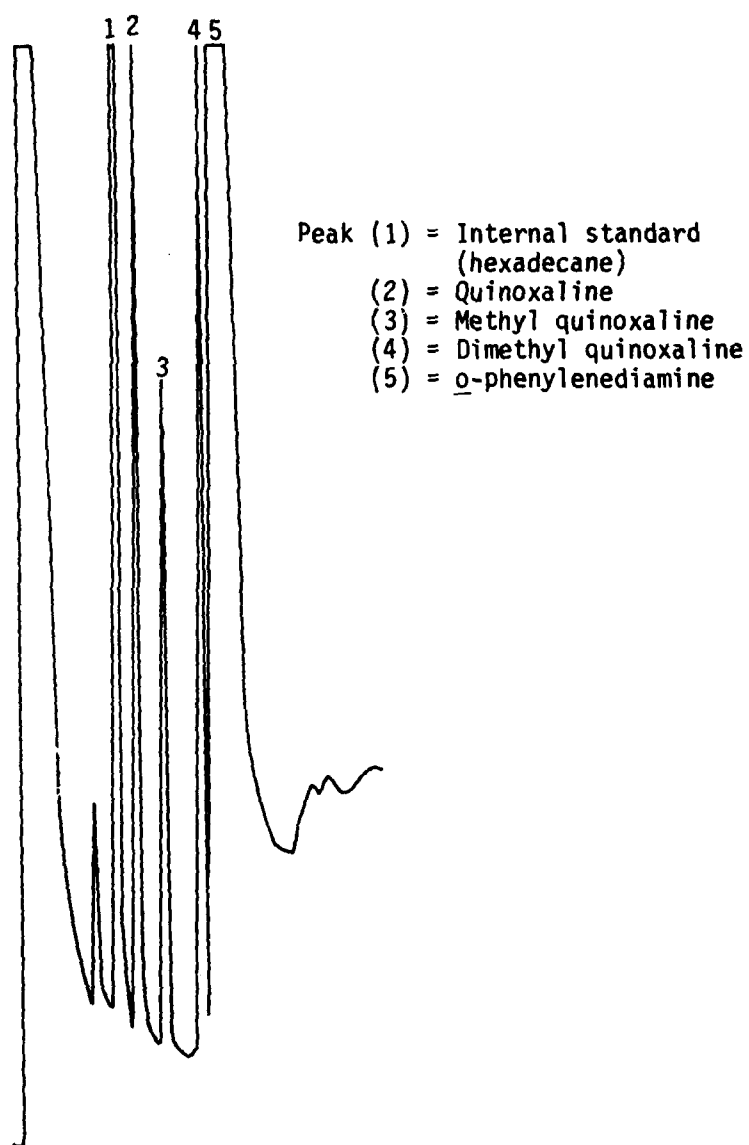   (4) = Dimethyl quinoxaline
     (5) = o-phenylenediamine

Figure 20.   Gas chromatogram of quinoxaline mixture on OV-225.

45
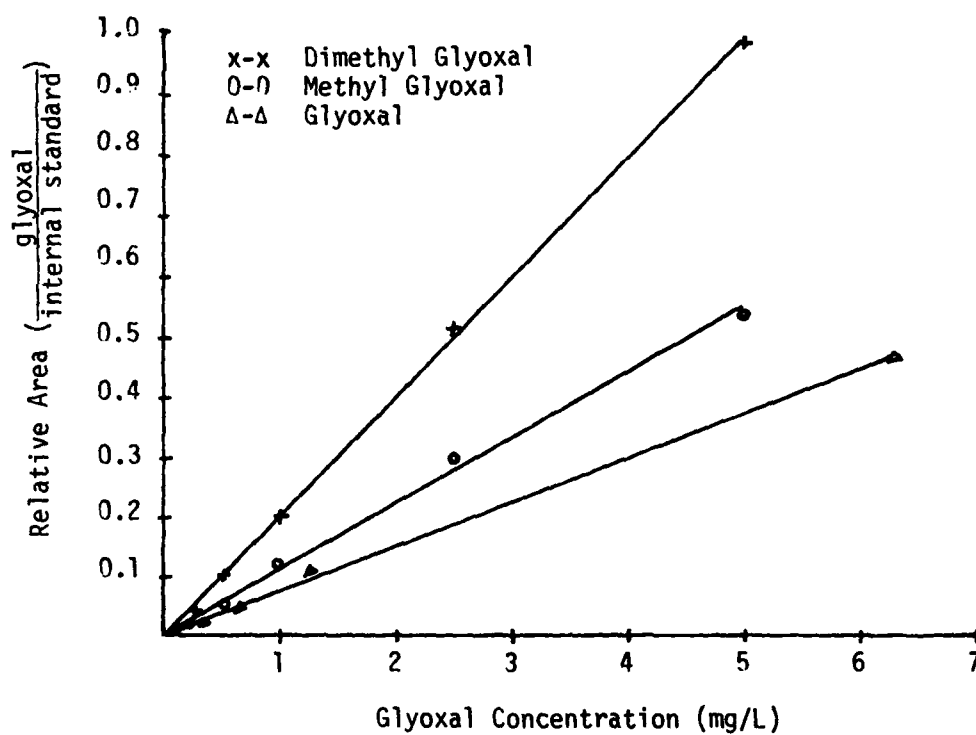
Figure 21. Standard curves for glyoxals.

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A. Literature Review

Formaldehyde has been determined in low ppm amounts by gas chromatography and colorimetric/fluorimetric methods. Because of the low FID detector response to formaldehyde, TC detectors have had to be used, with limited sensitivity. Hammarstrand[22] let formaldehyde react with dimedone (5,5-dimethyl-1,3-cyclohexanedione) to form a derivative from one molecule of formaldehyde and two of dimedone, thus increasing the FID response. Kallio et al.[23] demonstrated that the 2,4-dinitrophenylhydrazone of formaldehyde could be detected in less than 100- to 200-pg amounts by electron capture detection on an SE-30 column. However, the derivatives were prepared in milligram amounts, not at the low levels expected in wastewaters.

Colorimetric analyses of formaldehyde have often been performed with chromotropic acid in hot, strong sulfuric acid. A milder reaction was found to be the Hantzsch reaction, in which ammonia reacts with formaldehyde and a β-diketone or a β-keto ester to form a dihydropyridine derivative. An example is the method used by Nash[24] with acetylacetone (equation 3):

(DDL)
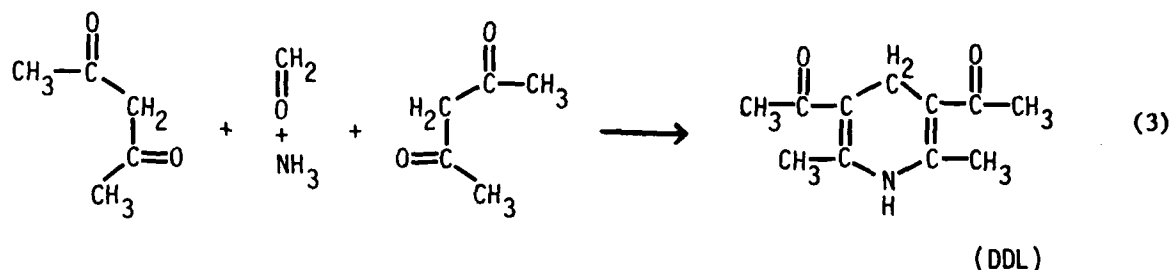
The product, diacetyldihydrolutidine (DDL), was found to have a molar extinction of 8,000 L/mole•cm at 412 nm. Belman[25] applied this reaction to the assay of formaldehyde at sub-ppm levels, using excitation at 410 nm and fluorescence at 510 nm. In an extensive literature review by Bartos and Pesez,[26] the uses of ethyl acetoacetate, 2,4-pentanedione, and dimedone for fluorimetric analysis of formaldehyde were described. In addition, J-acid (6-amino-1-naphthol-3-sulfonic acid) has been used to form a fluorescent derivative of formaldehyde. These reagents have been utilized in the determination of alpha glycolic compounds via analysis of the formaldehyde generated on reaction of the compound with permanganate or periodate.[27] Because of their specificity and ease, the methods of Belman and Nash were chosen for use with ozonated RO-Lab wastewaters.

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B. Materials and Methods

Color-developing reagent was prepared by combining 150 g of ammonium acetate and 3 mL of glacial acetic acid with 2 mL of redistilled acetylacetone and diluting the mixture to 1 liter with water.

Ten mL of color-developing reagent was mixed with 10 mL of formaldehyde standard or 10 mL of a sample containing less than 8 mg/L of formaldehyde. The mixture was heated in glass test tubes with Teflon-lined screw caps at 58°C for 10 minutes and then allowed to cool.

Fluorescence was read on a Perkin-Elmer Model 204 fluorescence spectrophotometer with 1-cm quartz cuvettes, using λ(excitation) = 440 nm, λ(emission) = 510 nm.

Absorbance was read on a Bausch & Lomb Spectronic 700 UV-visible spectrophotometer at 410 nm with 1-cm or 5-cm quartz cells.

Standard formaldehyde solution was prepared by weighing approximately 6.0 g of 40% (wt/vol) formaldehyde (ACS grade) solution and transferring to a 100-mL volumetric flask. Ten mL of 1 N sulfuric acid (H_2SO_4) were added and the solution allowed to stand 10 minutes. Then 10 mL of 1 N sodium hydroxide were added and the mixture was diluted to 100 mL. This stock solution was standardized by measuring 25 mL of the solution into an Erlenmeyer flask, adding 2 drops of thymolphthalein indicator solution (1.0 g dye/L ethanol) and neutralizing with dilute acid or base. In another flask, 25 mL of sodium sulfite solution (125 g/L in water) was similarly neutralized with thymolphthalein indicator. The formaldehyde and sodium sulfite solutions were combined and titrated with standard 1 N sulfuric acid (1 mL 1 N H_2SO_4 is equivalent to 30.03 mg formaldehyde).

C. Results and Discussion

Figure 22 shows the linear standard curve obtained from 0 to 8 mg/L of formaldehyde, analyzed after the Hantzsch reaction with a spectrophotometer. A standard curve from 0 to 0.4 mg/L formaldehyde, analyzed by fluorimetry, is shown in Figure 23. Standards of 0.005 and 0.010 mg/L did not show a detectable response at the settings used (10 x 1), but the response to 0.050 mg/L was readily seen. The interference from a 10-mg/L glyoxylic acid standard was equivalent to only 0.004 mg/L of formaldehyde with this method.

It should be noted that Belman[25] reported an excitation maximum at 410 nm, whereas the maximum with the Perkin-Elmer unit was found to be at 440 nm, perhaps because of mechanical problems with the instrument wavelength settings. The lower limit of 0.01 mg/L formaldehyde by fluorimetric analysis reported by Belman[25] was not achieved here. A practical limit of detection should probably be about 0.02 mg/L. With a 5-cm cell, spectrophotometric analysis showed an absorbance of 0.095 for a 0.1-mg/L standard of formaldehyde.
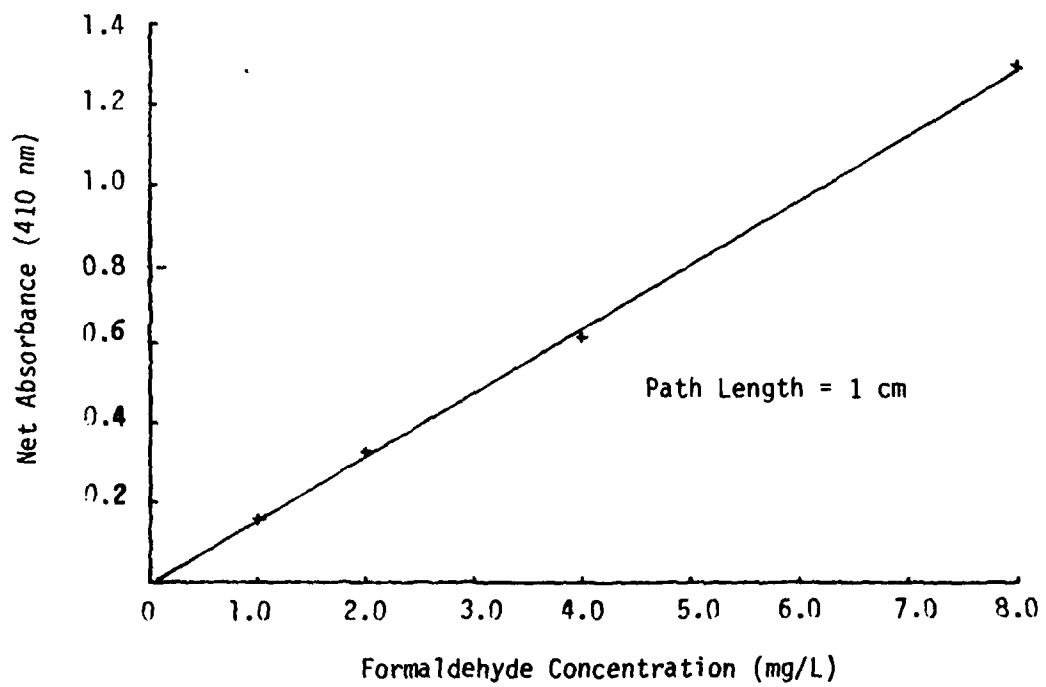
48

Figure 22.   Standard curve for formaldehyde in a test using
             spectrophotometric detection.

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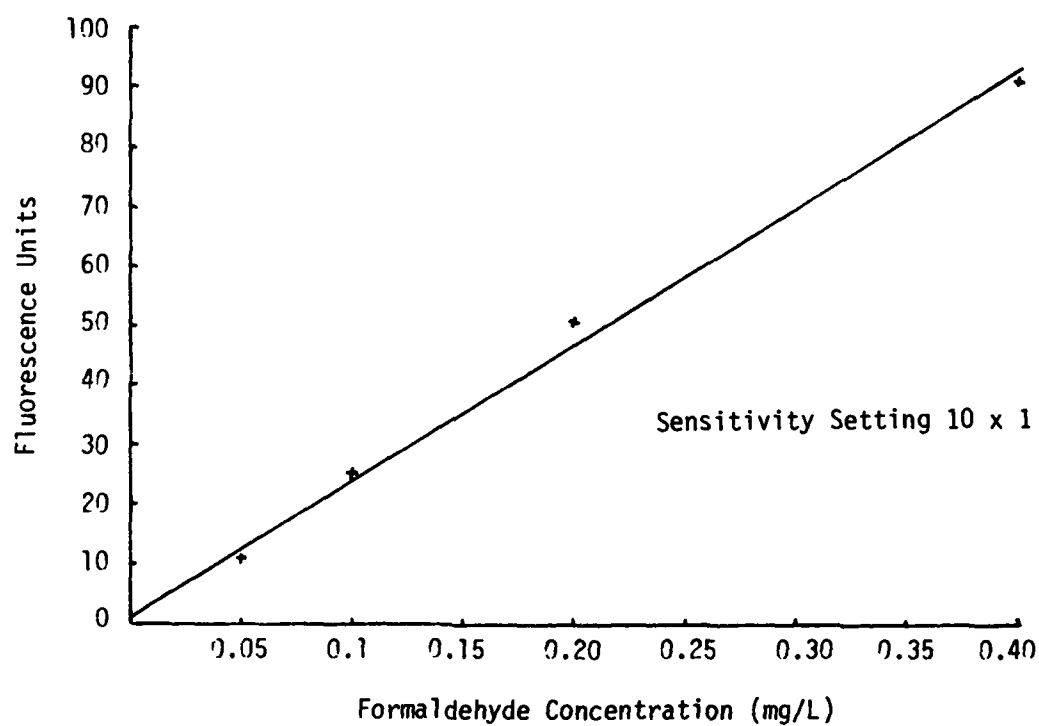
Figure 23. Standard curve for formaldehyde in a test using
fluorescence detection.

The precision of the spectrophotometric method was tested on replicates of an ozonated synthetic laboratory wastewater RO permeate (RO-Lab) that was diluted by 1/10 with water for analysis. Table 10 shows that the coefficient of variation was only 4%.

Table 10. Precision of Colorimetric Determination
of Formaldehyde with Diluted O_3/RO-Lab Permeate

Sample No.	Formaldehyde (mg/L)
1	4.49
2	5.09
3	4.54
4	4.60
5	4.57
6	4.63
7	4.55
Mean $\bar{x}$	4.64
Variance	4.16×10^{-2}
Standard deviation	2.04×10^{-1}
Coefficient of variation	4%

Addition of a formaldehyde spike to the ozonated RO-Lab solution (Table 11) showed that an average of 83% of the expected value was found. As in the precision test, all solutions were diluted by 1/10 before an analysis to get the concentrations into the range of 0 to 8 mg/L.

Table 11. Accuracy of Colorimetric Determination
of Formaldehyde with Spiked O_3/RO-Lab Permeate[a]

Sample No.	Formaldehyde (mg/L)
1	6.08
2	6.19
3	6.08
4	6.02
5	6.08
6	6.08
7	6.13
Mean $\bar{x}$	6.09
Variance	2.795×10^{-3}
Standard deviation	5.29×10^{-2}
Spike recovered	1.45
Percent recovery	83%
Coefficient of variation	1%

a. Initial concentration = 4.64 mg/L; added spike = 1.74 mg/L.

The progress of ozonation of synthetic RO-Lab wastewater was followed with fluorimetric analyses for formaldehyde, although the levels were much higher than expected and dilutions of 1/20 or 1/40 were necessary to permit work within the linear standard curve from 0 to 2.0 mg/L. These analyses could have been performed much more easily with the spectrophotometric methods outlined previously. Table 12 shows the progress of two ozonations on separate days. In both runs, the formaldehyde peaked at around 180 minutes. Hence, the chemical analyses appeared able to track the progress of the ozonation with respect to formaldehyde.

Table 12. Formaldehyde Formation During Two Ozonations
of RO-Lab Wastewater

Time (min)	Formaldehyde (mg/L)	
	Test 1	Test 2
0	0.8	0.8
60	53.6	16.8
120	56.0	49.2
180	59.2	56.4
240	44.0	34.8
300	24.2	23.6
360	0.2	10.6
420	<0.1	<0.1
450	0.8	No data
480	<0.1	1.0

VII. GLYOXYLIC ACID

A. Literature Review

Gas chromatography of glyoxylic acid requires the formation of volatile derivatives. Among those that were synthesized for possible GC applications have been: (a) O-benzyl oxime trimethylsilyl esters, (b) O-methyl oxime trimethylsilyl esters, and (c) 1,3-diphenylimidazolidine-2-carboxylic acid trimethylsilyl ester.[28] Kallio and Linko[29] synthesized the 2,4-dinitrophenylhydrazone of glyoxylic acid and then made the methyl ester of that product. However, in these cases, there was no attempt to analyze for glyoxylic acid in dilute aqueous (1 to 10 ppm) solution.

A colorimetric method using oxidation of glyoxylic acid phenylhydrazone to form 1,5-diphenylformazancarboxylic acid was proposed by Kramer et al.,[30] but formaldehyde was an interfering compound.

A relatively specific and sensitive procedure was reported by Zarembski and Hodgkinson,[31] who permitted glyoxylic acid to react with resorcinol and measured the product by its fluorescence. Their method appeared to be the best candidate for evaluation in the studies reported here.

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B. Methods and Materials

Potassium Carbonate-Bicarbonate Buffer (pH 9.6). Two hundred mL of 0.1 M K_2CO_3 and 800 mL of 0.1 M $KHCO_3$ were mixed.

Glyoxylic Acid Stock. A 1,000-mg/L solution was prepared with glyoxylic acid in distilled water. This solution was diluted to a 100-mg/L working solution.

Reagents. HCl, resorcinol, K_2CO_3, and ascorbic acid were prepared as aqueous solutions.

Procedure. Each sample (2 mL) or standard was placed in a screw-cap test tube capable of holding 20 mL. The cap was lined with a Teflon disk. To each sample or standard was added 1 mL of 3 N HCl, 0.1 mL of 5% (wt/vol) resorcinol, and 1.5 mL concentrated HCl. The tubes were capped and heated in boiling water for 5 minutes; then they were cooled in an ice bath. Five mL of isobutanol were added and the tube was shaken for 2 minutes and allowed to stand for phase separation. The isobutyl alcohol phase was transferred to a 25-mL stoppered tube, 10 mL of 25% (wt/vol) K_2CO_3 were added, and the tube was shaken again for 2 minutes. After settling, the aqueous phase was transferred to a 25-mL volumetric flask, 1 mL of 10% (wt/vol) ascorbic acid was added, and the volume was made up to 25 mL with potassium carbonate-bicarbonate buffer. After mixing, the solution was allowed to stand for 30 minutes. The fluorescence was measured on a Perkin-Elmer Model 204 spectrofluorimeter with 1-cm quartz cuvettes at an excitation wavelength of 490 nm and an emission wavelength of 530 nm.

C. Results and Discussion

A linear calibration curve was found for the range of 0 to 5 mg/L glyoxylic acid (Figure 24). The detection limit was no higher than 0.1 mg/L for the method.

The precision of the glyoxylic acid procedure was checked with replicate analyses of an ozonated RO-Lab permeate, as shown in Table 13. The coefficient of variation was about 15% at the low level tested. When a spike was added to increase the concentration to 0.88 mg/L, the precision as expressed by coefficient of variation was about 8% (Table 14). The percent recovery of the spike was 96%.
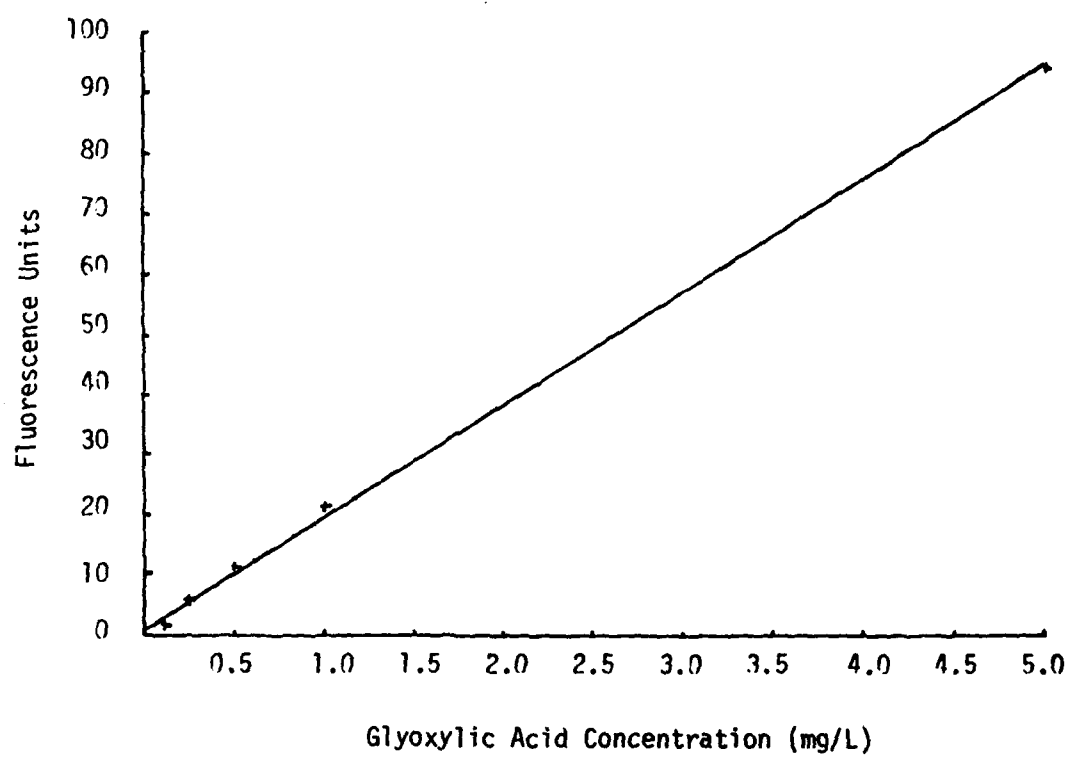
Figure 24. Standard curve for glyoxylic acid.

Table 13. Precision of Glyoxylic Acid Method (Low-Level Sample)

Sample No.	Glyoxylic Acid (mg/L)
1	0.16
2	0.21
3	0.14
4	0.14
5	0.16
6	0.16
7	0.16
Mean $\bar{x}$	0.16
Variance	0.0005
Standard deviation	0.0234
Coefficient of variation	15%

Table 14. Recovery of Glyoxylic Acid Added to Ozonated RO-Lab Permeate[a]

Sample No.	Glyoxylic Acid (mg/L)
1	0.89
2	0.84
3	0.74
4	0.84
5	0.92
6	0.92
7	0.79
Mean $\bar{x}$	0.85
Variance	0.0045
Standard deviation	0.0674
Spike recovered	0.69
Percent recovery	96%
Coefficient of variation	8%

a. Unspiked concentration = 0.16 mg/L; added spike = 0.72 mg/L.

Analysis of samples from ozonation of RO-Lab permeate showed very low levels of glyoxylic acid (Table 15). A maximum of only 0.4 mg/L was seen after 180 to 240 minutes of ozonation. These values may be low due to decreases in glyoxylic acid during 12-day storage at 4°C. The effect of storage time was not tested in these studies.

Table 15. Production of Glyoxylic Acid During Ozonation of Synthetic RO-Lab Wastewater

Time (min)	Glyoxylic Acid (mg/L)
0	<0.1
60	0.2
120	0.2
180	0.4
240	0.4
300	0.2
360	<0.1
420	<0.1
480	<0.1
485	0.1

VIII. CONCLUSIONS

A. Acetic and Formic Acids

The method of Bethge and Lindstrom[6] was found to be effective for analysis of acetic and formic acids. There was no advantage to the use of pentafluorobenzyl bromide over benzyl bromide, even though the Hall detector showed very good sensitivity to the PFB esters. The key disadvantage of the PFB-Br system was the more difficult GC resolution of the PFB-acetate and formate esters from the unreacted PFB-Br. In both the PFB-Br and benzyl bromide procedures, the carbonate alkalinity of the samples interfered more with the esterification of formic acid than acetic acid. The interference could be reduced by purging the samples with inert gas after passage of the samples through acidic ion exchange resin or by adding an excess of benzyl bromide reagent to cover the carbonic acid formed with the ion exchange resin. Although Bethge and Lindstrom[6] suggested computation of the amount of benzyl bromide reagent needed for each sample from equation (1), a more practical procedure would be to spike a control sample (containing no ozonated products) with 10 mg/L of each of the acids to be determined, plus internal standard, then to titrate this sample to calculate the amount of reagent to be added to all samples and standards, with equation (1). Since 0- to 10-mg/L calibration curves prepared with one level (1.5 molar excess over the 10-mg/L level) of reagent were linear, the presence of excess benzyl bromide reagent did not interfere at the lower concentrations. Acetic and formic acid levels as low as 0.25 mg/L were easily reached when the benzyl bromide method was used. Recoveries were essentially quantitative and the precision was 1 to 4% of the mean (relative standard deviation) at 4 to 5 mg/L of added formic or acetic acids.

B. Oxalic Acid

Attempts to quantitatively derivatize 0- to 10-mg/L solutions of oxalic acid with pentafluorobenzyl bromide were not successful. Although a TMS diester was easily formed, it apparently was not very stable during gas chromatography and TMS esterification was discarded as a method for low levels of oxalic acid. The dimethyl ester, however, was readily prepared and chromatographed, although care still had to be taken to avoid losses of oxalic acid during rotary evaporation of water. A temperature of 30°C was found to be a good compromise temperature for rotary evaporation. A lower level of detection of about 2 mg/L was seen with the procedure described. The spike recovery was 97% with a 7% coefficient of variation at 9.9 mg/L added oxalic acid.

C. Glyoxals

The modification of Chian and Kuo's method[21] showed linear standard curves for glyoxal, methylglyoxal, and dimethylglyoxal. The sensitivity increased with the number of methyl groups in the series. Although the method

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gave reasonable precision for methylglyoxal and dimethylglyoxal at about 2 mg/L levels, the precision for glyoxal was not acceptable at this level. The spike recoveries for all glyoxals were in the range of 79 to 88%. A multiple extraction procedure should be tried to obtain more consistent recoveries of the quinoxalines formed in the reaction step.

D. Formaldehyde

Formaldehyde was readily determined by colorimetry[24] for concentrations up to 8 mg/L and by fluorimetry[25] for levels up to 2 mg/L. As little as 0.05 mg/L was detected by the fluorimetric method. Glyoxylic acid did not interfere with the formaldehyde determination, and recovery of an added spike of less than 2 mg/L was about 83%, with a precision of about 1% relative standard deviation using the colorimetric method. For most studies, the extra sensitivity of the fluorimetric method may not be necessary; colorimetry with 1- to 5-cm cells would be recommended in such cases.

E. Glyoxylic Acid

The resorcinol procedure of Zarembski and Hodgkinson[31] was found to work well for 0- to 5-mg/L levels of glyoxylic acid. At least 0.1 mg/L could be detected. The precision was shown to be about 7% relative standard deviation, and a spike of 0.8 mg/L was recovered at 96% of theoretical. Because of the multiple extractions used, this method was more time-consuming than most colorimetric procedures and it did require the use of a spectrofluorimeter.

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APPENDIX A

1. Synthesis of Dipentafluorobenzyl Oxalate

Oxalic acid dihydrate (0.5 g) was titrated to pH 8 with 0.386 M tetra-n-butylammonium hydroxide (TBAOH) in a small volume of water. The water was removed by rotary evaporation at 43°C. The residue was a yellow oil.

To the residue was added 270 μL of pure pentafluorobenzyl bromide, and the mixture was allowed to stand about 15 minutes until white crystals formed. About 10 mL of benzene was added to dissolve the crystals, and the TBA bromide salts were extracted with two portions of water. The benzene was removed from the organic layer by rotary evaporation, and the crystals were recrystallized from ethyl ether and air dried (melting point = 133°-134°C).

Figure A-1 shows the single nuclear magnetic resonance (NMR) proton peak expected for the four equivalent $-CH_2$-protons. An infrared spectrum (Figure A-2) showed a carbonyl band at 1,770 cm^{-1}, but no OH absorption in the 2,500- to 3,000-cm^{-1} range, indicating that a free-COOH group was not present, as expected for an ester.

2. Mass Spectrometry of PFB Acetate, Formate, and Oxalate

The mass spectra were recorded at 70 electron volts ionizing potential on a DuPont 21-490B gas chromatograph/mass spectrometer and 21-094 data collection system.

a. Dipentafluorobenzyl Oxalate

The crystals of dipentafluorobenzyl oxalate were introduced into the mass spectrometry source through the probe.

The major fragments were:

$(C_6F_5CH_2)^+$, m/e 181, as a base peak;

$(C_6F_5CH_2CH_2)^+$, m/e 195;

$(C_6F_5CH_2(OCO)_2)^+$, m/e 269;

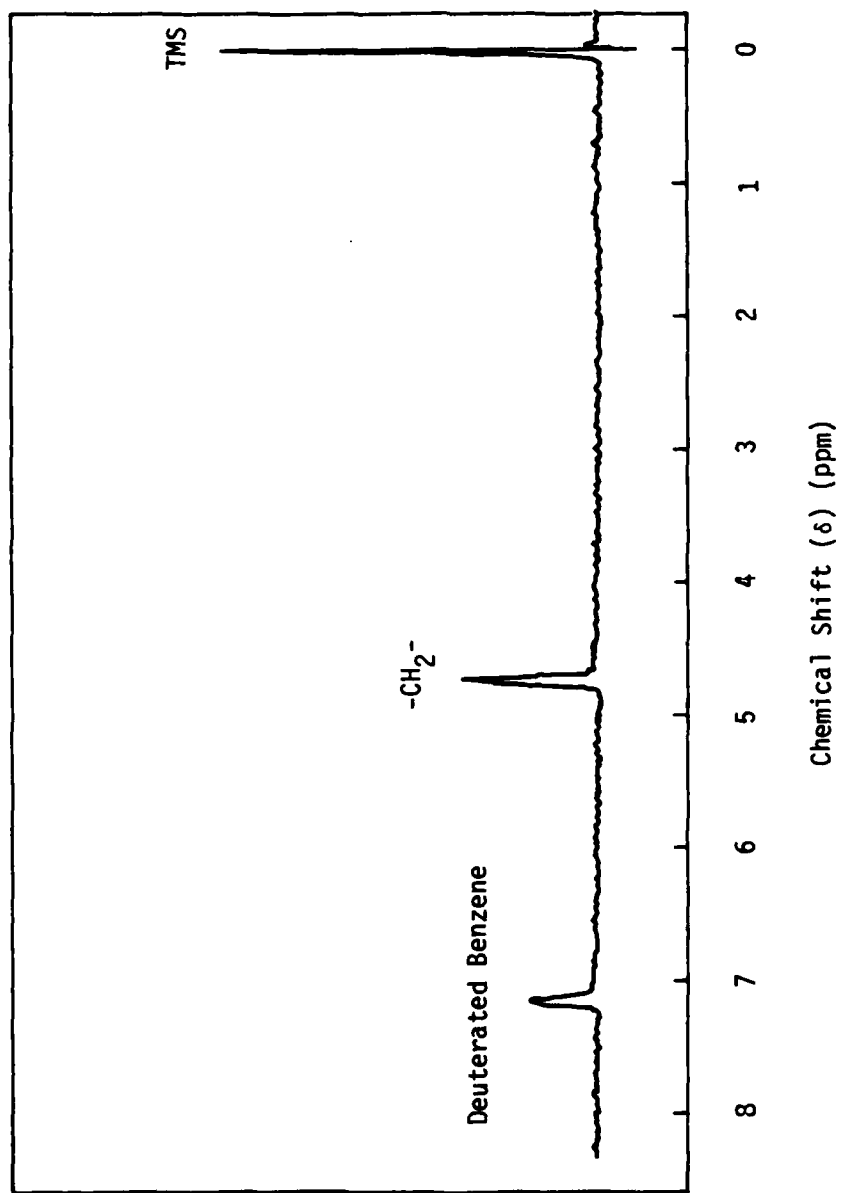and $(C_6F_5CH_2OCO)_2^{+}$, m/e 450, as a parent ion.
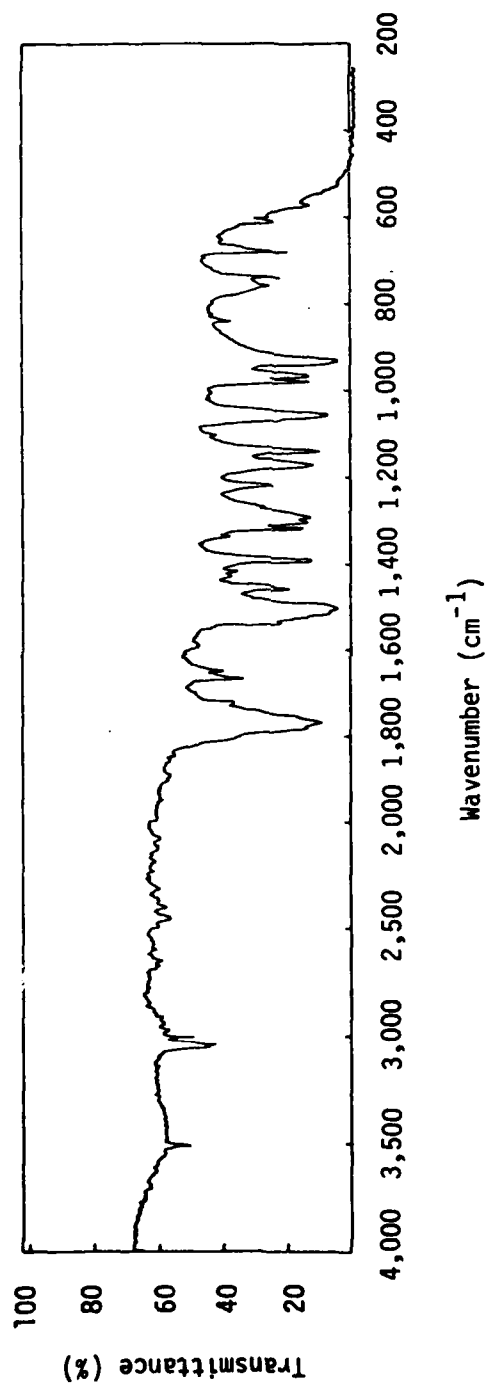
Figure A-1. NMR spectrum of dipentafluorobenzyl oxalate.

Figure A-2. Infrared spectrum of dipentafluorobenzyl oxalate.

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b. <u>Pentafluorobenzyl Formate</u>

The GC conditions were:

GC column:	10 ft x 2 mm I.D. glass, packed with 10% OV-1 on 100/120 mesh Gas Chrom Q (Applied Science Laboratories, Inc.)
Carrier:	Ultra high purity grade helium, 20 psi
Column temperature:	100°C to 250°C (2 minutes), 12°C/minute
Injection port temperature:	150°C
Detector temperature:	225°C

The major fragments were:

$(C_6F_5CH_2)^+$, $\underline{m}/\underline{e}$ 181, as a base peak, and

$(C_6F_5CH_2\text{-}OCHO)^{\ddot{+}}$, $\underline{m}/\underline{e}$ 226, as a parent ion.

c. <u>Pentafluorobenzyl Acetate</u>

The GC conditions were:

GC column:	10 ft x 2 mm I.D. glass, 10% OV-1 on 100/120 mesh Gas Chrom Q (Applied Science Laboratories, Inc.)
Carrier:	Ultra high purity grade helium, 20 psi
Column temperature:	100°C to 250°C (2 minutes), 8°C/minute
Injection port temperature:	150°C
Detector temperature:	220°C

The major fragments were:

$(C_6F_5CH_2)^+$, $\underline{m}/\underline{e}$ 181;

$(C_6F_5CH_2CH_2)^+$, $\underline{m}/\underline{e}$ 195;

and $(C_6F_5CH_2\text{-}O\text{-}CO\text{-}CH_3)^{\ddot{+}}$, $\underline{m}/\underline{e}$ 240, as a parent ion.

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