THE FATE OF THE CYANIDE ION IN THE AQUATIC

ENVIRONMENT

by

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ABSTRACT OF THE THESIS

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The fate of the cyanide ion in aqueous environments is an area of concern as exampled by an accident on the Danube River in 2000. A mining company accident in Romania spilled thousands of cubic meters of waste slurry that included cyanide and unspecified heavy metals into the Sasar River in Romania, which eventually flowed into the Danube River in Hungary. Thousands of fish died in the incident and agriculture was damaged. The population that depended on the rivers for their livelihood was devastated. There was no investigation into the fate of the spill, and eventually life forms near the origination of the spill began to re-emerge.

This thesis investigates hydrolysis and volatilization as possible fates of the cyanide ion in the aqueous environment. Ion selective electrodes were used to analyze the disappearance of cyanide and the appearance of ammonia (the end product of hydrolysis). If these could be measured simultaneously the rate of the hydrolysis of cyanide in a neutral aqueous environment could be determined.

The hydrolytic pathway for the cyanide ion has been investigated for the reaction catalyzed with cyanidase, and enzyme found in some bacteria and fungi. The products of the enzyme-catalyzed hydrolysis are formate and carbon dioxide or formate and ammonia, depending on the organism. A two-step reaction has been proposed for cyanide hydrolysis without a catalyst in which formamide is an intermediate. The limiting step in this reaction is the initial hydrolysis of cyanide to formamide and investigations into the appearance of ammonia were used as a surrogate to the formation of this intermediate.

Hydrogen cyanide gas is extremely volatile and will escape into the environment at room temperature. The experiments conducted for this thesis showed a consistent loss of cyanide during a timed reaction from an aqueous solution without a corresponding increase in an equivalent amount of ammonia. From additional experiments on the effects of temperature, ultraviolet radiation and aeration, it was concluded that volatilization was the most probable fate of cyanide in these investigations. Confirmation of volatilization was achieved by infrared spectrometry of the vapor phase above aqueous potassium cyanide solutions.

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LIST OF ACRONYMS

ATP   Adenosine triphosphate

CNS   Central nervous system

DTGS   Deuterated triglycine sulfate

EPA   Environmental Protection Agency

FT-IR   Fourier transform infrared spectroscopy

ISAB   Ionic strength adjustment buffer

ISE   Ion selective electrode

LD_{50} Lethal dose per 50% tested

MCLG   Maximum Containment Level Goals

OSHA   Occupational Safety and Health Administration

REHS   Rutgers Environmental Health and Safety

RfD   Recommended oral dose

1. INTRODUCTION

1.1. Cyanide General Information

Cyanide is a naturally occurring toxin. In its gaseous form, it associates with a hydrogen ion to form hydrogen cyanide, an extremely toxic gas that smells slightly like almonds. Cyanide, in its solid form, is combined with various metal ions such as potassium and sodium to form white crystalline substances, which are stable. Hydrogen cyanide in the liquid state can be a blue or clear liquid called hydrocyanic acid. This liquid form is also stable except when it is exposed to atmosphere, in which case it rapidly turns to hydrogen cyanide gas and escapes from the liquid phase. All forms of these cyanide compounds can be extremely dangerous and highly toxic.

1.2. Toxicity

According to the Environmental Protection Agency (EPA), the MCLG (Maximum Contaminant Level Goals), for cyanide in water has been set at 0.2 parts per million (ppm). The EPA standards for exposure to hydrogen cyanide, is 0.003 mg/m^3, based on central nervous system (CNS) symptoms and thyroid effects in humans (EPA 2006). The Occupational Safety and Health Administration (OSHA) has set a limit for hydrogen cyanide and cyanide salts of 10 ppm in the workplace atmosphere. (Habeck 2008). The EPA also recommends an oral reference dose (RfD) of no more than 3.8 mg/day for potassium cyanide. The LD$_{50}$ for potassium cyanide is 5-10 mg/kg in rats and mice and for sodium cyanide it is 6.64 mg/kg in rats and mice (EPA 2006).

Cyanide is rapidly absorbed from the stomach, lungs, mucosal surfaces, and unbroken skin. Effects begin within seconds of inhalation and within 30 min of

ingestion of solution or solid, depending on the dose. There are two ways in which cyanide interferes with normal metabolism. Initially it enters the bloodstream and binds with the ferric form of hemoglobin, methemoglobin (Chin and Calderon 2000, Hillman and Smith 2007). Cyanomethemoglobin cannot transport oxygen, so body cells become oxygen deprived. Secondly, and most importantly, it inhibits the enzyme cytochrome c oxidase. Cytochrome c oxidase is the last enzyme in the electron transport chain, found in the cell mitochondria. Cyanide interferes with the binding of oxygen to the enzyme and blocks the reduction of oxygen. This results in decreased oxidative metabolism and oxygen utilization. The cell reverts to anaerobic metabolism and a buildup of lactic acid occurs. Production of ATP is inhibited (Hillman and Smith 2007).

The initial effects of poisoning include headache, faintness, vertigo, excitement, anxiety, a burning sensation in the mouth and throat, breathing difficulty, increased heart rate, and hypertension. Nausea, vomiting, and sweating are common. A bitter almond odor may be detected on the breath. Later effects include coma, convulsions, paralysis, respiratory depression, pulmonary edema, and death (small red spots are visible on the skin at death) (Manbir 2007). The treatment of cyanide poisoning is complicated because the symptoms that occur are similar to those of carbon dioxide poisoning. It appears at first that the patient is suffering the symptoms of a heart attack, but they quickly progress to the former mentioned effects. The first step to treatment is to remove the patient from the source of exposure and administer 100% oxygen. Activated charcoal absorbs cyanide when administered at the recommended dosage, but is not the most effective treatment. Antidotes that are approved for use in

the United States are provided in kits. They contain several ingredients including nitrate compounds, which produce methemoglobin. Methemoglobin competes with the cytochrome oxidase system for the binding of cyanide and cyanomethemoglobin is formed, restoring the activity of cytochrome c oxidase. However, the main process for eliminating cyanide from the body is done in the mitochondria by the enzyme rhodanase. This enzyme converts cyanide to thiocyanate. Another treatment for cyanide poisoning is the administration of thiosulfate. It is believed that this provides the sulfur that the rhodanase enzyme needs to convert cyanide to thiocyanate (Chin and Calderon 2000).

1.3. Occurrence in Plants, Foods and Organisms

Cyanide is found in more than a thousand plants and foods such as: almonds, millet sprouts, lima beans, soy, spinach, bamboo shoots, cassava roots (tapioca), and corn, to name a few. It is also found in the pits of chokecherries, black cherries, apricots, and peaches. Some societies use the pits of these fruits to make sweet food treats. Apricot pits, as well as bitter almonds, peach pits, plums, apples, pears and some kinds of cherries, contain a cyanogenetic glycoside, amygdalin also known as laetrile. This glycoside hydrolyzes to form cyanide, benzaldehyde and glucose. The cyanide then hydrolyzes to hydrocyanic acid. Amygdalin is harmless by itself, but these pits contain another enzyme, emulsin that catalyzes the hydrolytic reaction. The reaction takes place slowly in acidic conditions, i.e. the stomach, but occurs quickly in an alkaline environment, i.e. the small intestine (Lasch and Shawa 1981).

Some bacteria, fungi and algae contain cyanide, but these organisms have an enzyme called cyanidase that degrades cyanide to ammonia and formate or ammonia

and carbon dioxide. It is believed that these organisms may use the cyanide as a nitrogen source (Watanabe et al. 1998).

1.4. Industrial Uses

Large amounts of cyanide are used in the metal plating and metal finishing industry, in gold mining, iron and steel mills, agricultural industries, organic chemical industries and the pharmaceutical industry. Wastewaters from these industries must be treated to conform to governmental standards before they are released to the environment (Watanabe et al. 1998).

1.5. Routes of Chemistry

The environmental fate of the cyanide ion has three common routes of chemistry. It can complex with metal ions, which may or may not precipitate out of a neutral aqueous medium. This characteristic of cyanide is used in the mining industry to extract silver and gold from rock. The cyanide ion is a strong ligand, which can complex at low concentrations to many heavy metals (Young and Jordan 1995). Water-soluble cyanide compounds include $NaCN$, KCN, $Ca(CN)_2$ and $Hg(CN)_2$. Relatively insoluble compounds are $Zn(CN)_2$, $Cu(CN)$, $Ni(CN)_2$, and $Ag(CN)$. Strong complexes having soluble sodium salts include $Fe(CN)_6^{3-}$, $Co(CN)_6^{4-}$ and $Au(CN)_2^-$ (Fuller 1984).

Cyanide can hydrolyze with or without a catalyst consistent with a hydrolytic pathway according to the two-step hydrolysis equation:

$$HCN \xrightarrow{H_2O} HCONH_2 \xrightarrow{H_2O} HCOOH + NH_3 \,.$$ (Fallon 1992, Krieble and McNally1929). Hydrogen cyanide will hydrolyze in the first reaction to formamide and further hydrolysis of formamide will produce formic acid/formate and ammonia.

Formamide is a metabolite of cyanide that is common to many aerobic organisms. Studies by Robert Fallon done in 1992 using an unnamed cell material showed that cells rapidly convert formamide to formate. He observed that the rate of formamide hydrolysis was three times faster than the maximum rate of the direct hydrolysis of cyanide to formate. He concluded that both the direct hydrolysis of cyanide to formate and the two-step hydrolysis of cyanide with formamide as an intermediate are both possible. However, formamide would not be observed in the two-step process because the first step of the hydrolysis reaction appears to be the rate-limiting step. Since formamide hydrolysis to formate is much quicker than hydrolysis of cyanide to formamide, it would be difficult to detect this as an intermediate (Fallon 1992).

Hydrogen cyanide gas can volatilize. Free cyanide ions hydrolyze in water to produce hydrogen cyanide. The aqueous hydrogen can then volatilize as hydrogen cyanide gas. Hydrogen cyanide has a vapor pressure of .99 atm at 26°C, which is above that of water (.34 atm at 26°C). Pure hydrogen cyanide has a boiling point of 26°C, and therefore has a higher vapor pressure than water at 26°C. Hydrogen cyanide gas can be released from an aqueous medium at elevated temperatures and/or reduced pressures. Agitation rates, the air/solution ratio and the surface area at the air/solution interface can increase the rate of evaporation (Young and Jordan 1995).

1.6. Accident on the Danube

It is important to understand what happens to cyanide in the aquatic environment as exampled by an accident involving a gold processing plant in Baia Mare, Romania on 30 January 2000. A foreign mining company, Esmeralda Exploration

based in Australia, was employed to partner with Romania's state mining company to introduce innovative cyanide technology to an old mining site. Although 'state of the art' plant technology was used to construct the site, regulations and oversight mandates for safety were not well enforced. During a period of heavy precipitation, one of the holding ponds formed a fissure and a section of a retaining wall collapsed, allowing an overflow of 10,000 cubic meters of slurry, including 50-100 tons of cyanide and unspecified amounts of heavy metals to escape. The contamination entered the Sasar River, then flowed into the Lapus River, then downstream to the Somes River in Hungary. From the Somes River, it traveled to the Tisza River, which flows into the Danube River and from the Danube River, it returned to Romania and then finally entered the Black Sea. The plume was estimated to have been approximately 60-70 kilometers long, and traveled downstream at a rate of 2.1-2.4 kilometers per hour. The spill was attributed to killing tens of thousands of fish and other forms of wildlife, destroying the livelihoods of the fishing and agriculture population, and to contaminating drinking water supplies. Exact measurements of the toxins both cyanide and heavy metal related, were not conclusive and the tributaries had an initial level of contamination before the accident, but as the spill traveled downstream, cyanide levels decreased in the immediate area. It was not know to what extent the cyanide degraded, complexed with metals or volatilized (Cunningham 2005).

1.7. Previous Work on Cyanide Degradation

Some bacteria and fungi contain the enzyme cyanidase used to degrade cyanide. Specifically, the bacterium *Pseudomonas fluorescens* has been shown to degrade

cyanide to ammonia and carbon dioxide. Another *Pseudomonas* species degraded

cyanide to ammonia and formate under both aerobic and anaerobic conditions. The

two bacteria, *Alcaligenes xylosoxidans dentrificans* and *Bacillus pumilus* have both

been found to degrade cyanide by cyanidase to ammonia and formate. Fungi species,

Gloeocercospora sorghi, Fusarium lateritium and *Stemphylium loti* have been

reported to degrade cyanide by cyanidase catalyzed hydrolysis to formamide

(Watanabe et al. 1998).

In a study done by S.L Baskin, S.A. Katz and H. Salem it was demonstrated that

in the presence of cyanidase, cyanide degradation was significant. They used the

hydrolytic enzyme cyanidase and monitored the degradation of cyanide in a buffered

solution. During this study, however, control experiments were done and it was noted

that cyanide degraded even without the presence of cyanidase. It was concluded in

this study that indeed cyanidase had a significant effect on the rate of degradation of

cyanide, but the loss of cyanide in the absence of cyanidase could be due to

hydrolysis or volatilization. Further tests were not done to determine to what the loss

was attributed (Baskin et al. 1998).

1.8. Reasoning for ISE Methodology

The experiments that are presented in this thesis are an attempt to determine to

what extent and to what end, cyanide degradation occurs in neutral aquatic

conditions. The focus was on methodology and the ability to determine if hydrolysis

was occurring using ion selective electrodes (ISE), by observing the rate of cyanide

loss and ammonia appearance in solution. The hypothesis would determine that if

cyanide were being hydrolyzed, the product, ammonia, would appear in mass balance.

Ion selective electrodes were chosen for these experiments because they are relatively inexpensive and simple to use compared to other methods of analysis. The intention for using these electrodes was that methodology could be developed to make simultaneous measurements of cyanide loss and ammonia presence through cyanide hydrolysis for these experiments.

Ion selective electrodes are membrane electrodes that respond selectively to ions. In some cases, as in solid-state electrodes, they are not ion specific and care must be taken to minimize interference by other ions. Two different kinds of ISEs were used in these experiments. A combination ammonia gas sensing ISE was used to detect ammonia in solution and a solid state cyanide ISE in conjunction with a double junction reference electrode were used to detect cyanide in solution.

Gas sensing electrodes consist of an ISE electrode, (usually a glass membrane pH electrode) which is in contact with an thin layer of solution held in place by a gas permeable membrane. When the gas passes through the membrane, there is a change in the solution pH and glass membrane portion of the electrode senses the change. This change is measured against a stable reference electrode with constant potential (in combination electrodes, this reference electrode is incorporated into the body of the electrode), and the potential difference between the two electrodes is related to the activity of the ion in solution. This activity is read using a voltmeter. The voltage is related to the activity of the ion through the Nernst equation and calculations of ion concentration can be determined.

A solid-state electrode consists of a solid membrane containing relatively insoluble inorganic salts specific to the ion to be detected. These salts are mobilized by the ion to be detected, and a flow of new salts proceeds to the membrane surface. The solid-state electrode is connected to a reference electrode with constant potential, and this flow of salts to the electrode surface is measured as a difference in potential. The difference in potential is related to the activity of the ion being detected, and this activity (a) is related to concentration $a = fC$, where f is the activity coefficient. Debye – Hückel limiting law can be used to calculate the activity coefficient. The concentration of the ion being detected can be calculated through this relationship. A more detailed discussion of the activity – concentration relationship is presented on page 12.

2.   MATERIALS AND METHODS

2.1. General Information

All solutions were made and washings done using water that was distilled twice. Conductivity testing of the double distilled water verified that there were no detectable ions present that might interfere with potential measurements of the solutions in this experiment.

Experiments were conducted under a fume hood and at 21° C ± 2° C unless otherwise stated. Three separate 100 mL standard cyanide solutions of 0.1M were made by mass from solid KCN and one 500 mL standard ammonia solution of 0.1M was made by mass from NH_4Cl.

2.2. Ion Selective Electrodes and Volt Meter

An Orion 720Aplus pH/ISE Meter was used with all of the electrodes in these experiments (Orion Research Inc.). Corning's Ammonia Combination Electrode model 477341 was used throughout the experimentation (Corning Science Products).

The Corning ammonia electrode is a combination pH electrode, a silver/silver chloride reference electrode and gas permeable membrane mounted on a replaceable tip. A specialized solution (internal filling solution) is placed between the membrane and the pH glass and this changes pH in response to changes in partial pressure of dissolved ammonia. Ammonia diffuses across the membrane until equilibrium between the gas and the internal filling solution is obtained.

At equilibrium, $NH_{3\ gas} \xleftrightarrow{K_p} NH_{3\ diss} + H_2O \xleftrightarrow{K_H} NH_4^+ + OH^-$

$$K_p = \frac{[pNH_3]}{[NH_3]} = \text{Henry's Law Constant}$$

$$K_H = \frac{[NH_4^+][OH^-]}{[NH_3]} = \text{Hydrolysis Constant}$$

Then, $pNH_3 = \dfrac{K_p \cdot [NH_4^+][OH^-]}{K_H}$

The internal filling solution keeps the $[NH_4^+]$ constant (K_2).

$$K_1 = \frac{K_p[NH_4^+]}{K_H} = \frac{K_p \cdot K_2}{K_H}$$

So, $pNH_3 = K_1[OH^-]$ and since $[OH^-] = \dfrac{K_w}{[H^+]}$, then $pNH_3 = \dfrac{K_1 \cdot K_w}{[H^+]}$

The changes in proton activity in the solution are related to ammonia concentration using the Nernst equation: $E = E° - S \cdot \log[NH_3]$ where :

E = measured electrode potential

E_0 = reference potential

$[NH_3]$ = ammonia in solution

S = electrode slope   (Corning 1983).

Orion Cyanide solid-state ISE model 94-06 and Orion Double Junction silver/silver

chloride reference electrode model 90-02 were used throughout the experimentation

(Orion Research Inc.).

    The cyanide electrode consists of a solid membrane containing a mixture of

inorganic silver compounds. When the membrane is in contact with a cyanide

solution, the silver ions dissolve from the membrane surface and silver ions within the

membrane move to the surface to replace the dissolved ones. This sets up a potential

difference that depends on the concentration of cyanide in solution. The potential is

measured against a constant reference potential that is simultaneously connected to a

digital mV meter. The measured potential that corresponds to the activity of the

cyanide ion in solution is described by the Nernst equation: $E = E_0$ - S log (A) where:

E = measured electrode potential

E_0 = reference potential

A = cyanide activity

S = electrode slope

The activity of cyanide ion in solution (A) is related to its concentration, $A = fC$, and

the ionic strength by

the Debye-Hückel Limiting Law: $- \log f = 0.512\, Z_i^2\, \sqrt{\mu}$

where: $\mu = \dfrac{1}{2} \Sigma\, m_i\, z_i^2$

μ = ionic strength

f = activity coefficient

Z = charge on the ion

m = molarity

2.3. Ammonia Ion Selective Electrode (ISE) Testing

Preliminary tests were performed to verify the Nernstian behavior of the ammonia ISE. A new ammonia ISE gas penetrable membrane was assembled and prepared for the ammonia ISE before experimentation began according to the preparation procedure described in the Corning Ammonia Electrode instruction manual (Corning 1983).

Four standard solutions of ammonium chloride were made in concentrations of 1000 ppm, 100 ppm, 10 ppm, and 1 ppm. The millimole conversions for the ammonia standard solutions in the solution are respectively, .54 x 10^1 mmol, 5.54 mmol, 5.54 x 10^{-1} mmol and 5.54 x 10^{-2} mmol in the ammonium ion. Using four separate glass beakers, 100 mL of each standard solution was measured into each. One at a time, the beakers with magnetic stir rods were placed on a stirring plate, which was set at 300 rpm. Standard solution potential readings were made from low concentration to high concentration as recommended by the Corning ammonia ISE instruction manual. The ammonia ISE was submerged into the solution to approximately one half inch from the bottom of the beaker, and it was allowed to equilibrate for one minute. One mL of 10N sodium hydroxide, ionic strength adjustment buffer or ISAB was added to the standard solution, according to procedure recommended by EPA Method 350.3 (EPA 1974), and the Corning ammonia electrode instructions to maintain a constant ionic strength in the solution, and also to convert ammonium ions to ammonia gas. The

solution was left to equilibrate for another minute, to allow the voltmeter to adjust to the potential, and the millivolt output for each standard solution was recorded. The ammonia ISE was rinsed after each measurement, and stored in 0.05M solution of ammonium chloride when trials were finished. Six separate trials were conducted. Calibration curves were made from each set of data.

2.4. Ionic Interference Testing

Additional preliminary tests were performed using the ammonia ISE to determine if potassium or sodium ions in solution would interfere with the potentials measured by the electrode. These interferences were cited as potential problems with the solid-state ammonium ISE in accordance with the Beginner's Guide to ISE Measurement, Chapter 6 (Rundle 2000). These tests were done to verify that there was no effect on the ammonia gas sensing ISE, since the experiments would be done using a potassium phosphate buffer system.

Standards of ammonium chloride were made using molarity instead of parts per million, to simplify the procedure and calculations. Standard solutions were made in concentrations of 1.0×10^{-6}M, 1.0×10^{-5}M, 1.0×10^{-4}M, and 1.0×10^{-3}M. The volume for each standard was 100 mL. The millimole conversions for the standard solutions are: 1.0×10^{-4}mmol, 1.0×10^{-3}mmol, 1.0×10^{-2}mmol and 1.0×10^{-1}mmol. The same procedure was used for the interference tests as was used for the initial ammonia ISE tests, except before the 1.0 mL of ISAB was added, increasing concentrations of potassium chloride or sodium chloride was injected into the solutions. The ISAB itself contributes a significant sodium ion concentration to the system. Potentials were measured without the addition of interference ions as a base-

line measurement. Additions of the potassium chloride and the sodium chloride solutions were added, so the concentration of the interference ion in the standard ammonia solution was 1.0×10^{-4}M, 1.0×10^{-3}M and 1.0×10^{-2}M per 100 mL or in millimoles, 1.0×10^{-2}mmol, 1.0×10^{-1}mmol and 1.0 mmol.

Potentials of each solution were measured as per the standard ammonia solution procedure previously mentioned and the data was recorded. Three separate trials for each interference ion were conducted. Calibration curves were made comparing the ammonia standards and the potassium ion or sodium ion interference.

2.5. Simultaneous Cyanide Ion Selective Electrode and Ammonia ISE Testing

Methods for determining the quantity of cyanide in solution were referenced from the instruction manual included with the Orion cyanide ISE and from EPA Method 9213 (EPA 1996).

Calibration curves were made each day testing was done using 0.1M potassium cyanide and 0.1M ammonium chloride standard solutions. Standards were placed in 100.0 mL of 0.01M potassium phosphate buffer solutions at 37°C ± 2°C and at 7.4 pH with constant stirring at 300 rpm. Initial calibration standard concentrations were 1.0 x 10^{-3}M, 8.0×10^{-4}M, 6.0×10^{-4}M, 4.0×10^{-4}M, and 2.0×10^{-4}M. The contents in terms of millimoles for both standard solutions in 100.0 mL of potassium phosphate buffer solution were 1.0×10^{-1} mmol, 8.0×10^{-2} mmol, 6.0×10^{-2}mmol, 4.0 x 10^{-2}mmol and 2.0×10^{-2}mmol. Standards for both cyanide and ammonia were placed in the same beaker. The cyanide ISE, silver/silver chloride reference electrode and the ammonia ISE were submerged into the buffer solution to approximately one half inch from the bottom of the beaker. After the addition of the cyanide and ammonia

standards, a 1.0 mL aliquot of 10N NaOH (ISAB) was placed in the solution and the solution was allowed to equilibrate for one minute. Millivolt readings for each electrode were taken and recorded. A separate beaker with 0.01M potassium phosphate buffer solution was used for each set of calibration points.

A reaction mixture was prepared using 100.0 mL of 0.01M phosphate buffer solution with constant stirring at 300 rpm. The ammonia ISE, cyanide ISE and reference electrode were submerged into the volume. A 1 mL aliquot of 0.1M cyanide standard solution was placed in the reaction vessel with 1 mL of ISAB and initial millivolt readings were taken for the ammonia and cyanide after one minute equilibration time. All electrodes were then raised out of the solution, rinsed and dried and the reaction was maintained for one hour. Millivolt readings for both ammonia and cyanide were taken at ten minute intervals, each time submerging the electrodes, allowing one minute equilibration time and then the removing, rinsing and drying the electrodes. Millivolt readings were recorded and a calibration and a decay curve were made for each trial.

Calibration curves and reaction mixtures for the simultaneous measurement of cyanide and ammonia were repeated using new standard solution dilutions and no ISAB. Calibration curves were made each day testing was done using 0.1M potassium cyanide and 0.1M ammonium chloride standard solutions. Standards were placed in 100.0 mL of 0.01M potassium phosphate buffer solutions at 7.4 pH with constant stirring at 300 rpm. The calibration curves and reaction mixtures were maintained at 21° C ± 2° C (ambient hood temperature) and constant stirring at 300 rpm. The contents in terms of millimoles for ammonia and cyanide in the calibration curve

solutions were: 1.0×10^{-2} mmol, 2.0×10^{-2} mmol, 3.0×10^{-2} mmol, 4.0×10^{-2} mmol, 5.0×10^{-2} mmol, 6.0×10^{-2} mmol, 7.0×10^{-2} mmol, 8.0×10^{-2} mmol, 9.0×10^{-2} mmol, and 1.0×10^{-1} mmol. Standards for both cyanide and ammonia were placed in the same beaker. The cyanide ISE, silver/silver chloride reference electrode and the ammonia ISE were submerged into the buffer solution to approximately one half inch from the bottom of the beaker. Millivolt readings for each electrode were taken after the solution was allowed to equilibrate for one minute and they were recorded.

A reaction mixture was prepared using 100.0 mL of 0.01M phosphate buffer solution with constant stirring at 300 rpm. Reaction mixture solutions were maintained at $21°$ C $\pm$ $2°$ C . The ammonia ISE, cyanide ISE and reference electrode were submerged into the solution. A 1 mL aliquot of 0.1M cyanide standard solution was placed in the reaction vessel and initial millivolt readings were taken for the ammonia and cyanide after one minute equilibration time. All electrodes were then raised out of the solution, rinsed and dried and the reaction was maintained for one hour. Millivolt readings for both ammonia and cyanide were taken at ten minute intervals, each time submerging the electrodes, allowing one minute equilibration time and then the removing, rinsing and drying the electrodes. Millivolt readings were recorded and a calibration and a decay curve were made for each trial.

2.6. Effect of Ultra Violet (UV) Radiation on Cyanide Degradation

The exact procedure for the cyanide degradation testing using a 0.01M phosphate buffer solution was used to test for degradation of cyanide by UV light. Measurements were taken the same day that the calibration curves were made. The calibration curve

for the simultaneous measurement of cyanide and ammonia without ISAB were used for calculations. A UV light model UVSL-55 made by Ultra-Violet Products, Inc. was mounted on a ring stand and lowered to approximately 15 cm from the top of the beaker and remained fixed during the entire degradation reaction. The UV light emitted short UV light at 254 nm with a calculated power output of 20.7 Watts. Three separate trials with calibration curves and reaction mixtures were performed.

2.7. Effect of Stirring Rate on Cyanide Degradation

The effects of reducing the rate of stirring the reaction system from 300 to 150 rpm on cyanide degradation were evaluated. Measurements were taken the same day that the calibration curves were made. The calibration curve for the simultaneous measurement of cyanide and ammonia without ISAB were used for calculations. Three separate trials with calibration curves and reaction mixtures were performed.

2.8. Effect of Temperature on Cyanide Degradation

The effect of lowering the temperature of the reaction system from $21°$ C ± $2°$ C, to $2°$ C ± $2°$ C on the degradation of cyanide was evaluated. A calibration curve was made at constant stirring at 300 rpm and at $2°$ C ± $2°$ C. Three separate trials with calibration curves and reaction mixtures were performed

2.9. Ammonia Spike

A test was done to confirm the detection of ammonia in the reaction mixture. Calibration curves and reaction mixtures for the simultaneous measurement of cyanide and ammonia were performed with no ISAB. A calibration curves was made using 0.1M potassium cyanide and 0.1M ammonium chloride standard solutions. Standards were placed in 100.0 mL of 0.01M potassium phosphate buffer solutions at 7.4 pH

with constant stirring at 300 rpm. The calibration curve and reaction mixture were maintained at $21°\ C \pm\ 2°\ C$ (ambient hood temperature) and constant stirring at 300 rpm. The contents in terms of millimoles for ammonia and cyanide in the calibration curve solution was: 1.0×10^{-2} mmol, 2.0×10^{-2} mmol, 3.0×10^{-2} mmol, 4.0×10^{-2} mmol, 5.0×10^{-2} mmol, 6.0×10^{-2} mmol, 7.0×10^{-2} mmol, 8.0×10^{-2} mmol, 9.0×10^{-2} mmol, 1.0×10^{-1} mmol and 1.1×10^{-1} mmol. Standards for both cyanide and ammonia were placed in the same beaker. The cyanide ISE, silver/silver chloride reference electrode and the ammonia ISE were submerged into the buffer solution to approximately one half inch from the bottom of the beaker. Millivolt readings for each electrode were taken after the solution was allowed to equilibrate for one minute and they were recorded.

A reaction mixture was prepared using 100.0 mL of 0.01M phosphate buffer solution with constant stirring at 300 rpm. Reaction mixture solutions were maintained at $21°\ C \pm\ 2°\ C$. The ammonia ISE, cyanide ISE and reference electrode were submerged into the solution. A 1 mL aliquot of 0.1M cyanide standard solution was placed in the reaction vessel and initial millivolt readings were taken for the ammonia and cyanide after one minute equilibration time. All electrodes were then raised out of the solution, rinsed and dried and the reaction was maintained for one hour. Millivolt readings for both ammonia and cyanide were taken at ten minute intervals, each time submerging the electrodes, allowing one minute equilibration time and then the removing, rinsing and drying the electrodes. At the end of the one hour reaction, a 0.1 mmol spike of ammonia standard solution was added and allowed to equilibrate for

one minute. Millivolt readings were recorded and a calibration and a decay curve were made.

2.10. Cyanide Degradation Testing

The cyanide ISE alone was used for the degradation testing. The calibration curve and reaction mixture were maintained at 21° C ± 2° C (ambient hood temperature) and constant stirring at 300 rpm. Calibration curve standards for cyanide were prepared in 100 mL of 0.01M phosphate buffer solution in millimole concentrations. The contents in terms of millimoles for cyanide in the calibration curve solution were: 1.0×10^{-2} mmol, 2.0×10^{-2} mmol, 3.0×10^{-2} mmol, 4.0×10^{-2} mmol, 5.0×10^{-2} mmol, 6.0×10^{-2} mmol, 7.0×10^{-2} mmol, 8.0×10^{-2} mmol, 9.0×10^{-2} mmol, 1.0×10^{-1} mmol and 1.1×10^{-1} mmol. The cyanide ISE was submerged into each solution, with no ISAB and allowed to equilibrate for one minute. Millivolt readings were taken for each dilution and recorded. The reaction mixture was made using 100 mL of 0.01M phosphate buffer solution and a 1 mL aliquot of 0.1M cyanide standard was added to the solution. The cyanide ISE was submerged into the solution and an initial millivolt reading was taken. The reaction mixture was maintained for one hour, with millivolt readings for cyanide taken every ten minutes.

Readings were recorded and a calibration curve and decay curve for cyanide were made for each trial.

2.11. Titration of Cyanide with Silver Nitrate Solution

Titrations of cyanide using silver nitrate were performed periodically to verify the stability of the standard solution. Single trials were performed because of the limitations of the cyanide standard solution volumes that were left. The method for

cyanide titration was modified from Denigés to titrate a volume of 10 mL of 0. 1M

cyanide standard solution in 50mL of double distilled water (Denigés 1895).

Turbidity is observed at the equivalence point of the titration according to the

equation:

$$Ag^+ + 2\,CN^- \rightarrow Ag(CN)_2^-$$

$$Ag(CN)_2^- + Ag^+ \rightarrow 2\,AgCN$$

$$2\,AgCN + 2\,NH_3 \rightarrow Ag(NH_3)_2^+ + Ag(CN)_2^-$$

$$Ag(NH_3)_2^+ + I^- \rightarrow AgI + 2\,NH_3$$
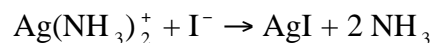
2.12.   Colorimetric Determination of Ammonia in Cyanide Standard Solutions

The standards for cyanide solutions were tested for the presence of ammonia

using Nessler's reagent $K_2[HgI_4]$ according to the method described by Williams,

P.C. (Williams 1964). Reagent volumes were modified to minimize waste.

Ammonia complexes according to the reactions:

$$HgCl_2 + 4\,KI \rightarrow K_2[HgI_4] + 2\,KCl$$

$$2\,[HgI_4]^{2-} + 3\,OH^- + NH_3 \rightarrow [HgNH_2]I \cdot H_2O + 2\,H_2O + 7\,I^- \qquad \text{(ETH 2007)}$$
$$\text{orange-brown}$$
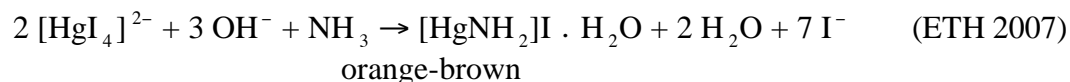
Standards for a calibration curve were made in concentrations of 1.0×10^{-3} M,

1.0×10^{-4} M and 1.0×10^{-5} M. Absorbances were determined at 430nm and 520nm

(Williams 1964) using Vernier Lab Pro® Spectrometer. Samples from the three

cyanide calibration standard solutions were tested using 0.2 mL of Nessler's reagent

with 0.1 mL of 0.1M cyanide standard in 10.0 mL of double distilled water.

Absorbances were read at 430nm and 520nm. Millimoles of ammonia were calculated

using the calibration curve.

2.13.   Ninhydrin Test for Formamide

Preliminary tests were performed to detect formamide in the cyanide standard

solutions. Reference to this test as an indicator of ammonia production was proposed

by Miyakawa et al. 2002, but methods that were referenced did not provide detail

about the procedure. Attempts to try the ninhydrin test as a preliminary indicator

were executed despite the lack of formal methods and doubt that this procedure

would detect formamide without the hydrolysis of cyanide occurring.   A ninhydrin

solution was made using 0.35g ninhydrin solid in 100.0 mL of ethanol. Standard

concentrations of 0.1M fomamide and 0.1M glycine were prepared to see if the

ninhydrin solution was viable. In three separate test tubes, the following volumes and

concentrations of samples were prepared; 10.0 mL of 0.1M formamide, 10.0 mL of

0.1M glycine and 10.0 mL of 0.1M cyanide standard solution. The test tubes were

place in an 80° C water bath for 10 minutes, and cooled using an ice bath. The

presence of formamide was supposed to be indicated by the purple-blue indicator

color of ninhydrin.

2.14.   Identification of Hydrogen Cyanide in the Vapor Phase

To investigate the possibility that the apparent degradation of cyanide without a

corresponding appearance of ammonia was due to volatilization or out gassing of

hydrogen cyanide, 1.0 mL of the 0.1 M potassium cyanide standard solution was

introduced into an 10-cm spectrophotometer cell with KBr windows, and the infrared

spectrum of the vapor phase above the liquid was recorded every 10 minutes for an

hour. A final scan was done an hour after the initial scans were done. Analyses of

the hydrogen cyanide in the vapor phase above the 0.1M aqueous potassium cyanide

solution was done using a Varian (formerly BioRad) FTS 6000 FT-IR spectrometer.

Collection of data was done at ambient temperature in a closed cell. The operating

range was from 4000 to 400 cm^{-1} with a resolution of 0.25 cm^{-1} using a Deuterated

triglycine sulfate detector (DTGS). In all scans, 8 inferograms per 10-minute sample

time were coadded and averaged for each spectrum.

2.15.   Safety Considerations

All experiments involving cyanide were done under a hood. Rutgers

Environmental Health and Safety (REHS) monitored the maintenance of hood

velocity. Gloves and automatic pipettes were used to minimize exposure of cyanide

to the skin. The amount of cyanide at any given time was limited to no more than

three 100mL 0.10M solutions of potassium cyanide. The waste cyanide solutions

were oxidized to cyanate solution by using an excess of ammoniacal copper sulfate

solution in a 2-liter container (Baskin et al. 1998).

$$CN^- + 2[Cu(NH_3)_4]^{+2} + H_2O \rightarrow CNO^- + 2[Cu(NH_3)_2]^+ + 2NH_4^+$$
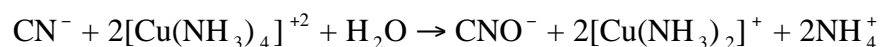
Solutions of silver nitrate and Nessler's reagent were collected separately to

minimize heavy metal waste. Waste containers were collected by REHS as they were

filled.

Image 1: Experimental setup in hood.

Image 2: Detail of experimental setup including electrodes, digital analyzer and standard solutions.

Image 3: Detail of electrodes immersed in reaction solution.

Image 4. Apparatus for examining infrared spectrum of vapor phase above 0.1 M potassium cyanide solution.

3. RESULTS

3.1. Ammonia ISE Testing

The activity of an electroactive species and potential are related according to the

Nernst equation: $E = E° + (\dfrac{2.303RT}{nF})$log A, where E is the potential between the

sensing and reference electrode, E° is the constant characteristic of a particular

ISE/reference pair, $(\dfrac{2.303RT}{nF})$ is the slope of the curve, and log A is the activity of

the measured ion. The slope of the calibration graph is equal to the millivolt response

per decade of concentration change. A typical Nernstian response should result in a

calibration curve with a slope between -50 mV to -60 mV according to the ammonia

ISE manual (Corning 1983). All plots are done with a line of best fit and the

significant figures for the slope and intercept are only known to three significant

figures.
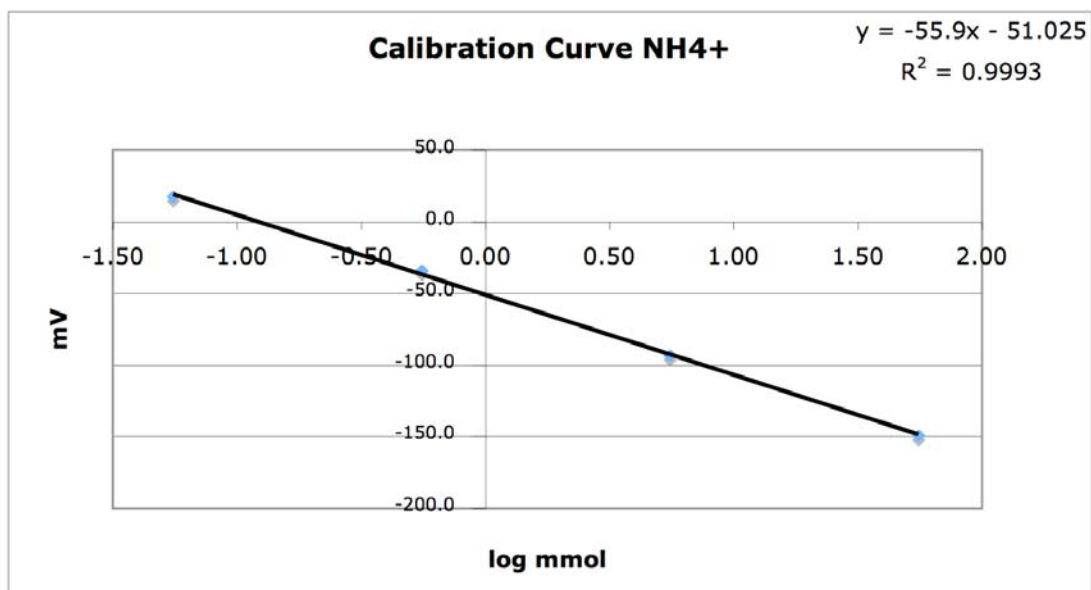
Figure 1: Typical calibration curve for the ammonia ISE.

mmol NH4+	log mmol	mV
5.54E-02	-1.26	17.6
5.54E-01	-0.256	-33.9
5.54E+00	0.744	-93.4
5.54E+01	1.74	-149

Table 1: Typical electrode responses to increasing concentrations of ammonia.

The six trials produced slopes between -53.0 mV/mmol to -55.9 mV/m

3.2. Ionic Interference Testing

Potassium and sodium ions were tested with ammonium standard solutions to determine if they would cause interference in the millivolt readings for the ammonia ISE. All charts presented are typical results from trials. The lower limit mV/mmol readings taken during the potassium ion and sodium ion calibrations were omitted because the lower limit of 1.0×10^{-4} mmol ammonia was exhibiting false readings because of the ammonia ISE detection limitations. These limits are defined between 10^{-1}M to 10^{-5}M (Corning 1983), which translates into 1.0×10^{2} to 1.0×10^{-2} mmol.
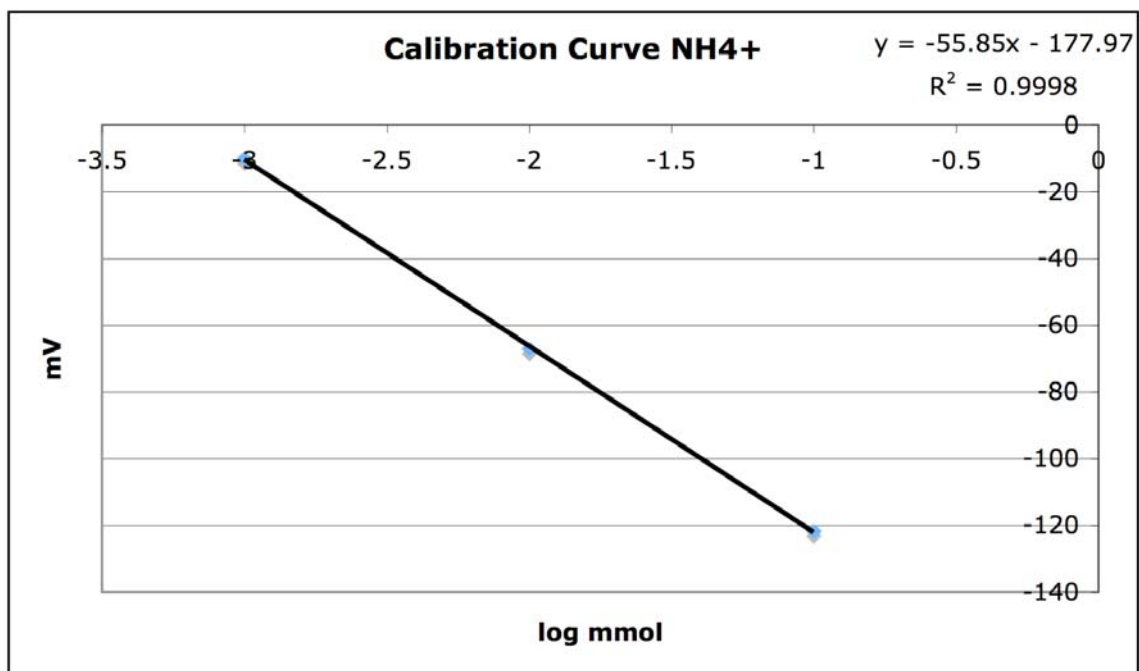
Figure 2: Reference curve with only ammonia for ion interference testing for potassium ions with ammonia.

mmol NH4+	log mmol	NH4+ mV
1.00E-04	-4.00	3.27E+01
1.00E-03	-3.00	-1.00E+01
1.00E-02	-2.00	-6.71E+01
1.00E-01	-1.00	-1.22E+02

Table 2: Electrode responses for reference curve for ammonia/potassium ion interference.

Potassium Ion Interference:
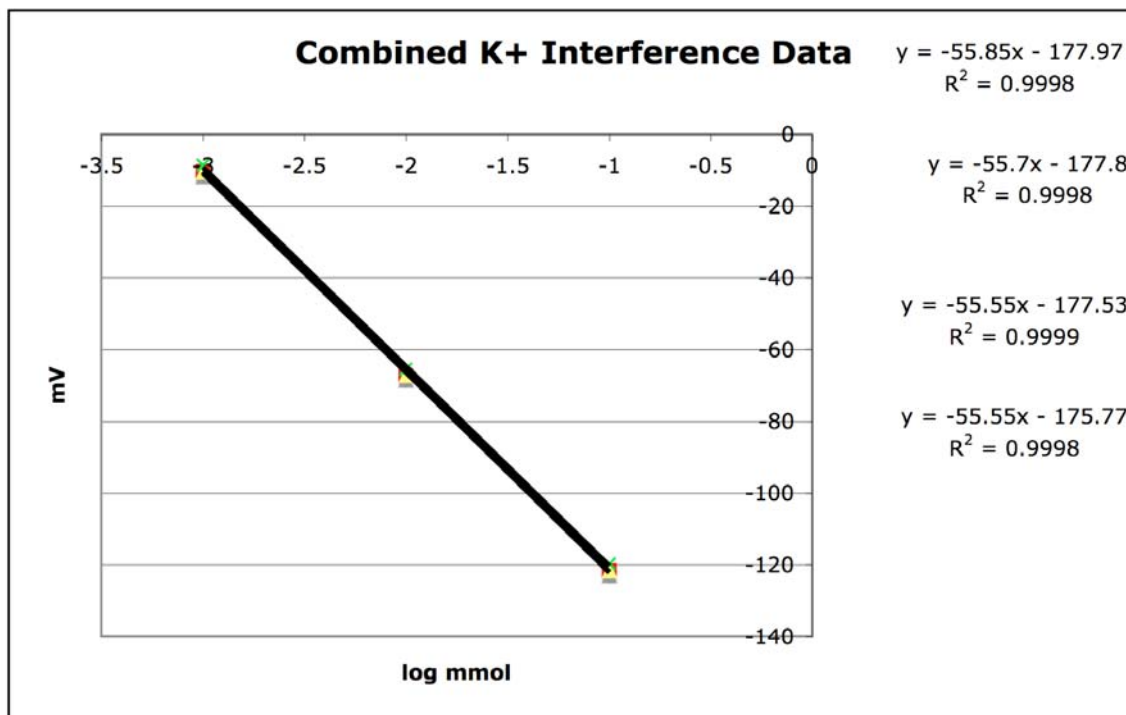
Figure 3: Combined slopes for interference testing for ammonia with potassium ions. Slopes in descending order are for ammonia alone, ammonia with the addition of 0.01mmol of potassium, ammonia with the addition of 0.1mmol of potassium and ammonia with the addition of 1mmol of potassium.

mmol NH4+	log mmol	NH4+ mV	mV w/0.01 mmol K+	mV w/ 0.1 mmol K+	mV w/ 1.0 mmol K+
1.00E-04	-4.00	3.27E+01	3.25E+01	3.24E+01	3.29E+01
1.00E-03	-3.00	-1.00E+01	-1.03E+01	-1.05E+01	-8.70E+00
1.00E-02	-2.00	-6.71E+01	-6.72E+01	-6.72E+01	-6.55E+01
1.00E-01	-1.00	-1.22E+02	-1.22E+02	-1.22E+02	-1.20E+02

Table 3: Electrode responses for increasing addition of potassium ions to ammonia.

Calibration curve slopes for all trials ranged from -53.0 mV/mmol to -55.85 mV/mmol.
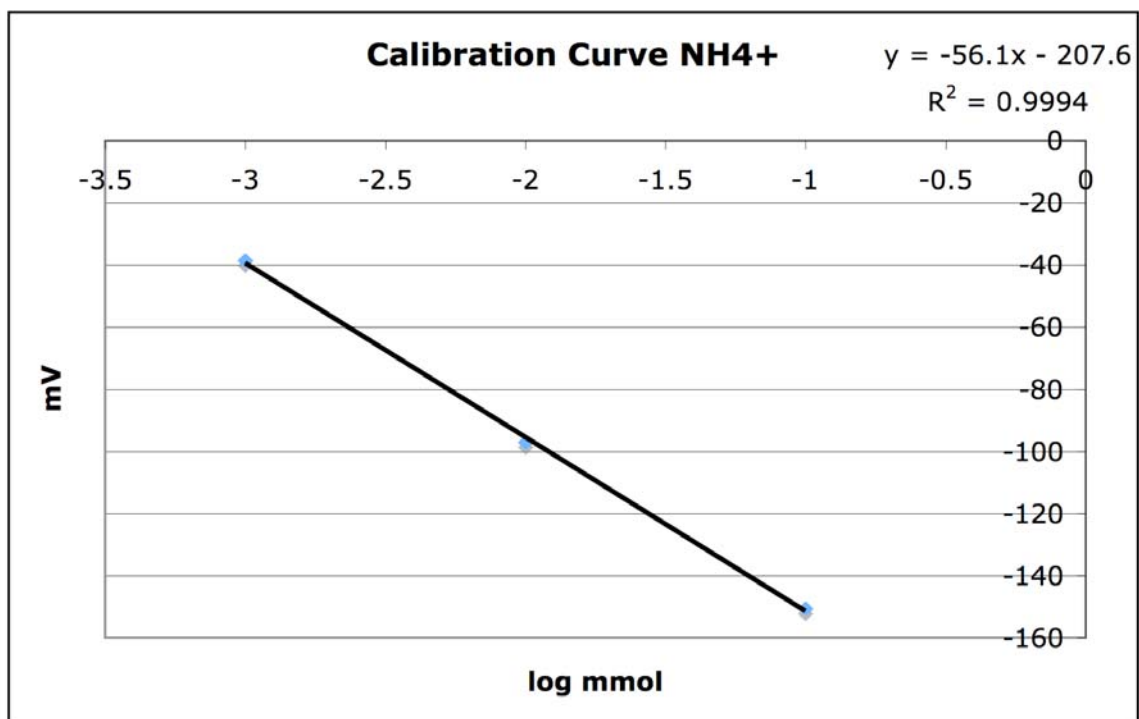
Figure 4: Reference curve with only ammonia for ion interference testing for sodium ions with ammonia.

mmol NH4+	log mmol	NH4+ mV
1.00E-04	-4.00	-2.30E+00
1.00E-03	-3.00	-3.85E+01
1.00E-02	-2.00	-9.70E+01
1.00E-01	-1.00	-1.51E+02

Table 4: Electrode responses for reference curve for ammonia/sodium ion interference.

Sodium Ion Interference:
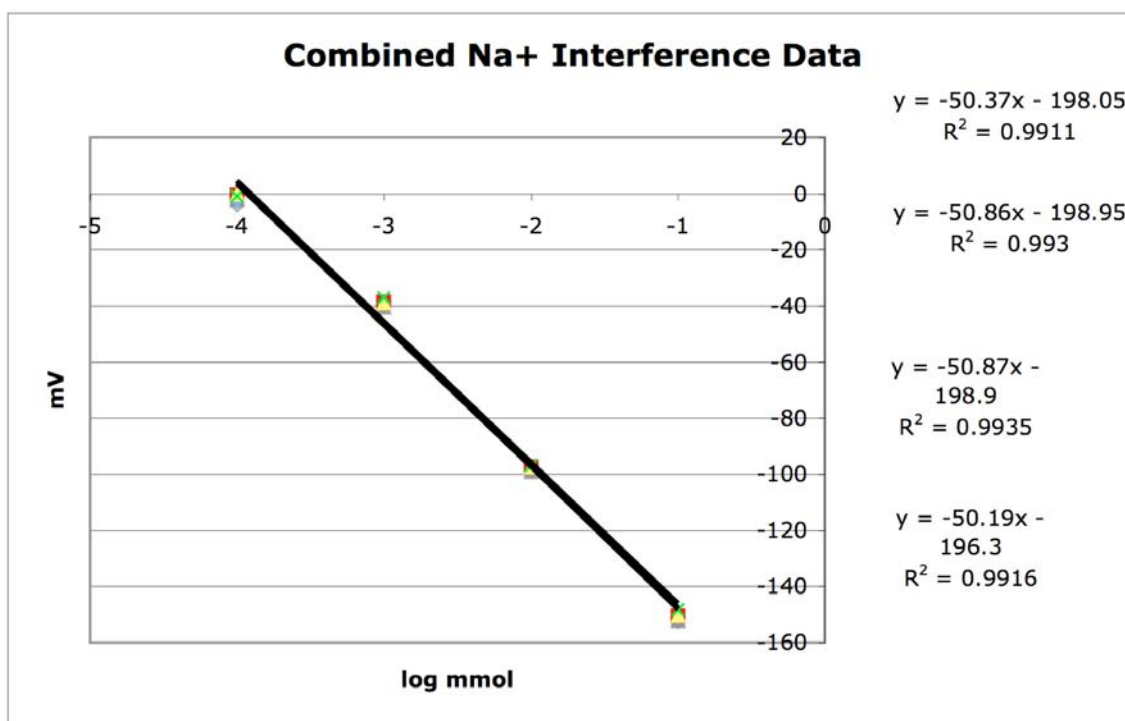
Figure 5: Combined slopes for interference testing for ammonia with sodium ions. Slopes in descending order are for ammonia alone, ammonia with the addition of 0.01mmol of sodium, ammonia with the addition of 0.1mmol of sodium and ammonia with the addition of 1mmol of sodium.

mmol NH4+	log mmol	NH4+ mV	w/0.01 mmol Na+	w/0.1 mmol Na+	w/1.0 mmol Na+
1.00E-04	-4.00	-2.30E+00	-6.00E-01	-3.00E-01	-9.00E-01
1.00E-03	-3.00	-3.85E+01	-3.87E+01	-3.89E+01	-3.72E+01
1.00E-02	-2.00	-9.70E+01	-9.73E+01	-9.73E+01	-9.69E+01
1.00E-01	-1.00	-1.51E+02	-1.51E+02	-1.50E+02	-1.48E+02

Table 5: Electrode responses for increasing addition of sodium ions to ammonia.

Calibration curve slopes for all trials ranged from -50.2 mV/mmol to -56.1 mV/mmol.

3.3. Simultaneous Cyanide Ion Selective Electrode and Ammonia ISE Testing

Initial tests were done using 0.01M phosphate buffer pH 7.4 with the addition of

1.0 mL of ISAB to the calibration curve standards and reaction mixture. Initial tests

were performed at 37° C ± 2° C. A typical calibration curve for cyanide ions should

result in a -57 ± 3 mV per decade slope (Orion Cyanide ISE).
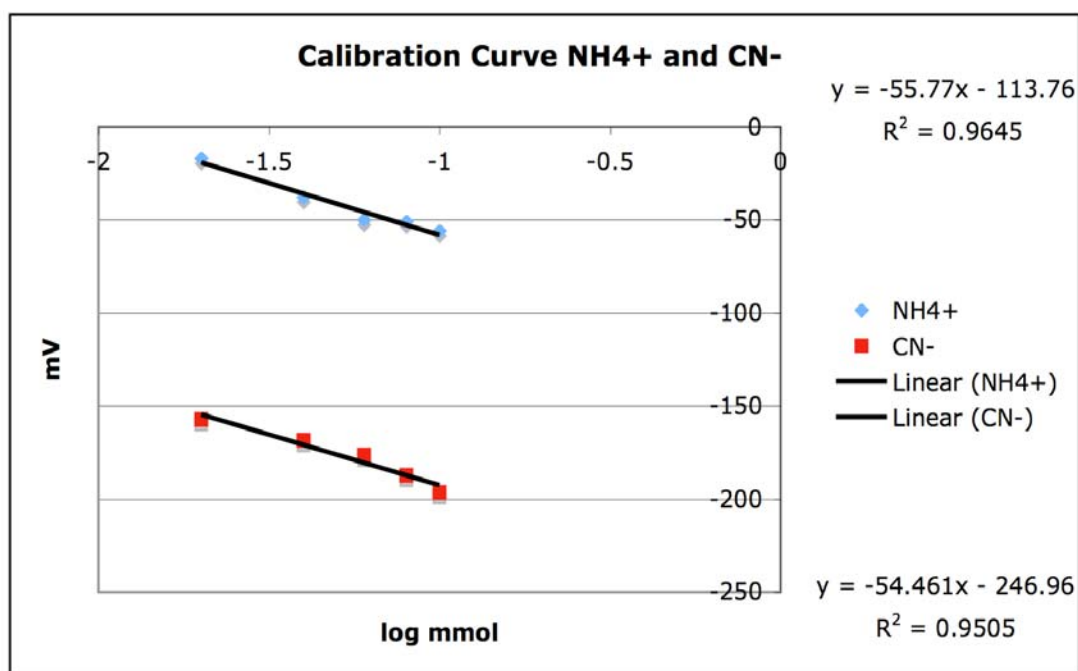
Figure 6: Typical calibration curve for simultaneous cyanide ISE and ammonia ISE
with ISAB. Upper slope is for ammonia and lower slop is for cyanide.

mmol NH4+ and CN-	log mmol	mV NH4+	mV CN-
1.00E-01	-1.00	-5.57E+01	-1.96E+02
8.00E-02	-1.10	-5.09E+01	-1.87E+02
6.00E-02	-1.22	-4.99E+01	-1.76E+02
4.00E-02	-1.40	-3.77E+01	-1.68E+02
2.00E-02	-1.70	-1.68E+01	-1.57E+02

Table 6: Typical electrode responses for increasing concentrations of cyanide and
ammonia with ISAB.

Calibration curve slopes for all trials ranged from -36.90 mV/mmol to -55.77

mV/mmol for NH_4^+ and between -49.55 mV/mmol to -63.72 mV/mmol for CN^-.
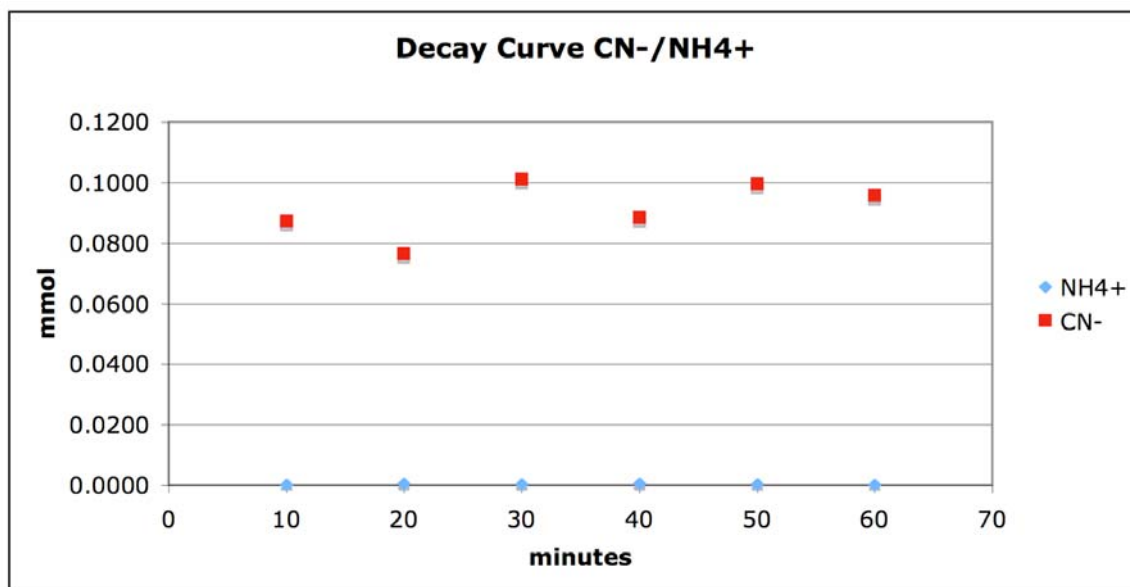
Figure 7: Decay curve for a one-hour reaction mixture measuring cyanide degradation and ammonia formation with ISAB.

min	mV NH4+	mV CN-
10	94.5	-1.89E+02
20	65.1	-1.86E+02
30	84.6	-1.93E+02
40	65.0	-1.90E+02
50	81.4	-1.92E+02
60	88.5	-1.91E+02

Table 7: Electrode responses during a one hour reaction with cyanide ISE and ammonia ISE with ISAB.

min	log NH4+	mmol NH4+	min	log CN-	mmol CN-
10	-3.73	1.84E-04	10	-1.06	8.73E-02
20	-3.21	6.21E-04	20	-1.12	7.66E-02
30	-3.56	2.77E-04	30	-0.99	1.01E-01
40	-3.21	6.23E-04	40	-1.05	8.85E-02
50	-3.50	3.17E-04	50	-1.00	9.96E-02
60	-3.63	2.36E-04	60	-1.02	9.59E-02

Table 8: Millivolt conversions to millimoles of cyanide and ammonia with ISAB.

min	% CN- in soln	% NH4+ in soln
10	87.3	0.184
20	76.6	0.621
30	100	0.277
40	88.5	0.623
50	99.6	0.317
60	95.9	0.236

Table 9: Percent CN- and NH_4^+ in solution with ISAB.

Since there was no evidence to confirm the degradation of cyanide or the presence of ammonia in the previous reactions, new standards were developed and the calibration curve and reaction mixture were done without the addition of ISAB. These tests were performed at 21° C ± 2° C.
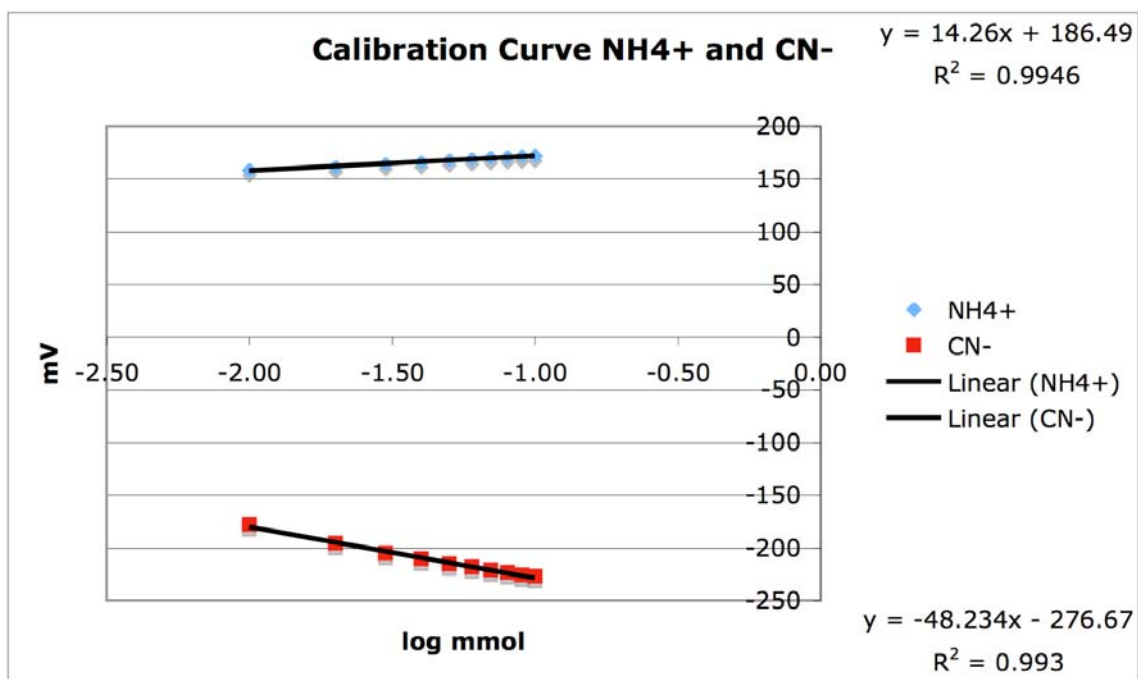
Figure 8: Typical calibration curve for simultaneous cyanide ISE and ammonia ISE without ISAB.

mmol NH4+/CN-	log mmol	mV NH4+	mV CN-
1.00E-02	-2.00	1.59E+02	-1.78E+02
2.00E-02	-1.70	1.62E+02	-1.96E+02
3.00E-02	-1.52	1.65E+02	-2.05E+02
4.00E-02	-1.40	1.66E+02	-2.10E+02
5.00E-02	-1.30	1.68E+02	-2.15E+02
6.00E-02	-1.22	1.69E+02	-2.18E+02
7.00E-02	-1.15	1.70E+02	-2.21E+02
8.00E-02	-1.10	1.71E+02	-2.23E+02
9.00E-02	-1.05	1.72E+02	-2.26E+02
1.00E-01	-1.00	1.72E+02	-2.27E+02

Table 10: Typical electrode responses to increasing concentrations of cyanide and ammonia without ISAB.

Calibration curve slopes varied from -14.26 mV/mmol to -24.77 mV/mmol for

NH_4^+ and -40.77 mV/mmol to -48.23 mV/mmol for CN-.
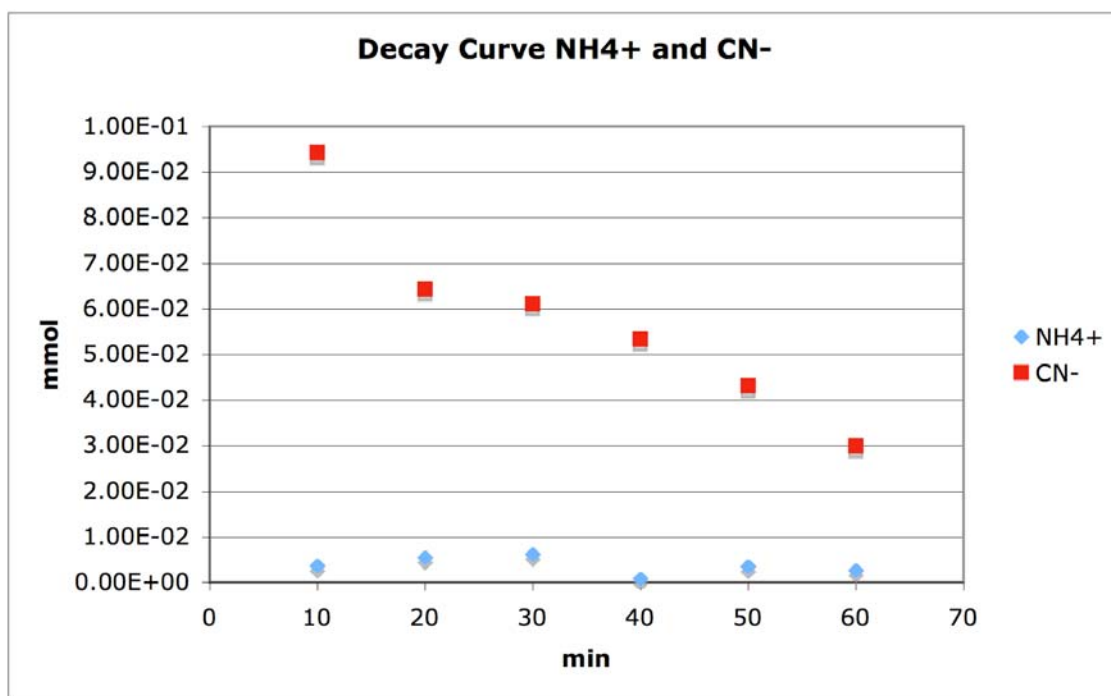
Figure 9: Decay curve for a one-hour reaction mixture measuring cyanide degradation and ammonia formation without ISAB.

min	mV NH4+	mV CN-
10	1.52E+02	-2.27E+02
20	1.54E+02	-2.19E+02
30	1.55E+02	-2.18E+02
40	1.43E+02	-2.15E+02
50	1.52E+02	-2.11E+02
60	1.50E+02	-2.03E+02

Table 11: Electrode responses during a one-hour reaction with cyanide ISE and ammonia ISE without ISAB.

min	log NH4+	mmol NH4+	min	log CN-	mmol CN-
10	-2.43	3.69E-03	10	-1.03	9.43E-02
20	-2.26	5.53E-03	20	-1.19	6.43E-02
30	-2.21	6.19E-03	30	-1.21	6.11E-02
40	-3.04	9.06E-04	40	-1.27	5.34E-02
50	-2.45	3.58E-03	50	-1.37	4.31E-02
60	-2.57	2.67E-03	60	-1.52	3.00E-02

Table 12: Millivolt conversions to millimoles of cyanide and ammonia without ISAB.

min	% CN- in soln	% NH4+ in soln
10	94.3	3.69
20	64.3	5.53
30	61.1	6.19
40	53.4	0.904
50	43.1	3.58
60	30	2.67

Table 13: Percent CN- and NH_4^+ in solution without ISAB.

3.4. The Effect of UV Radiation on Cyanide Degradation

Experiments were done with exposure to UV light at 254nm, to see if this

stimulus would contribute to cyanide degradation.
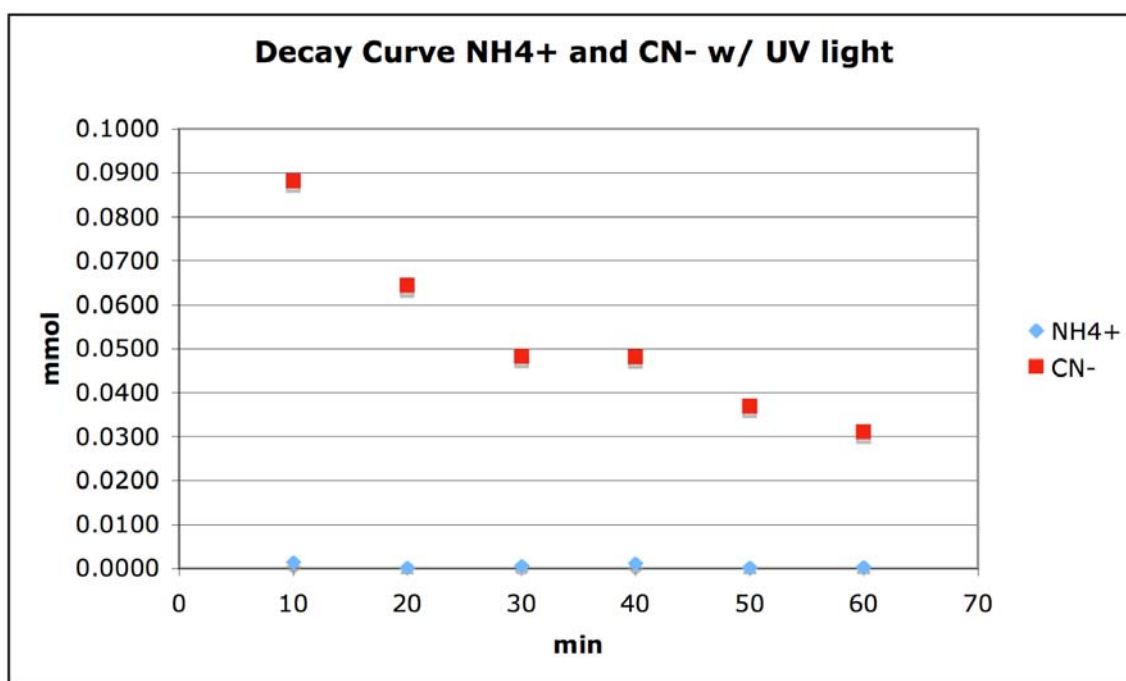
Figure 10: Typical decay curve of cyanide exposed to UV light in reaction mixture.

min	mV NH4+	mV CN-
10	1.46E+02	-2.26E+02
20	1.34E+02	-2.19E+02
30	1.41E+02	-2.13E+02
40	1.45E+02	-2.13E+02
50	1.31E+02	-2.08E+02
60	1.35E+02	-2.04E+02

Table 14: Typical electrode responses of ammonia and cyanide exposed to UV light.

min	log NH4+	mmol NH4+	min	log CN-	mmol CN-
10	-2.85E+00	1.42E-03	10	-1.05E+00	8.82E-02
20	-3.67E+00	2.12E-04	20	-1.19E+00	6.43E-02
30	-3.23E+00	5.96E-04	30	-1.32E+00	4.83E-02
40	-2.94E+00	1.14E-03	40	-1.32E+00	4.81E-02
50	-3.87E+00	1.35E-04	50	-1.43E+00	3.70E-02
60	-3.60E+00	2.49E-04	60	-1.51E+00	3.11E-02

Table 15: Millivolt conversions to millimoles of ammonia and cyanide exposed to UV light.

min	% CN- in soln	% NH4+ in soln
10	88.2	1.42
20	64.3	0.212
30	48.3	0.596
40	48.1	1.14
50	37	0.135
60	31.1	0.249

Table 16: Percent CN- and NH_4^+ in solution with UV light.

3.5. Effect of Stirring Rate on Cyanide Degradation

Experiments were done using a slower stirring speed (150 rpm) to see if this stimulus retards the decay of cyanide.
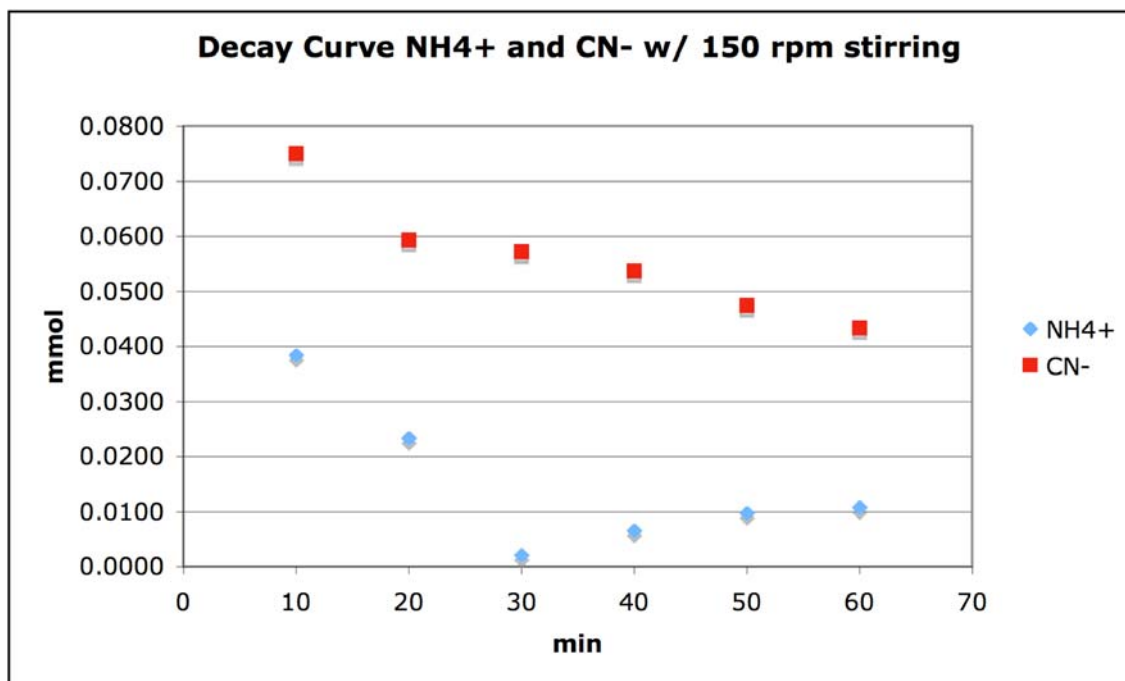
Figure 11: Typical decay curve of cyanide with slow stirring in reaction mixture.

min	mV NH4+	mV CN-
10	1.66E+02	-2.22E+02
20	1.63E+02	-2.18E+02
30	1.48E+02	-2.17E+02
40	1.55E+02	-2.15E+02
50	1.58E+02	-2.13E+02
60	1.58E+02	-2.11E+02

Table 17: Typical electrode responses of ammonia and cyanide with slow stirring.

min	log NH4+	mmol NH4+	min	log CN-	mmol CN-
10	-1.42E+00	3.84E-02	10	-1.13E+00	7.50E-02
20	-1.63E+00	2.33E-02	20	-1.23E+00	5.93E-02
30	-2.69E+00	2.06E-03	30	-1.24E+00	5.71E-02
40	-2.19E+00	6.50E-03	40	-1.27E+00	5.37E-02
50	-2.01E+00	9.73E-03	50	-1.32E+00	4.74E-02
60	-1.97E+00	1.07E-02	60	-1.36E+00	4.33E-02

Table 18: Millivolt conversions to millimoles for ammonia and cyanide with slow stirring in reaction mixture.

min	% CN- in soln	% NH4+ in soln
10	75	38.4
20	59.3	23.3
30	57.1	2.06
40	53.7	6.5
50	47.4	9.73
60	43.3	10.7

Table 19: Percent CN- and NH_4^+ in solution with slow stirring (150 rpm).

3.6. Effect of Temperature on Cyanide Degradation

Calibration curves were made at 2°C ± 2°C and a cyanide degradation experiment was done at the same temperature to see if temperature change retards the decay of cyanide.
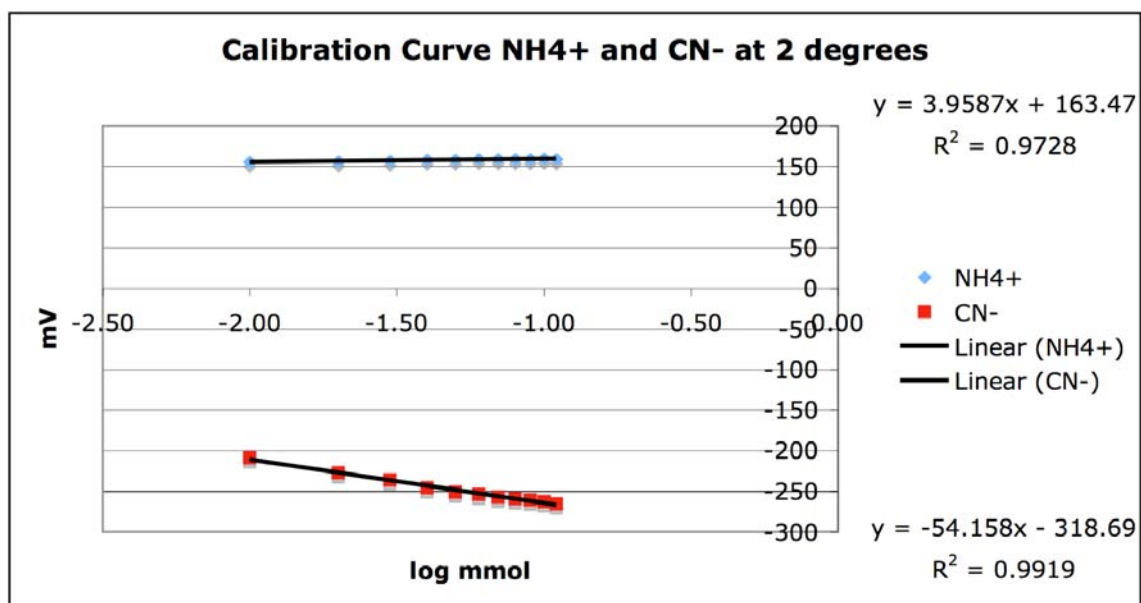
Figure 12: Typical calibration curve of ammonia and cyanide at 2°C ± 2°C . Upper slope is for ammonia and lower slope is for cyanide.

mmol	log mmol	mV NH4+	mV CN-
1.00E-2	-2.00	1.56E+02	-2.08E+02
2.00E-2	-1.70	1.57E+02	-2.27E+02
3.00E-2	-1.52	1.57E+02	-2.36E+02
4.00E-2	-1.40	1.58E+02	-2.45E+02
5.00E-2	-1.30	1.58E+02	-2.51E+02
6.00E-2	-1.22	1.59E+02	-2.54E+02
7.00E-2	-1.15	1.59E+02	-2.57E+02
8.00E-2	-1.10	1.59E+02	-2.59E+02
9.00E-2	-1.05	1.59E+02	-2.61E+02
1.00E-1	-1.00	1.60E+02	-2.63E+02
1.10E-1	-0.96	1.59E+02	-2.66E+02

Table 20: Typical electrode responses of ammonia ISE and cyanide ISE at 2°C ± 2°C.

Calibration curve slopes varied from 3.96 mV/mmol to 16.02 mV/mmol for NH_4^+ and -40.77 mV/mmol to -54.158 mV/mmol for CN-.
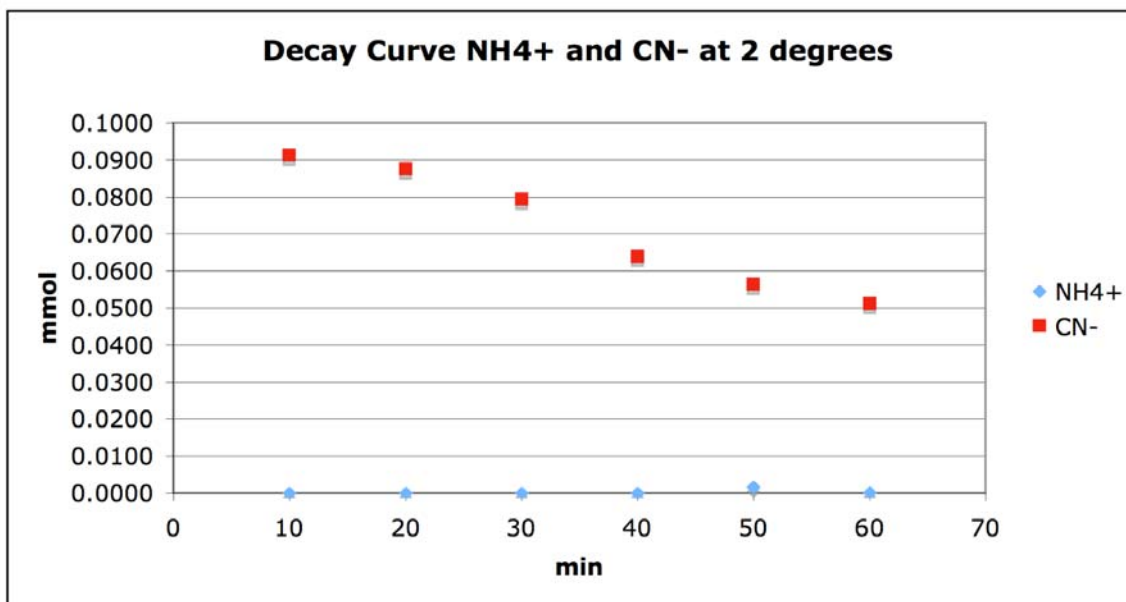
Figure 13: Typical decay curve of ammonia and cyanide at 2°C ± 2°C in reaction mixture.

min	mV NH4+	mV CN-
10	1.46E+02	-2.62E+02
20	1.44E+02	-2.61E+02
30	1.46E+02	-2.59E+02
40	1.37E+02	-2.54E+02
50	1.52E+02	-2.51E+02
60	1.49E+02	-2.49E+02

Table 21: Typical electrode responses of ammonia ISE and cyanide ISE at 2°C ± 2°C in reaction mixture.

min	log NH4+	mmol NH4+	min	log CN-	mmol CN-
10	-4.54	2.89E-05	10	-1.04	9.13E-02
20	-4.97	1.07E-05	20	-1.06	8.75E-02
30	-4.54	2.89E-05	30	-1.10	7.94E-02
40	-6.66	2.18E-07	40	-1.19	6.39E-02
50	-2.80	1.60E-03	50	-1.25	5.65E-02
60	-3.68	2.09E-04	60	-1.29	5.12E-02

Table 22: Millivolt conversions to millimoles of ammonia and cyanide at 2°C ± 2°C.

min	% CN- in soln	% NH4+ in soln
10	91.3	2.89E-02
20	87.5	1.07E-02
30	79.4	2.89E-02
40	63.9	2.18E-04
50	56.5	1.60E+00
60	51.2	2.09E-01

Table 23: Percent CN- and NH_4^+ in solution at 2°C ± 2°C.

3.7. Combined Decay Curves for NH_4^+ and CN- at 21° C ± 2° C, with UV Light,

Slow Stirring (150 rpm) and at 2°C ± 2°C.
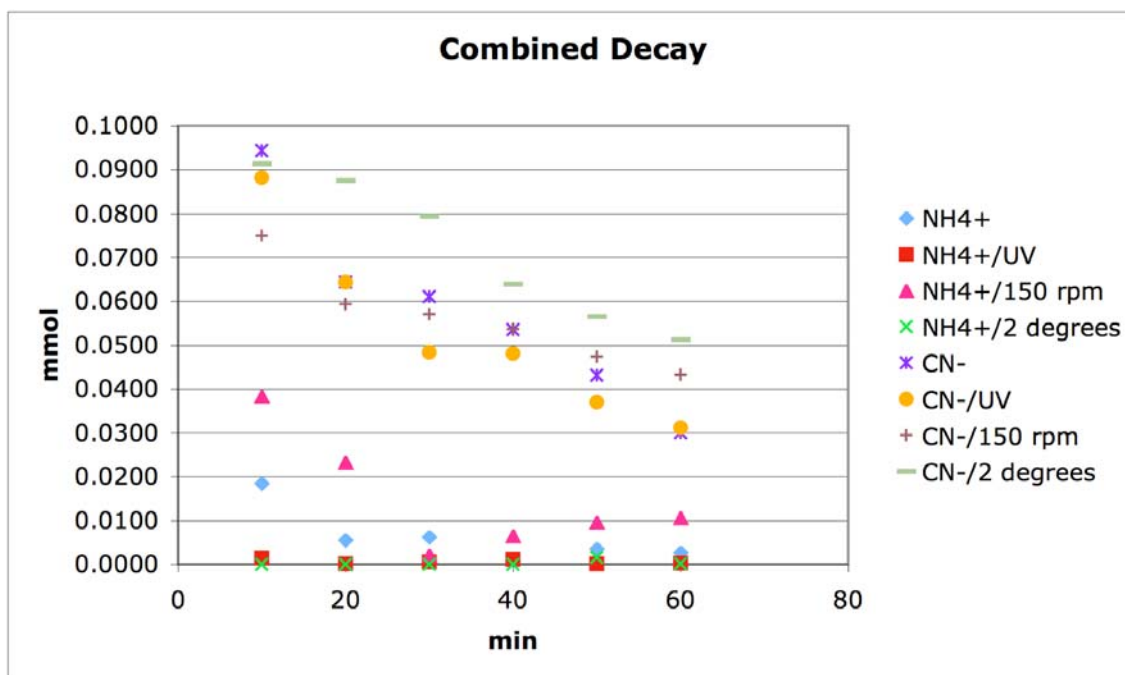
Figure 14: Combined decay curve for cyanide and ammonia at 21° C ± 2° C, with UV light, slow stirring (150 rpm) and at 2°C ± 2°C.

min	mmol NH4+	w/ UV light	w/ 150 rpm	w/ 2 degrees
10	1.86E-02	1.42E-03	3.84E-02	2.89E-05
20	5.53E-03	2.12E-04	2.33E-02	1.07E-05
30	6.19E-03	5.96E-04	2.06E-03	2.89E-05
40	9.06E-04	1.14E-03	6.50E-03	2.18E-07
50	3.58E-03	1.35E-04	9.73E-03	1.60E-03
60	2.67E-03	2.49E-04	1.07E-02	2.09E-04

Table 24: Combined millimole data for ammonia during reactions at 21° C ± 2° C, with UV light, slow stirring (150 rpm) and at 2°C ± 2°C.

min	mmol CN-	w/ UV light	w/ 150 rpm	w/ 2 degrees
10	9.43E-02	8.82E-02	7.50E-02	9.13E-02
20	6.43E-02	6.43E-02	5.93E-02	8.75E-02
30	6.11E-02	4.83E-02	5.71E-02	7.94E-02
40	5.34E-02	4.81E-02	5.37E-02	6.39E-02
50	4.31E-02	3.70E-02	4.74E-02	5.65E-02
60	3.00E-02	3.11E-02	4.33E-02	5.12E-02

Table 25: Combined millimole data for cyanide during reactions at 21° C ± 2° C, with UV light, slow stirring (150 rpm) and at 2°C ± 2°C.

| min | w/UV light | w/ 150 rpm | w/ 2 degrees |
	% CN- in soln	% CN- in soln	% CN- in soln	% CN- in soln
10	94.3	88.2	75	91.3
20	64.3	64.3	59.3	87.5
30	61.1	48.3	57.1	79.4
40	53.4	48.1	53.7	63.9
50	43.1	37	47.4	56.5
60	30	31.1	43.3	51.2

Table 26: Combined data for percent CN- in solution for reactions at 21° C ± 2° C, with UV light, slow stirring (150 rpm) and at 2°C ± 2°C.

| min | w/UV light | w/ 150 rpm | w/ 2 degrees |
	% NH4+ in soln	% NH4+ in soln	% NH4+ in soln	% NH4+ in soln
10	3.69	1.42	38.4	2.89E-02
20	5.53	0.212	23.3	1.07E-02
30	6.19	0.596	2.06	2.89E-02
40	0.904	1.14	6.5	2.18E-04
50	3.58	0.135	9.73	1.60E+00
60	2.67	0.249	10.7	2.09E-01

Table 27: Combined data for percent NH_4^+ in solution for reactions at 21° C ± 2° C, with UV light, slow stirring (150 rpm) and at 2°C ± 2°C.

3.8. Two-Sample T- Tests

T-tests were done using the values obtained from all trials at the 40-minute interval. The 40-minute interval was selected because it was well into the hour-long reaction time. It was determined that there was no significant difference between the results obtained by any of the different exposure methods (data not shown).

3.9. Ammonia Spike

A calibration curve for both NH_4^+ and CN- was prepared and a reaction mixture was run. At the end of the reaction, a 1.0 mL spike of ammonia standard solution was added to determine if the electrode would have detected ammonia if it were present in an amount corresponding to the loss of cyanide.
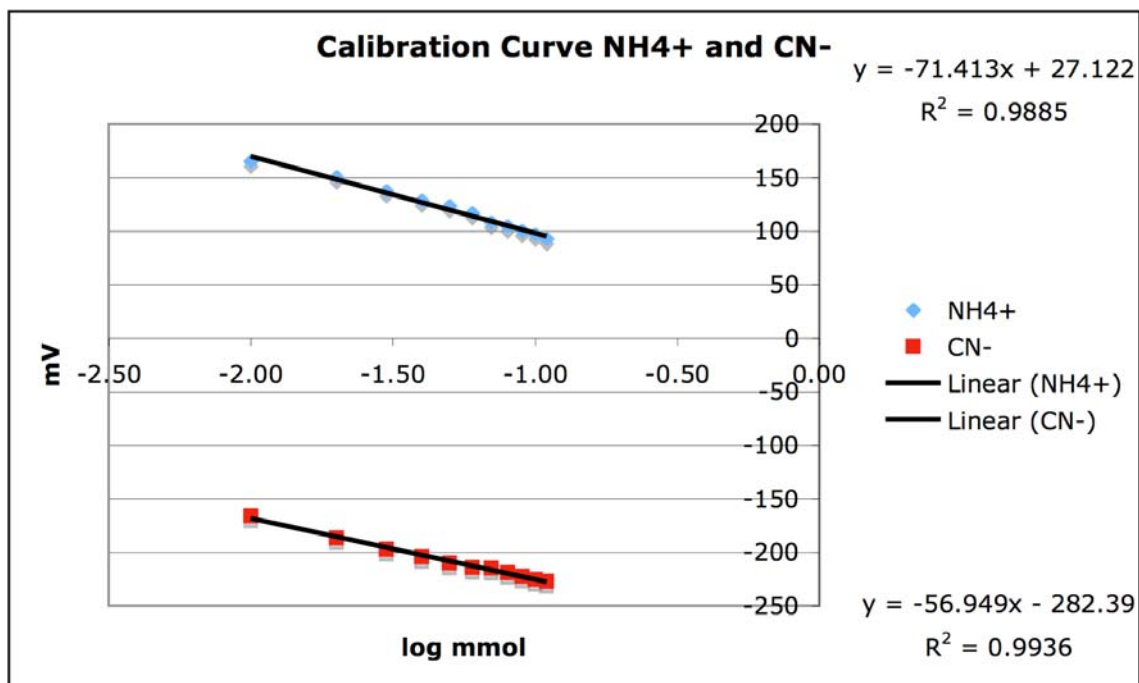
Figure 15: Calibration curve for ammonia and cyanide to determine ammonia spike value. Upper slope is for ammonia and lower slope is for cyanide.

Mmol NH4+/CN-	log mmol	mV NH4+	mV CN-
1.00E-02	-2.00	1.65E+02	-1.66E+02
2.00E-02	-1.70	1.51E+02	-1.87E+02
3.00E-02	-1.52	1.37E+02	-1.97E+02
4.00E-02	-1.40	1.29E+02	-2.04E+02
5.00E-02	-1.30	1.24E+02	-2.10E+02
6.00E-02	-1.22	1.17E+02	-2.14E+02
7.00E-02	-1.15	1.08E+02	-2.15E+02
8.00E-02	-1.10	1.05E+02	-2.19E+02
9.00E-02	-1.05	1.01E+02	-2.22E+02
1.00E-01	-1.00	9.70E+01	-2.25E+02
1.10E-01	-0.96	9.32E+01	-2.27E+02

Table 28: Electrode responses of ammonia ISE and cyanide ISE for ammonia spike value.
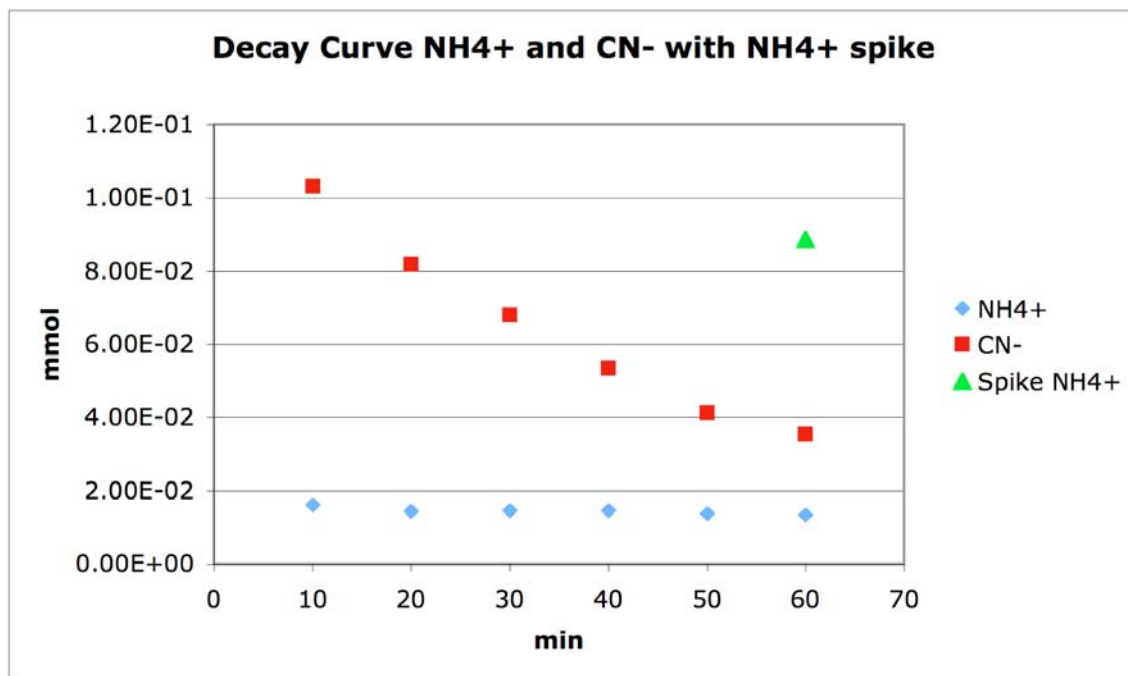
Figure 16: Decay curve of cyanide and ammonia with ammonia spike.

min	mV CN-	log mmol	mmol CN-
10	-2.26E+02	-0.987	1.03E-01
20	-2.21E+02	-1.09	8.19E-02
30	-2.16E+02	-1.17	6.80E-02
40	-2.10E+02	-1.27	5.36E-02
50	-2.04E+02	-1.38	4.14E-02
60	-2.00E+02	-1.45	3.55E-02

Table 29: Millivolt to millimole conversion of cyanide with ammonia spike.

min	mV NH4+	log mmol	mmol NH4+
10	1.55E+02	-1.79	1.61E-02
20	1.59E+02	-1.84	1.44E-02
30	1.58E+02	-1.84	1.46E-02
40	1.58E+02	-1.83	1.47E-02
50	1.60E+02	-1.86	1.38E-02
60	1.61E+02	-1.87	1.34E-02

Table 30: Millivolt to millimole conversion of ammonia with ammonia spike.

mV	log mmol	mmol NH4+
1.02E+02	-1.05	8.89E-02

Table 31: Ammonia spike at 60 minutes.

min	% CN- in soln	% NH4+ in soln
10	103.1	16.1
20	81.9	14.4
30	68	14.6
40	53.6	14.7
50	41.4	13.8
60	35.5	13.4

Table 32: Percent CN- and NH_4^+ in solution with ammonia spike.

3.10.   Cyanide Degradation Testing

It seemed that there was no evidence for ammonia production in the experiments thus far, so the focus of the experiment shifted to examining the loss of cyanide in the reaction mixtures more closely. These experiments were done in a potassium phosphate buffer pH 7.4, 300 rpm stirring at 21° C ± 2° C with no addition of ISAB. A typical calibration curve and decay curve are presented.
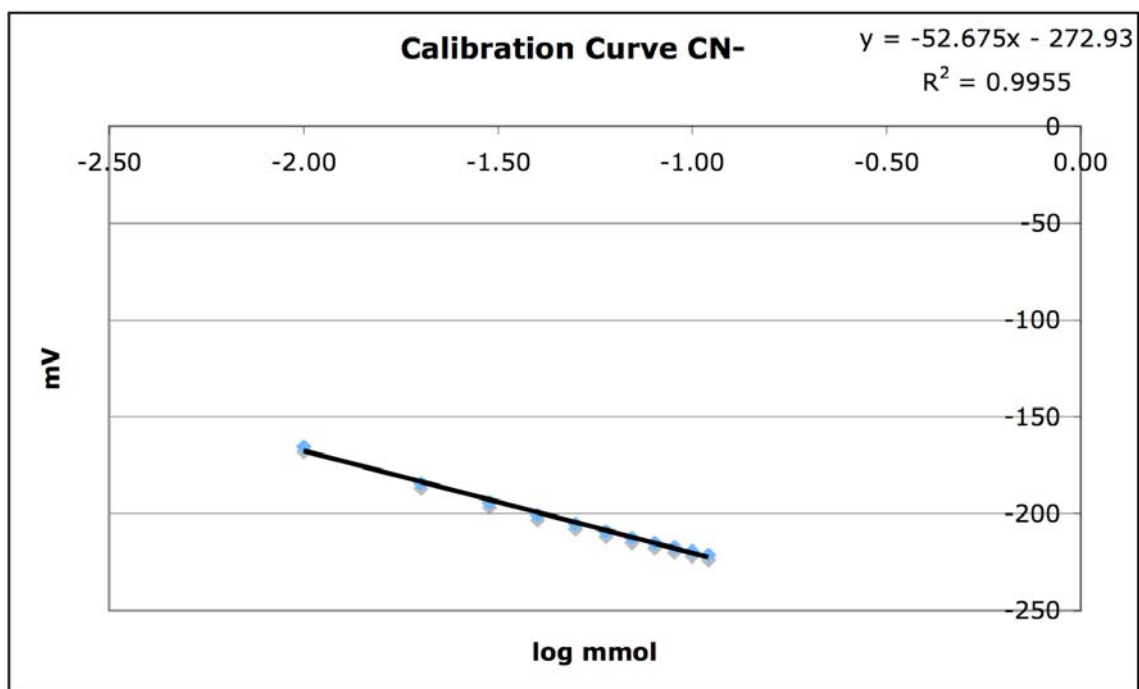
Figure 17: Typical calibration curve for cyanide.

mmol	log mmol	mV CN-
1.00E-02	-2.00	-1.65E+02
2.00E-02	-1.70	-1.84E+02
3.00E-02	-1.52	-1.94E+02
4.00E-02	-1.40	-2.01E+02
5.00E-02	-1.30	-2.05E+02
6.00E-02	-1.22	-2.09E+02
7.00E-02	-1.15	-2.12E+02
8.00E-02	-1.10	-2.15E+02
9.00E-02	-1.05	-2.17E+02
1.00E-01	-1.00	-2.19E+02
1.10E-01	-0.959	-2.21E+02

Table 33: Typical electrode responses for cyanide calibration curve.
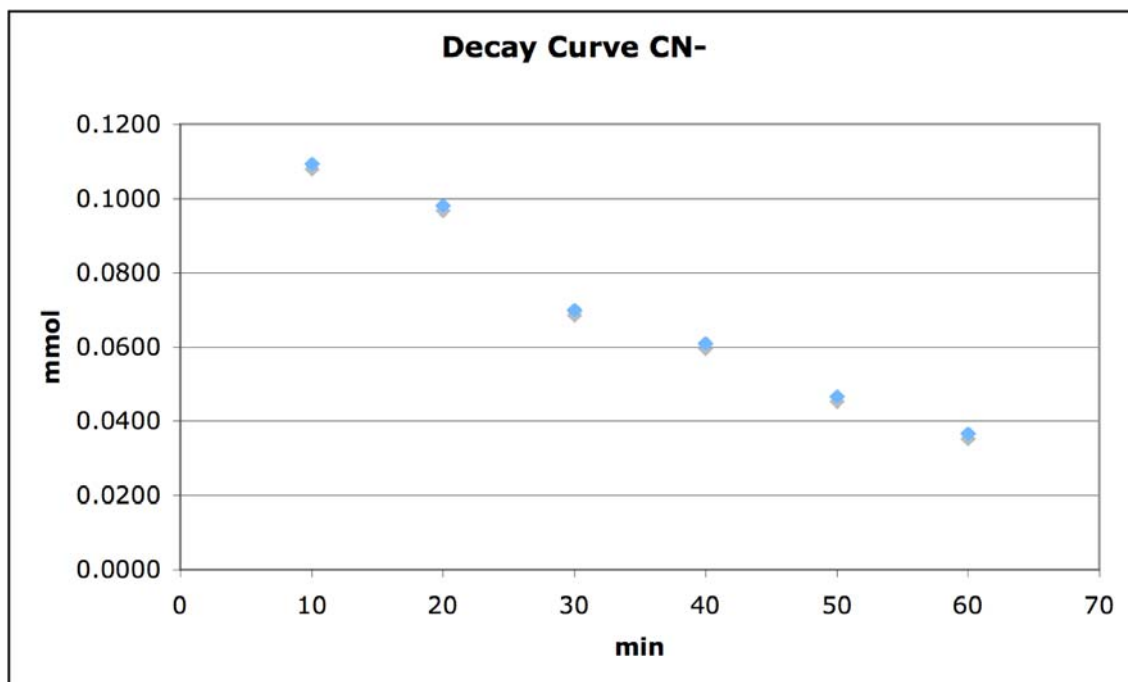
Figure 18: Typical decay curve for cyanide.

min	mV	log mmol	mmol CN-
10	-2.22E+02	-0.961	1.09E-01
20	-2.20E+02	-1.01	9.80E-02
30	-2.12E+02	-1.15	7.00E-02
40	-2.09E+02	-1.22	6.09E-02
50	-2.03E+02	-1.33	4.66E-02
60	-1.97E+02	-1.44	3.67E-02

Table 34: Typical millivolt to millimole conversion for cyanide.

min	% CN- in soln
10	109
20	98
30	70
40	60.9
50	46.6
60	36.7

Table 35: Percent CN- in solution.

Calibration curve slopes of all the trials for cyanide alone ranged from -49.29

mV/mmol to -61.34 mV/mmol.

3.11.   Titration of Cyanide with Silver Nitrate

All solutions tested were initially 0.1M cyanide standards.

10 mL of 0.1M cyanide standard was titrated with 0.1N silver nitrate.

Initial titration solutions should have contained 1.0 mmol of cyanide if none was lost

in the containment vessel by hydrolysis, leaching or volatilization.

Solution 1: made on 8/06/07

Date tested

<u>11/29/07</u>   used 1.6 mL of $AgNO_3$
            calculated: 0.32 mmol of CN^- in solution
            lost: 0.68 mmol of CN- in 16 weeks

<u>1/16/08</u>   used 0.30 mL of $AgNO_3$
            calculated: 0.06 mmol of CN^- in solution
            lost: 0.94 mmol of CN- in 20 weeks

Solution 2: made on 9/08/07

Date tested

<u>11/29/07</u>   used 3.5 mL of $AgNO_3$
            calculated: 0.70 mmol of CN^- in solution
            lost: 0.30 mmol of CN- in 12 weeks

<u>1/09/08</u>   used 3.47 mL of $AgNO_3$
            calculated: 0.69 mmol of CN^- in solution
            lost: 0.31 mmol of CN- in 17weeks

<u>2/19/08</u>   used 3.27 mL of $AgNO_3$
            calculated: 0.65 mmol of CN^- in solution
            lost: 0.35 mmol of CN- in 23 weeks

<u>3/05/08</u>   used 3.21 mL of $AgNO_3$
            calculated: 0.64 mmol of CN^- in solution
            lost: 0.36 mmol of CN- in 25 weeks

Solution 3: made on 12/04/07

Date tested

1/09/08   used 5.88 mL of $AgNO_3$
                 calculated: 1.18 mmol of CN^- in solution
                 lost: 0.0 mmol of CN- in 4 weeks

2/19/08   used 5.58 mL of $AgNO_3$
                 calculated: 1.16 mmol of CN^- in solution
                 lost: 0.02 mmol of CN- in 9 weeks

3/05/08   used 5.41 mL of $AgNO_3$
                 calculated: 1.10 mmol of CN^- in solution
                 lost: 0.06 mmol of CN- in 11 weeks

Percent CN- Lost with Titration

Titration

Solution 1	Percent CN- lost	Percent lost/week
16 weeks	68%	4.25%/week
20 weeks	94%	4.7%/week

Solution 2	Percent CN- lost	Percent lost/week
12 weeks	30%	2.5%/week
17weeks	31%	1.82%/week
23 weeks	35%	1.52%/week
25 weeks	36%	1.44%/week

Solution 3	Percent CN- lost	Percent lost/week
4 weeks	0%	0%/week
9 weeks	2.00%	.22%/week
11 weeks	6.00%	.55%/week

Table 36: Percent CN- lost in each solution determined by titration.

3.12.   Colorimetric Determination of Ammonia in Cyanide Standard Solutions

A typical calibration curve using ammonia standard solutions is presented:
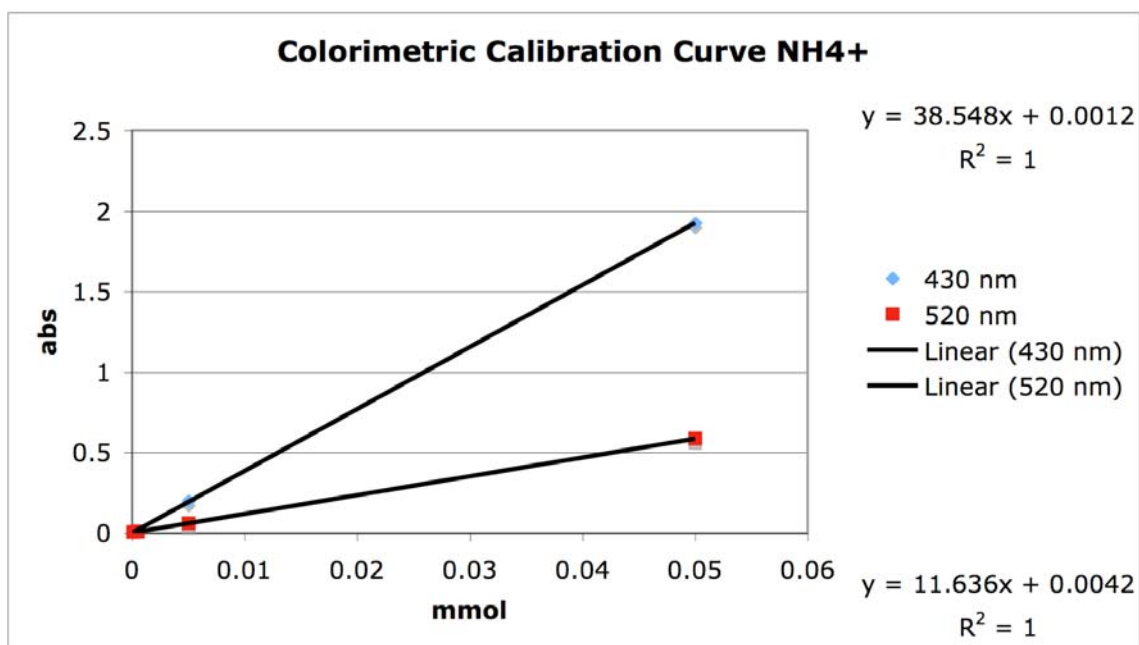
Figure 19: Typical calibration curve used for colorimetric determination of ammonia in cyanide standard solutions.

All sample solutions contained 0.1mmol CN^-

Determination of ammonia in cyanide samples:

Solution 1: made 8/06/07

Solution 2: made 9/18/07

Solution 3: made 12/04/07

Solution 1: made 8/06/07		Solution 2: made 9/18/07		Solution 3: made 12/04/07	
Abs	Abs	Abs	Abs	Abs	Abs
430 nm	520 nm	430 nm	520 nm	430 nm	520 nm
0.009	0.000	0.032	0.000	0.000	0.000
Calculated:		Calculated:		Calculated:	
mmol NH4+	mmol NH4+	mmol NH4+	mmol NH4+	mmol NH4+	mmol NH4+
6.20E-04	0.00E+00	1.00E-03	0.00E+00	0.00E+00	0.00E+00

Table 37: Colorimetric test for ammonia for three standard solutions tested on: 12/06/07.

Solution 1: made 8/06/07		Solution 2: made 9/18/07		Solution 3: made 12/04/07	
Abs	Abs	Abs	Abs	Abs	Abs
430 nm	520 nm	430 nm	520 nm	430 nm	520 nm
0.089	0.052	0.150	0.074	0.012	0.001
Calculated:		Calculated:		Calculated:	
mmol NH4+	mmol NH4+	mmol NH4+	mmol NH4+	mmol NH4+	mmol NH4+
2.28E-03	4.11E-03	3.86E-03	6.00E-03	2.80E-04	-2.75E-04

Table 38: Colorimetric test for ammonia for three standard solutions tested on 1/16/08.

Solution 2: made 9/18/07		Solution 3: made 12/04/07	
Abs	Abs	Abs	Abs
430 nm	520 nm	430 nm	520 nm
0.163	0.070	0.036	0.022
Calculated:		Calculated:	
mmol NH4+	mmol NH4+	mmol NH4+	mmol NH4+
4.09E-03	5.59E-03	8.48E-04	1.64E-03

Table 39: Colorimetric test for ammonia for two standard solutions tested on 2/20/08.

Solution 2: made 9/18/07		Solution 3: made 12/04/07	
Abs	Abs	Abs	Abs
430 nm	520 nm	430 nm	520 nm
0.250	0.093	0.173	0.057
Calculated:		Calculated:	
mmol NH4+	mmol NH4+	mmol NH4+	mmol NH4+
5.38E-03	7.16E-03	3.60E-03	4.63E-03

Table 40: Colorimetric test for ammonia for two standard solutions tested on 3/05/08.

Combined results in mmol of NH_4^+ at each wavelength.

Date tested:	Solution 1		Solution 2		Solution 3	
	430 nm	520 nm	430 nm	520 nm	430 nm	520 nm
12/6/07	6.00E-04	0.00E+00	1.00E-03	0.00E+00	0.00E+00	0.00E+00
1/16/08	2.30E-03	4.10E-03	3.90E-03	6.00E-03	3.00E-04	0.00E+00
2/20/08			4.10E-03	5.60E-03	8.00E-04	1.60E-03
3/5/08			5.40E-03	7.20E-03	3.60E-03	4.60E-03

Table 41: Combined results of millimole recovery of ammonia in three standard solutions.

Solution 1		8/6/07	
Date tested:	430 nm	520 nm	
12/6/07	6.00E-04	0.00E+00	17 weeks
1/16/08	2.30E-03	4.10E-03	22 weeks
2/20/08			
3/5/08			

Solution 2		9/8/07	
Date tested:	430 nm	520 nm	
12/6/07	1.00E-03	0.00E+00	13 weeks
1/16/08	3.90E-03	6.00E-03	18 weeks
2/20/08	4.10E-03	5.60E-03	24 weeks
3/5/08	5.40E-03	7.20E-03	26 weeks

Solution 3		12/4/07	
Date tested:	430 nm	520 nm	
12/6/07	0.00E+00	0.00E+00	0 weeks
1/16/08	3.00E-04	0.00E+00	6 weeks
2/20/08	8.00E-04	1.60E-03	11 weeks
3/5/08	3.60E-03	4.60E-03	13 weeks

Table 42: Millimoles of ammonia detected at 430 nm and 520 nm.

Solution 1   8/6/07

	Percent ammonia detected		Percent detected/week	
	430 nm	520 nm	430 nm	520 nm
17 weeks	0.6	0	0.035	0
22 weeks	2.3	4.1	0.1	0.19

Solution 2   9/8/07

	Percent ammonia detected		Percent detected/week	
	430 nm	520 nm	430 nm	520 nm
13 weeks	1	0	0.076	0
18 weeks	3.9	6	0.22	0.33
24 weeks	4.1	5.6	0.17	0.23
26 weeks	5.4	7.2	0.21	0.28

Solution 3   12/4/07

	Percent ammonia detected		Percent detected/week	
	430 nm	520 nm	430 nm	520 nm
0 weeks	0	0	0	0
6 weeks	0.3	0	0.05	0
11 weeks	0.8	1.6	0.073	0.15
13 weeks	3.6	4.6	0.28	0.35

Table 43: Percent ammonia detected per week at 430nm and 520 nm.

3.13.   Ninhydrin Test for Formamide

Ninhydrin solution was used as a preliminary indicator of the formation of

formamide. The experiment only yielded one positive result with the glycine. The

test tube with the glycine in it turned a medium violet color, indicative of the presence

of an amino group, but both the test tube with the formamide and the test tube with

the cyanide standard solution did not change color to the indicator. This method was

abandoned and no further investigation into the presence of formamide was done.

3.14.   Identification of Hydrogen Cyanide in the Vapor Phase using

Spectroscopy

To investigate the possibility that the apparent degradation of cyanide without a

corresponding appearance of ammonia was due to volatilization or out gassing of

hydrogen cyanide, the following infrared spectra of the vapor phase above the 0.1 M

potassium cyanide aqueous solution were recorded and compared to a reference

spectrum for hydrogen cyanide. The wavenumber that hydrogen cyanide gas should

be detected is 712 cm^{-1} (Madarász and Pokol 2007, Li et al. 2007). Other

wavenumbers for hydrogen cyanide gas are expected at 3289 cm^{-1} and approximately

2100 cm^{-1} (Choi and Barker 1932), but these peaks are obscured by the water

molecule spectra in the sample.

Figure 20: Infrared spectrum of hydrogen cyanide with peak at 712 cm^{-1} used as reference (Li et al. 2007).
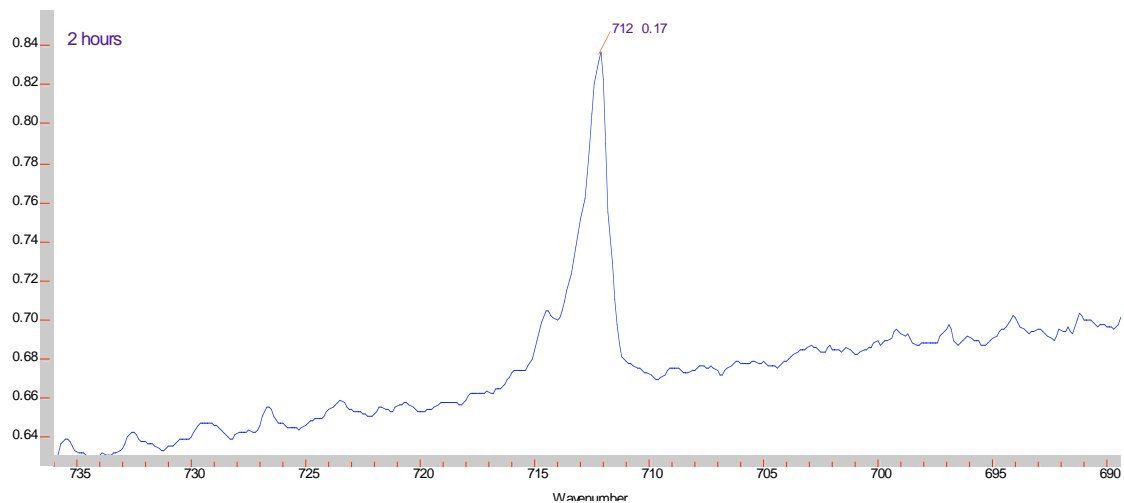
Figure 21: Experimental infrared spectrum of vapor phase above 0.1 M aqueous potassium cyanide solution with hydrogen cyanide gas peak labeled at 712 cm^{-1}. The peak height is labeled at 0.17 absorbance units.

4. DISCUSSION

    4.1. Ammonia ISE Testing

    A series of calibration curves were established to determine the Nernstian behavior of the ammonia ISE. Solutions were initially made in ppm, but converted later to molarity and mmols. The sensitivity of the ammonia ISE for uncomplexed ammonia activity ranges from 10^{-1} to 10^{-5} M as stated in the manual for the electrode (Corning 1983).

    Repeated testing of these standard solutions revealed that the lower limit for the experiment, (1 ppm or 5.54 x 10^{-5}M) gave inconsistent results but it was used for the first calibration curve because the correlation coefficient was close to one. As stated in the ammonia ISE manual (Corning 1983), a reading of -100 mV is equivalent to a NH_3 concentration of 5.0 x 10^{-3} M solution. Standard solutions of 5.54 x 10^{-3}M gave activity readings averaging -96 mV, so Nernstian behavior was accepted for the electrode.

    4.2. Ionic Interference Testing

    Though interference by potassium and/or sodium ions was only referenced for the ammonium solid state ISE (Rundle 2000), testing was done to confirm that these interferences would not effect the ammonia gas sensing ISE, since the experiments were done using a potassium phosphate buffer solution. Standard solutions were made for ammonia in concentrations of 1.0 x 10^{-6}M, 1.0 x 10^{-5}M, 1.0 x 10^{-4}M, and 1.0 x 10^{-3}M. The conversions to millimole for the standard solutions are: 1.0 x 10^{-4}mmol, 1.0 x 10^{-3}mmol, 1.0 x 10^{-2}mmol and 1.0 x 10^{-1}mmol. Each standard solution for ammonia was tested using a separate beaker and the concentrations of

potassium ions and sodium ions were added to each of the concentrations of standard

solutions for ammonia in the following increments: 0.01mmol, 0.1mmol and

1.0mmol. The absence of interference by these ions was confirmed. The addition of

potassium and sodium ions to the standard solutions of ammonium chloride produced

near identical slopes for the calibration curves.

4.3. Simultaneous Cyanide ISE and Ammonia ISE Testing

A calibration curve for ammonia and cyanide was made using ISAB in a

potassium phosphate buffer solution. The slopes for both components displayed near

Nernstian behavior.

A reaction mixture containing a 1.0 mL volume of 0.1M cyanide standard

solution was made, and the mixture was stirred constantly at 300 rpm. The solution

was maintained in a water bath at 37° C ± 2° C until measurements were taken every

10 minutes. The concentrations of cyanide ion that were calculated from the

calibration curves fluctuated from 1.0×10^{-1} mmol to 7.66×10^{-2} mmol. There was

no apparent degradation in the cyanide concentration and the fluctuations could be

attributed to drift in the ISE. The solution was basic, ~ 11 pH with the added ISAB,

so it was not expected that there would be cyanide loss due to volatilization of HCN.

The millivolt readings for the ammonia in solution were values that were beyond the

detection limits for the ammonia ISE based on the calibration curve data that was

obtained for that experiment. The millivolt readings for the calibration curve ranged

from -5.57×10^{1} to -1.68×10^{1} mV and the millivolt readings for the reaction mixture

ranged from 9.45×10^{1} to 6.50×10^{1} mV. Ammonia was not detected in the reaction

mixture solution and the millimole data was derived from the linear equation of the calibration curve.

Another series of tests were done without the use of ISAB. The difference between activity and concentration is small at low ionic strengths and can be ignored (Rundle 2000), so the ISAB was omitted. This design more closely resembled that of a natural aquatic environment. The pH of the buffer solution was 7.4, and it was assumed that a natural aquatic environment would have somewhere close to a neutral pH. Of course, this may not always be the case, depending on the many variables that accompany different aquatic conditions. These tests were performed at $21°$ C $±$ $2°$ C to more closely resemble the temperature relating to environmental conditions. Calibration curves were prepared for ammonia and cyanide. The ammonia slope showed non-Nernstian behavior and had a positive slope. The calibration curve was linear, and the correlation coefficient was close to 1, so the slope was used for calculations. The cyanide slope was less than Nernstian, but again, the correlation coefficient was close to 1, so the curve was used to calculate concentrations using the linear equation of best fit. A reaction mixture was made with constant stirring of 300 rpm, and millivolt readings were obtained every ten minutes for the ammonia and cyanide ions. In the one-hour reaction, there was virtually no detection of ammonia, but the cyanide concentration diminished by 70 percent. Tests were done using several different variables to determine if the cyanide loss was dependent on other factors, such as photolysis, slower stirring or lower temperature.

4.4. Effects of UV Radiation on Cyanide Degradation

A reaction mixture was made as described previously with 300 rpm stirring at 21° C ± 2° C. A UV light source was mounted on a ring stand and UV light was administered to the reaction at 254 nm with 20.7 Watts, approximately 15 cm from the top of the reaction solution. The UV light exposure was continuous during the hour-long experiment. Millivolt readings for cyanide and ammonia were taken at 10-minute intervals, and then compared to the calibration curve to calculate millimoles of ammonia and cyanide ions in the solution. The results were similar to those done without UV light, with no detection of ammonia and a 68.9 percent loss of cyanide. UV light was expected to increase the loss of HCN if photolysis occurred, but results from this one-hour experiment did not reveal those results.

4.5. Effects of Stirring Rate on Cyanide Degradation

A reaction mixture was made as previously done with 150 rpm stirring at 21° C ± 2° C. Millivolt readings for cyanide and ammonia were taken at 10-minute intervals, and then compared to the calibration curve to calculate millimoles of ammonia and cyanide ions in the solution. It was expected at the slower stirring speed, the volatilization of hydrogen cyanide from the solution would be decreased. The lower stirring rate degraded the cyanide in solution as the two previous experiments, but to a lesser degree. There was a 56.7 percent loss of cyanide ions at the end of the experiment and no ammonia detected.

4.6. Effect of Temperature on Cyanide Degradation

A calibration curve and reaction mixture was done at 300 rpm stirring and 2°C ± 2°C. The calibration curve was done at the low temperature, because the electrode

potentials can be affected by temperature. Again, the ammonia slope was sub-Nernstian. The correlation factor however was close to 1, so the slope was used for calculations. The cyanide slope was less than Nernstian, but, the correlation factor was close to 1, so the curve was used to obtain millimole values using the linear equation. The reaction at this temperature showed a 48.8 % loss of cyanide ions, which was less than the reactions at room temperature. This would be expected because at lower temperatures, HCN gas is more soluble in water, and the potential for it to volatilize would be lower. There was no reliable detection of ammonia in the solution based on millivolt readings.

Two sample T-tests were done to compare the values obtained by the entire variable testing at the 40-minute interval. P values for all the samples were well above the 0.05 and 0.1 critical values, so it was concluded that the experiments done using different stimuli with the reaction mixture, were not significantly different in their results.

Another test was done to determine if indeed ammonia could be detected in the reaction mixture. A calibration curve was made with 300 rpm stirring at 21° C ± 2° C. A reaction mixture was made as previously without ISAB and millivolt readings were taken at 10-minute intervals. A 65.5 percent loss of cyanide ions was detected, and no reliable amount of ammonia was in the solution. At the end of the experiment, a 0.1 mmol spike was added to the solution and the millivolt reading was taken and 88.9 percent of the spike was recovered according to the calibration slope for ammonia. This indicated that if hydrolysis was happening and there was ammonia

forming in the reaction mixture during the one-hour reaction time, the ammonia ISE would have detected it.

4.7. Cyanide Degradation Testing

It was apparent that the cyanide loss was not due to hydrolysis.   There was no mass balance between the loss of cyanide and the appearance of ammonia, so the experiment focused on strictly the loss of cyanide in the reaction solutions. A calibration curve with 300 rpm stirring at 21° C ± 2° C was made and a reaction mixture was made in a pH 7.4 phosphate buffer solution without ISAB. Cyanide loss for the reaction mixture was 63.3 percent.

The reproducibility of the results was demonstrated with all the cyanide degradation and ammonia detection experiments. It would be expected; if cyanide were hydrolyzing in neutral pH, that a mass balance of ammonia would be detected in the reaction solution. Multiple trials were done for each experiment and ammonia was not detected with the simultaneous loss of cyanide. In some instances the detection of cyanide was over 100 percent. These calculations were based on the millivolt readings for cyanide after a one-minute equilibration time. Fluctuations in the millivolt reading from the cyanide ISE during the experiments would be responsible for this error.

4.8. Titration of Cyanide with Silver Nitrate

Three 0.1M solutions of cyanide were made over the course of these experiments. After the realization that hydrolysis was not occurring during the one hour reaction mixture experiments, the original standard solutions were tested for cyanide loss. The original cyanide standard solutions were in closed containers, but they were accessed

and opened during the course of the experiments. All three solutions exhibited some loss of cyanide over time. The first solution was tested 16 and 20 weeks after it was made, and the silver nitrate titration showed that the solution had lost 94.0 percent of the original cyanide concentration. This loss was estimated to be approximately 4.70 percent per week. The second solution was tested at 12, 17, 23, and 25 weeks and through the titration experiment it showed a loss of 36.0 percent of its total cyanide concentration. This loss was estimated to be approximately 1.44 percent per week. The third solution was tested at 4, 9 and 11 weeks, and the total loss for this solution was 6.00 percent of the cyanide concentration, and this loss was estimated to be approximately 0.55 percent per week. These losses cannot be attributed primarily to volatilization, as there was some indication of ammonia appearance using colorimetric testing, implying that slow hydrolysis may have been occurring in the standard solutions.

The loss of cyanide in the standard solutions was presumed to be from either hydrolysis or volatilization. The pH of all three solutions in their bottles was between 9 and 10, making the standard solutions basic. It appeared that the loss per week of cyanide increased with the age of the standard solution. The older the solution, the more it was accessed for experimentation, so if volatilization occurred, there would be a greater loss with age.

4.9. Colorimetric Determination of Ammonia in Cyanide Standard Solutions

Since there was a definite loss of cyanide in the three standard solutions, colorimetric testing was done to determine if the loss was due to hydrolysis or volatilization. Samples from the three cyanide standard solutions were tested against

ammonia standard solutions. The absorbances were read at 430 and 520 nm. A percent recovery of ammonia was calculated with the assumption that if there were a mass balance of cyanide loss and ammonia recovery, the total amount of ammonia that would be produced would equal the 0.1M original cyanide concentration in the standards solutions. Ammonia was indeed detected in some of the cyanide standard solutions but not in a mass balance. The first solution (made on 8/06/07) was tested and at 22 weeks the ammonia concentration was 2.30 percent at 430 nm and 4.10 percent at 520 nm. This was a recovery of approximately 0.10 percent per week at 430 nm and 0.19 percent per week at 520 nm.

The second solution (made on 9/08/07) was tested and at 26 weeks showed a recovery of ammonia of 5.40 percent at 430 nm and 7.20 percent at 520 nm. This was a recovery of approximately 0.21 percent per week at 430 nm and 0.28 percent per week at 520 nm. The third solution (made on 12/04/07) was tested and at 13 weeks showed a recovery of ammonia of 3.60 percent at 430 nm and 4.6 percent at 520 nm. This was a recovery of approximately 0.28 percent per week at 430 nm and 0.35 percent at 520 nm.

The recovery of ammonia from the cyanide standard solutions was not consistent throughout the three solutions tested. Ammonia is also a volatile gas that may have been escaping from the containment vessels when they were being accessed for the experiments. If hydrolysis was occurring with the standard solutions, and the cyanide was escaping as hydrogen cyanide gas while the containers were accessed, there would be less cyanide in solution to hydrolyze and thereby less ammonia formed.

4.10.   Ninhydrin Test for Formamide

The ninhydrin test for formamide was not pursued because the initial results did

not give a positive result for formamide via ammonia detection. Colorimetric

detection of ammonia using Nessler's reagent was used instead.

4.11.   Infrared Spectrum of Vapor Phase above the Aqueous Solution.

The final test was conducted to determine whether the fate of cyanide in the

aquatic environment was due to volatilization by the detection of hydrogen cyanide in

the vapor phase above the aqueous solution. This test was positive. The loss of

cyanide from the aqueous phase was due to volatilization as hydrogen cyanide. This

may be the major cause of its degradation.

4.12.   Synopsis

Ion selective electrodes are not the only methods that can be used to detect

cyanide. Cyanide can be detected by gas chromatography (GC) high performance

liquid chromatography (HPLC), ion chromatography (IC), atomic absorption

spectrometry (AAS), infrared spectroscopy (IR) and fluorometric methods just to

name a few, but the simplicity of the electrochemical methods was most appealing for

the investigation of cyanide.

Cyanide degradation in a neutral aqueous solution can occur several different

ways. Addition of a cyanide-degrading enzyme, cyanidase, can be added to a cyanide

solution to hydrolyze it to ammonium formate. This occurs naturally in some plants,

bacteria and fungi.

Without cyanidase, cyanide can hydrolyze in an aqueous solution to formamide and then to ammonia and formic acid as presented in experiments by Robert Fallon (Fallon 1992).

Cyanide can also react with metals such as iron(III), cobalt(II), copper(II) , nickel(II), sodium, potassium and calcium to form soluble complexes and/or precipitates.

Probably the greatest loss of cyanide from solution is through volatilization. Henry's law constant for hydrogen cyanide is 5.1×10^{-2} atm•m^3/mol which is relatively large compared to other volatiles such as acetone which is 3.88×10^{-5} atm•m^3/mol and chloroform which is 3.67×10^{-3} atm•m^3/mol . This means that the partial pressure of hydrogen cyanide above the solution is greater than that of acetone or chloroform making hydrogen cyanide more volatile than acetone or chloroform.

This is probably the most predominant fate of cyanide in these experiments. The presence of hydrogen cyanide in the vapor phase above the aqueous cyanide solutions was confirmed by infrared spectrometry. The loss of cyanide did not balance with the appearance of ammonia as a hydrolysis product, and all of the solutions were subject to constant stirring, increasing the energy of the solution and allowing volatilization to occur easily.

Acids and bases can catalyze hydrolysis of hydrogen cyanide. In acidic conditions, the time it takes to hydrolyze 25 percent of hydrogen cyanide to formamide compared to the time it takes to hydrolyze 25 percent of formamide to ammonium formate was found to be in a ratio of 1400 hours to 6 minutes (Krieble

1929). So, in acid catalyzed hydrolysis of hydrogen cyanide, clearly the hydrolysis to formamide is slow and rate limiting.

Base catalyzed hydrolysis of hydrogen cyanide also has an initial rate-limiting step, with the attack of the hydroxide ion on the cyanide ion to form formamide and then eventually formate and ammonia. In the alkaline hydrolysis of nitriles such as cyanide, amides rapidly hydrolyze as intermediates with the initial step of the hydroxide attack on the nitrile being the slow step.   (Wiegand and Tremelling 1969).

A study was done on using five different transition states for the neutral hydrolysis of formamide. It was found that each one had a high activation free energy barrier with the most stable structure having a barrier of 48.7 kcal/mol. It was claimed in this study that the neutral hydrolysis of formamide does not occur in aqueous solutions (Almerindo and Pliego 2007) and that "hydrolyses of carboxylic acid amides generally do not take place in neutral conditions" (Hine et al. 1981).

These papers are in agreement with our studies. The neutral hydrolysis of hydrogen cyanide to formamide and to formate and ammonia were not observed with the experiments in this thesis. There was no mass balance of cyanide loss and ammonia appearance in neutral conditions, however a small amount of ammonia was recovered from the cyanide standard solutions, which were alkaline. Hydrolysis may have been occurring slowly in the cyanide standard solutions.

The major loss of cyanide from a neutral solution seems to be from volatilization with a loss in an hour of between 60 to 70 percent under variable neutral conditions. Baskin, et. al. had similar results with cyanide degradation and a loss of 60 percent of cyanide from a neutral solution. They did not confirm what the cyanide loss was

from, but hypothesized that it was either from hydrolysis or volatilization (Baskin et al. 1998). G. Charlot has included the hydrolysis and volatility of cyanides in his description of their analytical chemistry (Charlot 1954). Similarly, volatilization is among the factors discussed by Garcia in his considerations of cyanides in the environment (Garcia 2003).

5. CONCLUSION

Ion selective electrodes were used to determine the fate of the cyanide ion in neutral aquatic conditions. Results for the spontaneous hydrolysis of cyanide in a neutral medium were not confirmed. Cyanide was lost in the reaction solution and ammonia was not detected as a hydrolysis product in the trials. Titrations and colorimetric methods were used to determine what was happening to the cyanide in solution and if any ammonia was being formed. A mass balance of cyanide loss and ammonia presence was not discovered. Titrations showed that there was a constant loss of cyanide from the standard solutions used in the experiments, and colorimetric determination of ammonia showed minimal production of ammonia from alkaline hydrolysis in the cyanide standard solutions. Hydrolysis in neutral aquatic environments happens slowly and the loss of cyanide in environmental conditions would more likely be a result of volatilization, metal complexing or degradation by organisms using cyanidase. The IR spectroscopy experimental data demonstrated that the cyanide ion was escaping from solution through volatilization.

Further work on the rate of volatilization should be done using a closed system and collecting the headspace gases of reaction mixtures. GC or HPLC could be used to quantify the amount of hydrogen cyanide that was being lost through the reaction.

6. APPENDIX

42 min
712.257 0.161

52min
712.257 0.167

62 min
712.261 0.173
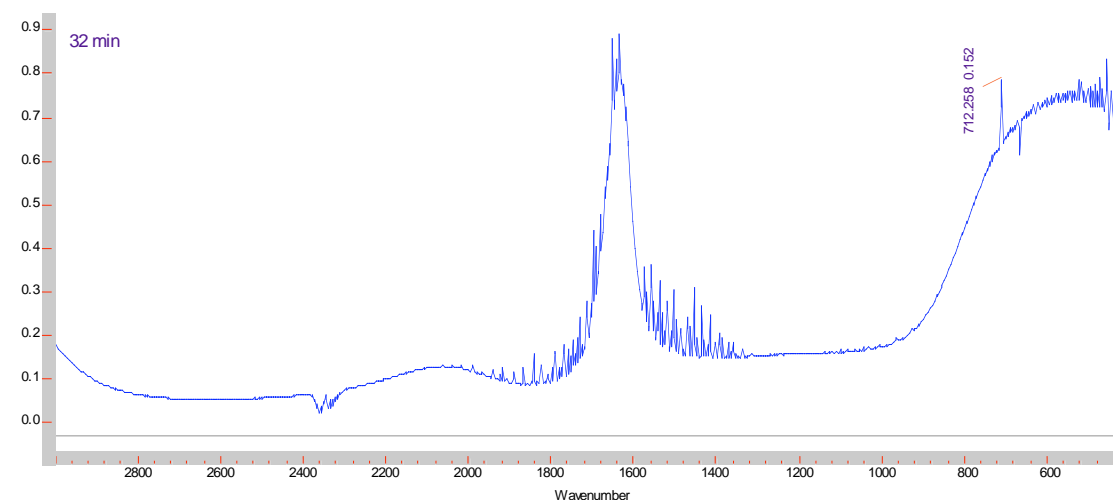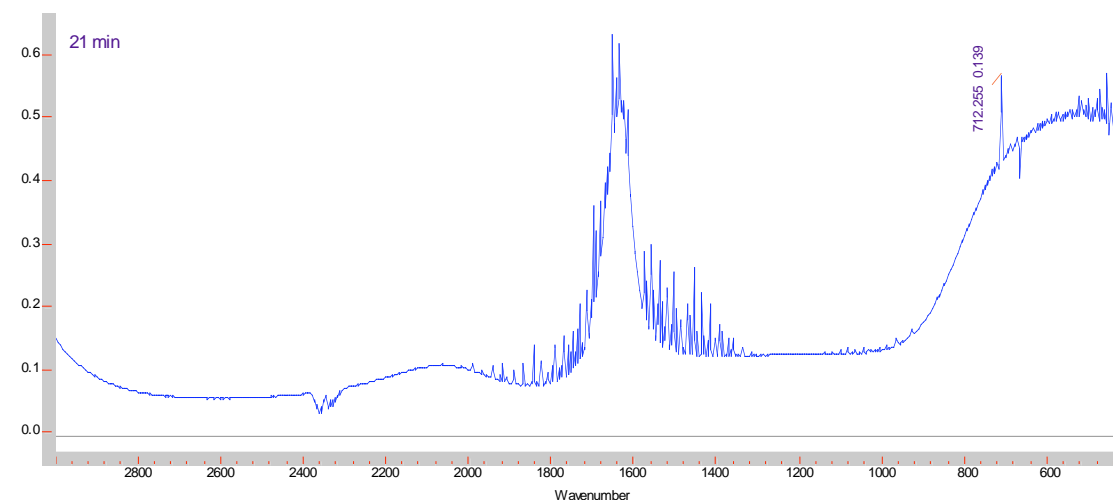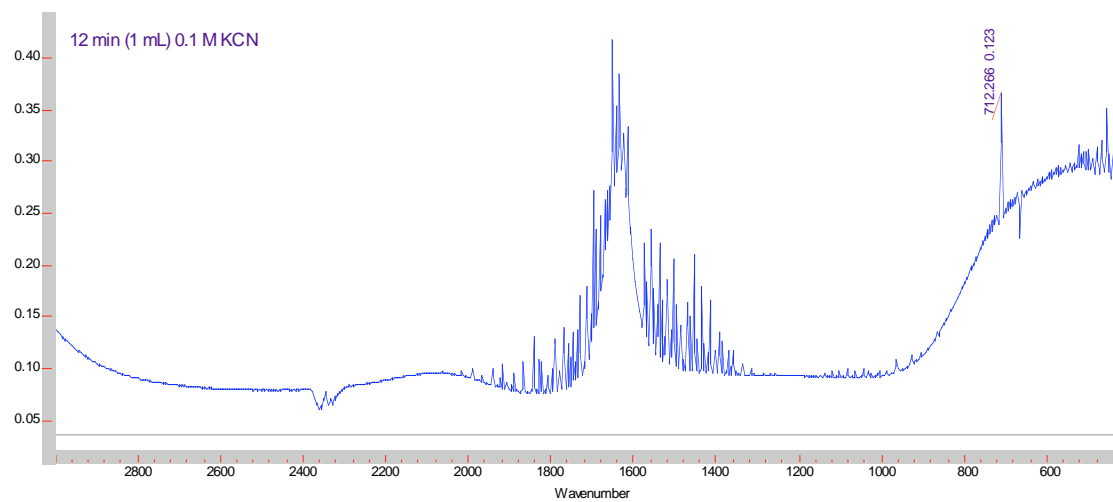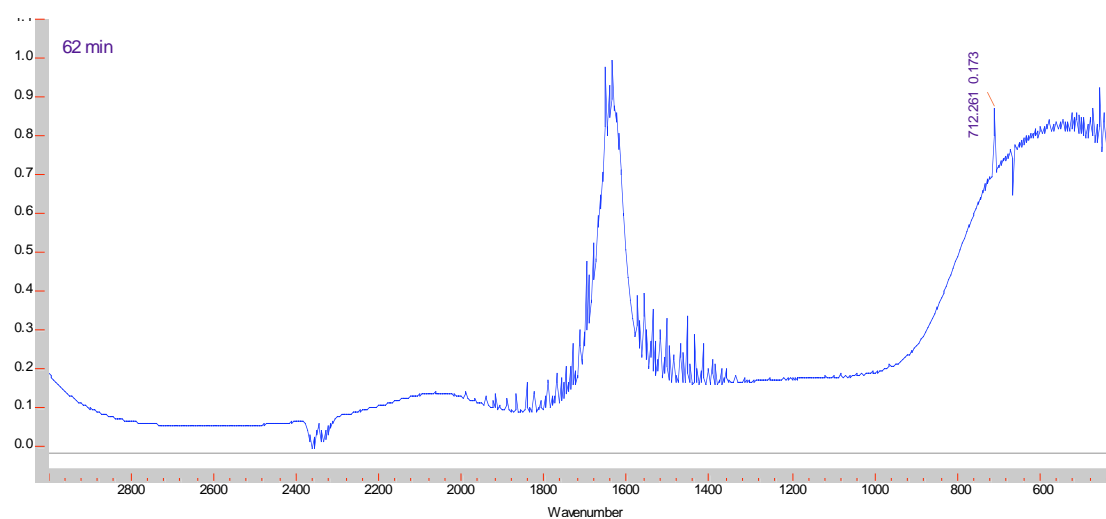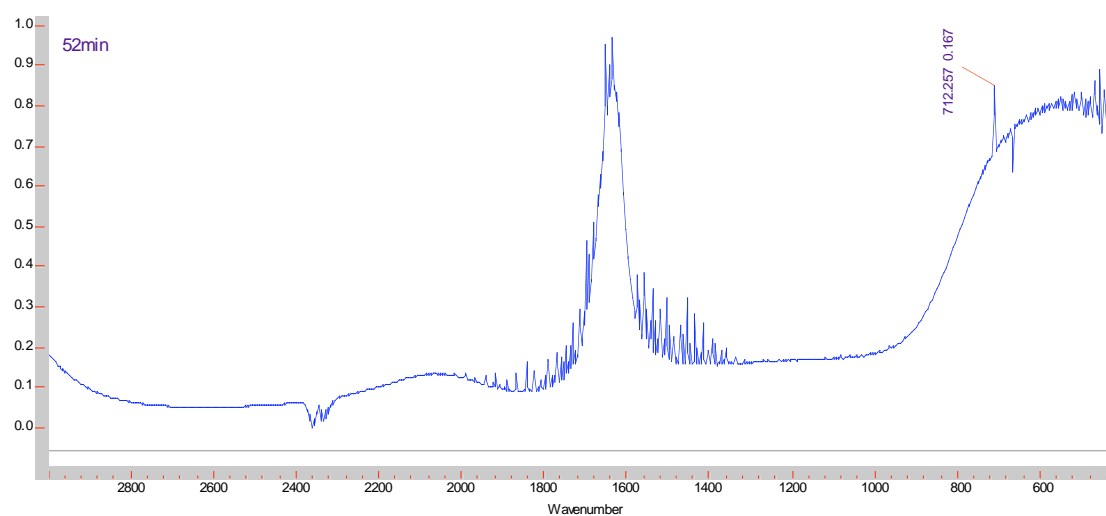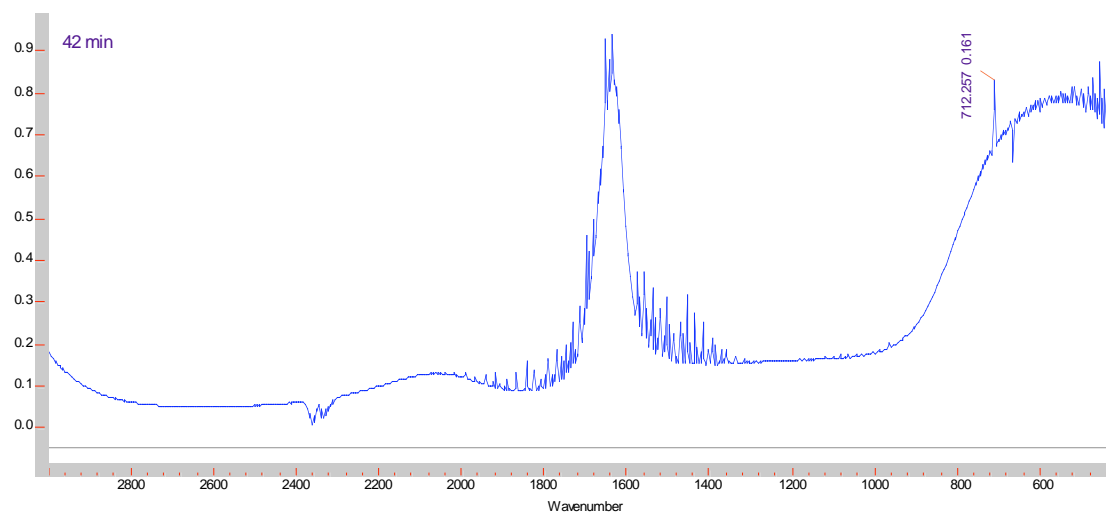
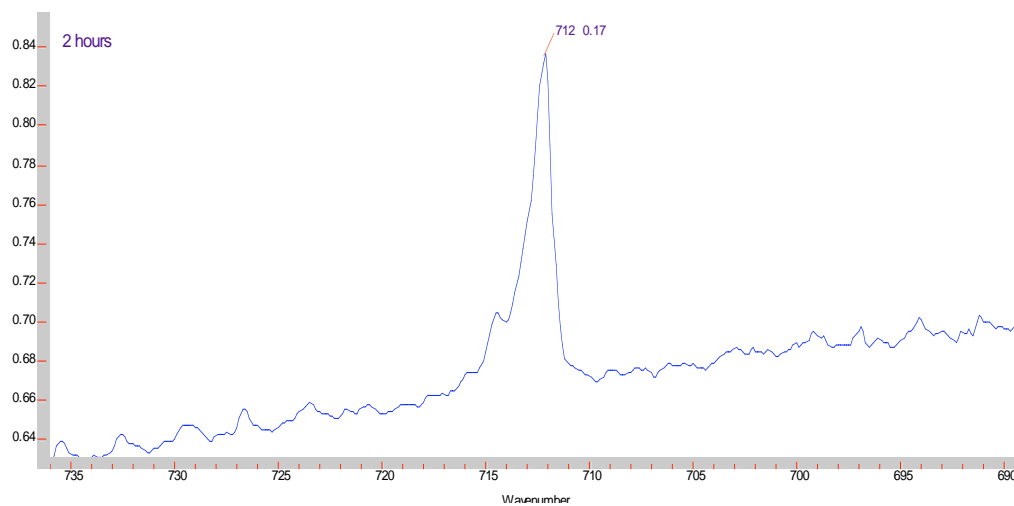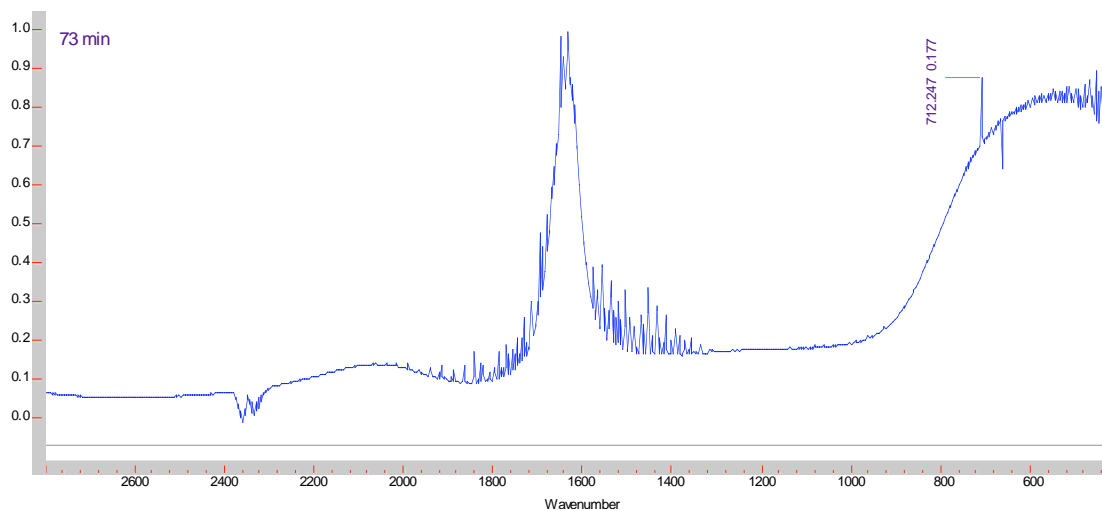Figure 22: IR spectroscopy scans for hydrogen cyanide gas showing an increasing peak height at 10-minute intervals at 712 cm^{-1} wavenumber.
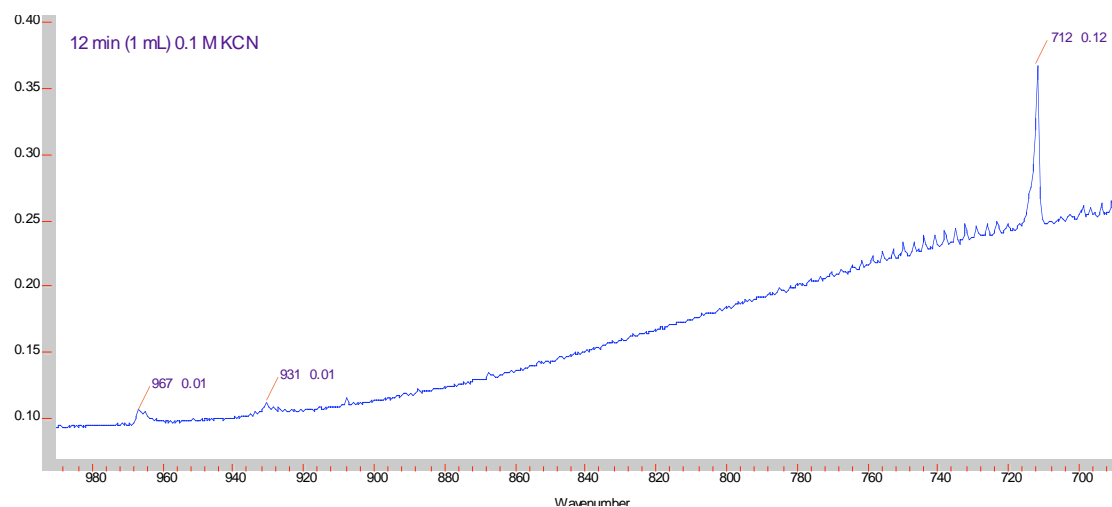
Figure 23: IR spectroscopy scan for hydrogen cyanide gas showing a 712 cm^{-1} wavenumber for HCN and two small peaks at 967 cm^{-1} and 931 cm^{-1} that were identified as ammonia by the distance between the peaks according to Wang et al. (Wang et al. 2007).

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