METHANOL, FORMALDEHYDE, AND ACETALDEHYDE IN RAIN

DEVELOPMENT OF A METHOD TO DETERMINE $\delta^{15}N$- NO_2^- and NO_3^- IN FRESH AND BRACKISH WATERS

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ABSTRACT

Methanol, formaldehyde, and acetaldehyde concentrations were measured in 49 rain events in Wilmington, NC from August 2007 to August 2008. The first methanol concentrations in rainwater are reported. Methanol concentrations ranged from below the detection limit (<0.10 μM) up to 9.3 μM with a volume weighted average concentration of 1.2 $\pm$ 0.2 μM and a simple average concentration of 1 $\pm$2 μM. Formaldehyde in the same rain events had a range of 0.1 μM to 5.5 μM with a volume weighted average concentration of 1.5 $\pm$0.2 μM and a simple average concentration of 2 $\pm$2 μM. Acetaldehyde in the rain events had a volume weighted average concentration of 0.140 $\pm$ 0.002 μM and a simple average concentration of 0.2 $\pm$ 0.2 μM with concentrations ranging from 0.01 to 0.72 μM. Additional rainwater components including hydrogen peroxide, hydrogen ion, nitrate, formate, acetate, and non sea salt sulfate (NSS) were measured in an attempt to correlate these components with methanol, formaldehyde, and acetaldehyde. Methanol only correlated well with acetaldehyde which may be due to both having strong biogenic sources. Formaldehyde correlated well with H^+, NO_3^-, and NSS suggesting common anthropogenic sources. Acetaldehyde correlates well with methanol, formaldehyde, nitrate, NSS, and hydrogen peroxide suggesting substantial anthropogenic and biogenic sources. When methanol, formaldehyde, and acetaldehyde weighted average concentrations were grouped according to rain event origin, methanol and acetaldehyde concentrations were highest in rain events originating strictly over land, supporting previous observations of methanol having a large biogenic source. Formaldehyde concentrations in rain events originating over land were also higher, supporting the idea of formaldehyde having anthropogenic sources but also suggesting a significant biogenic source. When methanol,

formaldehyde, and acetaldehyde volume weighted concentrations were sorted according to seasons (winter, fall, spring, summer) and to growing and non-growing seasons, concentrations of all three increased during the spring, summer, and growing seasons, suggesting an increase in biogenic sources and photochemical production. Methanol, formaldehyde, and acetaldehyde concentrations also increased during rain events occurring between 12pm – 6pm. Increases may be due to increases in photochemical production, plant activity, and activity from anthropogenic sources during the day.

ABSTRACT

A method for determining $\delta^{15}N$- NO_2^- and NO_3^- with a precision of 0.6‰ that is appropriate for low level enrichment and many natural abundance applications has been presented. The method can be applied to any nitrogen containing compound in which nitrogen can be converted to nitrite. The $\delta^{15}N$ of $^{15}NO_2^-$ is determined by decomposing the nitrite derivative that results from the known reaction of nitrite and dinitrophenylhydrazine (DNPH). This decomposition produces N_2 (g). One nitrogen in the N_2 (g) is from nitrite and the other is from DNPH. N_2 (g) is analyzed by isotope ratio mass spectrometry. The method allows for minimal chemical manipulations, has no need for sustaining bacteria cultures, is relatively safe, and uses common inexpensive reagents. Currently 700 nmoles of N is needed for analysis, but this amount can be cut drastically by using smaller glassware during headspace sampling. The presented method is ideal for low level enrichment and some natural abundance applications and with further development and a better understanding of blank corrections it may be used for many natural abundance applications.

ACKNOWLEDGMENTS

I would like to thank Dr. Jones for serving as my advisor, always supporting me, and answering all of my many questions. I would also like to thank Dr. Tobias, Dr. Seaton, Dr. Kieber, and Dr. Avery for serving on my committee and lending me their knowledge. I'd like to thank Dr. Willey for her many ideas on how to analyze my data. All of these people served as an advisor to me in some way and I could not have finished this work without them.

I would like to extend special thanks to my parents and Melinda Greenland for supporting me during my time in Wilmington.

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Introduction

Methanol is present in the environment through a variety of anthropogenic and biogenic sources which allow for methanol to be the second most abundant organic gas in the atmosphere after methane (Singh et al., 2001). Anthropogenic sources include alternative fuels, gasoline additives, aerosol sprays, paint strippers, solvent use, vehicle exhaust, decomposition of biological waste and many industrial processes (Howard 1990) whereas nonanthropogenic and biogenic sources of methanol include atmospheric production, plant growth, plant decay, and marine production (Millet et al., 2008). Biogenic sources of methanol account for 80 – 89% leaving anthropogenic sources to account for 11-20% (Heikes et al., 2002) although whether biogenic sources actually account for this large percentage of methanol is still under debate (Millet 2008). Using a global transport model Tie et al. (2003) showed these combined surface emissions of methanol produce approximately 1-2% increase in O_3, 1-3% decrease in OH, 3-5% increase in HO_2, and a 3-9% increase in formaldehyde. Methanol is a significant source of formaldehyde in the atmosphere (Palmer et al 1998) because it reacts with hydroxyl radicals to form formaldehyde as its major product. Methanol reacting with hydroxyl radicals can also lead to primary formation of formic acid (Monod 2000) in turn adding to the acidification of rain. Five to ten percent of the methanol in the atmosphere reacts with sulfuric acid to produce methyl sulfate (Allen 2003). Methyl sulfate is more stable than methanol, creating a base for cloud formation (Allen 2003). Clouds trap heat and light in the atmosphere and also reflect heat implicating methanol as a factor in global warming and cooling.

Until now atmospheric methanol concentrations have consisted of surface air analysis (Heikes 2002) and have helped lead to wide discrepancies in global budget models of methanol. The first rainwater methanol data is reported in this study. Methanol was determined in rain using a recently developed method involving enzymatic oxidation of short chain alcohols to their corresponding aldehydes with alcohol oxidase (Magolan and Jones 2004). This study includes 12 months of rainwater events (49 events) collected at a coastal site in Wilmington, North Carolina. Methanol data was investigated in correlation with formaldehyde, acetaldehyde, and other rainwater components including hydrogen peroxide, H^+, nitrate, formate, acetate, and non sea salt sulfate. Methanol, formaldehyde, and acetaldehyde concentrations compared to rain event origin, season, and diurnal variation are also reported in this study.

Experimental Procedure

Reagents and Standards:

Alcohol oxidase (100 units) from the yeast *Hansenula sp* was purchased from Sigma (St. Louis, MO). Water was purified using a Millipore Q-water system (Millipore Corp., Bedford, MA) and used to prepare all solutions. Reagent grade 2,4-dinitrophenylhydrazine (DNPH) was purchased from Sigma (St. Louis, MO), triply recrystallized from acetonitrile and kept refrigerated in the dark. Acetonitrile (HPLC grade, Burdick and Jackson, Muskegon, MI), 12 M hydrochloric acid (Reagent Grade, VWR International, West Chester, PA), and carbon tetrachloride (HPLC grade 99.9%, Sigma, St. Louis, MO) were used in preparation and purification of DNPH reagent solution.

Formaldehyde (37.69% CH_2O, 12.37% MeOH) and paraformaldehyde (94.19%, containing no methanol) were obtained from Wright Chemical Company (Wilmington, NC). A 1M formaldehyde stock solution was prepared before each rain event.

Methanol (HPLC grade, Burdick and Jackson, Muskegon, MI), ethanol (200 proof, AAPER Alcohol and Chemical Co., Shelbyville, KY), 1-propanol (Fisher Scientific, Fair Lawn, NJ) and Milli-Q water was used to prepare a 1M alcohol stock solution before each rain event.

ACS grade (99.0%) potassium dihydrogen phosphate and reagent grade potassium hydrogen phosphate (Alfa Aesar, Ward Hill, MA) were used in preparation of all buffer solutions.

General Procedures

Procedures are done according to Magolan (2005). Polyethylene disposable gloves (VWR International) were worn when handling all reagents, buffers, and samples. Gloves were changed between all aldehyde and alcohol samples, (including stock, dilution, and sample preparation) as well as prior to enzyme handling, to avoid cross-contamination. All alcohol, aldehyde, buffer, and DNPH reagent solutions were stored in different locations to prevent contamination.

All digital pipet tips (1-200 μL, 200-1000 μL, and 1-5mL, VWR International) were placed in a 10% hydrochloric acid bath (700mL concentrated HCl diluted with 7.0L of DI water) and allowed to soak for a few hours. After soaking, they were rinsed thoroughly with Milli-Q water in a clean room, allowed to dry under positive flow hoods, packaged in acid rinsed Ziploc Bags, and stored in a room free of alcohol and aldehyde contamination. Pipet tips were changed between samples of varying concentrations and between use of aldehyde and alcohol solutions.

All volumetric glassware and caps were rinsed several times with Milli-Q water prior to making solutions. All HPLC vials were heated in a muffle furnace for 6 hours at 550 degrees Celsius prior to use.

On days when lab work was conducted, no perfume, nail polish, hair products, or any other sources of alcohols or aldehydes were worn to prevent contamination. Alcohol was not consumed on days prior to conducting lab work.

All sinks in rain sample prep room were flushed with water because dry sink traps a source of sample contamination by alcohols and aldehydes from other lab rooms in the building.

Dinitrophenylhydrazine Reagent Preparation

Reagent was prepared according to Kieber and Mopper (1990). The 2,4-dinitrophenylhydrazine (DNPH) reagent was prepared on a weekly basis in a 30 mL Teflon vial by dissolving 20mg of triply recrystallized DNPH in 4 mL of concentrated hydrochloric acid (HCl), 10mL of Milli-Q water, and 2 mL of acetonitrile (ACN). DNPH reagent was then shaken for 1 hour on a wrist action shaker. To reduce background signal, reagent was extracted with 2 mL of carbon tetrachloride, shaken for 10 minutes on a wrist action shaker, and centrifuged for 2 minutes. After the initial extraction, the lower organic layer was removed and the process was repeated. After the second extraction, the organic layer was left in the reagent vial and removed prior to successive extractions. DNPH reagent was extracted twice on the first day of use, and once for each following day used, for up to one week.

Buffer Preparation

0.1M potassium phosphate buffer (KPB) at pH 9.0 was prepared in a 1 L volumetric flask by adding 0.2177 g of potassium dihydrogen phosphate (KH_2PO_4) and 22.4578 g of potassium hydrogen phosphate trihydrate ($K_2HPO_4 \bullet 3H_2O$) and diluting with Milli-Q water. This solution was stored in a refrigerator free of alcohol and aldehyde contamination when not in use.

Enzyme Solution Preparation (Magolan and Jones 2004).

Twenty, 25 mL Teflon enzyme vials and caps were rinsed several times with Milli-Q water in a clean room and allowed to dry under a positive flow hood before use. Alcohol oxidase (100 units) was dissolved in 5 mL of 0.1M KPB at pH 9.0 resulting in a concentration of 20 units/mL. Since alcohol oxidase does not readily dissolve, 1.0, 1.5, and 2.5 mL portions of the 5mL of 0.1 M KPB at pH 9.0 were added to the enzyme separately by digital pipet. Each portion was drawn up into a digital pipet and dispensed quickly into a 25 mL Teflon vial several times to dissolve the oxidase. Nineteen aliquots of 250 μL were removed from this initial vial, placed in separate 25 mL Teflon vials which were in an ice water bath, labeled, and stored at –80 degrees Celsius. The resulting amount of enzyme in each 25 mL vial was 5 units. One vial of enzyme was removed from the freezer on a daily basis and diluted with 2.5 mL of 0.1M KPB at pH 9.0 resulting in an enzyme concentration of 2 units/mL. 100 μL of this enzyme solution was added to all enzyme blanks, alcohol solutions, and rain samples that required enzyme. Final enzyme concentration in each sample was 0.18 units/mL.

Sample Preparation (Magolan and Jones 2004).

1.0 M formaldehyde and methanol working stock solutions were prepared before each rain event in 50 mL volumetric flasks. Formaldehyde and methanol dilutions were made in a separate area that was free of alcohol and aldehyde vapors. Note that separate Teflon containers were used for each concentration of working stock or diluted solution for both aldehydes and alcohols. To make a 1.0 mM solution, 100 μL of 1.0 M formaldehyde (or methanol) working stock was dispensed into a 100 mL volumetric flask, and diluted with Milli-Q. 10 μM formaldehyde (and methanol) solutions were made by diluting 1000 μL of the 1.0 mM solution with Milli-Q in a 100 mL volumetric flask. 1.0 μM solutions were prepared in an HPLC vial from 100 μL of 10 μM formaldehyde (methanol) solution and 900 μL of 1.0 mM KPB at optimum pH. All HPLC samples were 1.0 μM unless otherwise noted.

Reagent blanks consisted of 1000 μL of 1.0 mM KPB at pH 9.0. HPLC samples containing 1.0μM formaldehyde were combined with 10μL of DNPH reagent. Enzyme blanks were made using 1000 μL of 1.0 mM KPB at pH 9.0 and 100 μL of enzyme followed by 10 μL of DNPH reagent after enzyme reaction was complete. 1.0 μM methanol samples (1000 μL) were also inoculated with 100 μL of enzyme followed by 10 μL of DNPH reagent after enzyme reaction was complete. All results were corrected for dilution.

Rain Sample Preparation (Magolan 2005)

Rain samples were collected at the University of North Carolina Wilmington rain collection site from August 28, 2007 to April 10, 2008 on an event basis. The collection site is located at 34º 13.9'N, 77º 52.7'W and is about 8.5 km from the Atlantic Ocean. Since the collection site is near the laboratory, rain samples were analyzed within hours of collection,

minimizing loss of alcohols. If it wasn't possible to analyze the rain samples within hours, they were frozen immediately and stored in a -80^{o}C freezer.

Rainwater samples were collected using four Aerochem-Metrics (ACM) Model 301 Automatic Sensing Wet/Dry Precipitation Collectors. Rain for alcohol analysis was collected in sample collectors consisting of a Teflon funnel connected by Tygon tubing to a 2 L trace metal cleaned Teflon bottle. Rainwater for alcohol analysis was then poured into a clean 30 mL Teflon container, labeled with the event number, and placed in a refrigerator. Teflon vials were rinsed well with Milli-Q and allowed to dry in a positive flow hood prior to use. Information such as pH, hydrogen peroxide concentration, rain amount and duration, time of day, and storm origin were recorded for each event. Real time precipitation maps were used to indicate the beginning and end of each rain event.

Collected rainwater was analyzed for formaldehyde (Kieber and Mopper 1990) and methanol. To determine aldehydes, 1000 μL of rain was combined with 10 μL of 0.1M KPB at pH 9.0 and 10 μL of DNPH and allowed to react for 30 minutes before HPLC analysis. To determine both aldehydes and alcohols in rainwater, 1000 μL of rain was combined with 10 μL of 0.1M KPB at pH 9.0, 100 μL of enzyme, and allowed to react at 40^{o}C for 40 minutes before addition of 10 μL of DNPH. Peak areas from rain samples with DNPH were subtracted from those in rain samples with added enzyme and DNPH to obtain the signal generated from alcohols alone. Rain samples were also spiked with 0.813 μM methanol. This was done by combination of 1000 μL of rain, 20 μL of 0.1 M KPB at pH 9.0, 100 μL of the 10 μM methanol, and 100 μL of enzyme, and was allowed to react at 40^{o}C for 40 minutes prior to addition of 10 μL of DNPH. Resulting peak areas for this sample indicated the aldehydes and alcohols present in the rain as well as the alcohol spike. By taking the difference between signals generated from this sample

and the sample containing rain with enzyme, the resulting peak area was that of the methanol spike alone. Peak areas of the spike were compared to the signal from methanol alone in buffer, to test for method accuracy. All results were corrected for dilution.

Procedures for analysis of Nitrate, nSS, Acetate, Formate, H+, and Hydrogen Peroxide

Hydrogen peroxide

Hydrogen peroxide was analyzed by a fluorescence decay technique involving the peroxidase mediated oxidation of the fluorophore scopoletin by H_2O_2 in a phosphate buffered (0.1 M) sample at pH 7 (Kieber and Helz, 1986; Kieber and Helz, 1995). Each sample was analyzed at least three times. Calibration curves were obtained by recording the decrease in fluorescence upon addition of dilutions of hydrogen peroxide stock solution to the sample. The method has an analytical precision of 2% RSD with a detection limit of 2 nM which was more than sufficient for rainwater samples.

$H+$, NO_3^-, SO_4^{2-}

Anions were determined using suppressed ion chromatography and quantified against synthetic rain standards with a precision of 3% RSD (EPA, 1981; Fitchett, 1983). Rainwater pH was determined using a Ross Model 81-02 electrode calibrated with low ionic strength 4.10/6.97 buffers (Orion Research Incorporated, Boston, Mass.). pHix ionic strength adjuster (Orion) was added to each sample to match ionic strength of samples to buffers. Duplicate pH analyses agreed within 0.02 pH units.

Formate and acetate

Formate and acetate standards were prepared from sodium salts. Standards were prepared daily from a concentrated stock prepared every other month. Organic acid concentrations were measured with a Dionex 4000i/SP ion chromatograph with a SP4290 integrator, Dionex IonPacR

AS11 4mm analytical column, AG11 4mm Guard column and anion micromembrance Suppressor Model AMMS-11 (Avery, 2001). Under the conditions used, this column is capable of resolving 34 different anions of organic acids.

Season Definition:

The seasons in this study are defined as follows:

Fall: September 1, 2007 through November 30, 2007

Winter: December 1, 2007 through February 29, 2008

Spring: March 1, 2008 through May 31, 2008

Summer: August 28, 2007 and June 2008 through August 1, 2008

Growing Season: August 28, 2007 and April 1, 2008 through September 30, 2008

Non-growing Season: October 1, 2007 through March, 31 2008

Storm origin definitions:

Precipitation events were categorized using air-mass back-trajectories generated using version 4 of the Hybrid Single Particle Lagrangian Integrated Trajectory Model (HYSPLIT) developed at the National Oceanic Atmospheric Administration – Air Resources Laboratory (NOAA/ARL - http://www.arl.noaa.gov/ready/hysplit4.html). Trajectories were generated using a stand-alone PC version of the model; and calculated using pre-processed gridded horizontal and vertical wind fields generated at 6-hour intervals from the National Center for Environmental

Prediction's Global Data Assimilation System (GDAS) using the Medium Range Forecast model (MRF) to produce the forecast wind fields.

Single back-trajectories were run for each measured precipitation event collected at UNCW starting at the recorded onset of precipitation. Trajectories were run starting at the 500m level to represent the air-mass near the well mixed boundary layer likely to contribute more heavily to in-cloud processes contributing to wet deposition (Walker *et al.*, 2000). They were then visually categorized based on origin (compass direction) and path (terrestrial, oceanic, or mixed): (1) N-Mixed, (2) W/SW-Terrestrial, (3) N/NW-Mixed, (4) SW-Coastal, (5) E-Oceanic. Terrestrial air masses are those whose pathway for the 120 h period preceding the rain even was predominantly over a landmass, and like-wise over ocean for oceanic types. Mixed trajectories were determined to have the same potential for oceanic as terrestrial influence based on a visual analysis of their pathway (Kieber, Long, Willey 2005).

Diurnal variations: time period definitions:

Rain events were split into time periods only if the rain event occurred within one given time period.

Time Period I: 6 a.m. – 12 p.m.

Time Period II: 12 p.m. – 6 p.m.

Time Period III: 6 p.m. – 12 a.m.

Time Period IV: 12 a.m. – 6 a.m.

Results Discussion

The volume weighted averages and their standard deviations for formaldehyde, methanol, and acetaldehyde concentrations in 49 rain events collected at a site in Wilmington, NC from August 28, 2007 to August 01, 2008 are shown in Table 1. This data is presented for all events together, for each month, and for each of the seasons. Methanol concentrations for all 49 events ranged from below the detection limit (<0.10 μM, reported as 0.05 μM) up to 9.3 μM with a volume weighted average concentration of 1.2 $\pm$ 0.2 μM and a simple average concentration of 1 $\pm$2 μM. These concentrations of methanol found in rainwater suggest methanol rainout could be responsible for removing more than 10 -20% of atmospheric methanol abundance as predicted by Crutzen and Lawrence (2000). The volume weighted average falls within the gas phase atmospheric concentration of 1-10 ppbv (part per billion by volume) reported over land and 0.1-1.5 ppbv marine (Jacob et al., 2005; Singh et al., 2000). Formaldehyde in the same rain events had a range of 0.1 μM to 5.5 μM with a volume weighted average concentration of 1.5 $\pm$0.2 μM and a simple average concentration of 2 $\pm$2 μM. The volume weighted average concentration of formaldehyde falls within the range of 10 nM to 13 μM reported in an earlier study of the same rain site Kieber et al. (1999). The weighted average concentration of formaldehyde was about half that reported by Kieber et al. (1999) for the same rain site. This could be due partially to continually changing regulations in the Clean Air Act or due to formaldehyde deposition being affected by drought conditions that existed during the time of this study. Acetaldehyde in the rain events had a volume weighted average concentration of 0.140 $\pm$ 0.002 μM with a range of 0.01 μM to 0.72 μM and a simple average concentration of 0.2 $\pm$ 0.2 μM. These acetaldehyde concentration averages are similar to the 0.1 $\pm$ 0.15 μM average reported in a previous study in Yokohama, Japan (Matsumoto, 2005).

TABLE 1: Volume-weighted averages (vw ave) and standard deviations (vw s) for formaldehyde, methanol, and acetaldehyde concentrations in rainwater during months and seasons indicated. (n = 49 rain events)

	n	HCHO vw ave (μM)	HCHO vw s (μM)	MeOH vw ave (μM)	MeOH vw s (μM)	CH_3CH_2O vw ave (μM)	CH_3CH_2O vw s (μM)
All data (Aug-07 -Aug-08)	49	1.5	0.2	1.2	0.2	0.14	0.002
Aug-07	1	2.94	na	0.26	na		
Sep-07	6	3	0.7	0.2	0.1		
Oct-07	3	0.85	0.04	1	0.5		
Nov-07	3	2	0.7	1.3	0.2		
Dec-08	2	0.51	0.06	0.21	0.01		
Jan-08	8	0.64	0.05	0.9	0.2		
Feb-08	2	0.71	0.07	1.7	0.4		
Mar-08	7	1.4	0.3	0.26	0.06		
Apr-08	4	1.3	0.2	1.6	0.7		
May-08	3	1.6	0.9	1.5	0.7		
Jun-08	5	2.9	0.5	3.6	0.8		
Jul-08	4	2	0.5	1.7	0.2		
Fall (Sept-07 -Nov-07)	12	1.7	0.4	0.7	0.2	0.13	0.06
Winter (Dec-07 -Feb-08)	12	0.62	0.04	0.9	0.2	0.05	0.007
Spring (Mar-08 -May-08)	15	1.5	0.3	1	0.3	0.13	0.02
Summer (Aug 28, and June-08 - Aug-08)	10	2.5	0.3	2.3	0.5	0.34	0.06
growing season	24	1.9	0.3	1.5	0.3	0.21	0.04

Intercorrelations:

In addition to methanol, formaldehyde and acetaldehyde concentrations measured in this study, rain amount, H^+, NO_3^-, H_2O_2, NSS (non sea salt sulfate), formate, and acetate were recorded by the MACRL group at UNCW during the time period of this study. Methanol, formaldehyde, and acetaldehyde were all analyzed for correlation with each other and all other measured rainwater components (Table 2). Correlations among H^+, NO_3^-, and NSS suggest a common anthropogenic source of origin so they can be correlated with other rainwater components to suggest origin (Kieber et al. 1999). Methanol did not correlate strongly with any of these rainwater components except acetaldehyde, agreeing with previous observations that methanol sources are primarily biogenic. Its correlation with acetaldehyde is probably due to both components having a significant biogenic source (Custer and Schade, 2007) Acetaldehyde also correlates strongly with formaldehyde, nitrate, NSS, and H_2O_2 suggesting a substantial anthropogenic source of acetaldehyde. Formaldehyde had been highly correlated with H^+ ($r = 0.558$), NO_3^- ($r = 0 .499$), and NSS ($r = 0.532$) in a previous study of the same rain site which suggested common anthropogenic sources (Kieber et al. 1999). The current study yielded formaldehyde data which had similar correlations with H^+ ($r = 0.548$), NO_3^- ($r = 0.557$), NSS ($r = 0.604$), further implying common anthropogenic sources for these rain components.

Table 2: Intercorrelations between methanol, formaldehyde, acetaldehyde, and various other rainwater components.

	CH_3OH	CH_2O	CH_3CHO	NO_3^-	H_2O_2	H^+	NSS	Formate	Acetate
amount	0.057	−0.23	−0.152						
CH_3OH		0.166	**0.464**	0.176	0.307*	0.085	0.234	−0.142	0.0708
CH_2O			**0.643**	**0.557**	0.373*	**0.548**	**0.604**	**0.734**	**0.558**
CH_3CHO				**0.442**	**0.506**	0.357*	**0.699**	**0.609**	**0.779**
NO_3^-					**0.397**	**0.559**	**0.584**	0.371*	**0.630**
H_2O_2						**0.405**	**0.606**	**0.675**	**0.667**
H^+							**0.760**	**0.722**	**0.687**
NSS								**0.476**	**0.751**
Formate									**0.786**
Acetate									

Bold faced values indicate significance at $p < 0.001$. Asterisk (*) indicates significance at $p < 0.05$. Number of samples equals: 47 for acetaldehyde, nitrate, and sulfate; 27 for formate and acetate; 49 for others.

Methanol, formaldehyde, and acetaldehyde were analyzed for correlation with rain fall amount to see if there is any indication of rainout affecting their concentrations (figures 1a – 1c). Methanol concentrations were fairly consistent at all amounts of rain suggesting some re-supply of methanol occurring during rain events. Acetaldehyde concentrations were higher in lesser rain fall amounts and lower in greater rain fall amounts indicating rainout as affecting rain event concentrations. Formaldehyde concentrations were higher in lesser rain fall amounts and lower in greater rain fall amounts, but some concentrations did not follow this trend. This suggests a rainout affect along with some re-supply of formaldehyde to the atmosphere.

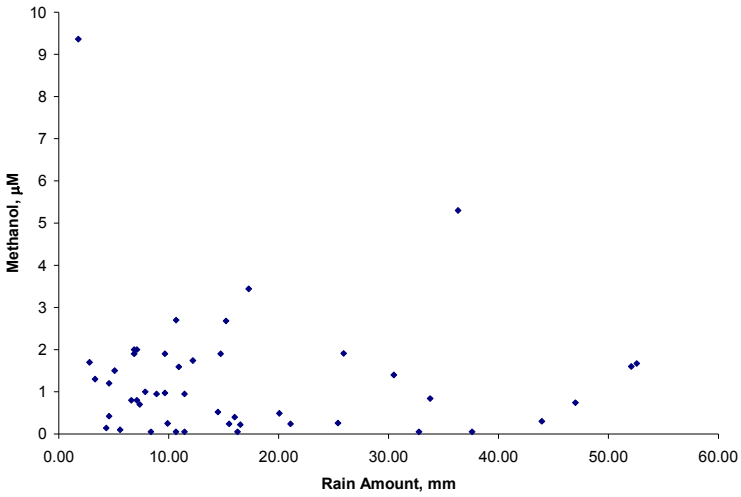


Figure 1a) Intercorrelation between methanol and rain amount. (n = 49)

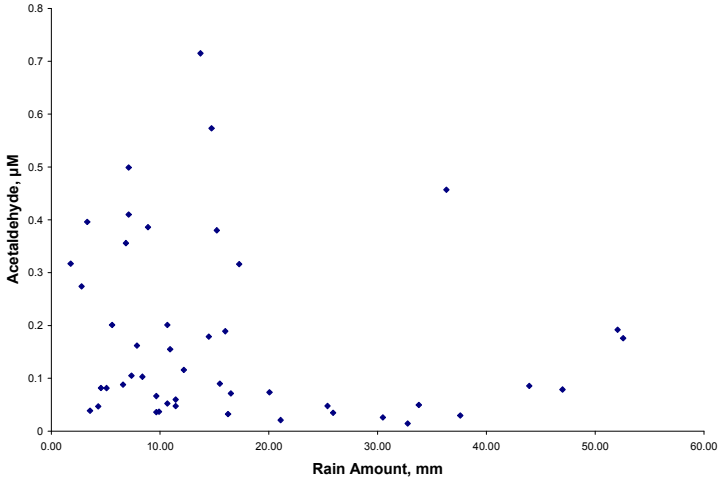


Figure 1b) Intercorrelation between acetaldehyde and rain amount. (n = 47)

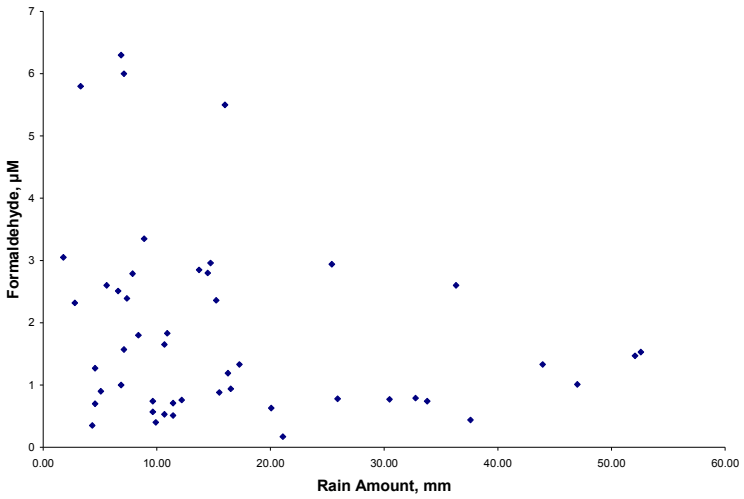


Figure 1c) Intercorrelation between formaldehyde and rain amount. (n = 49)

Multiple Linear Regressions

Methanol reacts with hydroxyl radicals to form formaldehyde so a multiple linear regression was used on the data set of formaldehyde, methanol, and H_2O_2 to create an equation to predict formaldehyde concentrations and to analyze any correlation between the three components. The resulting equation was used to predict formaldehyde concentration in rain:

$[\text{formaldehyde}] = 1.075 + 0.1008[\text{MeOH}] + 0.0209[H_2O_2]$

Predicted formaldehyde concentrations in rain were plotted against actual formaldehyde concentrations (figure 2).

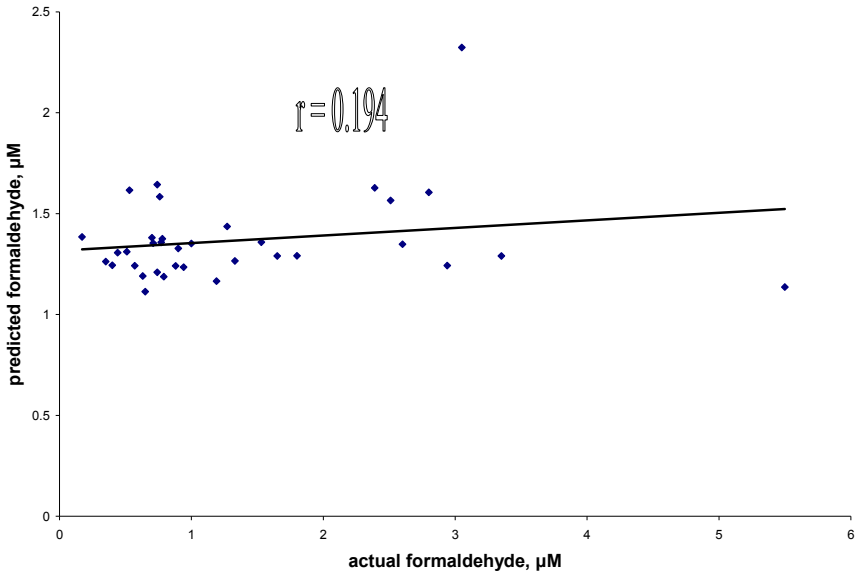


Figure 2) Predicted formaldehyde concentrations in rain were plotted against actual formaldehyde concentrations (n = 27).

The resulting linear regression had an r value of 0.194, showing no correlation between MeOH and H_2O_2 concentrations in rainwater with that of formaldehyde. This suggests atmospheric production of formaldehyde from MeOH is not a very significant source of formaldehyde in the atmosphere.

Formaldehyde was strongly correlated with formic acid (r = 0.734) which along with H_2O_2 is a product of the photolysis of formaldehyde (Gunz and Hoffman, 1990). Multiple linear regression was used on the data set of formaldehyde, formic acid, and H_2O_2 to create an equation to predict H_2O_2 concentrations and to analyze any correlation between the three components. The resulting equation used to predict H_2O_2 concentration in rain:

$[H_2O_2] = 6.649 + -0.500[\text{formaldehyde}] + 0.834[\text{formic acid}]$

Predicted H_2O_2 concentrations in rain were plotted against actual H_2O_2 concentrations (figure 3).

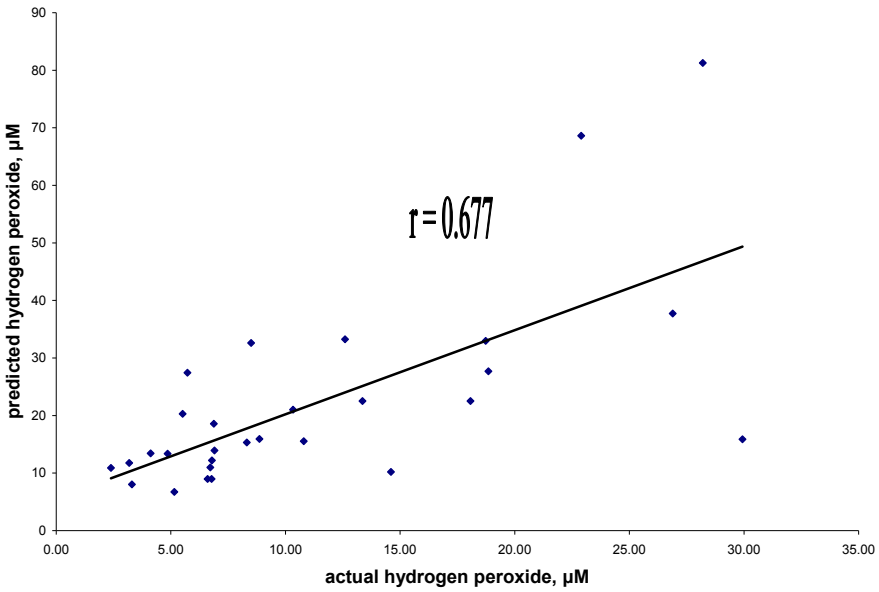


Figure 3) Predicted H_2O_2 concentrations in rain were plotted against actual H_2O_2 concentrations (n = 27).

The resulting linear regression had an r value of 0.677, and while there was weak correlation between formaldehyde and H_2O_2 (r = 0.373), the multiple linear regression provides evidence of a strong correlation between formaldehyde, formic acid, and the production of H_2O_2. Multiple linear regression was again applied to the data set of formaldehyde, formic acid, and H_2O_2 to create an equation to predict formic acid concentrations and to analyze any correlation between the three components. The resulting equation used to predict formic acid concentration in rain:

[formic acid] = -1.8729 + 0.386[H_2O_2] + 2.492[formaldehyde]

Predicted formic acid concentrations in rain and were plotted against actual formic acid concentrations (figure 4).

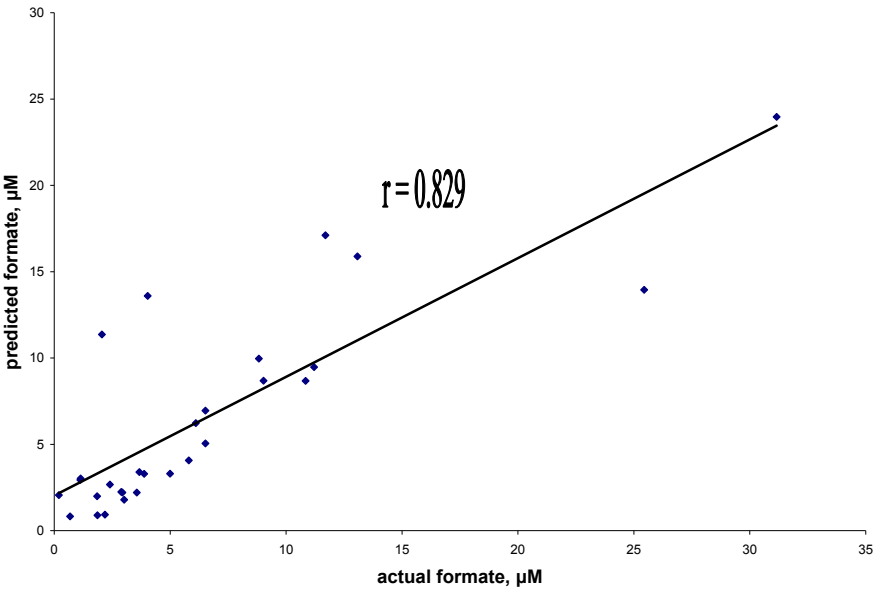


Figure 4) Predicted formic acid concentrations in rain and were plotted against actual formic acid concentrations (n = 27).

The resulting linear regression had an r value of 0.829, providing evidence of a strong correlation between formaldehyde, H_2O_2, and the production of formic acid.

Acetaldehyde was strongly correlated with acetic acid (r = 0.779) which along with H_2O_2 is and may be a product of the photolysis of acetaldehyde. Multiple linear regression was used on the data set of acetaldehyde, acetic acid, and H_2O_2 to create an equation to predict H_2O_2 concentrations and to analyze any correlation between the three components. The resulting equation used to predict H_2O_2 concentration in rain:

$[H_2O_2] = 6.124 + -1.248[\text{acetate}] + 44.383[\text{acetaldehyde}]$

Predicted H_2O_2 concentrations in rain were plotted against actual H_2O_2 concentrations (figure 5).

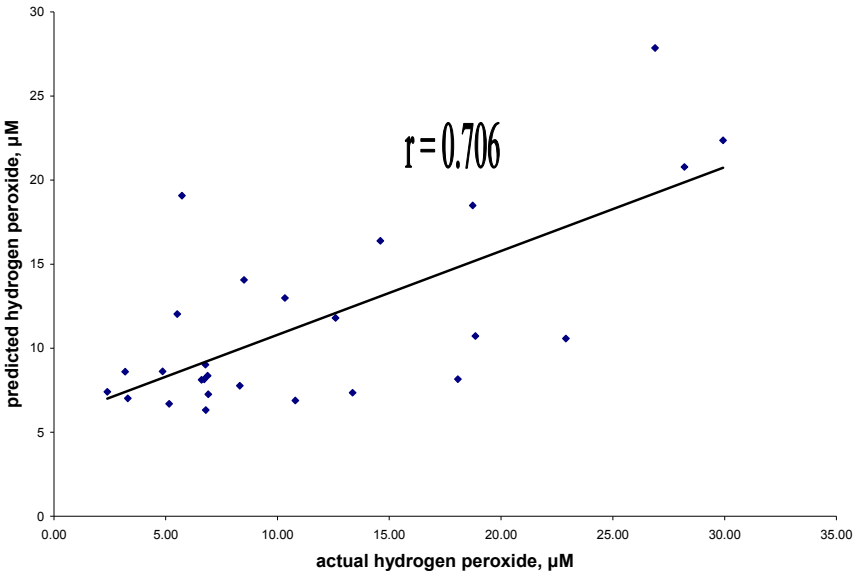


Figure 5) Predicted H_2O_2 rain concentration plotted against actual H_2O_2 concentration (n = 27).

The resulting linear regression had an r value of 0.706 providing evidence of a strong correlation between acetaldehyde, acetic acid, and the production of H_2O_2. Multiple linear regression was again applied to the data set of acetaldehyde, acetic acid, and H_2O_2 to create an equation to predict acetic acid concentrations and to analyze any correlation between the three components. The resulting equation used to predict formic acid concentration in rain:

[acetic acid] = 0.5732 + 8.913[acetaldehyde] + -0.0355[H_2O_2]

Predicted formic acid concentrations in rain were plotted against actual formic acid concentrations (figure 6).

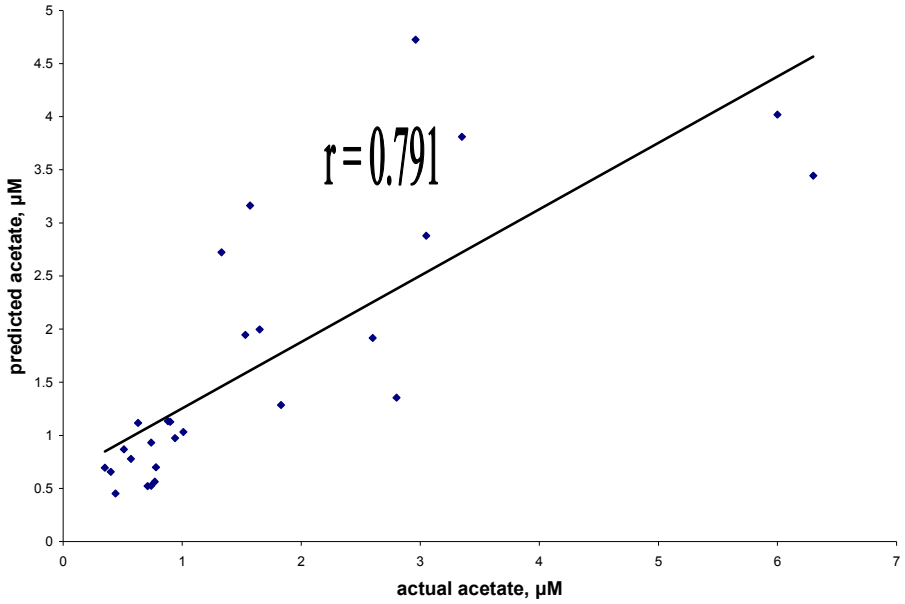


Figure 6) Predicted formic acid concentrations in rain were plotted against actual formic acid concentrations (n = 27).

The resulting linear regression had an r value of 0.791, providing evidence of a strong correlation between acetaldehyde, H_2O_2, and the production of acetic acid.

Seasonal Variations:

The monthly and seasonal data from table 1 is also presented as bar graphs in Figures 7a - 7c. The volume weighted average concentration of methanol during the winter rain events was 0.9 $\pm$0.2 µM. Sources of methanol are predominately biogenic, so it would be expected that methanol concentrations would peak during spring and fall in temperate climates (Singh et al., 1995) while decreasing during winter months. Higher than expected methanol concentrations during the winter may be due to the Henry's Law coefficient being temperature dependent, allowing for higher concentrations of methanol to be soluble in rainwater. The volume weighted

average concentration of formaldehyde during the winter months was 0.62 ±0.04 μM, a value which was close to the observed value of 1 ± 0.1 μM during the winter of 1997-1998. The 97-98 winter was an El Nino winter which saw greater than normal precipitation because El Nino winters in North America see a shift in the storm track from the northern part of the United States to the Southern part of the United States (www.cpc.noaa.gov/products/analysis_monitoring/ensocycle/nawinter.shtml). In contrast the winter under study was unusually dry with drought conditions described as moderate drought to extreme drought by the North Carolina Drought Management Advisory Council (http://www.ncdrought.org/archive/index.php). Since the formaldehyde concentrations of both time periods are similar it suggests the formaldehyde concentration following a seasonal trend rather than following wet/dry conditions. Acetaldehyde during the winter rain events had a relatively low volume weighted average concentration of 0.050 ± 0.007 μM which is expected since plant growth and decay is thought to be a major contributor to atmospheric acetaldehyde concentrations (Custer and Schade, 2007).

The volume weighted average concentration of methanol during the fall months was 0.7 ±0.2 μM. As stated earlier methanol concentrations are expected to peak during fall months. A possible explanation for lower than expected concentrations of methanol during the fall rain events may be that the drought conditions of the previous summer were severe to extreme (http://www.ncdrought.org/archive/index.php) possibly killing vegetation that would have decayed during the fall and emitted methanol. The volume weighted average concentration of formaldehyde and acetaldehyde during the fall months was 1.7 ±0.4 μM and 0.13 ±0.6 μM respectively. The fall acetaldehyde concentration may have shown an increase over that of the

winter because of increased plant activity specifically plant decay and increases in anthropogenic activities. The increase in formaldehyde concentrations may point to increase in acetaldehyde concentrations being mostly due to anthropogenic sources rather than biogenic sources.

The volume weighted concentration of methanol during the spring months was 1.0 $\pm$0.3 μM. Methanol concentrations are expected to increase in the spring because of plant growth occurring during this season. A slight increase was seen but methanol emissions from vegetation may have been hampered by an unusually dry spring. Formaldehyde volume weighted average concentration was 1.4 $\pm$0.2 μM. This is an increase from the winter events suggesting an increase in anthropogenic activity. Acetaldehyde concentrations in the spring (0.13 $\pm$0.02 μM) was double that of the winter concentrations possibly indicating an increase in anthropogenic sources of acetaldehyde rather than biogenic because a large increase in methanol concentrations wasn't seen.

Summer methanol and acetaldehyde concentrations are expected to be in the higher concentration range because the summer period is during the growing season leading to biogenic methanol/acetaldehyde emissions from vegetation. The volume weighted methanol and acetaldehyde concentrations for the summer under study was 2.7 $\pm$ 0.9 μM and 0.36 $\pm$ 0.08 μM which is a significant increase over the colder seasons. The volume weighted formaldehyde concentration for summer was 2.9 $\pm$ 0.4 μM. This increase in formaldehyde concentration during the summer agrees with a previous study (Keiber 1999) that suggested the increase may be from a combination of increased photochemical activity and increased biogenic and anthropogenic emissions.

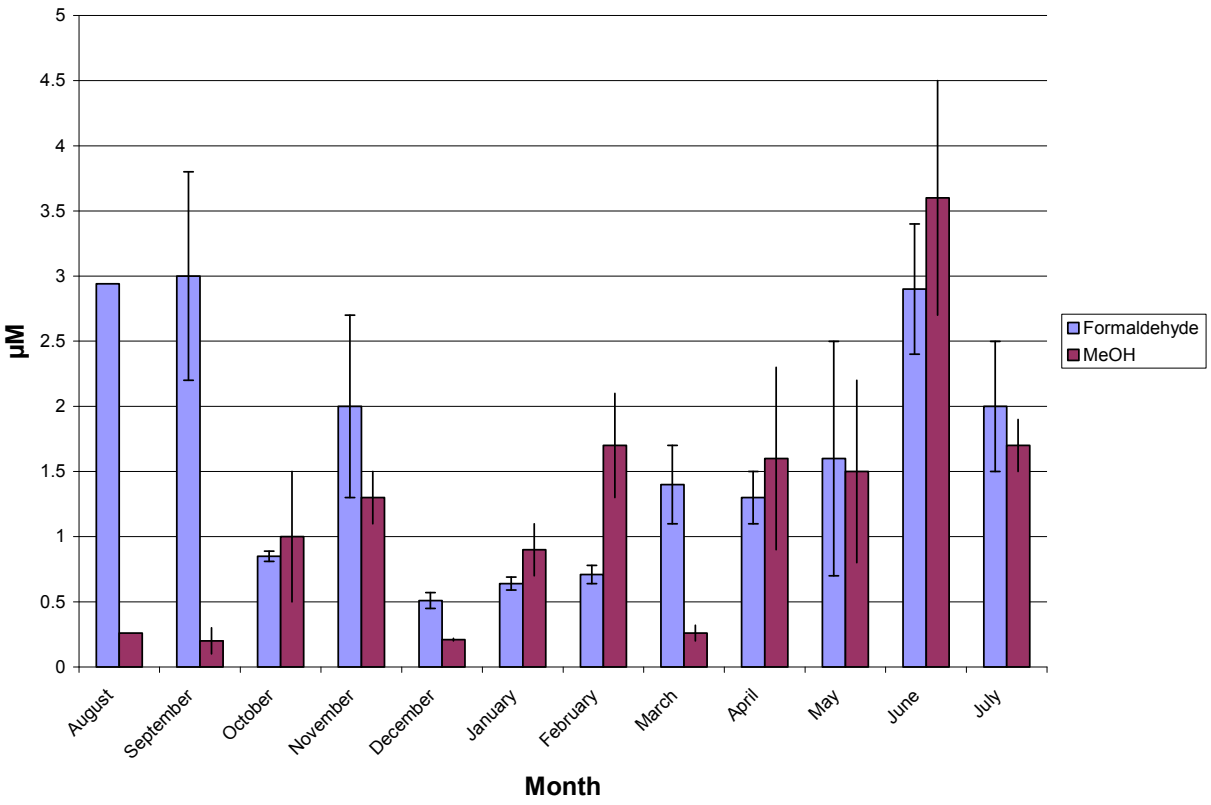


Figure 7a) Formaldehyde and methanol concentrations in rain by month (n = 49).

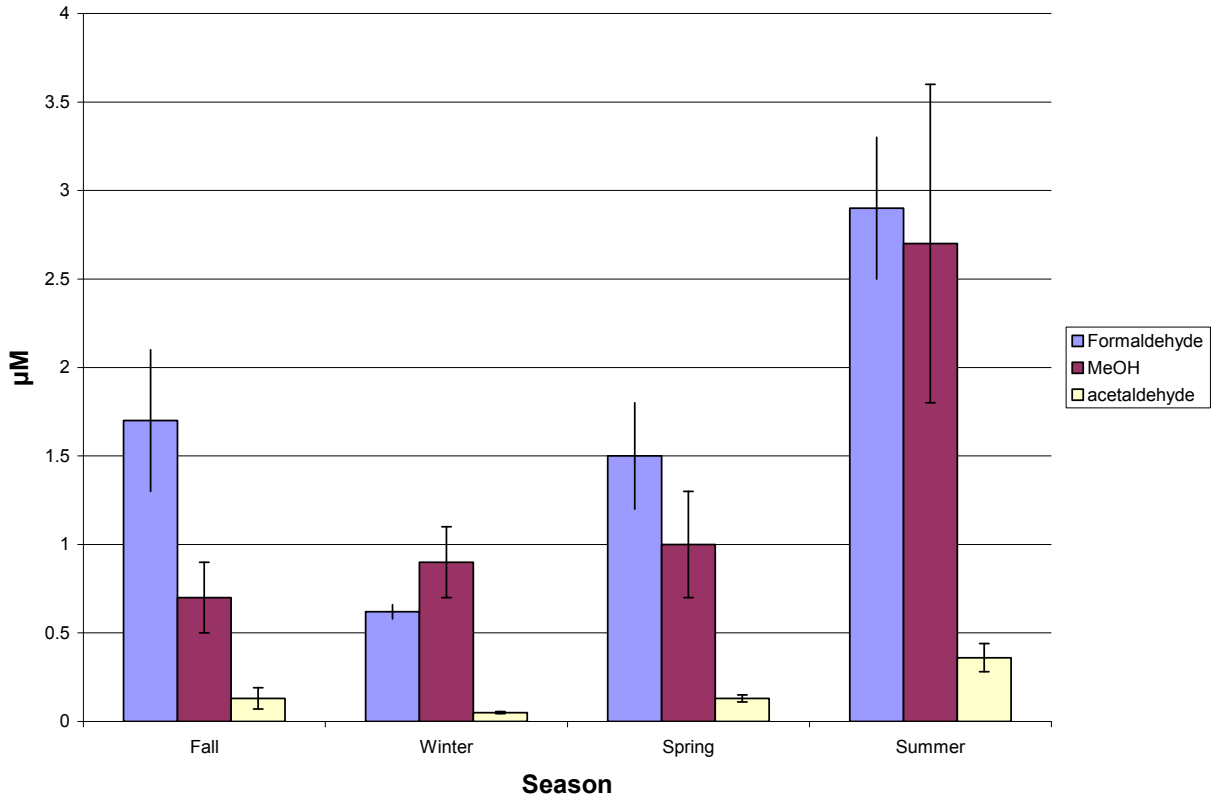


Figure 7b) Formaldehyde, methanol, and acetaldehyde concentrations in rain by season (n = 49).

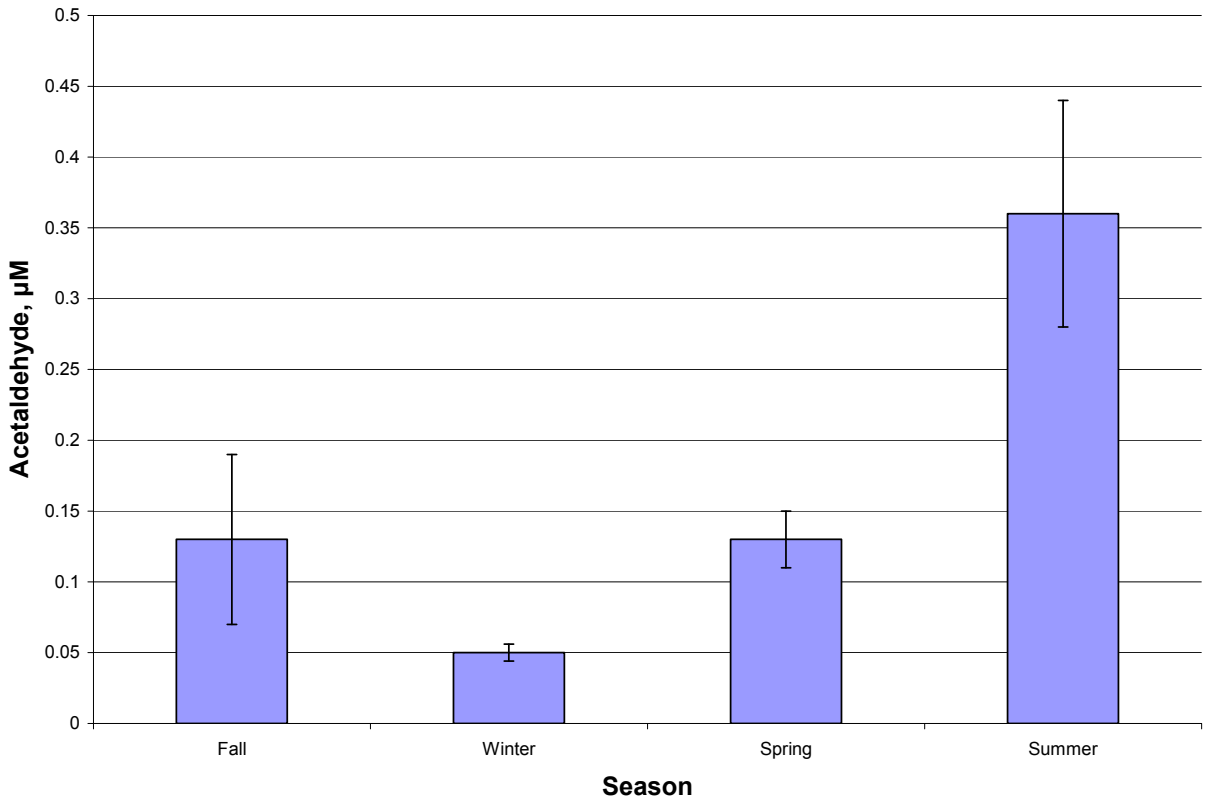
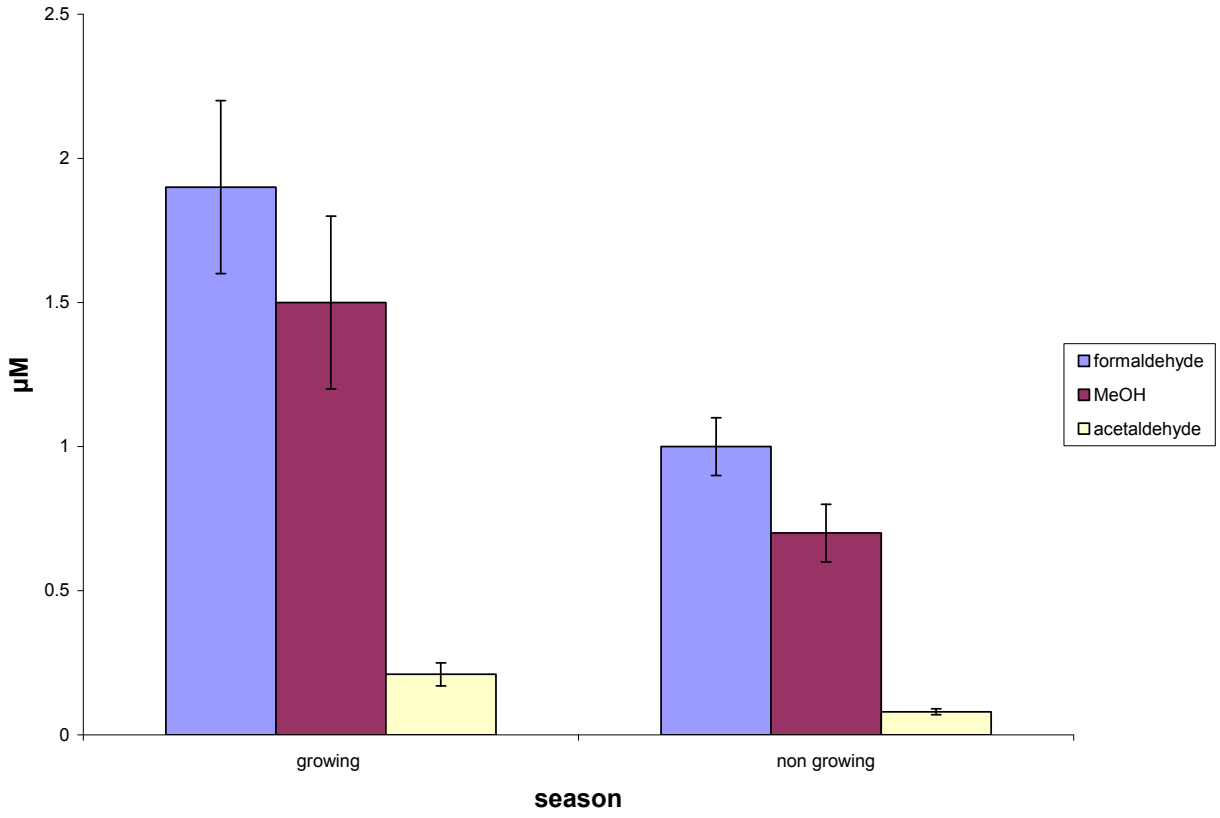


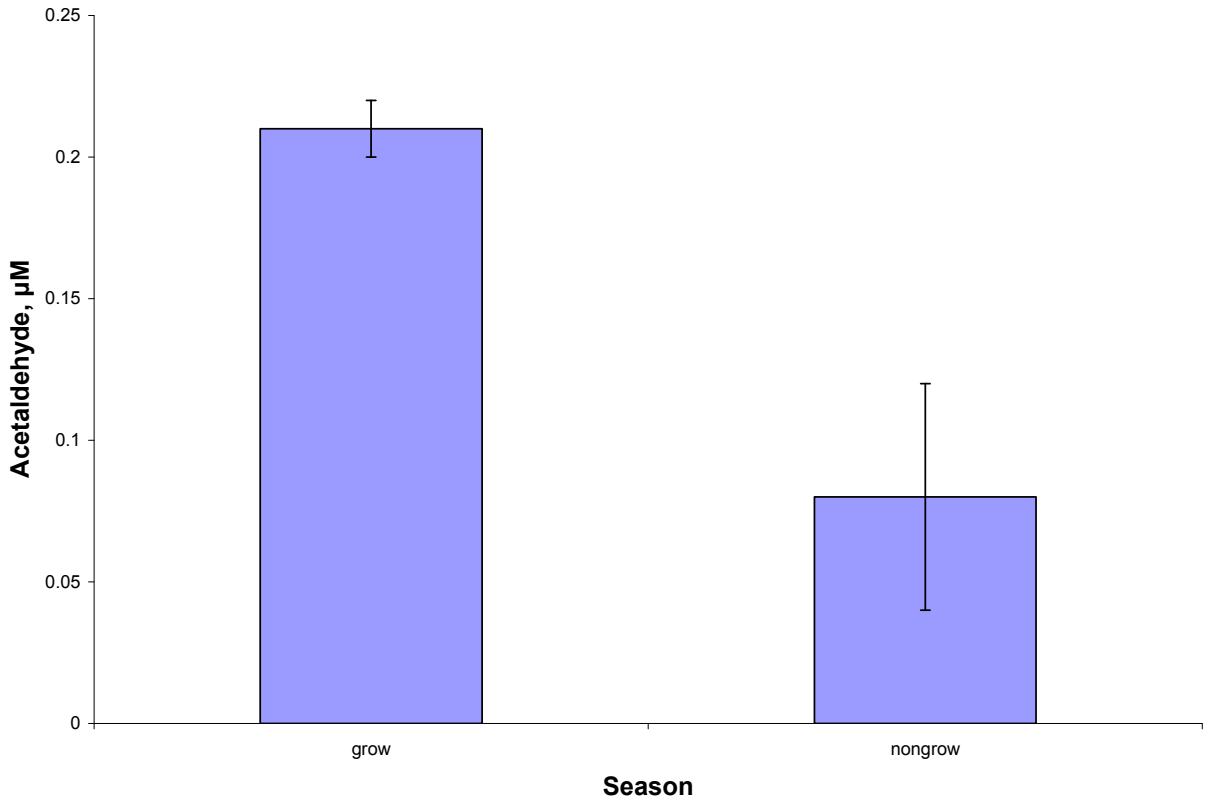
Figure 7c) Acetaldehyde concentrations in rain by month (n = 47).

Growing season rain events are defined as rain events occurring between April 1 and September 30 and the nongrowing season is defined as the remainder of the year. Results for methanol, formaldehyde, and acetaldehyde are summarized in figure 8a and acetaldehyde by itself in figure 8b. Methanol volume weighted concentration during the growing season was 1.5 $\pm$ 0.3 µM which was more than double the 0.7 $\pm$ 0.1 µM during the non growing season. This result was expected because of reported methanol sources being largely biogenic (Heikes et al., 2002). Acetaldehyde as expected followed the same trend as methanol with concentration increasing from 0.08 $\pm$ 0.02 µM in the non growing season to 0.21 $\pm$ 0.04 µM during the growing season. Formaldehyde volume weighted concentration during the growing season (1.9 $\pm$ 0.3

μM) was twice the concentration of the nongrowing season (1.0 ± 0.1 μM), suggesting a possible increase in biogenic sources of formaldehyde during the growing season. The growing season takes place during a period of increased anthropogenic activity (e.g. more vehicle activity), so increase in concentrations cannot be directly related to biogenic sources.



Figures 8a) Formaldehyde, methanol, and acetaldehyde concentrations in rain during growing and nongrowing seasons (n = 49).



Figures 8b) Acetaldehyde concentrations in rain during growing/nongrowing seasons (n = 47).

Storm Origin:

The volume weighted average concentration of methanol and formaldehyde in each storm type is given in figures 9a - 9b and table 3. The 1mixed, 2 terrestrial, 3 mixed, and 4 coastal storms have volume weighted average methanol concentrations of 0.9 $\pm$ 0.3 µM, 1.7 $\pm$ 0.6 µM, 1.0 $\pm$ 0.2 µM , and 1.5 $\pm$ 0.8 µM respectively and 5 marine storms have a noticeably lower MeOH concentration of 0.8 $\pm$ 0.23 µM. That methanol occurs in higher concentrations in rain over land is consistent with previous findings (Heikes et al. 2002) of greater than 80% biogenic source for gas phase methanol. Methanol that does occur in the more marine based storms may

be due to the ocean as source for atmospheric methanol or global transport of methanol from land masses to oceans due to methanol having an average atmospheric lifetime of 10 days (Jacob 2005). Methanol is readily produced in the ocean from the hydrolysis of alkyl halides (Singh, H. 2000). Millet et al 2008 observe the ocean as an overall methanol sink but consider it a large enough source to cause detectable concentrations in the atmosphere.

Formaldehyde volume weighted average concentrations in the storms more highly affected by land (1 mixed, 2 terrestrial, 3 mixed, and 4 coastal) were 1.8 ± 0.8 μM, 3.3 ± 0.8 μM, 1.3 ± 0.2 μM, and 1.4 ± 0.3 μM respectively. Overall these concentrations were higher than the 1.4 ± 0.2μM concentration of the type 5 marine storms. This agrees with the suggestion of Kieber 1999 that formaldehyde concentrations are due more to anthropogenic sources.

Sources of acetaldehyde over land include production from oxidation of alkanes and 2-alkenes of anthropogenic and biogenic sources (Holzinger et al., 1999). This along with sources from vegetation and biomass burning lead to 2 terrestrial storm types having highest acetaldehyde concentrations (0.32 ± 0.06μM). Acetaldehyde concentrations are a good indication of acetaldehyde being produced locally because of its average atmospheric lifetime of 1 day (Custer 2007). While not as high as acetaldehyde concentrations affected by land, 5 marine storm types still had significant acetaldehyde concentrations (0.12 ± 0.02μM). This is due to oceanic emissions being a substantial source of acetaldehyde (Singh et al., 2004).

TABLE 3: Volume-weighted averages (vwave) and standard deviations (vw s) for formaldehyde and methanol concentrations in rainwater according to rain event type. (n = rain events)

Event Type	n	HCHO vw ave (μM)	HCHO vw s (μM)	MeOH vw ave (μM)	MeOH vw s (μM)	CH_3CH_2O vw ave (μM)	CH_3CH_2O vw s (μM)
1Mixed	5	1.8	0.8	0.9	0.3	0.1	0.02
2Terrestrial	7	3.3	0.8	1.7	0.7	0.32	0.06
3Mixed	12	1.3	0.2	1	0.2	0.14	0.02
4Coastal	10	1.6	0.2	1.6	0.6	0.19	0.05
5Marine	14	1.4	0.2	0.8	0.2	0.12	0.02

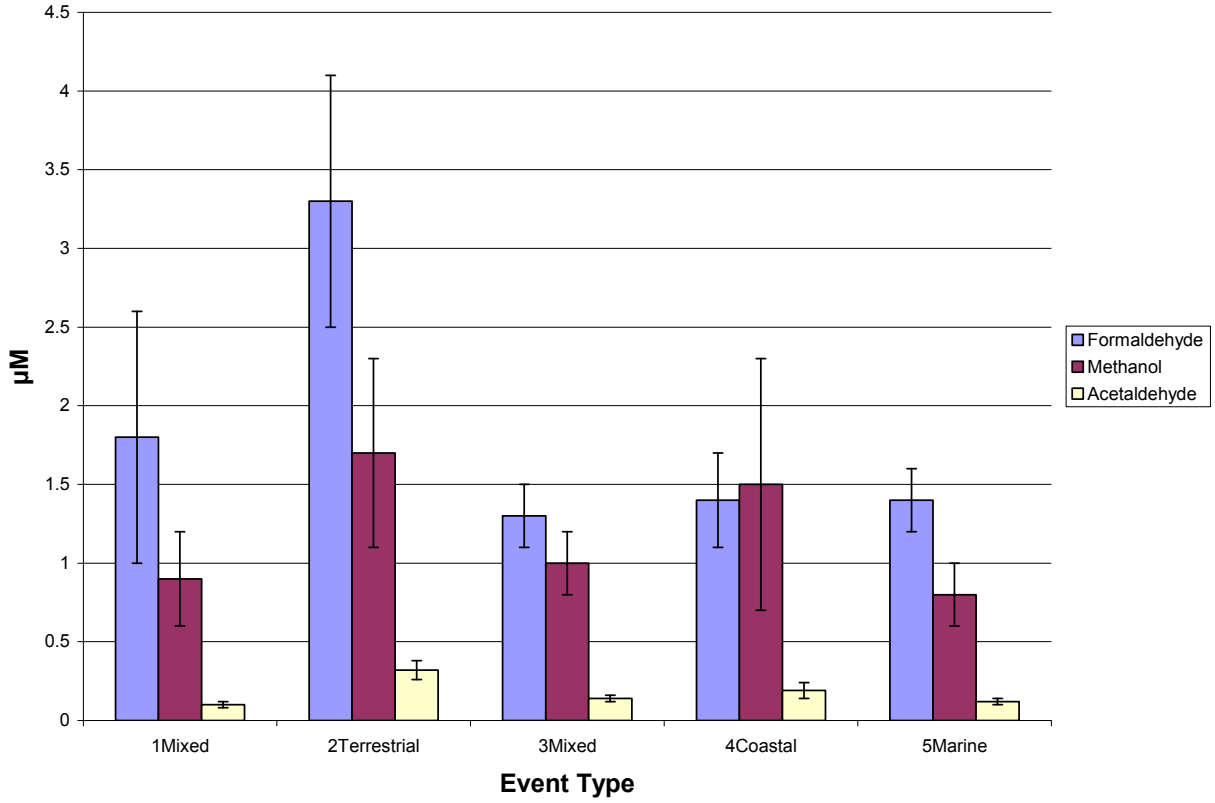


Figure 9a) Formaldehyde, methanol, and acetaldehyde concentrations according to rain event type (n = 49).

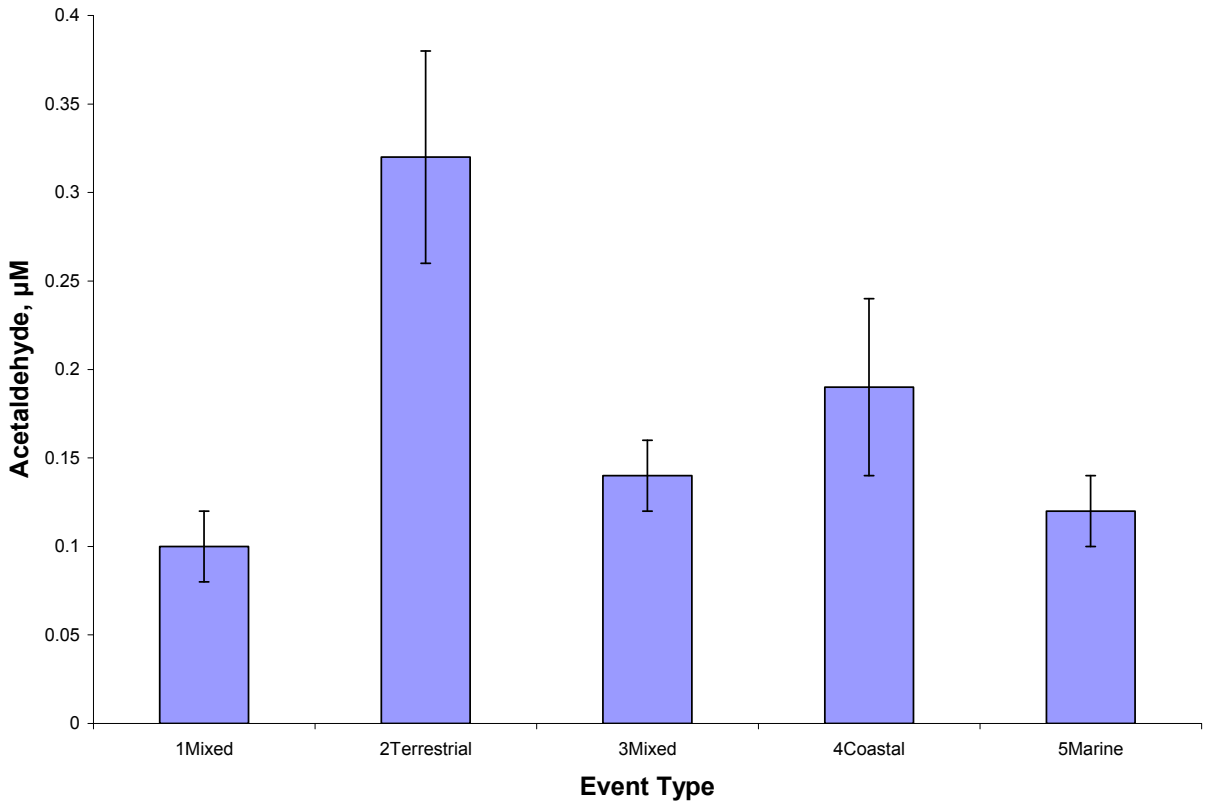


Figure 9b) Acetaldehyde concentrations according to rain event type (n = 47).

Diurnal Variations:

Rain events occurring during one of the given time periods without overlap were grouped together for analysis and presented in figure 10. Methanol, formaldehyde, and acetaldehyde concentrations all peaked during time period II (12pm – 6pm) with volume weighted concentrations of 2.6 $\pm$ 0.7µM, 2.2 $\pm$ 0.3µM, and 0.27 $\pm$ 0.06 µM respectively. All three components peaking during this time period of optimal sunlight may show a direct relationship to photochemical production, but more biogenic and anthropogenic activity also take place during this time period. A possible explanation for lower nighttime concentrations of all three components may be that concentrations of many of the rainwater components identified in dew

were similar to or greater than the concentrations seen in rainwater (Avery et al., 2001). This removal of components by dew would occur during the night and early morning leading to lesser amounts of each component to be available for rainwater.

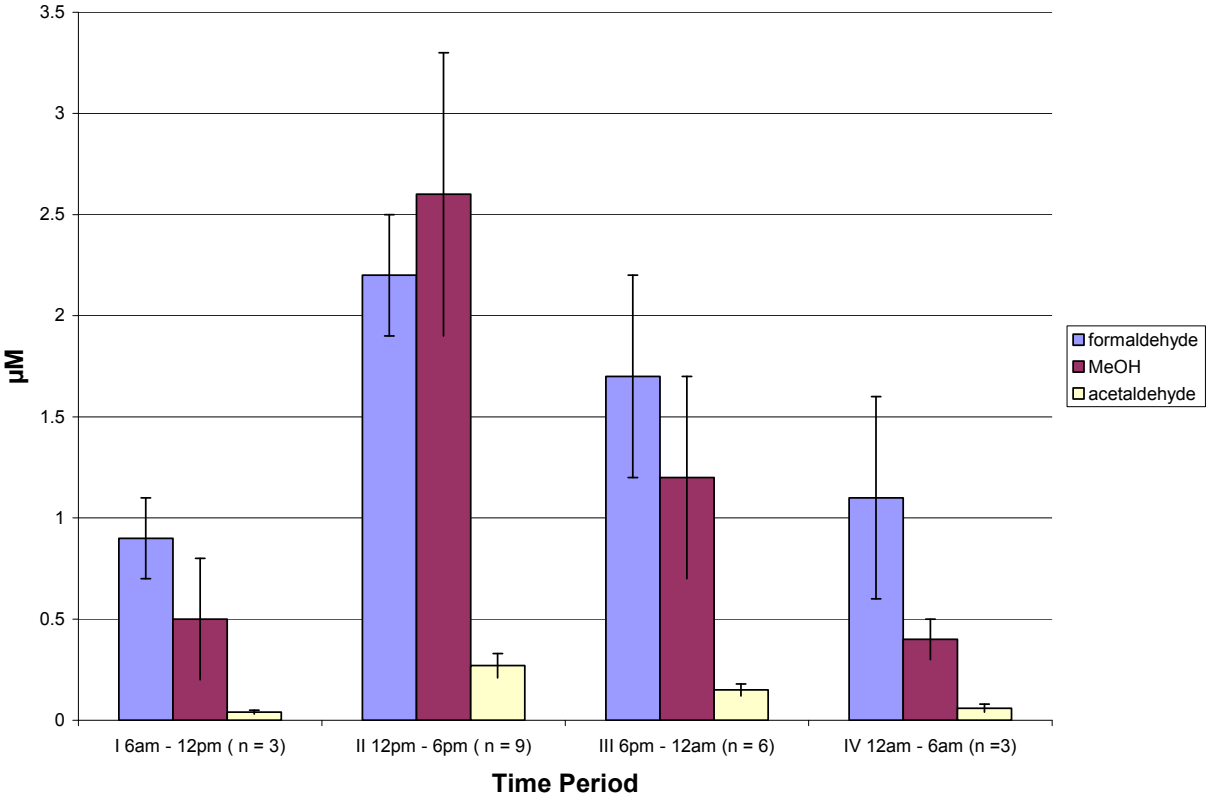


Figure 10a) Formaldehyde, methanol, and acetaldehyde concentrations according to time period of rain event.

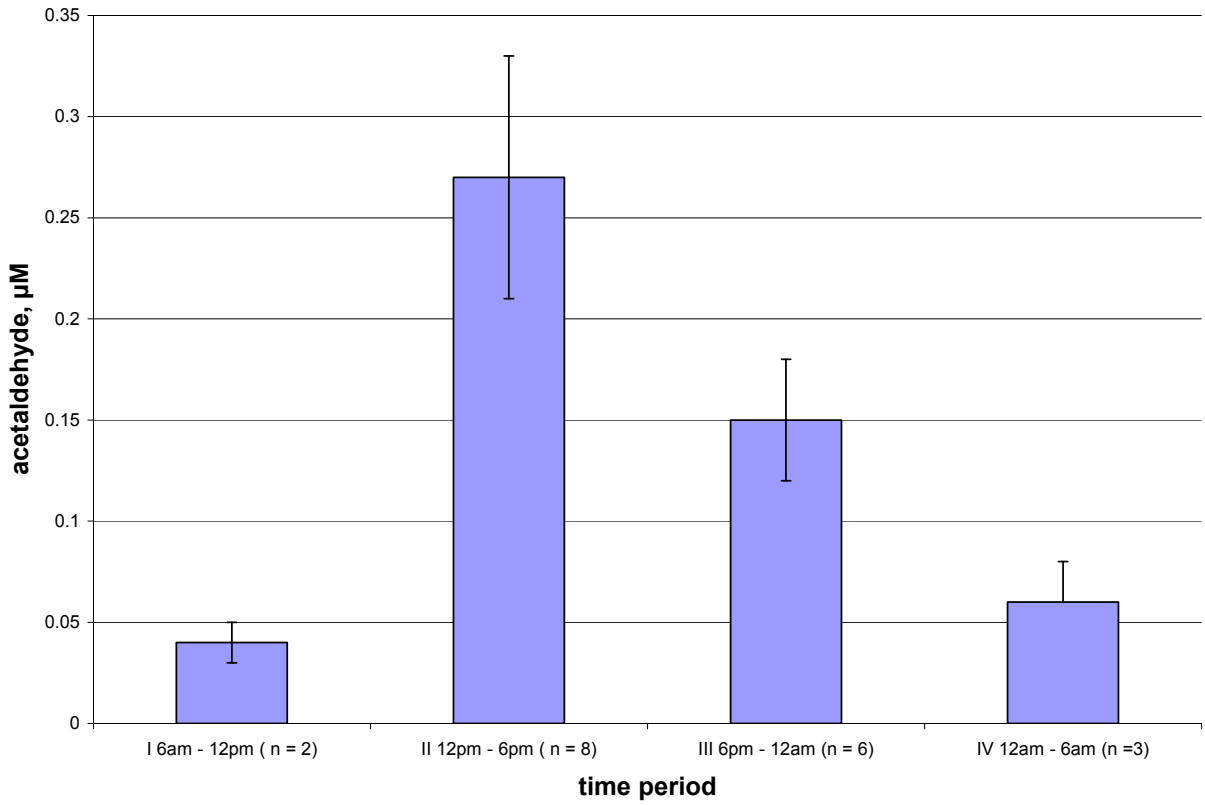


Figure 10b) Acetaldehyde concentrations according to time period of rain event.

Methanol and Formaldehyde %DOC

Methanol and formaldehyde in the rain events accounted for 1.46% and 2.08% of the dissolved organic carbon (DOC), respectively. This % DOC contribution from formaldehyde was similar to the ~3% seen by Kieber et al., (1999). %DOC methanol and formaldehyde contributed to each rain event was grouped according to storm origin (figure 11). %DOC contribution for both components was highest during marine dominated storms although both components had their lowest concentrations in rain during these storm types. This is because more DOC is seen in storms originated over land.

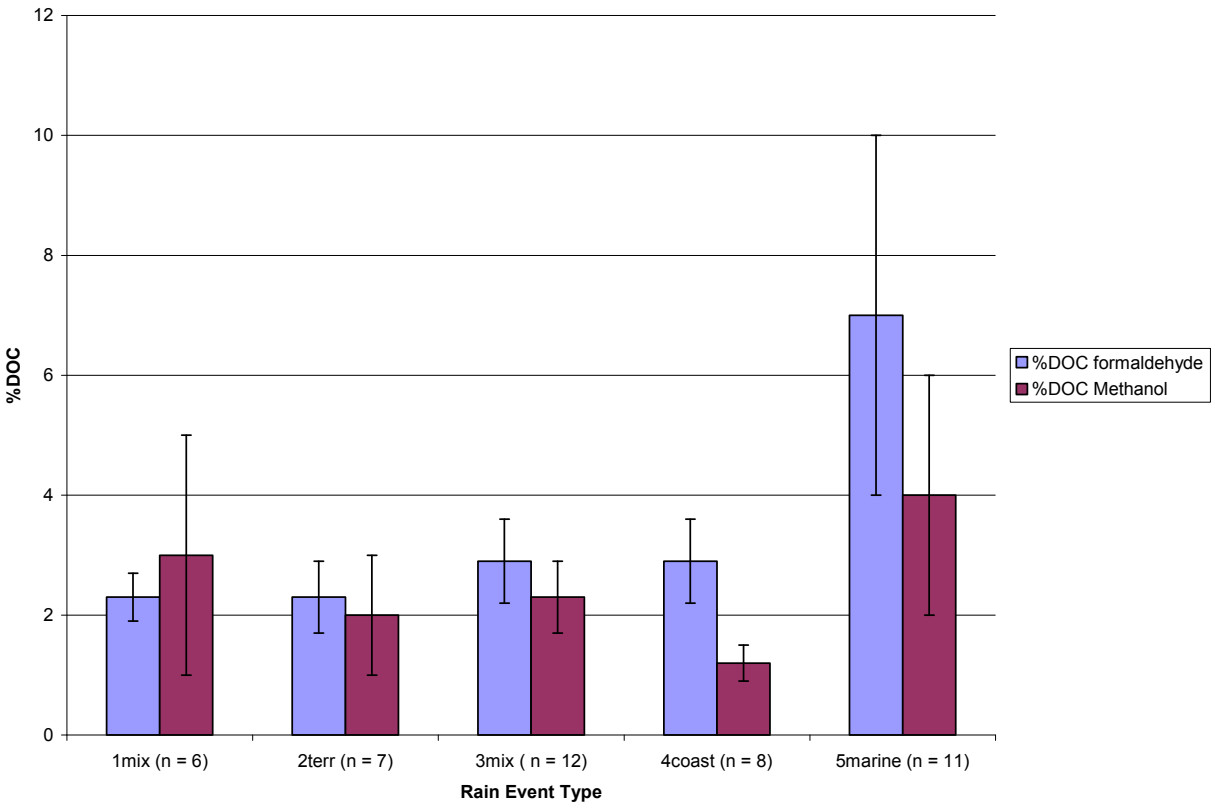


Figure 11) Methanol and formaldehyde contributions to dissolved organic carbon by rain event type.

Conclusions

The first methanol concentrations in rainwater are reported in this paper along with formaldehyde and acetaldehyde concentrations. Methanol, formaldehyde, and acetaldehyde concentrations were correlated with various other rainwater components, grouped according to storm origin, and grouped according to time period of storm in order to conclude if contributions to concentrations are being made by anthropogenic or biogenic sources. Evidence showed that methanol concentrations were affected primarily by biogenic sources, acetaldehyde was affected significantly by both anthropogenic and biogenic sources and formaldehyde was affected by both

anthropogenic and biogenic sources but primarily by anthropogenic sources. Diurnal variations indicated possible significant photochemical production of all three components under study.

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ready/hysplit4.html

Chapter 2

Introduction

Nitrogen inputs, specifically in the form of nitrate, besides being essential for plant growth can have a profound impact on the aquatic and marine environment including eutrophication of natural waters, acid rain, and phytoplankton blooms resulting in hypoxia. Nitrate influx to the environment has been linked to anthropogenic sources such as fertilizers, animal and human waste, and fossil fuel combustion from vehicles and stationary sources (Kendall et al., 2007). The isotopic composition of nitrate will vary depending on its source allowing $\delta^{15}N$ values to be a potentially valuable tool in tracing origin of nitrate. Typical nitrate natural abundance $\delta^{15}N$ values range between -15 and 25‰ (Kendal et al., 2007). $\delta^{15}N$ values of anthropogenic nitrate sources have been characterized and usually are positive but there are wide ranges of $\delta^{15}N$ values reported for each source depending on the study. The discrepancies between studies indicate a need for better characterization of nitrate sources and a greater variety of analyses to accomplish this characterization (Kendall et al. 2007).

There currently are several approaches to analyzing nitrogen isotopic ratios in aqueous environmental matrices and these methods focus on analysis of the isotopic ratio of nitrate. The bacterial denitrifier method is based on isotopic analysis of nitrous oxide produced from nitrate by denitrifying bacteria (Sigman et al., 2001). This method is useful because low concentrations are appropriate for marine samples but cannot be utilized for all environmental samples because some samples can kill the bacteria. A bacteria culture must be maintained when utilizing this method and this is labor intensive. An alternative is the silver nitrate method. The method involves nitrate in the sample being converted to silver nitrate then analyzed using EA-IRMS

(Silva et al., 1999). This method calls for large samples sizes, is time consuming, and limited to waters of low ionic strength. A method (Sigman et al. 1997) adapting the ammonia diffusion method has been used to obtain nitrate nitrogen isotopic composition but sample preparation can be time consuming and there is limited efficiency in the ammonia extraction and diffusion leading to considerable uncertainty when correcting for diffusion efficiency (McIlvin and Altabet., 2005). A recent chemical conversion method (McIlvin and Altabet., 2005) which includes the possibility for isotopic analysis of nitrite as well as nitrate is based on reduction of NO_2 to N_2O using sodium azide, a chemical which can decompose explosively and when in contact with water or acid produces a toxic gas.

An alternate method is presented for determining $\delta^{15}N$- NO_2^- and NO_3^- and is appropriate for low level enrichment and many natural abundance applications. The method can be applied to any nitrogen containing compound in which nitrogen can be converted to nitrite. Nitrate is reduced to nitrite using spongy cadmium and reacted with dinitrophenylhydrazine (DNPH). The $\delta^{15}N$ of $^{15}NO_2^-$ is determined by decomposing the nitrite derivative that results from the known reaction of nitrite and DNPH (figure 1). This decomposition produces N_2 (g). One nitrogen in the N_2 (g) is from nitrite and the other is from DNPH. N_2 (g) is analyzed by IRMS. This method allows for small sample volumes, relatively short time requirements, minimal chemical manipulations, and a low detection limit.

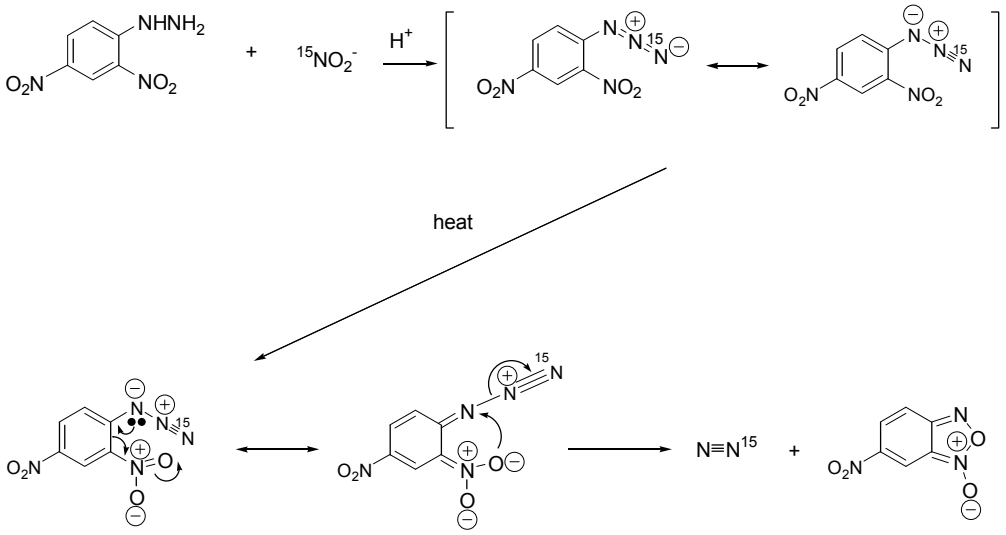


Figure 1) Reaction of nitrite and DNPH and decomposition of derivative to produce nitrogen.

Methods

Reagents/Standards

Derivatization

Water was purified using a Millipore Q-water system (Millipore Corp., Bedford, MA) and used to prepare all solutions. Reagent grade 2,4-dinitrophenylhydrazine (DNPH) was purchased from Sigma (St. Louis, MO), triply recrystallized from acetonitrile and kept refrigerated in the dark. Acetonitrile (HPLC grade, Burdick and Jackson, Muskegon, MI), sulfuric acid (Mallinckrodt, Phillipsburg, NJ), and carbon tetrachloride (HPLC grade 99.9%, Sigma, St. Louis, MO) were used in preparation and purification of DNPH reagent solution. Sodium nitrite (J.T. Baker, Baker analyzed A.C.S. Reagent) was used to prepare stock nitrite solution each day of an experiment.

Nitrate Reduction to Nitrite

20% w/v cadmium sulfate was prepared using DI water and cadmium sulfate (Certified A.C.S., Fisher Scientific Company, Fair Lawn, NJ). 0.7M ammonium chloride pH 8.5 buffer

was prepared using DI water, ammonium chloride (EMD Chemicals Inc., Darmstadt, Germany), and 10 M NaOH (VWR, West Chester, PA). Three separate sodium nitrate reagents were used to obtain varying $\delta^{15}N$ values ($\delta^{15}N = 0.63$; A.C.S. Reagent, Aldrich, Milwaukee, WI., $\delta^{15}N = 3.83$; EMD Chemicals Inc., Darmstadt, Germany., $\delta^{15}N = 7.20$; A.C.S. Reagent, New Jersey, USA).

General Procedures

Polyethylene disposable gloves (VWR International) or Kimberly-Clark powder free nitrile gloves were worn when handling reagents and samples. All volumetric glassware and caps were rinsed several times with Milli-Q water prior to making solutions.

Dinitrophenylhydrazine Reagent Preparation

Reagent was prepared according to the Kieber and Mopper method (Kieber, 1990). The 2,4-dinitrophenylhydrazine (DNPH) reagent was prepared on a weekly basis in a 30mL Teflon vial by dissolving 0.0375g of triply recrystallized DNPH in 4.2 mL of concentrated sulfuric acid (H_2SO_4) and 18 mL of 95% ethanol and mixing on a wrist shaker for 30min. To reduce background signal, reagent was extracted with 2 mL of carbon tetrachloride by shaking for 10 minutes on a wrist action shaker, followed by centrifugation for 2 minutes. After the initial extraction, the lower organic layer was removed and the process was repeated. After the second extraction, the organic layer was left in reagent vial and removed prior to successive extractions. DNPH reagent extraction was done twice on the first day of use, and once for each following day used, for up to one week.

Nitrite Derivative Preparation

Nitrite derivative preparation for solid state decomposition studies.

Decomposition of the derivative in solid state was studied to be sure the derivative was quantitatively decomposing and at what conditions. Quantitative decomposition is necessary to ensure no fractionation during decomposition. To obtain enough derivative to be used in solid state decomposition studies and to be analyzed using the Elemental Analyzer, derivatives of nitrite were prepared according to (Roberts, 1969). Specifically, 0.5000 g of triply recrystallized DNPH (a more concentrated DNPH reagent is used) was ground using a mortar and pestle. The DNPH reagent was made in an Erlenmeyer flask by adding 7 mL of concentrated H_2SO_4 to 30 mL of 95% ethanol while stirring. A nitrite solution was prepared by dissolving approximately 0.1500 g $NaNO_2$ in 4 mL of deionized water and this solution was cooled in a refrigerator. The nitrite derivative was prepared by adding 9 mL of cold DNPH reagent to the nitrite solution. Precipitation of derivative was immediate. The solution was placed in the dark and allowed to react further. The derivative was stored in the refrigerator at ~3^{o}C to finish reaction. The following day products were filtered using a Büchner funnel, rinsed with cold deionized water, and allowed to dry. The nitrite derivative sample was stored in a refrigerator at ~3^{o}C until time of analysis.

Nitrite derivative preparation for DNPH:Nitrite ratio fractionation study:

DNPH:nitrite ratio fractionation study was done to find the appropriate amount of excess DNPH to react with nitrite to ensure constant fractionation when preparing the nitrite derivative. To obtain nitrite derivative to be used in the DNPH:nitrite ratio fractionation study, derivatives

of nitrite were prepared according to (Roberts, 1969). After preparation of DNPH reagent, the nitrite derivative is made by reacting several different molar ratios (1:1 to 30:1) of DNPH and ~900 nmol nitrite in solution. The derivative product is extracted using $CHCl_3$, transferred to 12 mL Labco Exetainer vial, and $CHCl_3$ blown off using Argon. Resulting derivative sample is capped and stored in a refrigerator at ~3^oC or on ice to prevent decomposition. Just before analysis samples are purged with UHP He to rid sample container of any N_2 gas.

Spongy cadmium reduction for nitrate sample followed by sample preparation:

Nitrate must be reduced to nitrite using spongy cadmium before it can be derivatized. Spongy cadmium reduction technique was modified from Jones (1984). To produce spongy cadmium a Zn stick is placed in 40 mL of 20% w/v $CdSO_4$ and allowed to sit overnight. Cadmium produced is scraped off the Zn stick and the stick is removed from solution. The solution containing the cadmium is acidified with 6N HCl and drained from the cadmium. Cadmium is covered with 6N HCl and stirred for a minute then drained. Cadmium is rinsed with DI water until pH is above 5. The cadmium must remain wet at all times. A 15 ml nitrate sample is transferred to a 30 mL Teflon vial along with 3 mL NH_4Cl buffer and 0.6g Cd. The vial is put on a wrist shaker for 90 min to reduce the nitrate. The nitrite derivative was prepared by reacting DNPH and ~700 nmole nitrite in a molar ratio of at least 10:1 (DNPH:NO_2^-) to ensure consistent fractionation. Derivative product is extracted using $CHCl_3$, transferred to 12 mL Labco Exetainer vial, and the $CHCl_3$ is blown off using Argon. Resulting derivative sample is capped and stored in a refrigerator at ~3^oC or on ice to prevent decomposition. Just before analysis samples are purged with UHP He to rid sample container of any N_2 gas.

Natural Samples Preparation:

Natural samples in this study were brackish water samples that have tracer enrichment and high dissolved organic nitrogen. The samples were collected in Teflon bottles from Hewlett's Creek, a marsh dominated tidal creek in North Carolina, and frozen until day of sample prep. A sample volume was taken to obtain ~700nmole of nitrate and roto – vapped down to a suitable size (~30mL) for spongy cadmium reduction and extraction. After sample had been concentrated, nitrate in samples was reduced via spongy cadmium reduction. 30 mL reduced sample was transferred to a 70 mL Teflon vial along with 6 mL NH_4Cl buffer and 1.2g Cd (amount of Cd may be increased if there are high concentrations of humic material in the sample interfering with reduction). The vial was put on a wrist shaker for 4 hours to reduce nitrate. Nitrite derivative by reacting DNPH and ~700 nmole nitrite in a molar ratio of at least 10:1 ($DNP:NO_2^-$) to ensure consistent fractionation. Derivative product was extracted using ~ 6 mL $CHCl_3$, transferred to 12 mL Labco Exetainer vial, and $CHCl_3$ blown off using Argon. Resulting derivative sample capped and stored in a refrigerator at ~3^oC or on ice to prevent decomposition. Just before analysis samples were purged with UHP He to rid sample container of any N_2 gas.

HPLC Instrumentation and Conditions used to monitor nitrite derivative decomposition

HPLC instrument used was a Hewlett-Packard (Agilent) Model 1100 Series, equipped with an autosampler, a thermostatted column compartment, and a variable wavelength absorbance detector. Agilent ChemStation software for LC and LC/MS systems was used. Integration parameters were as follows; slope sensitivity set at 0.3402, peak width set at 0.1086, an area reject of 0.0282, a height reject of 0.0323, no shoulders, and an integration start time of

3.00 minutes with baseline integration at valleys from 3.2 to 7.322 min. A reversed phase Luna 100mm x 4.60mm 3μ C18(2) Phenomenex column with a pore size of 100 Angstroms was used. Mobile phase consisted of 48:52, Milli-Q water:filtered acetonitrile and 0.1% trifluoroacetic acid (TFA) in both solvents with a 1.00mL/min flow rate, and a 100μL injection volume. Column temperature was set at 10.0 degrees Celsius and detection was at 307 nm. Run times were 12min.

At the end of each experiment, a flush method was programmed to rinse the column. Conditions were as follows; flow rate of 1.250mL/min, 307nm detection, 10 degree Celsius column temperature, and a run time of 30 minutes. A mobile phase gradient program was used to ensure no acid was left on the column. Mobile phases were (A) Milli-Q with 0.1%TFA and (B) filtered acetonitrile, and the gradient was set up as follows: isocratic in 60% B for 6 minutes, 60% B to 80% B in 1.5 minutes, isocratic in 80% B for 6 minutes, 80% B to 100% B in 1.5 minutes, and then isocratic at 100% B for 15 additional minutes.

IRMS Instrumentation and Conditions:

All $\delta^{15}N$ analyses were performed on a Thermo-Fisher Delta V Plus Isotope Ratio Mass Spectrometer (IRMS). The instrument is equipped with multi collectors and simultaneously provides m/z ratios for 28, 29, 30 (N_2), and 40 and 36 (Ar) on headspace samples. The Gas Bench II interface introduced headspace gases (e.g. the N_2 produced during derivative decomposition) to the IRMS. 250 μl of headspace gas was chromatographically separated into Ar, and N_2 at 40 degrees C using a Mol Seive 5A capillary column prior to introduction to the IRMS. The $\delta^{15}N$ of solid samples (DNP, derivative, NO_3 salts) was determined using the Costech 4010 Elemental Analyzer (EA) interface for the IRMS. The samples were combusted at

1000 degrees C to CO_2 and NOx in the presence of chromium oxide. The NOx was reduced by Cu metal at 600 degrees C and the N_2 separated from CO_2 using a 2m Poropak Q GC column at 60 degrees C. The N_2 was subsequently introduced to the IRMS via a Conflo III open split interace. All $\delta^{15}N$ values were reported relative to atmospheric N.

Blank Correction of samples analyzed by combination of gas bench and IRMS:

Background nitrogen signal is seen so the following blank correction equation was used:

$(\delta^{15}N \text{ mix})(\text{Area mix}) = (\delta^{15}N \text{ blank})(\text{Area blank}) + (\delta^{15}N \text{ sample})(\text{Area sample})$

$\delta^{15}N$ mix = $\delta^{15}N$ of sample uncorrected for blank signal

$\delta^{15}N$ blank = $\delta^{15}N$ of blank from a vial purged with UHP He and spiked with air

$\delta^{15}N$ sample = $\delta^{15}N$ value of sample corrected for blank signal

Results and Discussion

Decomposition Nitrite derivative Studies

Decomposition of Nitrite derivative in Water

Effect of temperature and time on nitrite derivative decomposition were determined so proper conditions could be found for complete decomposition of the derivative to $N_2(g)$. Derivative was prepared in a 2 mL HPLC vial by adding 10 µL of DNPH reagent to a 1mL sample of 5 µM nitrite solution. The first derivative decomposition study was monitored while derivative was in water. Derivative was decomposed in a water bath at 75 (triplicate samples)

and 85°C (duplicate samples) over varying periods of time. Derivative samples were removed from water bath and decomposition was monitored using HPLC to observe decrease in derivative signal. Results show most of derivative decomposing in 60 minutes at 75 °C and all derivative decomposing in 30 minutes at 85°C. Isotopic composition was not determined during this experiment.

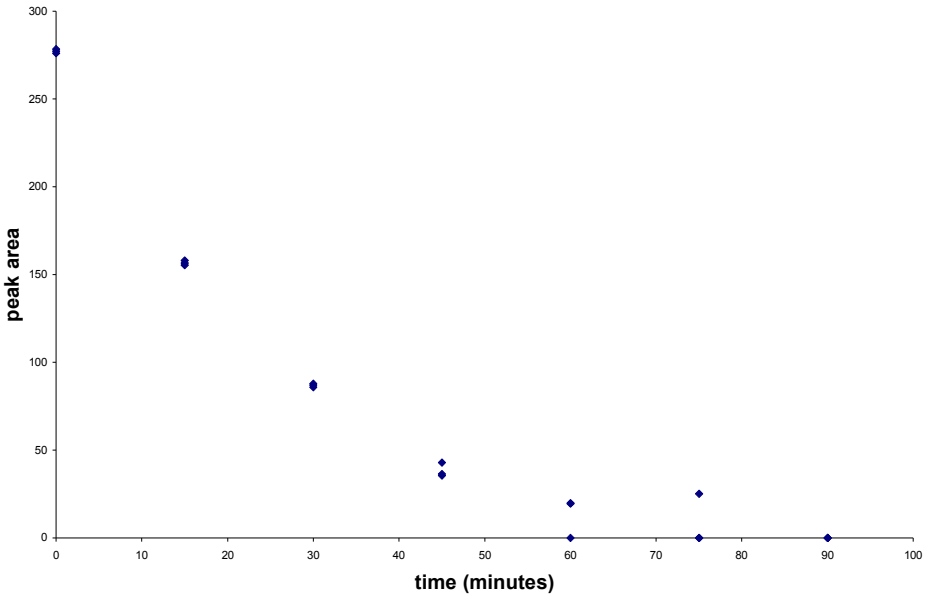
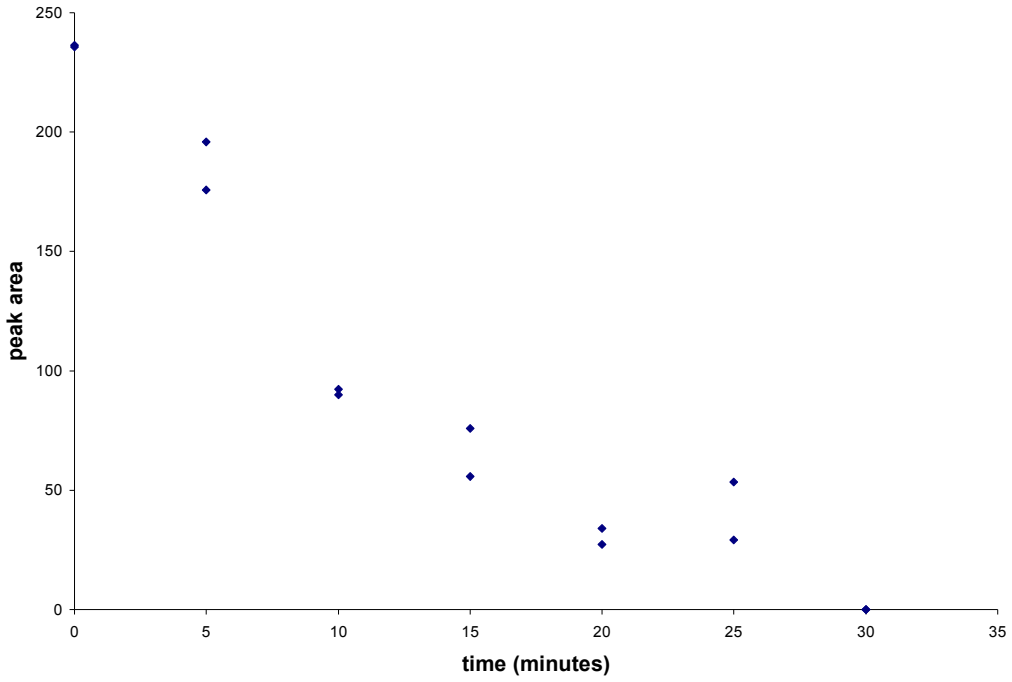


Figure 2) Timed Decomposition of nitrite derivative at 75 °C.



Figures 3) Timed Decomposition of nitrite derivative at 85 ^{o}C.

Decomposition of Nitrite derivative in Solid State

Decomposition of nitrite derivative in solid state was then studied. Derivative was prepared the same way as in the first study, but it was then extracted with $CHCl_3$ and transferred to a second 2 mL HPLC vial. The $CHCl_3$ was blown off using N_2 gas and derivative was then decomposed in a water bath at 70, 80, 90 ^{o}C for varying amounts of time with duplicate samples at each time. Decomposed derivative/derivative was dissolved in mobile phase and analyzed using HPLC. Results show total decomposition of derivative can be seen after it is heated at 80^{o}C for 30 min.

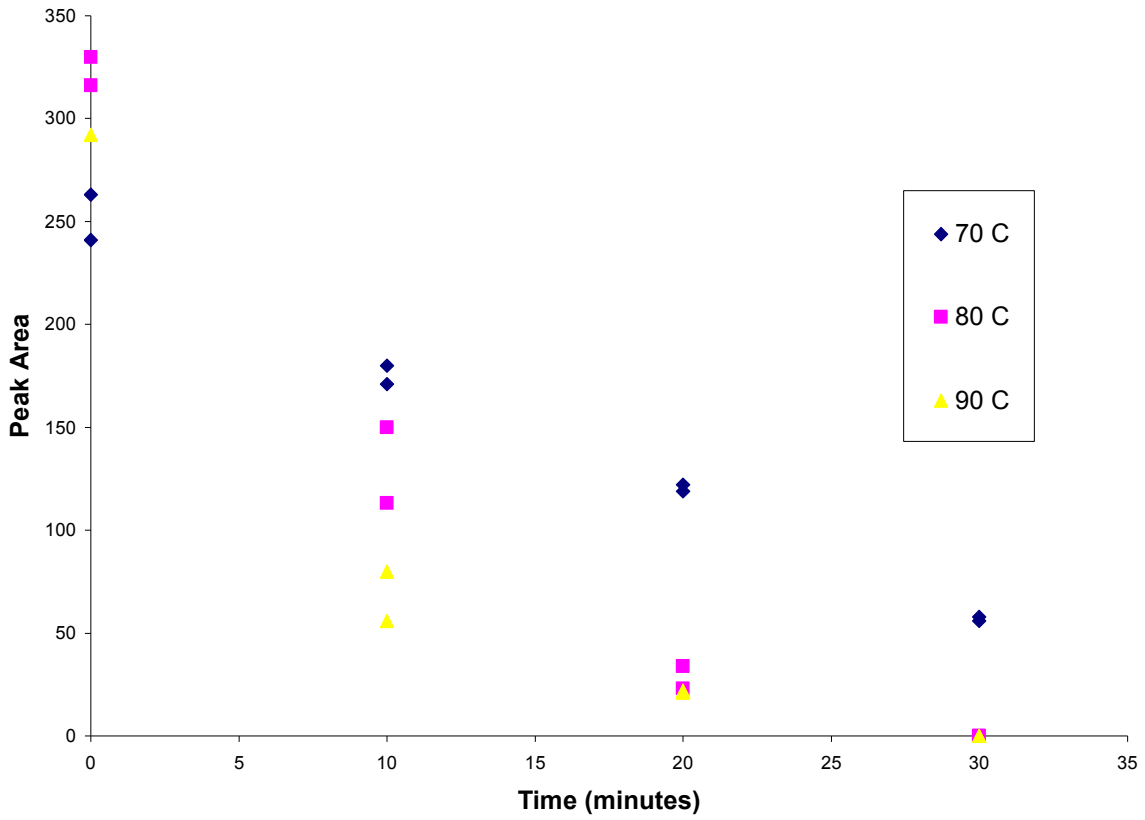


Figure 4) Timed solid state decomposition of nitrite derivative @ 70, 80, and 90 ^{o}C

Decomposition of derivative on the gas bench:

Derivative decomposition on the heating block was analyzed by IRMS to assess fractionation during decomposition. A 0.001M nitrite solution was prepared and 900µL aliquots of solution (~900 nmoles nitrite) were derivatized with a DNPH excess of at least 10 fold to ensure a constant fractionation. Derivative was extracted using chloroform and the chloroform was then driven off using argon. Resulting derivative was allowed to sit (in gas tight vial) in the heating block at 80^{o}C for varying amounts of time to monitor derivative decomposition. Amount of N_2 (g) being produced from decomposition of derivative becomes constant after 40 minutes in the heating block because decomposition is complete. A different sample was injected at each

time because once the septum of each sample container has been punctured it cannot be resampled. The $\delta^{15}N$ value of N_2 (g) decomposition product was also monitored to provide evidence of complete decomposition. The $\delta^{15}N$ values become less negative as decomposition continues because N_2 (g) with lighter isotopic composition is more likely to be the product at the beginning of decomposition. The $\delta^{15}N$ value also becomes constant after 40 minutes indicating complete decomposition. DNPH reagent was allowed to sit in the heating block for over 60 minutes to test for any nitrogen resulting from the decomposition of the reagent. None was seen.

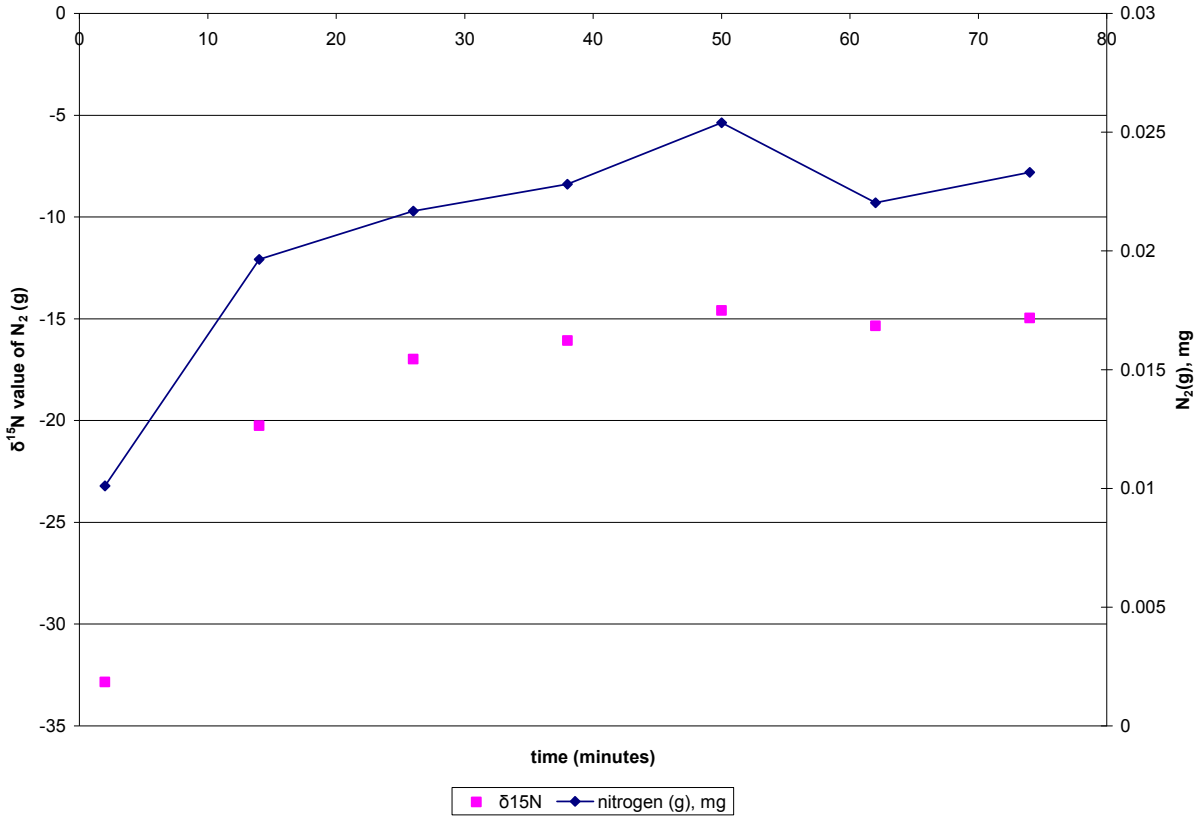


Figure 5) Milligrams of N_2 (g) and $\delta^{15}N$ value of N_2 (g) during timed decomposition of solid state nitrite derivative on heating block at 80°C (one sample per time)

DNPH:Nitrite Ratio Fractionation Study

DNPH/Nitrite derivative was prepared using several DNPH:$NaNO_2$ ratios to monitor nitrogen isotopic fractionation of the resulting N_2(g) decomposition product. Derivative was made by derivatizing an aqueous solution containing 700 nmoles of nitrite with various excesses (30:1, 20:1,10:1, 7.5:1, 5:1, and 2.5:1) and equimolar of DNPH reagent. The data showed when DNPH reagent was used in an excess of 5 and greater the fractionation is constant as seen in table 1 and figure 6.

Table 1: DNPH to nitrite ratio used to make each derivative and the average $\delta^{15}N$ values according to DNPH excess used.

DNPH:nitrite	Average $\delta^{15}N$	std dev
1 (n = 6)	-7	$\pm$2
2.5 (n = 6)	-12.4	$\pm$0.8
5 (n = 5)	-15.3	$\pm$0.9
7.5 (n = 4)	-15.0	$\pm$0.8
10 (n = 6)	-15.7	$\pm$0.6
20 (n = 3)	-15.3	$\pm$0.2
30 (n = 3)	-15.0	$\pm$0.3

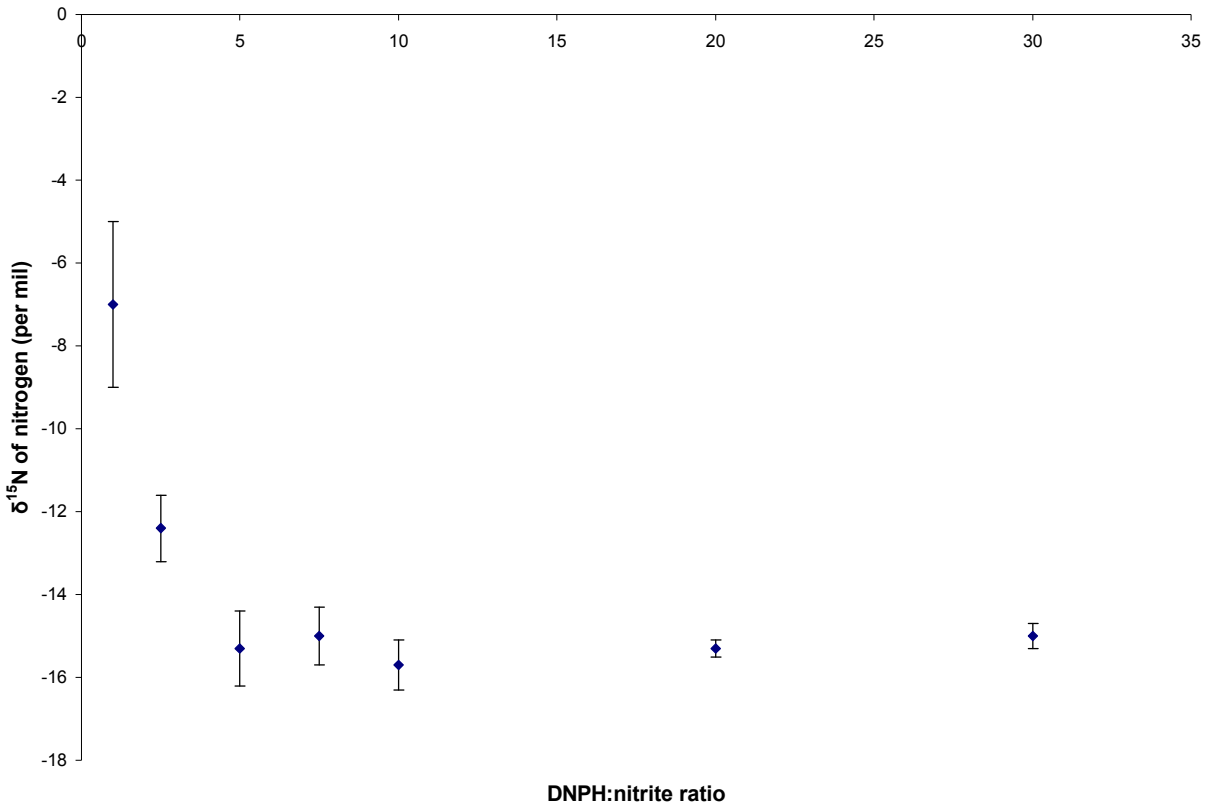


Figure 6) $\delta^{15}N$ values of nitrogen depending on DNPH:Nitrite ratio

Determination of $\delta^{15}N$ contribution from DNPH nitrogen to $\delta^{15}N$ value of $N_2(g)$:

It is not possible to buy DNPH with known isotopic labels on the nitrogen, so it must be back calculated using mixing equations. The $\delta^{15}N$ values of the reagents involved in producing the derivative must be found using EA-IRMS. Values of $\delta^{15}N$ determined in this study are: DNPH -4.56 $\pm$0.06, derivative -3.31 $\pm$0.03, derivative after heating @ 80^{o}C for 60min -2.1 $\pm$0.10. It is important to note that derivative in this study was prepared using excess $NaNO_2$ so

all DNPH is used up in the reaction. The follow mixing equations were used to calculate $\delta^{15}N$ value of the nitrogen in DNPH being contributed to the $\delta^{15}N$ of $N_2(g)$:

Calculation of $N_2(g)$ $\delta^{15}N$ value (X):

($\delta^{15}N$ Derivative) = (0.6)($\delta^{15}N$ Heated Derivative) + (0.4)(X)

(-3.31) = (0.6)(-2.1) + (0.4)(X)

X = -5.13 = $\delta^{15}N$ $N_2(g)$

There are five nitrogens in the nitrite derivative – four from DNPH and one from nitrite contributing to the $\delta^{15}N$ value of the derivative are from the heated derivative and 0.4 is used because 2 of the 5 nitrogens contributing to the $\delta^{15}N$ value of the derivative are from the nitrogen gas decomposition product. This information can be used to create a mixing equation to solve for $\delta^{15}N$ value of the $N_2(g)$ that occurs when all DNPH has been used up in reaction because of the use of excess nitrite in the reaction. All DNPH was used in reaction to ensure no fractionation from the DNPH end member.

Calculation of $NaNO_2$ $\delta^{15}N$ value contribution (X) to derivative:

($\delta^{15}N$ Derivative) = (0.2)(X) + (0.8)($\delta^{15}N$ DNPH)

(-3.31) = (0.2)(X) + (0.8)(-4.56)

X = 1.69 = $\delta^{15}N$ $NaNO_2$

0.8 is used here because 4 of the 5 nitrogens contributing to the $\delta^{15}N$ value of the derivative are from DNPH and 0.2 is used because 1 of the 5 nitrogens contributing to the $\delta^{15}N$ value of the derivative is from nitrite. This information can be used to create a mixing equation to solve for $\delta^{15}N$ value of the nitrite contribution to the $\delta^{15}N$ value of the derivative that occurs when all

DNPH has been used up in reaction because of the use of excess nitrite in the reaction. This is the $\delta^{15}N$ value of the nitrite contribution when excess nitrite has been used. All DNPH was used in DNPH/nitrite reaction to ensure no fractionation from the DNPH end member.

Calculation of $\delta^{15}N$ value from nitrogen in DNPH being contributed to $\delta^{15}N$ of N_2 (g):

$(\delta^{15}N\ N_2(g)) = (0.5)(NaNO_2) + (0.5)(X)$

$(-5.13) = (0.5)(1.69) + (0.5)(X)$

$X = -11.95 = \delta^{15}N$ value of the nitrogen on the DNPH being contributed to $N_2(g)$

The previous two calculations were used to solve for the $\delta^{15}N$ value of $N_2(g)$ that occurs when all DNPH has been used up in reaction and the nitrite contribution to the $\delta^{15}N$ value of the derivative that occurs when all DNPH has been used up in reaction. These calculated values can be used to calculate $\delta^{15}N$ value from nitrogen in DNPH being contributed to $\delta^{15}N$ of N_2 (g). $\delta^{15}N$ values were used in this series of equations that were calculated or measured from derivative that was made with excess nitrite, so all DNPH was used in reaction to ensure no fractionation from the DNPH end member. The 0.5 in the mixing equation was used because the DNPH and nitrite both contribute one nitrogen to the two nitrogens the nitrogen gas.

Nitrogen Isotopic Fractionation of Nitrate with known $\delta^{15}N$ *values:*

The nitrogen isotopic composition of three nitrate reagents ($\delta^{15}N = 0.63$; A.C.S. Reagent, Aldrich, Milwaukee, WI., $\delta^{15}N = 3.83$; EMD Chemicals Inc., Darmstadt, Germany., $\delta^{15}N = 7.20$; A.C.S. Reagent, New Jersey, USA) was determined by weighing out the nitrate salts in tin cups and analyzing with EA-IRMS. Nitrate samples were reduced to nitrite using spongy cadmium

and derivatized with a DNPH excess of at least 10 to ensure a constant fractionation. A line equation for the expected (no fractionation) $\delta^{15}N$ values of N_2 from decomposition of derivative was calculated using -11.95 $\delta^{15}N$ as the DNPH contribution and the following mixing equation:

$$(\delta^{15}N\ N_2(g)) = (0.5)(\delta^{15}N\ NaNO_3) + (0.5)(\delta^{15}N\ DNPH)$$

and then plotting the $\delta^{15}N\ NaNO_3$ vs $\delta^{15}N\ N_2(g)$.

A line equation was found for the actual $\delta^{15}N\ N_2(g)$ values by plotting the $NaNO_3$ reagent $\delta^{15}N$ values vs. the measured $\delta^{15}N\ N_2(g)$. The resulting plots are found in figure 7. The expected slope with 1:1 mixing is 0.5, but the actual slope is 0.6164. This is understandable since the standard deviation of the measured $\delta^{15}N$ value of the nitrate standard reagents is 0.6 ‰. Expected intercept is -5.975 and actual intercept is -17.146 suggesting a fractionation factor of 22.3 $\pm$ 1.2 ‰ from the reaction of nitrite with DNPH. This fractionation factor was determined by taking the difference between the intercepts and dividing by 0.5 because $\delta^{15}N\ N_2(g)$ value results from a 1:1 mixing.

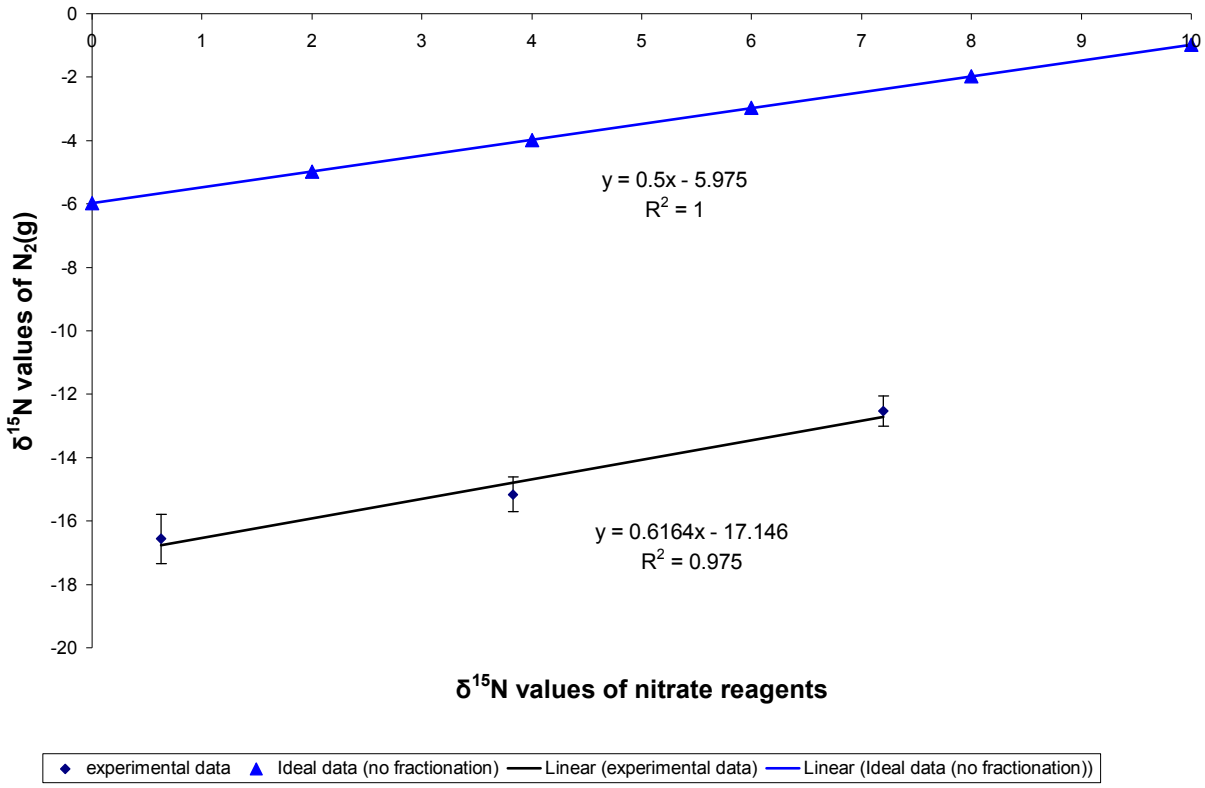


Figure 7) $\delta^{15}N$ values of nitrate standards plotted against $\delta^{15}N$ value of the nitrogen decomposition product of DNPH:Nitrite derivative

Determination of $\delta^{15}N$ of natural samples:

Natural samples enriched as part of an in-situ ^{15}N isotope addition experiment were collected in HDPE bottles from Hewlett's Creek in North Carolina and frozen until day of sample prep. The $\delta^{15}N$ nitrate/nitrite in these samples was determined by the ammonia diffusion method (Sigman et al., 1997). To obtain $\delta^{15}N$ values using the presented method, three nitrate standards of known $\delta^{15}N$ were also analyzed with the natural samples to obtain a reference slope. Resulting line equations produced from plotting $NaNO_3$ reagent $\delta^{15}N$ values vs. the measured

$\delta^{15}N$ values of $N_2(g)$ obtained on analysis days were y = 0.6164x - 17.146 and y = 0.6269x - 19.902 (*line equations vary depending on blank activity on day of analysis and standard deviation of the measured $\delta^{15}N$ values of $N_2(g)$). The y axis is the $\delta^{15}N$ values of $N_2(g)$ and the x axis is the $\delta^{15}N$ values of the nitrate reagents. Natural samples are blank corrected and actual $\delta^{15}N$ value is found by using measured $\delta^{15}N$ values as the y value in the line equation obtained from analysis of the nitrate standards. Actual $\delta^{15}N$ values were also found by using measured $\delta^{15}N$ values as the y value in the line equation with the slope value changed to an ideal value of 0.5 (ideal slope of 1:1 mixing). $\delta^{15}N$ values obtained from presented method matched the trend in $\delta^{15}N$ values seen using the ammonia diffusion method, but at larger enrichment levels the values obtained from presented method are consistently lower. There is large error in the ammonia diffusion method because of limited efficiency in the ammonia extraction and distillation, so many of $\delta^{15}N$ values derived from presented method fall with in error bars of each $\delta^{15}N$ values derived from ammonia diffusion (Figure 8).

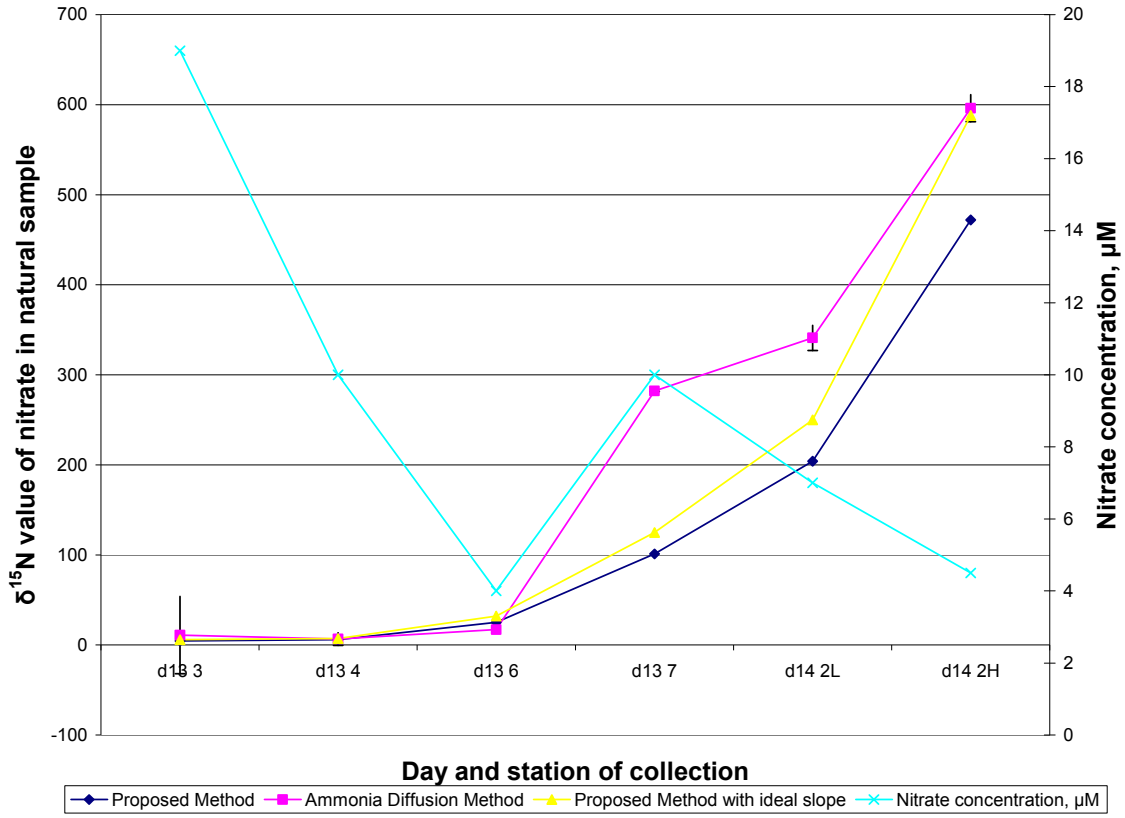


Figure 8) $\delta^{15}N$ values of enriched natural samples obtained from presented method using experimental slope and ideal slope and $\delta^{15}N$ values obtained from ammonia diffusion method.

Conclusions

A method for determining $\delta^{15}N$- NO_2^- and NO_3^- with a precision of 0.6‰ that is appropriate for low level enrichment and many natural abundance applications has been presented. The method can be applied to any nitrogen containing compound in which nitrogen can be converted to nitrite. The method allows for minimal chemical manipulations, has no need for sustaining bacteria cultures, is relatively safe, and uses common inexpensive reagents. Sample sizes to be analyzed by the method may be greatly reduced by using proper glassware. 12 mL vials which were on hand in our laboratory were used in sample analysis, if the

commercially available 3 mL vials are utilized the 700 nmole N needed for analysis can be cut to 175 nmole. A further complication is the inconsistency of the nitrogen blank, a problem which can be difficult to solve because of nitrogen's abundance in the atmosphere. This problem could be solved by monitoring the Argon peak in each sample. Since the argon to nitrogen ratio in air is constant, a blank correction can be made on a per sample basis for exactly how much air contamination is contributing to each signal. The presented method is ideal for low level enrichment and some natural abundance applications and with further development and a better understanding of blank corrections it may be used for many natural abundance applications.

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