



Instrument development for piezoelectric detection of the aqueous cyanide ion
by Cassandra Joy Graber

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in
Chemistry

Montana State University

© Copyright by Cassandra Joy Graber (1995)

Abstract:

Cyanide is present in many industrial effluents; however, standard methods of detection are often inadequate for regulating effluent waters. Piezoelectric cyanide detection as described in this paper presents an attractive alternative to the standard methods for cyanide determination due to its high sensitivity, simplicity, and lack of interference. A system was designed and built for cyanide detection. Electronic noise is eliminated by mixing two high frequency sine waves to produce a low frequency sine wave as input into a frequency counter. A reference crystal in the same environment as the sampling crystal is used to correct for environmentally-induced noise. Hardware used for building the instrument was obtained at low cost. The instrument frequency output is extremely stable over time. Analysis of this system's capabilities show this system is capable of ionic cyanide determinations in the sub-ppm range. Sensitivity is limited by poor reproducibility from the method of sampling, not by instrument capabilities.

INSTRUMENT DEVELOPMENT FOR PIEZOELECTRIC DETECTION OF THE
AQUEOUS CYANIDE ION

by

Cassandra Joy Graber

A thesis submitted in partial fulfillment of the requirements for the degree

of

Master of Science

in

Chemistry

MONTANA STATE UNIVERSITY
Bozeman, Montana

December 1995

N378
G 7524

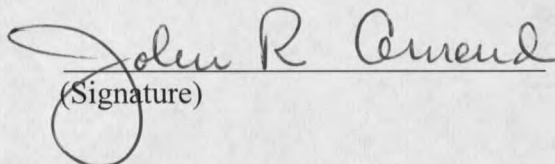
APPROVAL

of a thesis submitted by

Cassandra Joy Graber

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

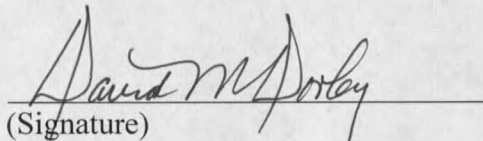
Dr. John Amend


(Signature)

JAN 18, 1996
Date

Approved for the Department of Chemistry

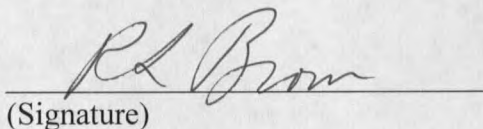
Dr. Dave Dooley


(Signature)

1/18/96
Date

Approved for the College of Graduate Studies

Dr. Robert Brown


(Signature)

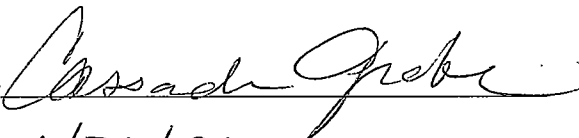
2/12/96
Date

STATEMENT OF PERMISSION TO USE

In presenting this thesis (paper) in partial fulfillment of the requirements for a master's degree at Montana State University, I agree that the Library shall make it available to borrowers under rules of the Library.

If I have indicated my intention to copyright this thesis by including a copyright notice page, copying is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright Law. Requests for permission for extended quotation from or reproduction of this thesis in whole or in parts may be granted only by the copyright holder.

Signature



Date

1/26/96

TABLE OF CONTENTS

	Page
CHAPTER 1: INTRODUCTION	1
Background.....	1
Industrial Use and Implications.....	2
Standard Methods of Cyanide Detection.....	4
Advances in Cyanide Detection.....	6
Quartz Crystal Microbalance.....	9
Theory.....	9
QCM Cyanide Detection.....	11
Literature Review.....	13
STATEMENT OF RESEARCH OBJECTIVE.....	16
CHAPTER 2: INSTRUMENT DEVELOPMENT.....	17
Initial Attempts.....	17
Single Crystal	17
Environmental and Aging Effects.....	20
Dual Crystal System for Corrected Frequency.....	22
Time Base Considerations.....	25
Characteristics of System Design.....	26
CHAPTER 3: EXPERIMENTAL.....	27
Data Collection.....	27
Instrument Stability.....	28
Sampling.....	29
Reagents.....	32
Noise from Sampling Procedure.....	33
Precision and Calibration	34
CHAPTER 4: RESULTS AND DISCUSSION.....	36
Instrument Stability.....	36
Response to Sampling Procedure.....	41
System Performance for Cyanide Determination.....	51
Observations Concerning Sampling and Reproducibility.....	58

CHAPTER 5: CONCLUSIONS	61
Conclusions.....	61
Instrument Stability.....	62
Response to Sampling Procedure.....	62
System Performance for Cyanide Determination.....	63
Observations Concerning Sampling and Reproducibility.....	64
Suggestions for Further Work.....	65
BIBLIOGRAPHY	66
APPENDIX	70

LIST OF TABLES

Table	Page
1. Sampling procedure.....	31
2. Frequency shifts for one and two crystals:.....	42
3. Signal to noise ratios	49
4. Data published by Rosentreter and Bunde of frequency shift from cyanide exposure to a silver-plated crystal.....	50
5. Data from table 3.....	51
6. Data from calibration curve presented in Figure 26.....	57
7. Statistical analysis of calibration data showing variation in number of observations.....	57
8. Statistical analysis of calibration data showing variations in frequency shift.....	57

LIST OF FIGURES

Figure	Page
1. Cyanide leaches gold from a gold-plated piezoelectric crystal.....	12
2. Block diagram and pictorial representation of a single crystal oscillating system.....	18
3. Example of frequency output for a single crystal system.....	20
4. Schematic of a dual-crystal piezoelectric system.....	23
5. Demonstration of frequency difference output.....	24
6. Demonstration of polling error induced with computer software.....	25
7. Schematic of complete dual-crystal system.....	26
8. Sampling schematic.....	30
9. Valve-controlled wash and sample positions.....	32
10. Frequency increases and then levels off after turning on the instrument.....	36
11. Frequency increases with an increase in temperature inside the instrument.....	37
12. Stability of the frequency difference output using a reference crystal to correct for environmentally-induced noise.....	38
13. Over time the frequency drifts up, but is stable to 4.13 Hz out of 10 MHz.....	38
14. Example of the correction capabilities for temperature using a reference crystal and monitoring the frequency difference of two crystals	40
15. Removing and replacing the crystal disturbs the fundamental frequency.....	41
16. When both crystals are removed and replaced, the extent of frequency shift is less.....	42
17. Increasing trend for frequency from drying crystal with compressed air.....	43

LIST OF FIGURES (CONTINUED)

Figure	Page
18. Scatter of frequency shifts induced from drying crystal	43
19. Initial changes in frequency for exposing crystal to water	44
20. Contrasting frequency shift behavior for drying and wetting/drying crystal.....	45
21. Scatter graph showing 23 frequency shifts from removing, wetting and drying a sample crystal.....	46
22. Instrument drift, blank response, and response to 5-minute exposure to 2 ppm cyanide.....	47
23. Demonstration of a blank shift observed in Figure 22	48
24. Demonstration of cyanide-induced frequency shift observed in Figure 22.....	48
25. Repeatability of leaching process under ambient temperature conditions.....	52
26. Frequency shifts for repeated treatment at constant temperature.....	52
27. Seven frequency shifts for a constant treatment of cyanide.....	53
28. The relationship between cyanide concentration and frequency shift is linear to 1 ppm.....	55
29. The relationship between cyanide concentration and frequency shift is linear to 1 ppm. The calibration is different for a different order of crystals.....	55
30. This relationship between cyanide concentration and frequency shift curves off fitting a polynomial equation for concentrations above 1 ppm.....	56

ABSTRACT

Cyanide is present in many industrial effluents; however, standard methods of detection are often inadequate for regulating effluent waters. Piezoelectric cyanide detection as described in this paper presents an attractive alternative to the standard methods for cyanide determination due to its high sensitivity, simplicity, and lack of interference. A system was designed and built for cyanide detection. Electronic noise is eliminated by mixing two high frequency sine waves to produce a low frequency sine wave as input into a frequency counter. A reference crystal in the same environment as the sampling crystal is used to correct for environmentally-induced noise. Hardware used for building the instrument was obtained at low cost. The instrument frequency output is extremely stable over time. Analysis of this system's capabilities show this system is capable of ionic cyanide determinations in the sub-ppm range. Sensitivity is limited by poor reproducibility from the method of sampling, not by instrument capabilities.

CHAPTER 1: INTRODUCTION

Background

The US Department of Health and Human Services describes cyanide as “one of the most rapidly acting poisons” (US 1991). The Agency for Toxic Substances and Disease Registry included cyanide in their list of the “hazardous substances most commonly found at superfund waste sights and which pose the most significant potential threat to human health” (Oak Ridge National Laboratories 1989). Cyanide can be inhaled, ingested, or absorbed through the skin. Lethal oral ingestion in humans has been reported as 0.56 mg/kg and death by inhalation can occur with exposure to 90 ppm hydrogen cyanide. Long term (5-15 years) exposure to concentrations as small as 6.4 ppm of hydrogen cyanide have been reported to cause serious health problems for the nervous and cardiovascular systems (El Ghawabi 1975). In freshwater, cyanide concentrations as low as 7.85 µg/L can cause death for certain species of fish (EPA 1986).

The cyanide ion is used extensively in industrial applications including the metal finishing and mineral processing industries; consequently, cyanide and cyanide complexes appear in the rinse water, effluent, gas scrub water, and other waste waters associated with these industrial processes. Severe toxic effects of cyanide and stringent environmental regulations present a serious waste management problem for these industries. Industrial efforts for regulation and treatment of cyanide-contaminated

effluents require dependable analytical procedures which can aid in monitoring and help provide a better understanding of the water chemistry associated with cyanide-containing waste.

Industrial Use and Implications

The cyanide ion consists of one carbon and one nitrogen triple-bonded together with a negative charge; it is polar and electropositive. The water chemistry associated with cyanide is complicated due to its high reactivity. Hydrogen cyanide, also referred to as molecular cyanide or hydrocyanic acid, is a gas and has a pKa of 9.31 at 25° C (CRC 1983) and therefore is present in aqueous cyanide-containing waters at a pH below 9.3. Simple and complex metal cyanides are present in many aqueous cyanide-containing waste. Simple cyanides are compounds containing the cyanide ion and following the formula: $A(CN)_x$ or $M(CN)_x$, where A is an alkali earth and M is a transition metal. Cyanide bonds ionically with the alkali earth metals, such as sodium and potassium, to form simple cyanides. In the presence of transition metals cyanide acts as a ligand, complexing with the metals to form compounds of high stability. Complexes contain both an alkali metal and a metal, the metal being iron, chromium, silver, or others (McGivney 1985). Some examples of cyanide complexes are $K_3[Cr(CN)_6]$ and $K[Au(CN)_2]$.

The unusual properties of cyanide make it a desirable reagent for industry. Over 350,000 tons of cyanide are produced in the United States each year for industrial

applications ranging from electroplating to the manufacturing of dyes and pigments (EPA 1988).

The usefulness of cyanide and its affinity for metals is exploited by the mineral processing industry. During a process called leaching cyanide is used to bring gold and other precious metals from low-grade ores into aqueous solution. Cyanide forms a soluble complex with the gold (Equation 1):



Gold present as sulfides, arsenides or tellurides requires tank leaching to maximize gold recovery by cyanide. After the precious metals are recovered both the cyanide and the tailings go to the waste pond (Von Michealis 1985).

As with many hazardous and highly utilized chemicals, cyanide poses a waste management problem for the mining industry and others who must dispose of cyanide-containing waste. Cyanide is regulated under the Clean Water Act Effluent Guidelines for many industrial point sources such as organic chemicals, inorganic chemicals, iron and steel manufacturing, mineral mining, aluminum forming, and others (Oak Ridge National Laboratories 1989).

The role of cyanide as a complexing agent for metals not only explains its industrial benefits, but its toxicity as well. Cyanide forms stable complexes with ferric iron, causing it to bind to cytochrome oxidase, the terminal component in the electron transport chain for the mitochondria. This prohibits the enzyme's ability to catalyze the reduction of oxygen by reduced cytochrome, resulting in impairment or cessation of oxygen respiration and possibly death by respiratory collapse (Way 1984).

The EPA Water Quality Criteria has set a limit for 4-day average natural water cyanide concentrations to not exceed 5.2 $\mu\text{g/L}$ more than once every three years. For salt water this value is even lower at 1.0 $\mu\text{g/L}$ (EPA 1986). Maximum recommended contamination level for cyanide is 0.01 mg/L in drinking water; rejection for treatment of raw water supplies is 0.2 mg/L (EPA 1980). Because of the difficulties involved in detecting cyanide, the maximum recommended levels are unfortunately similar to the sensitivity limits for the EPA-recommended standard methods of cyanide determination.

Standard Methods of Cyanide Detection

EPA-recommended standard methods for cyanide determination include colorimetric, titrimetric, and electrochemical methods (including potentiometry and the use of cyanide selective electrodes). Total cyanide analysis includes all free and complexed cyanides, but only free cyanide is capable of being determined by these three methods. By known methods, complexed cyanides can be further classified as weak complexes (weak-acid dissociable), moderate complexes (cyanides amenable to chlorination) or strong complexes (Standard Methods 1989). An acid distillation process outlined by standard methods as a required pretreatment serves to break these complexes down and allow for quantification of ionic cyanide.

The EPA recommended distillation also serves as a removal scheme for a number of interferences associated with the three determination methods. Unfortunately, this treatment can further complicate the effect of interferences. Acid distillation conditions produce erroneous results from colorimetric and titrimetric methods when any of the

following are present: Sulfide, nitrate, nitrite, thiocyanate, aldehydes, ketones, and certain oxidizing agents. In addition, if fatty acids are present they will co-distill and form soaps under alkaline titration conditions. Most serious interferences are caused by sulfide as well as thiocyanate and thiocyanate complexes which decompose to cyanide upon distillation. In subsequent determination steps sulfide ions give a positive interference for cyanide-selective electrodes. Sulfide ions can be air oxidized to polysulfides which react with cyanide to form thiocyanide (Oak Ridge National Laboratory 1989).

In addition to the interference problems, the EPA-recommended standard methods of cyanide determination are time-intensive and lack selectivity and/or adequate sensitivity. For example, in a study by Goulden and co-workers the colorimetric and titrimetric method recommended by ASTM did not allow cyanide to be quantitatively recovered in concentrations below 0.1 mg/L. In an effort to automate a cyanide detection method, Goulden determined that the release of molecular cyanide from an acidified solution is a relatively slow process and the distillation equipment commonly available for automated analysis does not provide satisfactory results below 0.2 mg/L (Goulden 1972).

To circumvent these problems considerable attention has been devoted to improving the overall effectiveness of metal-cyanide complex digestion and to finding alternatives to the titrimetric, colorimetric and electrochemical determination schemes. Following is a brief outline of the effort and creativity applied to this area of analytical chemistry.

Advances in Cyanide Detection

Over the past 20 years much effort has been devoted to the improvement of standard methods or development of new cyanide determination schemes. This is a result of the difficult analytical problems associated with the standard methods and because of the demand by industry and others for an efficient, specific and sensitive method.

“Only recently has there been much interest and progress in the development of reliable and sufficiently sensitive analytical methods for the determination of free cyanide, molecular cyanide, or cyanide ions in waters containing cyanide complexes of widely varying stability. Although several methods have now been developed and published, they are still little known and apparently have been profitably employed... it has long been obvious that the total cyanide determinations made and reported routinely in the past are often insufficiently instructive, if not meaningless, as measures of water quality” (Doudoroff, 1976).

The preparative distillation procedure outlined by standard methods allows for total cyanide analysis and serves to remove the cyanide from complex matrices, but it requires one hour of strong acid distillation. In an effort to improve the efficiency of this step, sequestering agents such as EDTA (Csikai 1983), Tiron and TEP (Inersoll 1981) have been added to displace cyanide from stable cyanide complexes with varying success. One advantage of adding sequestering agents is that the pH can be kept moderate during the distillation, avoiding the dissociation of SCN^- which would otherwise give a positive interference for cyanide. In some cases the distillation time can be shortened to 1/2 hour.

Efforts have been applied to the use of UV irradiation to induce photodecomposition of the cyanide-metal complexes. Kelada successfully developed an

automated method for total cyanide analysis using UV irradiation and following with acidification and thin film distillation (Kelada 1989). Rosentreter demonstrated the efficiency of this method, documenting up to 100% dissociation for all cyanide-metal complexes except ferrous cyanide in 33 minutes exposure time (Rosentreter 1991).

Sodium hypophosphite was added to prevent photo-oxidation of the free cyanide ion to cyanate under UV conditions.

In 1984 the development of resin-based anion-exchange column allowed the use of high pH eluents, extending the usefulness of ion chromatography to anions with pKa values greater than 7.0. Cyanide had been previously difficult to analyze because it is only weakly dissociated below a pH of 9.3. With the alkaline capabilities of ion chromatography, cyanide can be effectively separated from sulfide and other anions and detected via electrochemical detection (Thomas 1984).

Another method employs the use of Chloramine-T to oxidize cyanide to cyanogen chloride which is then extracted with hexane and quantified by electron capture detection. Detection limits are 0.25 ppm (Oak Ridge National Laboratory 1989).

Recent papers have reported the use of Surface-enhanced Raman spectroscopy (SERS) for the direct detection of trace cyanide in groundwater and wastewater. Cyanide has a linear response from 100 ppm to 10 ppb, with an estimated detection limit of 8 ppb (Shelton 1994). An advantage is that cyanide can be detected in high ionic solutions such as that of groundwater.

Detection limits in the sub part-per-billion range were achieved using an indirect atomic absorption method (Rosentreter 1991). In a flow-through configuration cyanide

solutions were treated with silver and subsequent analysis for AgCN allowed for quantification of the cyanide ion.

Enzyme-based amperometric sensors have been developed for the detection of cyanide utilizing its inhibitory effect in the electron transport process. The loss of the enzyme's ability to reduce oxygen in the presence of cyanide translates to a loss of current. The catalytic activity of the enzymes is very specific for cyanide, further eliminating the effect of interferences found in other detection methods. Smit, et. al., reported the use of horseradish peroxidase in a dual-working electrode which attained a sensitivity to cyanide at sub-ppb concentrations (Smit 1990). The enzyme tyrosinase has been incorporated into an electrode which detects the presence of hydrogen cyanide in air (Rechnitz 1993).

A simple cyanide determination method using the reaction of cyanide with hemoglobin was explored (Tomoda 1991). The change in absorbance of methameoglobin due to binding with cyanide are correlated to the concentration of cyanide, with sensitivity down to 2 ppm.

Many of these methods are extravagant and costly, labor intensive, and some still suffer from poor sensitivity. Sophisticated instrumentation and expertise required for atomic absorption spectroscopy and raman spectroscopy is not always available to industry. While some techniques have marked advantages such as specificity or automation capability, there remains a need for an inexpensive, simple, and most importantly, sensitive determination method for aqueous cyanide. It is envisaged that the quartz crystal microbalance and its piezoelectric effect, as described by Nomura, Bunde

and Rosentreter, can be used to establish such a method for cyanide determination (Nomura 1980, Bunde 1993).

Quartz Crystal Microbalance

Theory

In 1880 Jaques and Pierre Curie found that when pressing or applying a mechanical strain to a crystal, an electric potential is generated from the displacement of crystalline atoms and subsequent formation of dipoles. This phenomenon was named the piezoelectric effect, using the Greek root word *piezein* (to press). The Curie brothers experimented with the converse of this effect as well, in which the application of an electric field causes a mechanical strain on the crystal (Ward 1990).

The application of an electric field normal to the surface of the crystal results in what is called shear deformation. This phenomenon results from the individual structures of the crystal being shifted as stress is applied in one direction, then the other. The electric field is confined to the area between the two excitation electrodes and causes a vibration analogous to transverse waves traveling in a string of length (L). The string resonates when the applied frequency matches one of the fundamental frequencies (f_0) which is dependent on the mass per unit length (m_l) by the following equation where S is the tension on the string (Ward 1990):

$$f_0 = (S/m_l)^{1/2}/2L \quad (2)$$

The dependency of the frequency on the mass per unit length translates to a dependency on the density of quartz, and the previous equation can be re-written to include the shear modulus (μ) and density (ρ) (Equation 3):

$$f_0 = (\mu/\rho)^{1/2}/2T_q \quad (3)$$

An increase in the crystal thickness (T_q) results in an increase in the wavelengths ($\lambda = 2T_q$), decreasing the fundamental frequency. An increase in the mass without a change in the thickness of the crystal results in an increase in mass per unit length or density, and a subsequent decrease in the fundamental frequency.

Properties of the crystal such as the angle of cut, water solubility and temperature resistance affect piezoelectric oscillation. With these properties taken into account, instrumental use of a piezoelectric crystal detector is limited to alpha crystal, and the AT-cut is used for its superior temperature coefficient and mass sensitivity (Czanderna 1984). Typical oscillation frequencies are on the order of 1 to 10 megahertz (MHz) (Bard 1991).

Saurbrey was the first to set up a series of metal deposition experiments and prove the relationship between the fundamental frequency of oscillation of the crystal and its mass (Saurbrey 1959). The change in frequency due to the coating of metal (Δf in Hz), the frequency of the quartz plate (f in MHz), the mass of the deposited coating (Δm in g), and the area coated (A in cm^2) all need to be considered (Equation 4):

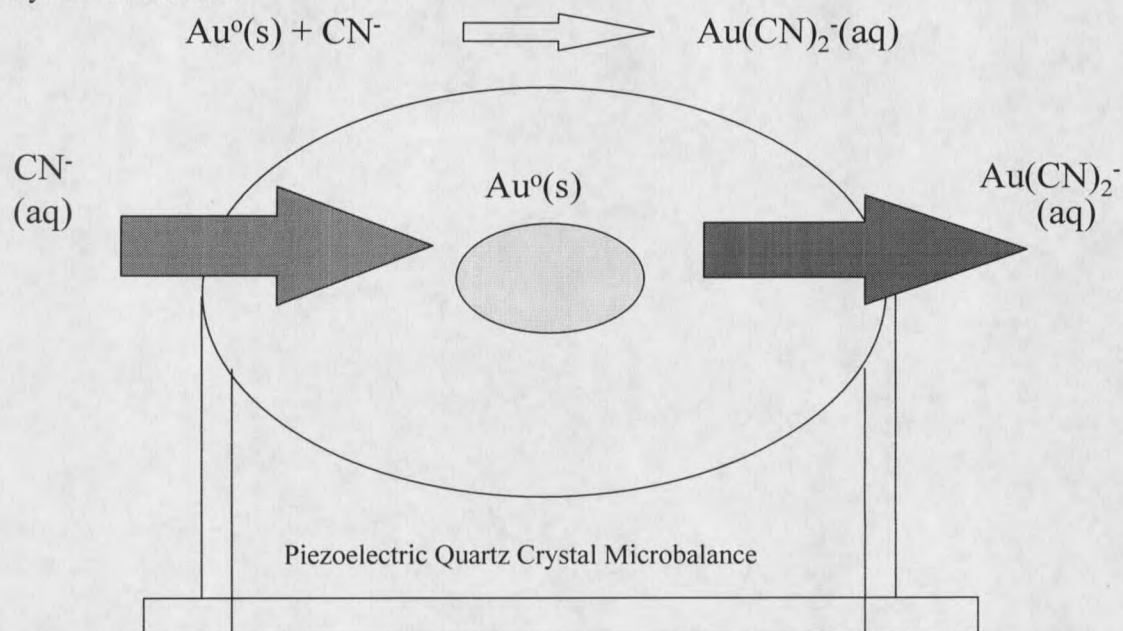
$$\Delta f = -2.3 \cdot 10^6 f^2 \Delta m / A \quad (4)$$

This equation presented is ideal, assuming any change of mass is a change in mass of the crystal itself (Czanderna 1984). According to this equation, a change in mass of 1 nanogram would result in a frequency change of 0.4 Hz for a 9 MHz crystal, or 2.6 Hz for a 15 M Hz crystal. The potential mass sensitivity of this device is therefore apparent. The now widely-used piezoelectric oscillating crystal is commonly referred to as a quartz crystal microbalance (QCM).

QCM Cyanide Detection:

Piezoelectric crystals are used almost exclusively for the detection of some mass gain or thin film deposition. An exception is the use of piezoelectric crystals for cyanide detection. A crystal plated with gold or other metal that complexes with cyanide will lose some of its mass upon exposure to cyanide by the same principle of cyanide leaching explained earlier as used in the mineral industry. This loss of plated gold translates to an increase in frequency proportional to the amount lost and; therefore, the amount of cyanide present (Figure 1).

Figure 1. Cyanide leaches gold from a gold-plated piezoelectric crystal and decreases the mass of the crystal, causing the fundamental frequency of oscillation exhibited by the crystal to increase.



In general, sampling or exposure of the crystal to cyanide is accomplished separately by dropping the cyanide solution on the crystal surface, immersing the crystal in a beaker of aqueous cyanide, or passing aqueous cyanide across the surface of the crystal in a flow cell. The frequency is monitored prior to sampling and the difference in frequency before and after sampling is considered the frequency shift due to mass loss by cyanide.

Literature Review

In 1980 Nomura and coworkers published a paper describing the application of a piezoelectric crystal for the detection of cyanide (Nomura 1980). Determination of cyanide was performed with an inexpensive single silver-plated crystal and oscillator system. The cyanide leached the silver from the silver-plated crystal by the same mechanism described earlier for gold. The frequency change was monitored by a digital counter and recorder. Since the system did not correct for temperature, the crystal, crystal holder, and oscillator were set in a thermostated air bath at 30° C.

The sampling procedure was as follows: Transfer a constant volume of 50 mL of standard solution adjusted to pH 9.3 to a beaker immersed in a water bath at 25 oC. Stir the solution at a constant rate (430 rpm) and immerse the crystal (frequency had previously been monitored) for exactly 15 minutes. Remove the crystal, wash it with water and then acetone. Set the crystal and oscillator in the air bath and measure the frequency after 1 minute.

Nomura claimed the calibration graph for cyanide concentration vs. frequency change to be linear over the range of 1×10^{-7} to 1×10^{-5} M. The standard deviation was 15.5 Hz (3.6%) for 5 determinations of 5×10^{-6} M cyanide with the same crystal. With a different crystal for each determination the standard deviation was 17.9 Hz (4%).

Interference effects were insignificant or non-existent for ions such as nitrate, perchlorate, carbonate, sulfate, aluminum, chromium, iron or ammonium. Iodide, thiosulfate and sulfide interfered by forming silver salts on the electrode. Metals such as lead (II), cadmium (II) and copper (II) and others interfered by forming stable cyanide

complexes, but most metals could be completely masked by adding EDTA to the sample solution.

In 1993 Bunde and Rosentreter (Bunde 1993) reported cyanide detection with a piezoelectric crystal in which a commercial microprocessor QCM unit was used. The commercial unit corrected for temperature and barometric pressure by subtracting the output of a reference crystal from a sampling crystal.

Bunde and Rosentreter used both gold and silver plated piezoelectric crystals and exposed the crystal to cyanide via two methods: a single-drop method and a flow cell method. The single drop method employed very small sample sizes and a 5 minute sample time. The flow cell method used a crystal mounted at 45° to the horizontal. The sample was introduced to the surface of the crystal for 15 minutes at a flow rate of 0.34 mL/min. Blank solutions (distilled water adjusted to pH 12) were run between each sample solution.

2 ppm solutions were used to test the precision of the experimental procedure. An average change in frequency was calculated to be 971 Hz and the RSD (relative standard deviation or coefficient of variation) as 3.7%. Bunde and Rosentreter claimed the precision of the system to be dependent on flow rates. For flow rates of 0.17 and 0.41 the RSD was calculated as 35% and 19.8% respectively. For use with a silver electrode the RSD was calculated as 14%.

Interference tests were performed with 100 ppm solutions. Results were similar to those for Nomura except that thiocyanate and iodide were not found to interfere with the gold plated crystals.

The cost of the commercial system used for these experiments is currently around \$7,000 (Universal Sensors price list). Fluctuations in power or other electronic noise which often interfere with high frequency electronics caused instrumental drift and instrument drift made analysis difficult. All experiments were conducted in a faraday cage, and even radiation from the lights was suspected to induce fluctuations in baseline frequency (Rosentreter).

Statement of Research Objective

The goal of this research is to design and develop a cost-effective, sensitive, stable instrument for piezoelectric cyanide detection. Construction of a dual crystal oscillating system that can be controlled with a laboratory interface and computer will be undertaken. The heterodyne principle will be used to mix the high frequency signals from the reference and sampling crystal in a manner which allows for maximum stability and correction of environmental parameters. The reference crystal will be configured to precisely gate the sampling period, in an effort to stabilize and simplify the piezoelectric system, and allow for the use of the interface counter without introducing additional error into the output. Experiments will be conducted to assess the stability and extent of correction of this instrument and the capability for cyanide detection. These experiments include the following: monitoring the output as a baseline over time; comparing temperature response of dual crystal system vs single crystal system; designing a sampling protocol and assessing noise induced from the protocol; determining the precision of the system for a constant treatment with cyanide; and calibrating the system output with cyanide concentration.

CHAPTER 2: INSTRUMENT DEVELOPMENT

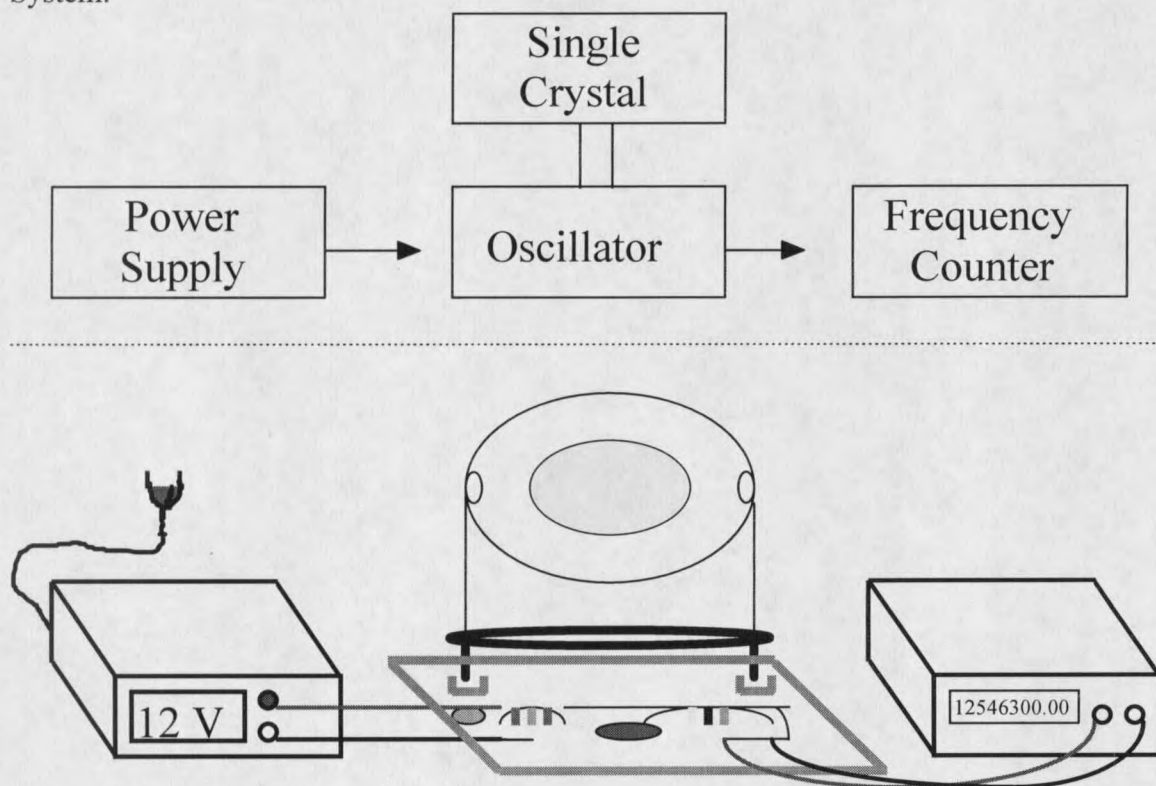
This chapter outlines some problems we encountered in developing a piezoelectric system. Two major modifications were made to the initial system and to instruments used previously for piezoelectric cyanide detection. The first is the use of the heterodyne principle for subtracting external noise. Second is the use of the reference crystal as a gate for the frequency counter.

Initial Attempts

Single Crystal

Initially we conceived and built the simplest working piezoelectric device (Figure 2). This is the type of system used by Nomura for cyanide detection (Nomura 1980), consisting of a crystal and an oscillator which applies a voltage to the crystal, and a frequency counter to monitor the oscillating crystal. A thermostated air bath to contain the apparatus was not available and was unwieldy to use in the possibility of a field situation.

Figure 2. Block Diagram and Pictorial Representation of Single Crystal Oscillating System.



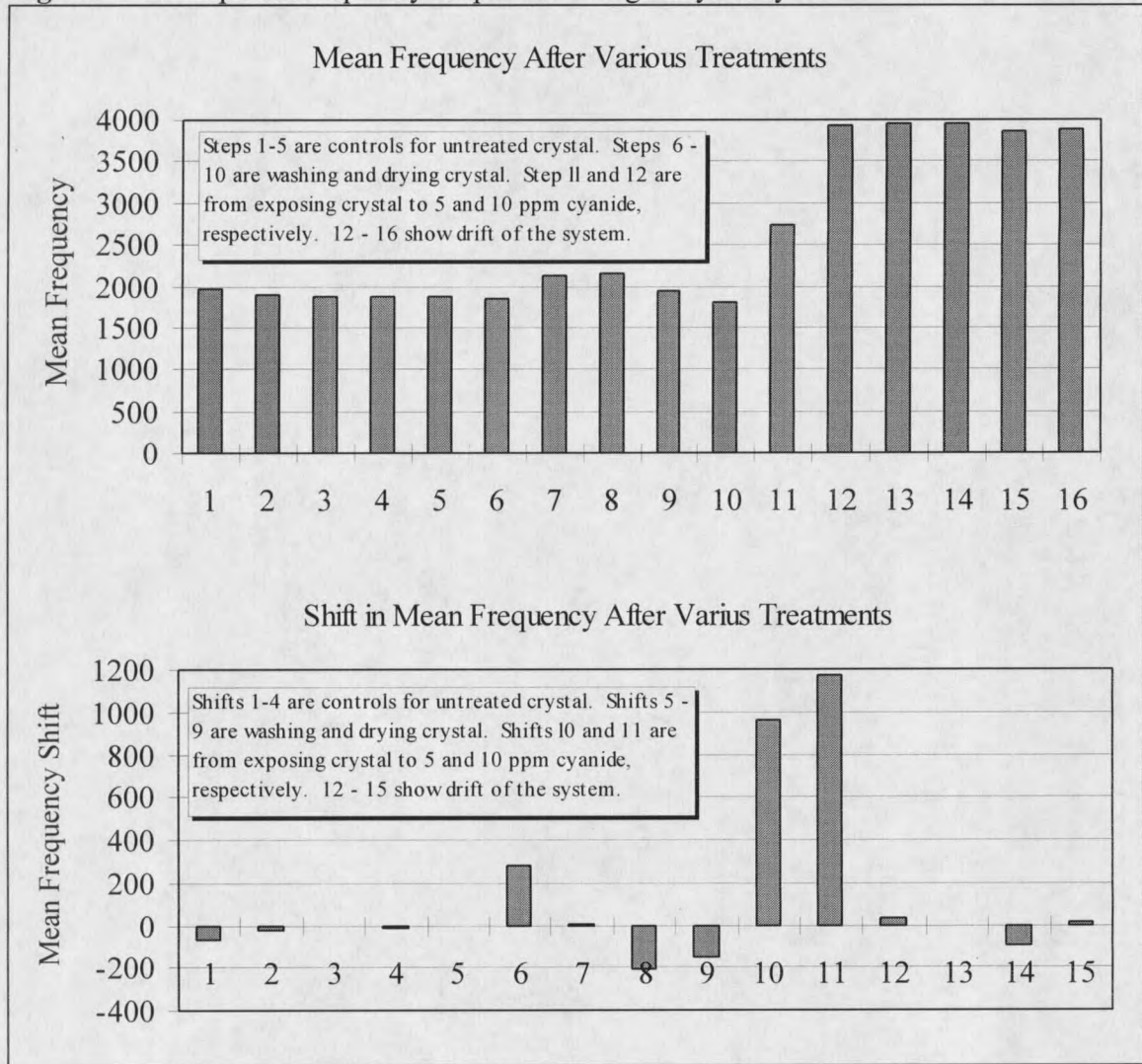
The frequency was counted either by a computer interface or by a conventional frequency counter. The conventional frequency counter was much more stable than the interface counter, drifting as little as 10 Hz out of 10 MHz over 120 minutes. The signal, however, appeared to be very susceptible to noise and it was cumbersome to record data manually.

The frequency output when using the interface counter was erratic and unpredictable, even the direction of drift after turning on the instrument was not constant. A stable baseline could not be achieved; standard deviations for 30 minutes were as high

as 17 Hz, and overnight experiments produced drifts in the order of 100 Hz. Following is a graph of mean frequencies measured with the interface after doing such things to the crystal as unplugging it, washing and drying it, exposing it via a drop on the surface to 5 and 10 ppm cyanide for 3 minutes (Figure 3). The mean values are from monitoring the frequency counts for a few minutes and determining a mean value. The recorded frequency is a few KHz because of limitations with high frequency counting by the interface and electronic manipulation of the high frequency signal. Standard deviations are in the order of 8 to 13 Hz.

The frequency output shifted up to 100 Hz (data point 14) just from drifting. Wetting and drying the crystal produced shifts in frequency that varied by a standard deviation of 217 Hz. When looking at signals around 1000 Hz for exposure to cyanide this background noise seemed unacceptable. Furthermore, the frequency shift did not double as would be predicted from the relationship defined by Sauerbrey (Equation 1) for sampling with 5 and 10 ppm cyanide. A stable baseline and a sampling procedure which produced greater signal to noise ratio must be achieved to make a useful system.

Figure 3. Example of frequency output for a single crystal system.



Environmental and Aging Effects

AT-cut quartz crystals are subject to several effects which produce noise or other undesired responses (Parzen 1983). Slow changes with frequency over time are

considered aging effects. The most common effect is positive aging, in which the frequency rises slowly. This is probably due to contamination on the crystal surface being redistributed to the other portions of the enclosure while the crystal is in motion. For crystals that are sealed in an enclosure (most are, but not in our case) changes in barometric pressure outside the enclosure can stress the enclosure material.

Environmental effects from temperature are divided into at least three categories: static temperature effects, thermal transients, and thermal-frequency hysteresis. Thermal-frequency hysteresis is poorly understood and occurs when cycling a quartz resonator over an extended temperature range. Static temperature effects, or effects from gradual changes in temperature, are significant and can be defined by temperature coefficients of frequency. Thermal transients "can cause substantial frequency departures in high precision AT oscillators". In addition, thermal noise in the quartz crystal itself causes instability in frequency output.

Other environmental effects include drive level effects such as variations in power supply, acceleration effects (from shock, vibration, turning the oscillator on and off or tipping the crystal over and changing the attitude), and radiation effects. Two types of radiation effects usually distinguishable are collision processes due to neutrons and alpha particles and ionizing radiation from x-rays, gamma rays, and electrons. Effects of ionizing radiation are more pronounced for crystals with ions present as impurities. Ionizing radiation causes ions to move through the crystal within the quartz and produce frequency changes.

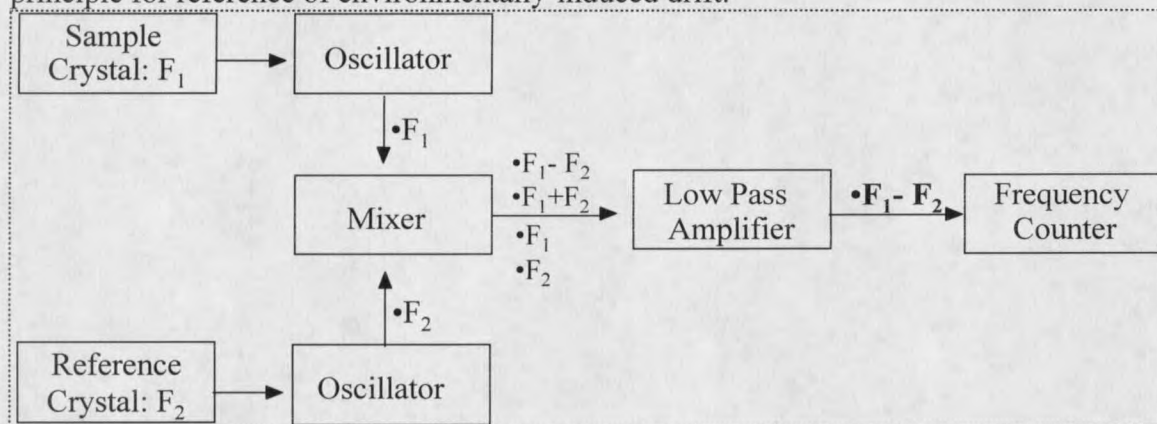
Dual Crystal System for Corrected Frequency:

It is possible to use two crystals which are placed with their respective oscillators in the same environment. Both crystals will experience frequency variations due to temperature and other external properties. One crystal may be used to sample with, and the other as a reference to environmentally-induced fluctuations in frequency. Thus, the only difference between the sampling and the reference crystal would be the frequency increase gained from sampling with cyanide. The difference between the two crystals is the corrected frequency output.

A dual crystal system incorporating a reference crystal correction was built by Universal Sensors, Inc. and used for cyanide detection. Although the piezoelectric oscillating system is electronically simple, a commercial dual crystal system is available for around \$7,000. It was deduced from the instruction manual and conversations with a company representative that this system uses expensive digital electronics to subtract the two frequency signals.

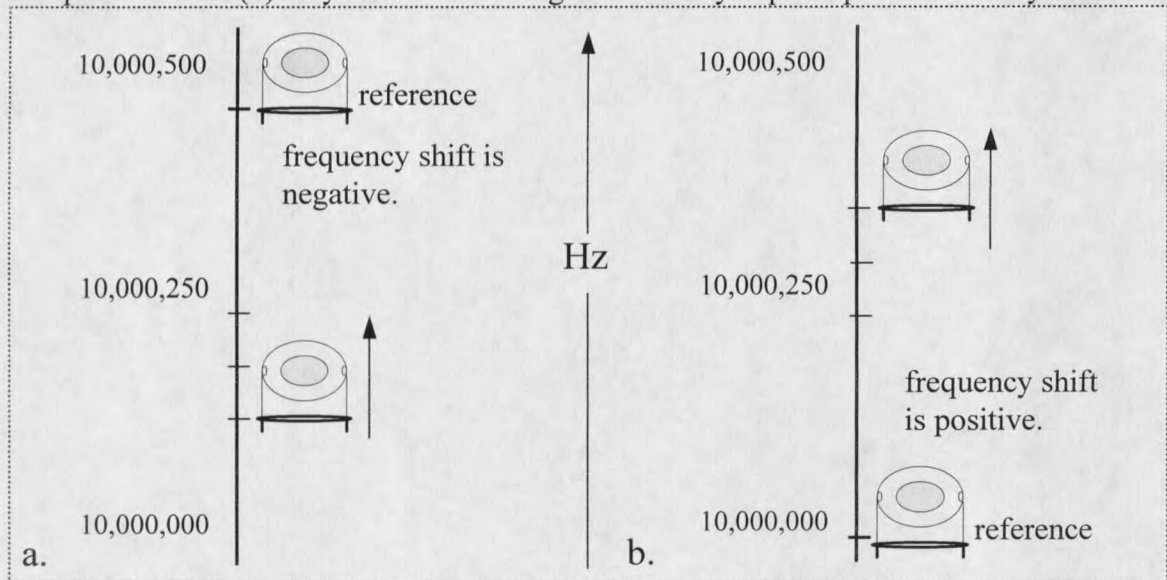
As an alternative to the use of expensive digital electronics a dual-crystal heterodyne mixing system was considered. The heterodyne principle is the mixing of two sine wave frequency signals in which the output is four frequencies: The addition, subtraction, and the two respective signals. This was configured into a piezoelectric device as follows, where F designates a frequency signal output (Figure 4).

Figure 4. Schematic of a dual-crystal piezoelectric system employing the heterodyne principle for reference of environmentally-induced drift.



The oscillator must put out a sine wave for heterodyne mixing to occur. A low pass amplifier serves to isolate the subtraction signal of the four signals. 10 MHz Crystals oscillate at around 10,000,000 Hz. For the heterodyne principle to work, one crystal must oscillate at a frequency at least slightly higher than the other crystal. When a mass change occurs, the frequency difference either increases or decreases from the original signal depending on whether the two respective frequencies are getting closer together or farther apart. The extent, and not the direction (positive or negative) of frequency change determines the frequency shift that is considered the instrument response to cyanide treatment (Figure 5).

Figure 5. Demonstration of frequency difference output. Negative frequency shift (a) and positive shift (b) may result from using the heterodyne principle and two crystals.



The frequency difference input is generally in the KHz range. There is less noise for this range as compared to a 10 MHz frequency input; yet any change in the 10 MHz frequency is reflected by the difference signal. In addition, the heterodyne mixer only accepts sine wave frequencies. Noise is not likely to be in the form of a sine wave, and the mixer serves as a filter to high-frequency noise. Therefore, the system has the stability of filtered low frequency input while maintaining the excellent sensitivity of a high frequency signal.

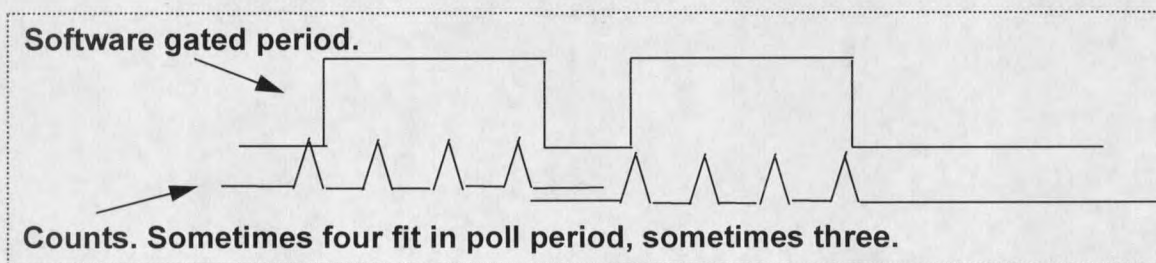
HSO-100 single stage oscillators with sine wave output were manufactured by International Crystal Manufacturing Co., Oklahoma City, OK. Crystals were 10 MHz, gold plated, obtained from Universal Sensors, Metairie, LA. A double balanced mixer

MS-83 obtained from Tele-tech Corporation, Bozeman, MT., was used to mix the sine wave output of the two crystals.

Time Base Considerations:

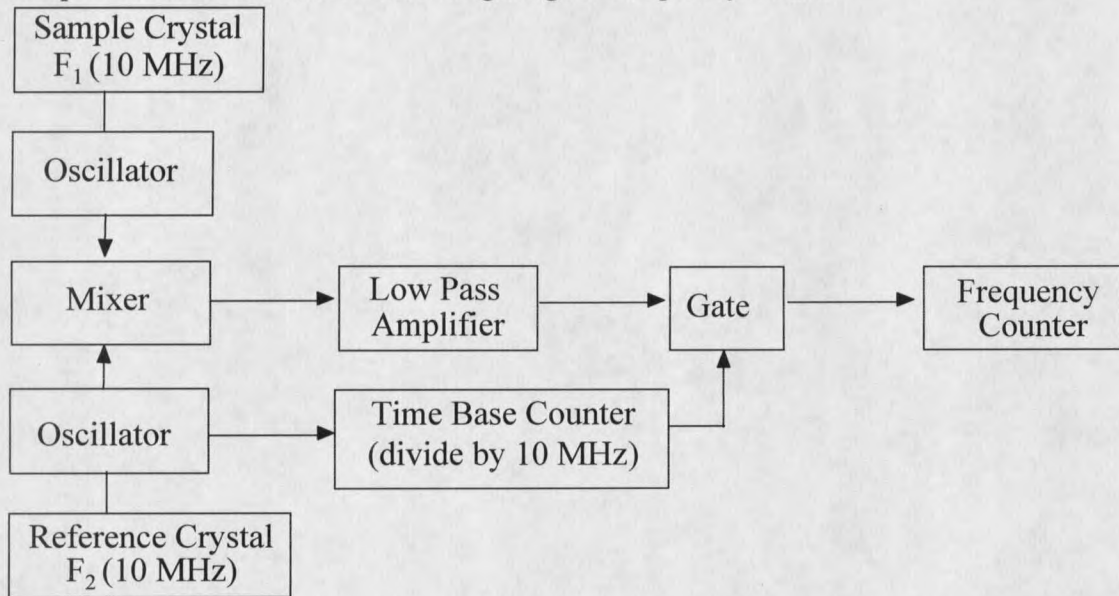
In the frequency counter a time base or additional counter is necessary to gate the counting period for the frequency input. For ease of data collection, the counter in a laboratory interface was used to record the frequency directly into a computer spreadsheet. However, this counter used software to gate the one-second time period required for recording Hz (counts per second) which induced a polling error, demonstrated as follows (Figure 6). Such a counter also introduced a third crystal for timing the gate period and its associated drift.

Figure 6. Demonstration of polling error induced with computer software.



If the computer software were used as a gate, the time period, and therefore the sampling period and frequency output, is not reproducible. To avoid this problem the reference crystal was used to gate the output. The reference crystal signal was divided from 10,000,000 Hz to 1 Hz. The time period was then accurate to at least 1.0000 seconds, gating a frequency difference signal in the KHz range or millisecond range.

Figure 7. Schematic of complete dual-crystal system employing the heterodyne principle and an internal time base for gating the frequency count.



Characteristics of the System Design (Figure 7):

- Simple: The system uses two crystals to accomplish sampling, reference crystal correction of environmental drift, and gating the frequency count.
- Stable: The heterodyne principle is used to mix the high frequency signals of two crystals in the same environment, the subtraction of the two frequencies is corrected for environmentally-induced drift. High frequency noise is filtered out by the heterodyne mixer, and the system output is a low frequency signal.
- Data collection: Data collection may be performed by a computer interface with no loss in stability of the gating period for counting the frequency.
- Inexpensive: Hardware costs for the instrument are around \$200.

CHAPTER 3: EXPERIMENTAL

This chapter is a discussion of the methods used to investigate the stability and capability for cyanide detection of the instrument designed in Chapter 2. Noise can be introduced into the system from two major sources: instrument instability or drift, and mass changes of the crystal not related to cyanide sampling.

Stability is inherent in the design of the instrument. Experiments were run to demonstrate instrument output drift with time and with extreme changes in temperature. The crystals are very sensitive to mass change, and for the procedure used in this thesis they are handled and exposed to water and 0.05 M NaOH solution (pH approximately 11). Experiments were run to determine the noise introduced into the system from sampling with the crystals.

The capability for cyanide detection investigated in this study includes repeatability for a similar treatment, calibration, and sensitivity. Experiments were run to obtain data for these parameters.

Data Collection

For all experiments a laboratory interface (SCI Technologies, Bozeman, MT) was used for counting the frequency output. The frequency count was actually a difference frequency output as explained in Chapter 2. For the remainder of the chapter, frequency difference output and frequency are used interchangeably.

The frequency difference output (frequency) was counted for one second and the counts were taken at 10-second intervals. Each count was imported directly into a spreadsheet. Frequency shifts were obtained for repeatability tests and for calibrating the frequency shift with cyanide concentration. Frequency shifts were observed as they occurred after sampling and logged into a lab book. In addition, the computer spreadsheet data was kept as raw data.

Instrument Stability

Warm-up Behavior, Drift

To determine warm-up behavior the frequency difference output was counted and recorded every 10 seconds until stabilization was achieved. In a second series of warm-up experiments, a thermistor was placed inside the instrument and the temperature recorded along with frequency. The frequency was monitored after stabilization by allowing a one to two hour warm up and then recording the one-second count (Hz) every 10 seconds for one to two hours. To determine long-term stability the frequency difference count was recorded every 10 minutes for 12 hours.

Temperature Effects

The effect of temperature on the frequency difference output for both crystals in the same dynamic temperature environment and for the crystals in different temperature environments was investigated with a thermostated air bath. The bath was comprised of a Styrofoam cooler to house a light bulb, fan, thermistor, and the appropriate electronics. A thermistor regulated a light bulb to turn on and off as needed for warming. A fan was

used for cooling and air circulation. The program written for running the air bath with the computer interface is included in the Appendix.

A single crystal was positioned in the air bath and connected to the oscillator in the instrument outside the bath with a cable. The air bath temperature was adjusted three times to 30, 34, and 38 °C. The crystal stabilized at each temperature for five minutes before counting the frequency difference output. The frequency was counted for two minutes at 10-second increments; there were 10 to 12 counts for each temperature point. Each experiment was run in triplicate.

Temperature of the crystals was the chosen parameter to investigate because the crystals could easily be placed in different temperature environments. According to Parzen (Parzen 1983) noise arising from the crystal itself is predominately associated with its temperature of operation, and this is the limiting factor for short-term stability.

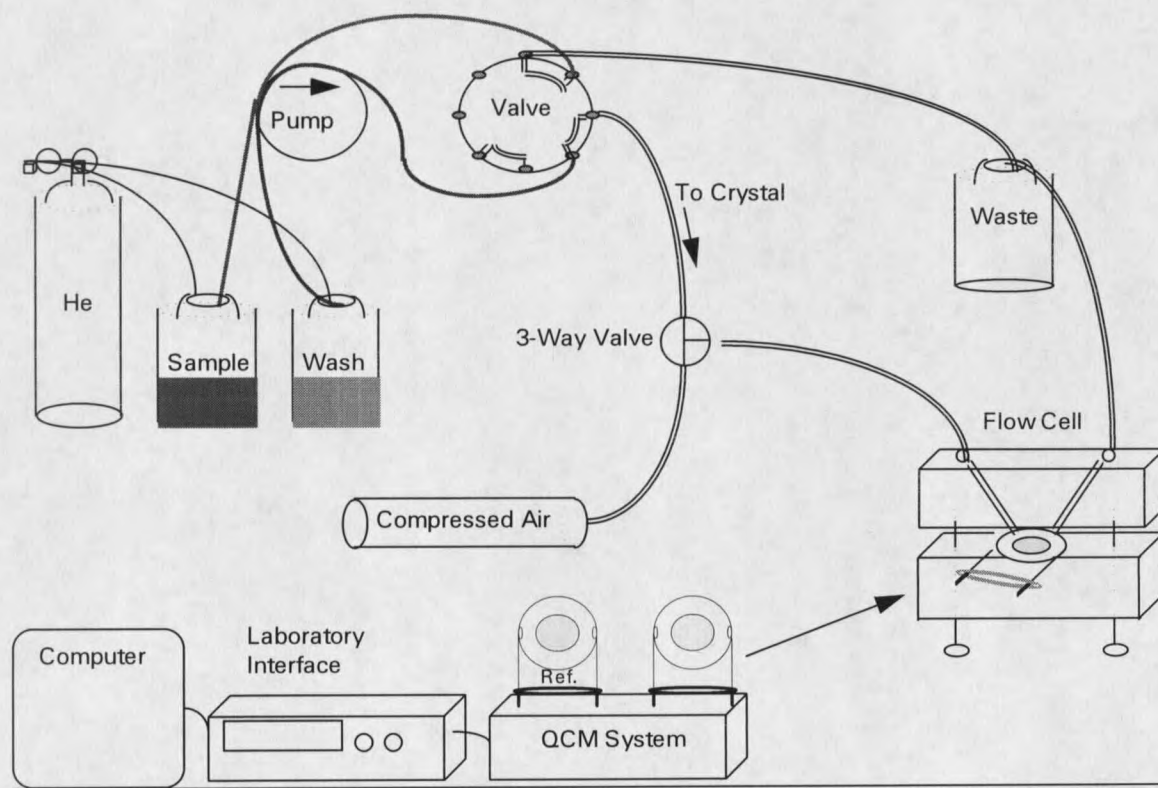
Sampling:

Sampling was performed only with the sampling crystal. A flow-through cell was implemented as the sampling device (Universal Sensors). In the cell the sampling crystal was secured between two o-rings, and wash or sample solution were passed directly over the surface of one side of the crystal. This method of sample delivery was chosen as opposed to methods used by previous authors because it could be adapted easily to an on-line situation. The sampling protocol for exposing cyanide to the crystals and monitoring the frequency shift was partially automated using a laboratory computer interface and

computer. A computer program written to accommodate the sampling steps is included in the Appendix.

The solutions were sparged with helium during sampling to rid them of carbon dioxide with formed bubbles in the flow lines and across the surface of the crystal in the flow cell. A peristaltic pump was used to pump the wash and sample solutions to the waste port or flow cell. Delivery of the wash and sample solutions was controlled with an 8-port air actuated valve (Valco, Inc.). The valve was switched using the laboratory interface. A solenoid, relay, and transformer were also incorporated so the cyanide sampling period could be timed between wash cycles. Compressed air was used to dry the crystal after sampling (Figure 8).

Figure 8. Sampling Schematic



The data acquisition and sampling procedure can be summarized in the following table (Table 1):

Table 1 . Sampling Procedure

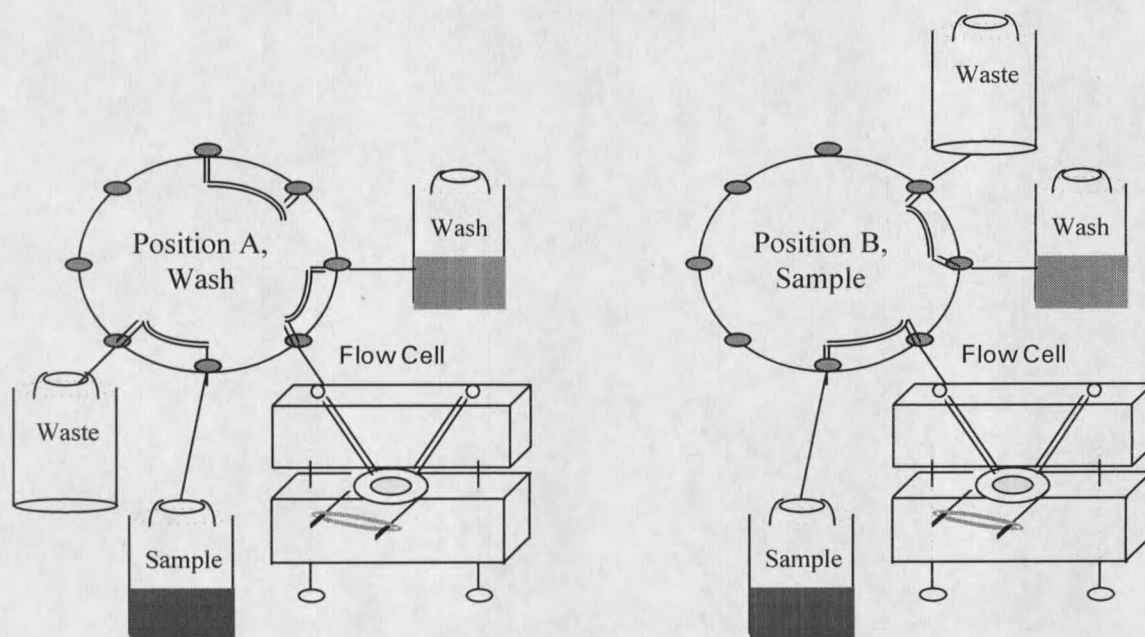
	<i>Step</i>	<i>Time</i>	<i>Computer Control</i>
1.	Frequency Difference Monitored	5 Minutes	Yes
2.	Crystal Inserted in Flow Cell		
3.	Crystal Pre-washed	2 Minutes	Yes
4.	Crystal Exposed to Sample Solution	15 Minutes	Yes
5.	Crystal Washed, Lines Cleaned	2 Minutes	Yes
6.	Crystal Dried with Compressed Air	3 Minutes	
7.	Crystal Inserted in System		
8.	Frequency Difference Monitored	5 Minutes	Yes

Sampling was performed as follows. The crystal was removed and placed in the flow cell, the proper tubing inserted into the sample and wash solutions, and the pump turned on. The computer timed a wash cycle for 2 minutes, then activated the valve to switch to the sample solution. The crystal was exposed to the sample solution for 15 minutes. A 2-minute wash cycle which served to wash the crystal prior to drying and to clean the flow lines followed the sample cycle. The computer paused so the compressed air could be turned on. The crystal was dried with compressed air for approximately 3 minutes and removed from the flow cell. Frequency was monitored for five minutes before and after sampling as described earlier in the data collection paragraph.

During the sample and wash cycle the sample and wash solutions were pumped continuously either to the crystal or to a waste port (Figure 9). This ensured that the line between the valve and the crystal was cleaned before and after sampling. In addition,

timing of the sampling period itself was performed by the computer, eliminating human error.

Figure 9. Valve-controlled Wash and Sample Positions.



Reagents

All cyanide solutions were prepared from a 100 mg/L stock solution that was standardized with silver nitrate (Standard Methods 1989). A 1.6 g/L NaOH solution was used for diluting the stock to the appropriate concentration. HPLC-grade NaOH was used for blank solutions. Volumetric flasks were used to prepare reagents and to make cyanide standards. Nanopure water was used to wash the crystals and to prepare

solutions. Stock cyanide solutions were kept in a refrigerator in a dark bottle, and discarded after 12 days as recommended by standard methods (Standard Methods 1989). All solutions were kept at pH above 11.0 for safe handling and because previous authors reported a greater reaction efficiency for high pH (Bunde 1990).

Noise from Sampling Procedure

To determine the response of the system to the sampling procedure, three steps of the procedure were isolated and system response evaluated. The three steps or combination of steps were chosen to isolate handling of the crystal and determine the effect, if any, on the oscillating frequency of the crystal. The frequency was monitored for at least 10 recorded counts in between these sampling steps. Sample #1: the crystal was removed for approximately three minutes and then replaced in the system. Sample #2: the crystal was removed, dried with air for approximately five minutes, then replaced. Sample #3: the crystal was removed, wetted with water, dried for five minutes, then replaced.

Blank runs were performed in between cyanide sampling in which the entire sampling procedure was executed, substituting a 1.6 g/L NaOH solution for the cyanide solution. A series of data points were taken before and after cyanide-induced shifts and before and after blank runs. The frequency counts before and after exposing the crystals to a blank NaOH solution were averaged and the difference regarded as noise. The signal was obtained from the average of data points before and after the cyanide-induced frequency shift.

Precision and Calibration

To determine the precision of the system to a constant treatment of cyanide the sampling crystal was exposed to 0.1 mg/L of cyanide for 15 minutes at a flow rate of 0.72 ml/min. This was repeated seven times.

External parameters that may affect precision were scrutinized. To ensure that fluctuations in ambient temperature did not affect the cyanide leaching process the sample cell was placed in the air bath described previously. Regulation of the light bulb during pre-washing and sampling of cyanide kept a constant temperature of 29 °C, stable to +/- 0.1 °C. To control the temperature of the sampling solutions they were placed in a constant temperature water bath maintained at about 29 °C. The temperature of the sampling solutions was monitored by a thermistor. The sampling period was 5 minutes and flow rate was 0.72 ml/min.

The results were compared to an experiment in which the same reaction conditions were implemented (1 mg/L exposure for 5 minutes at a flow rate of 0.72 ml/min) but the reaction occurred in ambient room temperature and the solution of cyanide was not in a temperature bath.

The system was calibrated to cyanide concentrations of 0.05, 0.1, 0.5, 1, 2 and 5 mg/L. The exposure time was 15 minutes at a flow rate of 0.72 ml/min. The leaching process was performed in a temperature bath thermostated at 29 °C, stable to +/- 0.1 °C. Three frequency shifts were obtained for each concentration up to 2 ppm and two frequency shifts were obtained for 5 ppm. In a second calibration experiment the system

was calibrated only to 1 ppm, with at least four points for each of five concentrations:

0.05, 0.1, 0.25, 0.5 and 1 ppm. Statistics were performed for this calibration to determine the standard deviation of cyanide concentration for a given frequency shift.

CHAPTER 4: RESULTS AND DISCUSSION

The results from experiments described in Chapter 3 are presented and discussed in this chapter. The results are divided into four sections: Instrument stability, sampling procedure noise, response to cyanide, and observations.

Instrument Stability

Warm-up behavior, drift. Figure 10 is a graph of the output (frequency difference) with respect to time immediately after turning on the instrument. The frequency difference increased steadily and leveled off after 15 minutes in this example. The time for the frequency to stabilize varied, sometimes it took as long as 70 minutes (Figure 11). The frequency behavior correlates well with temperature inside the instrument (Figure 11).

Figure 10. Frequency increases and then levels off after turning on the instrument.

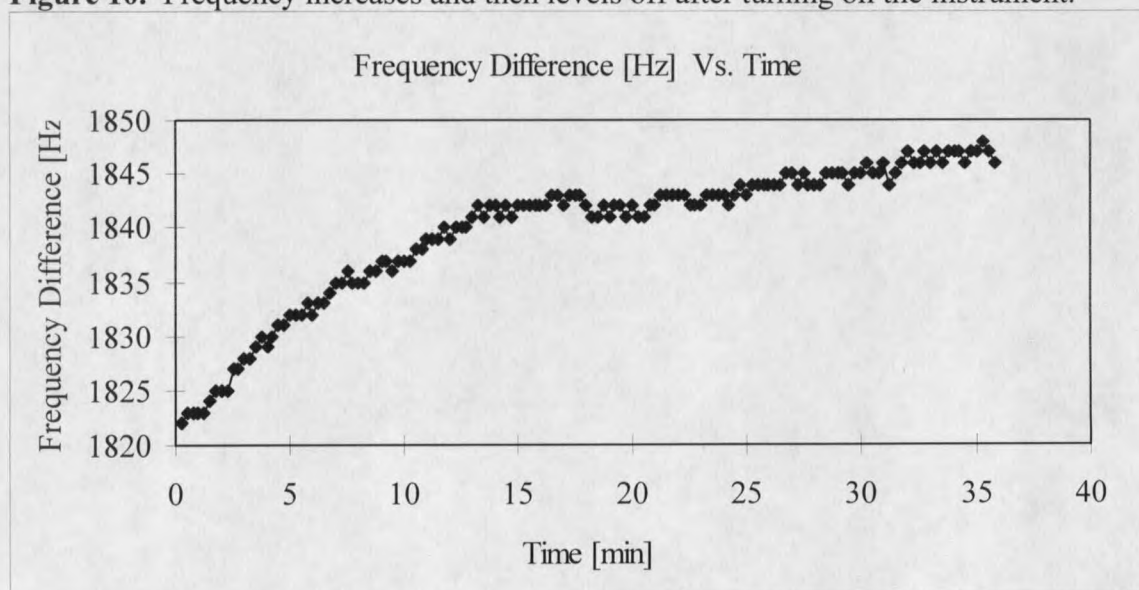
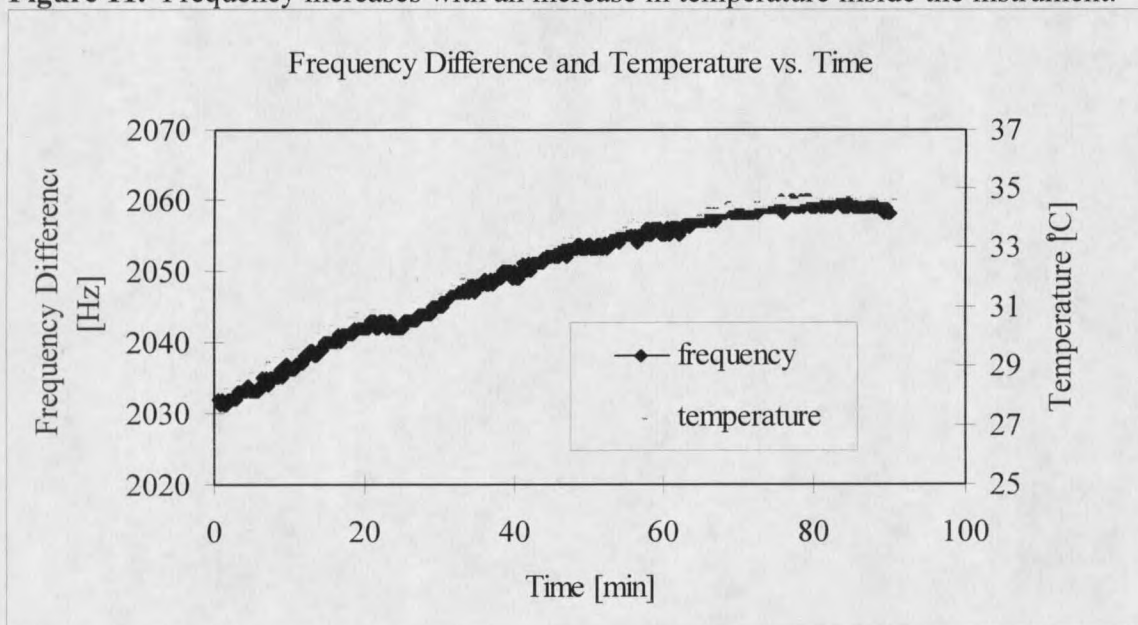


Figure 11. Frequency increases with an increase in temperature inside the instrument.



The increase and stabilization of temperature inside the instrument is due to the heat produced by a voltage regulator. The increase in temperature appears to cause a warm-up trend for the instrument output.

After a period of over one hour in which the instrument is allowed to warm up, the frequency remains stable within a few Hz out of 10 MHz. Figures 12 and 13 demonstrate the stability for one hour and 12 hours, respectively. The standard deviation for monitoring the frequency for one hour is 1.12 Hz (Figure 12) out of 10,000,000 Hz. Over a period of 12 hours the standard deviation becomes greater, at 4.13 Hz, but is still very small with respect to the 10,000,000 Hz frequency of the crystals. A gradual increase in frequency is observed (Figure 13).

Figure 12. Stability of the frequency difference output using a reference crystal to correct for environmentally-induced noise and crystal variation.

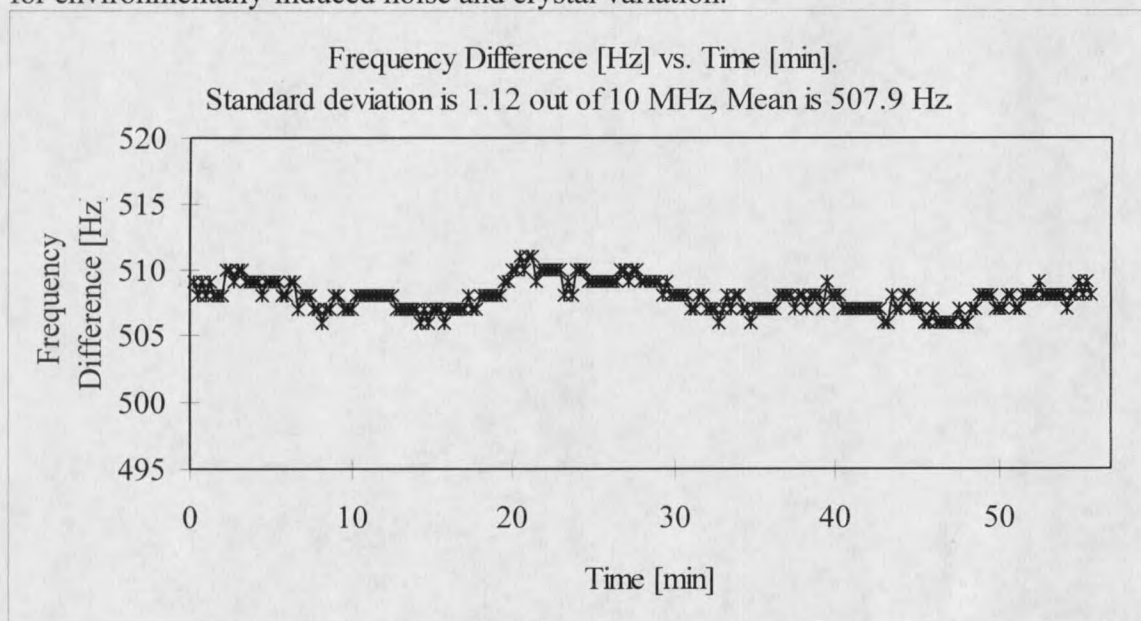
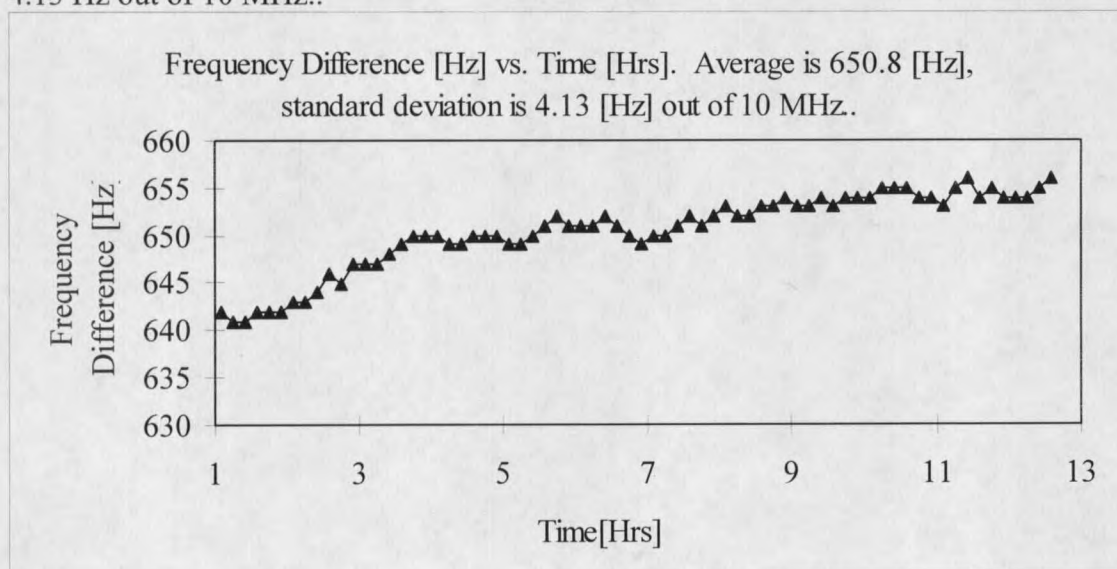


Figure 13. Over time the frequency drifts up but is stable with a standard deviation of 4.13 Hz out of 10 MHz..

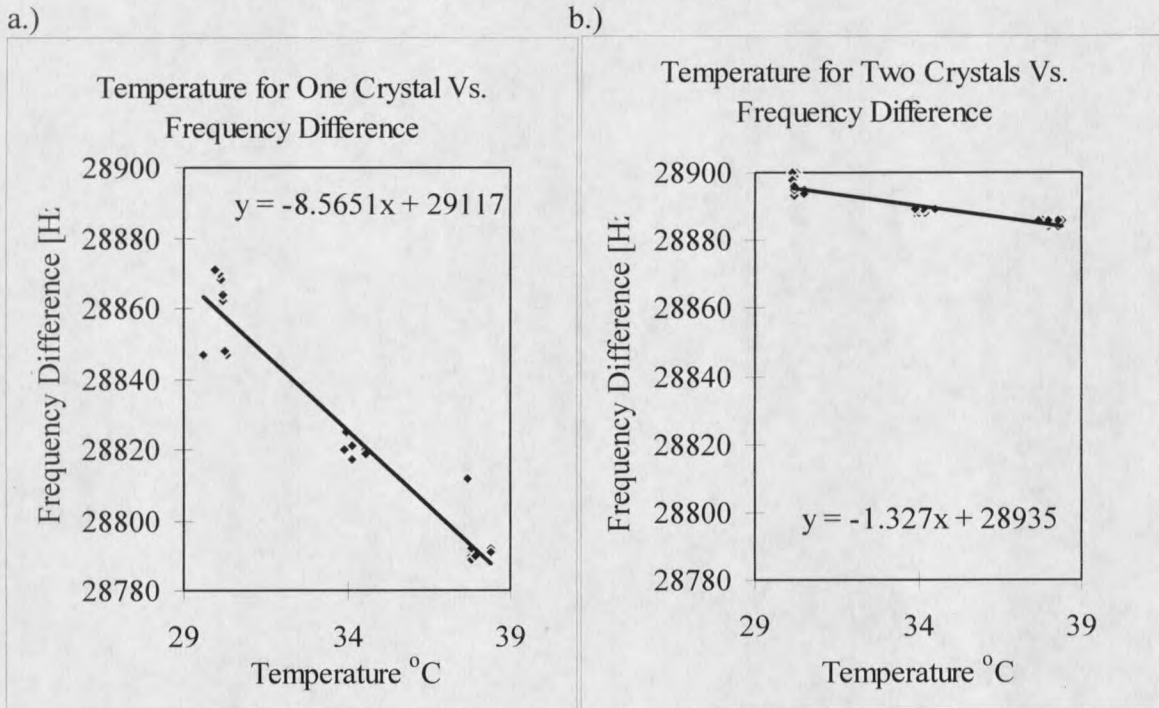


The exceptional stability of the instrument is demonstrated by these figures.

While we are unable to make any direct connection between single specific efforts in the instrument design and instrument stability, these results support our efforts to provide a stable instrument. Stability is a result of a number of factors in the instrument design: The output reflects the difference between two high-frequency signals subject to the same environmental drift; the mixer for the high frequency signals only accepts sine wave frequency and thereby serves as a filter to high frequency noise input; the difference signal output is low frequency and not susceptible to noise; the one second count is gated precisely by the reference crystal; the gated output count into the computer interface is entirely dependent on two crystals subject to environmental drift.

The ability of the reference crystal to correct for thermal noise of the crystals is demonstrated in Figure 14. Frequency difference output is graphed vs. three temperatures simulating environmental changes in temperature. When the reference crystal is in the same environment as the sampling crystal, the change in Hz is approximately 1.3 per degree temperature change. With only one crystal in the temperature bath and the crystals in different temperature environments the change is 8.5 Hz per degree temperature change.

Figure 14. Example of the correction capabilities for temperature using a reference crystal and monitoring the frequency difference of two crystals. a.) Frequency difference with crystals in different temperature environments. b.) Frequency difference with both crystals in the same temperature environment.



The effect of temperature correction for the crystals only is demonstrated here; the oscillators remained in the same environment during the experiment. It should be mentioned that correction of thermal noise arising from the oscillators is not demonstrated and may be occurring. In addition, correction of noise from the power supply and from the number of parameters discussed in the introduction may be occurring.

Response to Sampling Procedure:

The response to the three sampling steps which may introduce noise to the system is given in this section.

Sample #1. Removing and replacing the crystal disturbs its fundamental frequency. This effect is analogous to turning on and off the oscillators, which causes a thermal transient and frequency disturbance (Parzen 1983). The frequency is disturbed but the original frequency is generally recovered (Figure 15). The effect is less pronounced when the reference crystal and sampling crystal are treated the same, demonstrating some correction by the reference crystal (Figure 16, Table 2)

Figure 15. Removing and replacing the crystal disturbs the fundamental frequency.

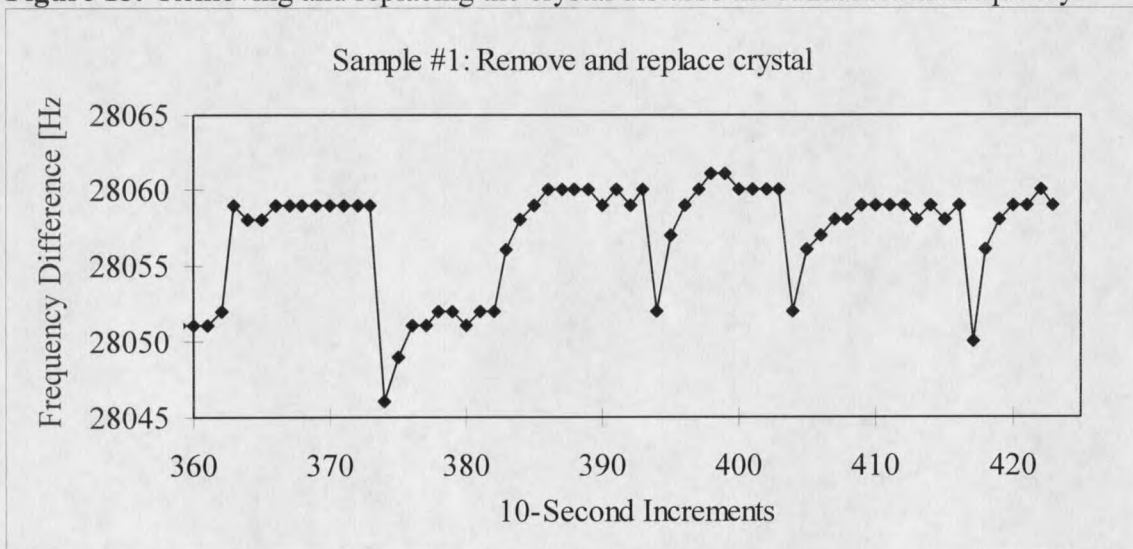


Figure 16. When both crystals are removed and replaced, the extent of frequency shift is less.

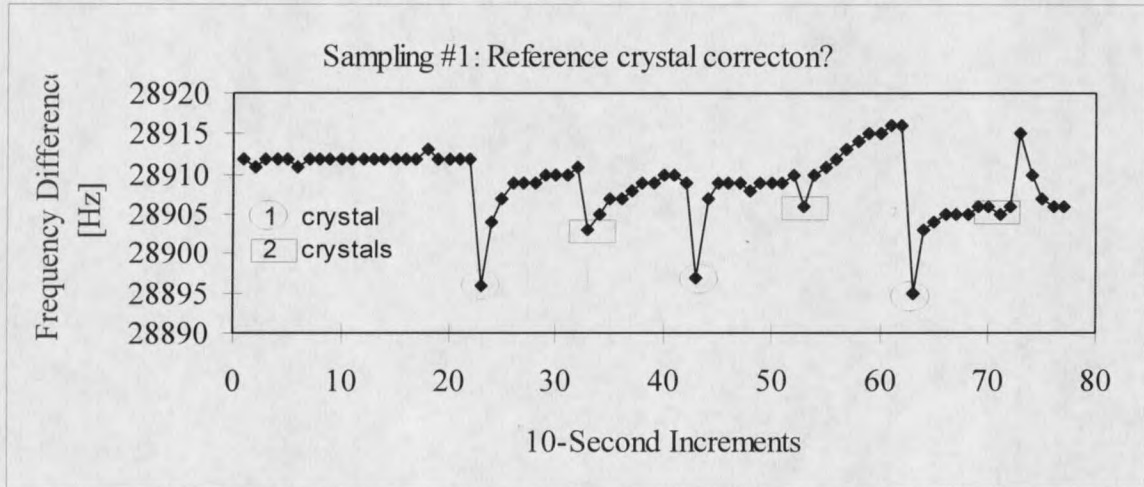


Table 2 . Frequency shifts for one and two crystals as demonstrated in Figure 15.

Shift [Hz] For One Crystal	Shift [Hz] For Two Crystals
-16	-7
-13	-4
-21	9

The disturbance in frequency output when the sampling and reference crystals were removed and replaced is less than half of that when only one crystal is disturbed. This curious behavior may demonstrate the correction ability of using two crystals in the same environment, but is not significant to the remainder of this study because only the sampling crystal is exposed to the sampling protocol.

Sample #2. The second sampling step investigated was removing, drying, and replacing the crystal. Figure 17 demonstrates the most common observed trend in which the frequency difference increased around 10 Hz with each drying. Figure 18 shows the results of 24 experiments in which the crystal was removed and dried for 5 minutes with

compressed air. The average change in frequency before drying and after drying was 8.2 Hz with a standard deviation of 6.1 Hz.

Figure 17. Increasing trend for frequency from drying crystal with compressed air.

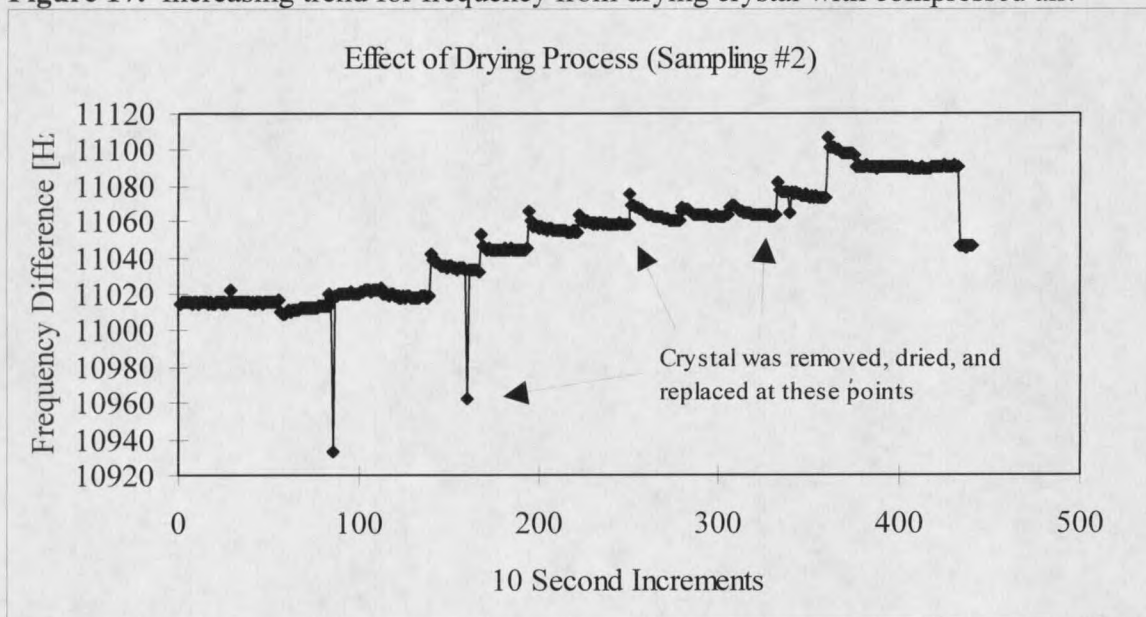
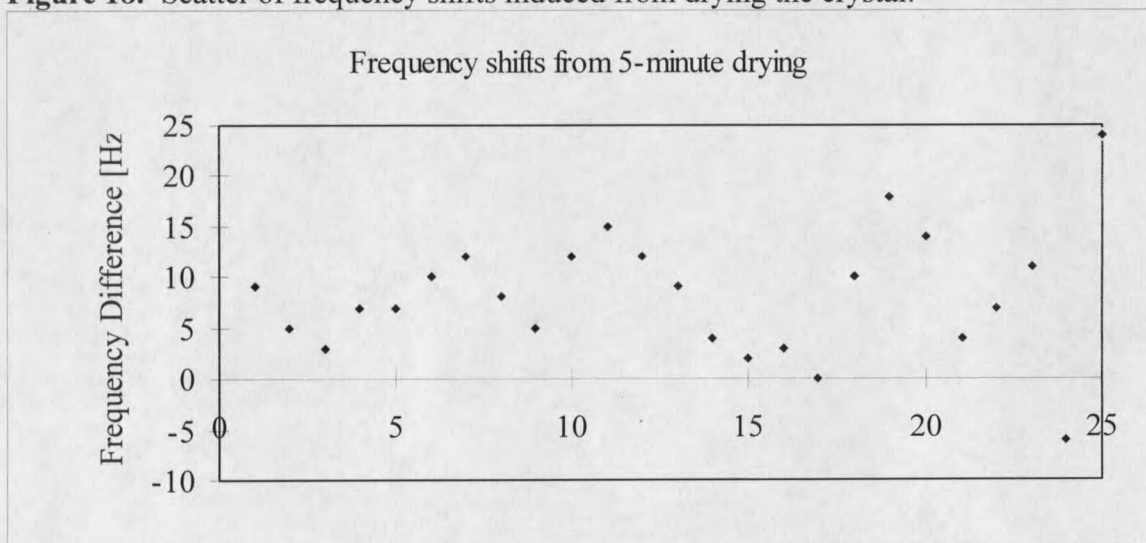


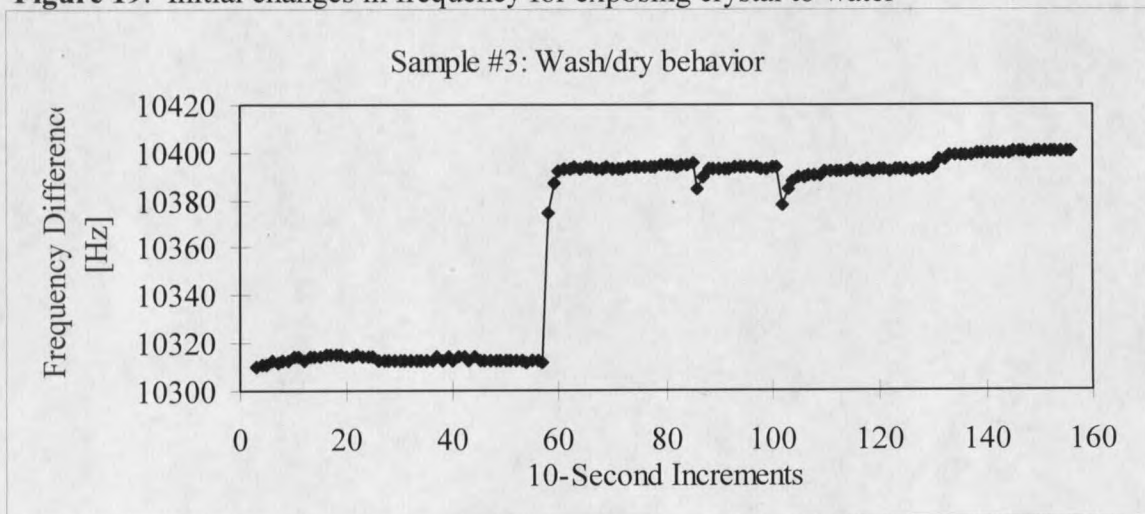
Figure 18. Scatter of frequency shifts induced from drying the crystal.



There is definitely a trend for the frequency to shift slightly upon drying the crystal. The shift from drying the crystal indicates that there is a frequency shift not likely related to a mass gain or loss. It is possible that, for instance, the crystal is dried of moisture adsorbed from the air and thereby shifts. This behavior could also be due to changes in temperature of the crystal or other phenomenon not related to mass change of the crystal.

Sample #3. The following graph demonstrates typical behavior observed for the first exposure of a crystal to liquid (Figure 19). The change in frequency was much greater for the first exposure of the crystal to water, at 63 Hz compared to -16 and then to 1. This could be due to some mass change of the crystal upon contact with water, perhaps formation of some oxide layer on the surface of the crystal, that remains stable after forming. Because of this phenomenon the crystals were “pre-treated” with water and air before being used to sample with.

Figure 19. Initial changes in frequency for exposing crystal to water



In one example a trend is observed in which wetting and drying the crystal causes the frequency to shift down by 10 to 20 Hz in contrast to behavior from drying the crystal (Figure 20). The average absolute value of frequency change for 23 wet/dry experiments is 18.3 Hz with a standard deviation of 16.2 Hz (Figure 21).

Figure 20. Contrasting frequency shift behavior for drying and wetting/drying crystal.

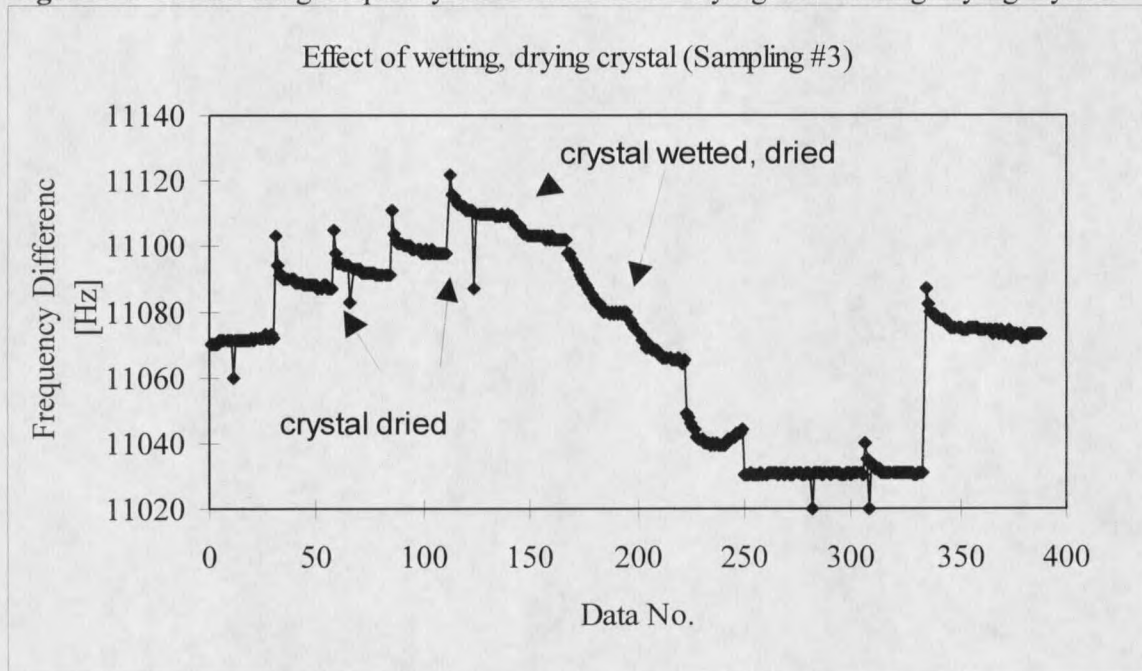
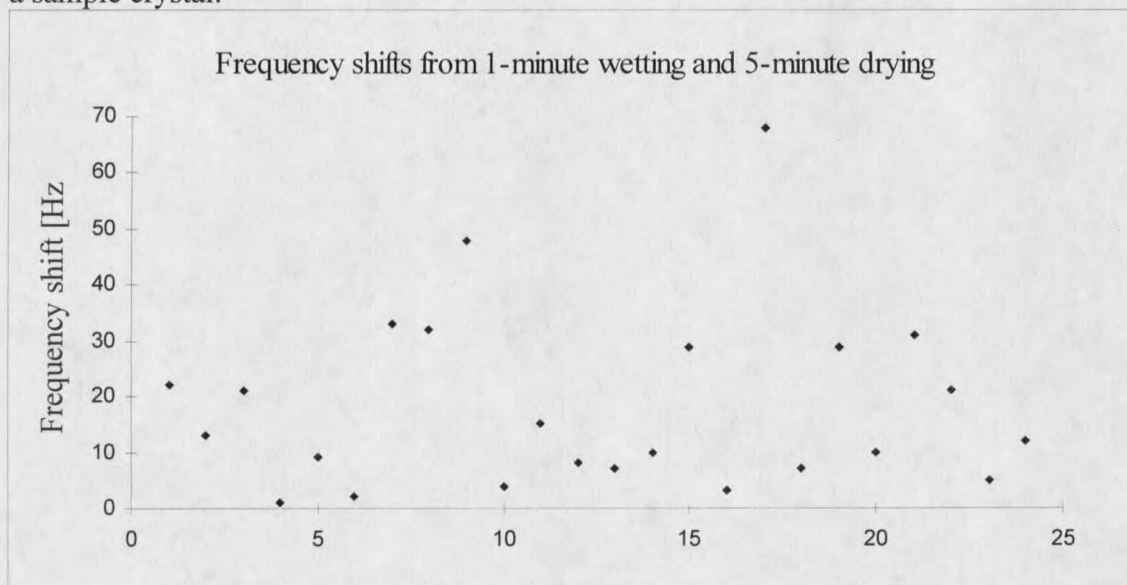


Figure 21. Scatter graph showing 23 frequency shifts from removing, wetting and drying a sample crystal.



The frequency shift for the process of wetting and drying the crystal is slightly greater than that for just drying the crystal. It is possible that there is some mass accumulation or mass loss to account for this as suggested by the behavior demonstrated in Figure 19.

Blank Runs. The results from blank runs performed in between cyanide sampling with 2 ppm solution are shown in Figure 22. Blank runs include the entire sampling procedure, substituting a “blank” solution of 0.05 M NaOH for cyanide solution (see Chapter 3). This figure illustrates instrument stability and sampling background noise in the context of sampling with cyanide. The circled portion is enlarged in the next two graphs, Figures 23 and 24.

Figure 22. Instrument drift, blank response, and response to 5-minute exposure to 2 ppm cyanide.

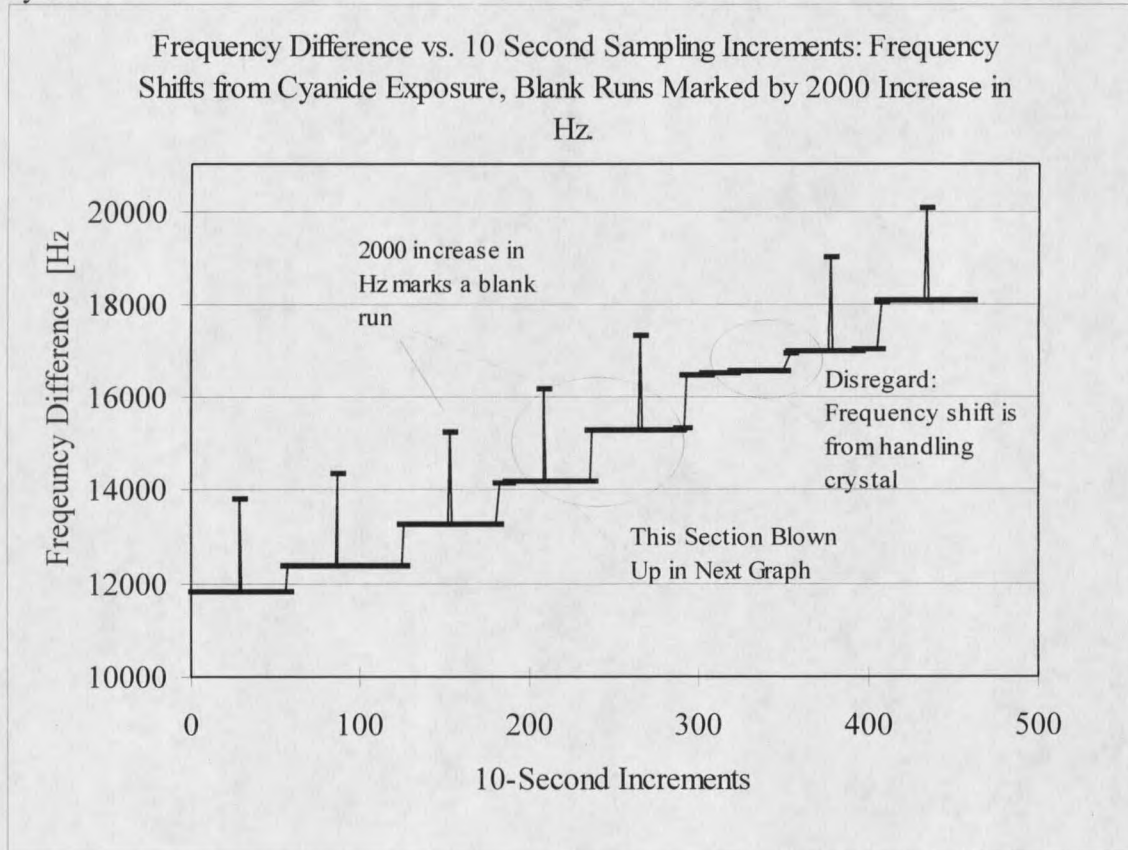


Figure 23. Demonstration of a blank shift observed in Figure 22.

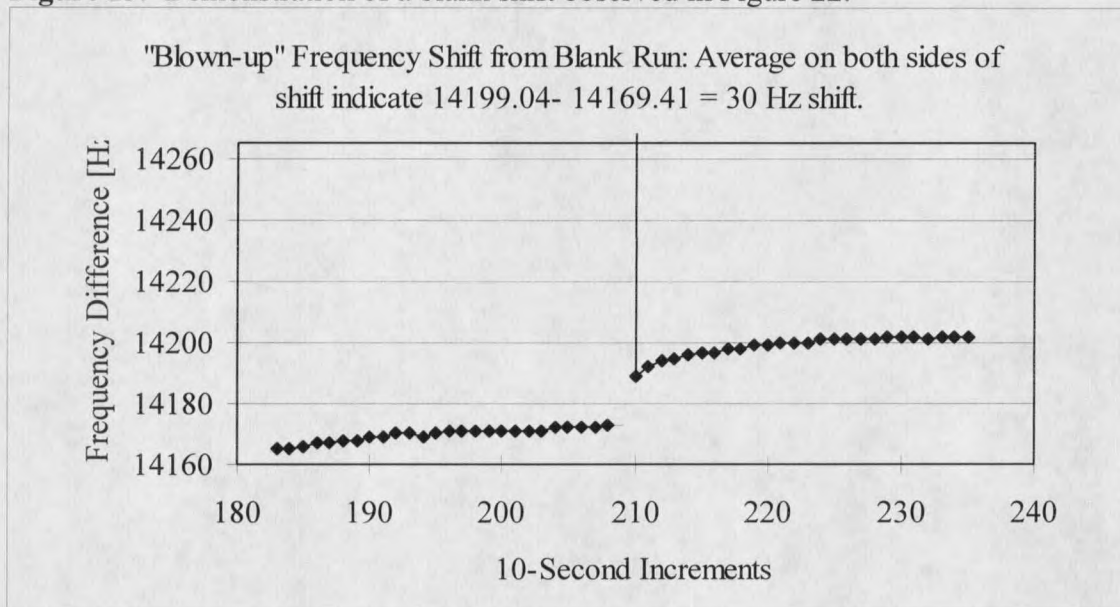
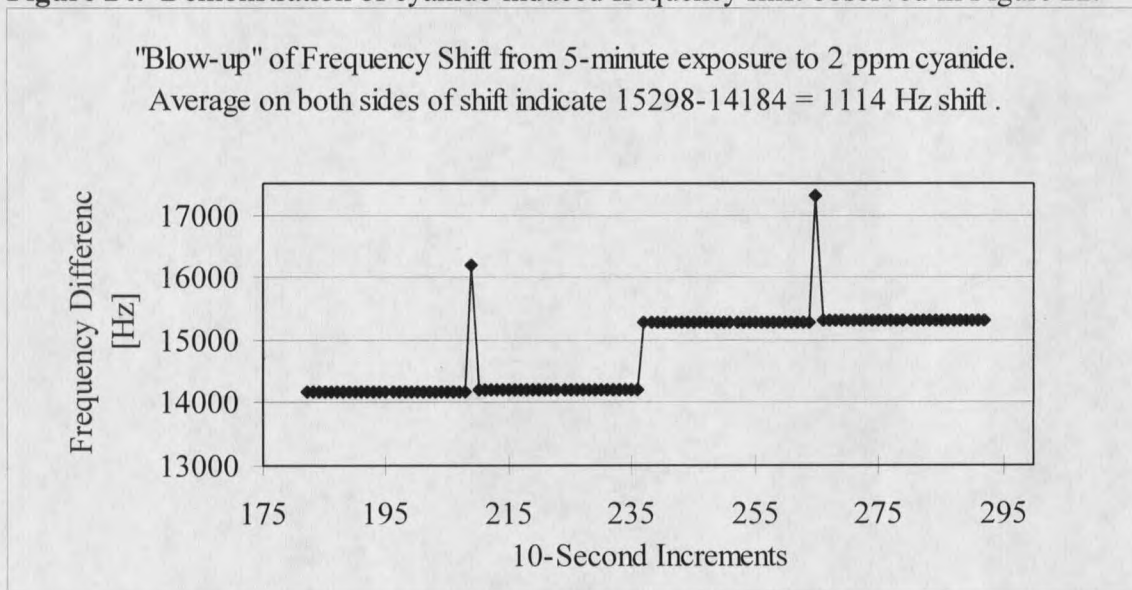


Figure 24. Demonstration of cyanide-induced frequency shift observed in Figure 22.



The shift in frequency (signal) and shift from the blank solution including instrument drift (noise) are calculated as demonstrated in Figure 23 and 24. The signal to noise ratio was calculated for each shift in Figure 22 and the results are presented in following table (Table 3).

This is the first time the system was exposed to cyanide. The first shifts are significantly lower than the last four for the same treatment (Table 3). This trend was not observed in subsequent experiments. The cyanide ion is polar and reactive and may adsorb to active sites on any surface it passes. Considering the low concentrations of cyanide it is reasonable to assume that during initial exposure of the system to the cyanide ion, the ion may be adsorbing to active sites on the plastic tubing used in the flow lines, the polycarbonate of the flow cell, or other unknown surface of the instrument (Neuman). From the data in Figure 22 the average ratio of signal to noise is 56.4.

Table 3. Signal to noise ratios for the shifts observed in Figure 22.

	<i>Signal</i> [Δ Hz]	<i>Noise (Blank)</i> [Δ Hz]	<i>S/N</i>
1.	531	11.8	45.0
2.	883	9.66	91.4
3.	900	17.1	52.7
4.	1090	29.6	36.8
5.	1187	17.5	67.8
6.	<u>1065</u>	<u>23.9</u>	<u>44.6</u>
Ave.	943	18.3	56.4

It is interesting that the average shift for the blank runs is 18.3 Hz, as is the average for the wet/dry experiments. There is definitely a response to blank treatment. The significance of this response would increase as one approaches the sensitivity limit

and could affect the precision. However, results from Rosentreter and Nomura show little or no loss in reproducibility from sampling (Table 4).

Table 4. Data published by Rosentreter and Bunde of frequency shift from cyanide exposure to a silver-plated crystal. The third column is the frequency shift less the shift from a blank run.

<i>Sample</i>	<i>Δ frequency (Hz)</i>	<i>Δ frequency (Hz)</i> <i>Blank</i>	<i>Δ frequency (Hz)</i> <i>Calculated</i>
		21	
1	992	10	977
2	845	7	837
3	1218	8	1211
4	1231	5	1225
5	1244	10	1237
6	1064	2	1058
7	1081	8	1076
St. Dev. (Hz)	147.24	2.85	148.58

As can be observed from the standard deviation, the poor reproducibility for Rosentreter and Bunde cannot be corrected for by subtracting the blank runs. Furthermore, it can be inferred that the sampling procedure is not causing poor reproducibility. This is in agreement with our data (Table 5). For this reason, blank runs were not performed for the remainder of the experiments.

Table 5. Data from table 3. The calculated frequency shift is the frequency shift minus a blank run frequency shift. Standard deviations are included. The standard deviation is calculated only for samples 2-6 because the first sample is not considered representative for this data series. If it is included, the standard deviation is 243 Hz for column one as compared to 130 Hz.

<i>Sample</i>	Δ frequency (Hz)	Δ frequency (Hz) <i>Blank</i>	Δ frequency (Hz) <i>Calculated</i>
1	531	11.8	519.2
2	883	9.66	873.34
3	900	17.1	882.9
4	1090	29.6	1060.4
5	1187	17.5	1169.5
6	1065	23.9	1041.1
St. Dev. (Hz)	130.24	7.54	126.16

System Performance for Cyanide Determination:

Repeatability. From the data in table 5 for the first repeated runs of a constant treatment of cyanide it is apparent that there is a standard deviation of 130 Hz. Although the poor precision presented in this table may be attributed to the loss of cyanide in the first few runs to surface adsorption in the flow lines it cannot be overlooked that there is a problem with precision. These results look similar to some of Rosentreter and Bunde's data. Over the course of the day the ambient temperature changed by as much as 10 degrees Celsius. It was hypothesized that poor precision could be due to very slight temperature-related changes in the extent of the reaction in which cyanide complexes gold. The next two graphs show reproducibility for the same treatment in which the experiments were run under conditions of ambient temperature (Figure 25) and conditions of constant temperature (Figure 26).

Figure 25. Repeatability of leaching process under ambient temperature conditions. Standard deviation is 27.4 Hz, average frequency shift is 523.3 Hz, coefficient of variation is 5.2%. Average blank shift is 5.5 Hz.

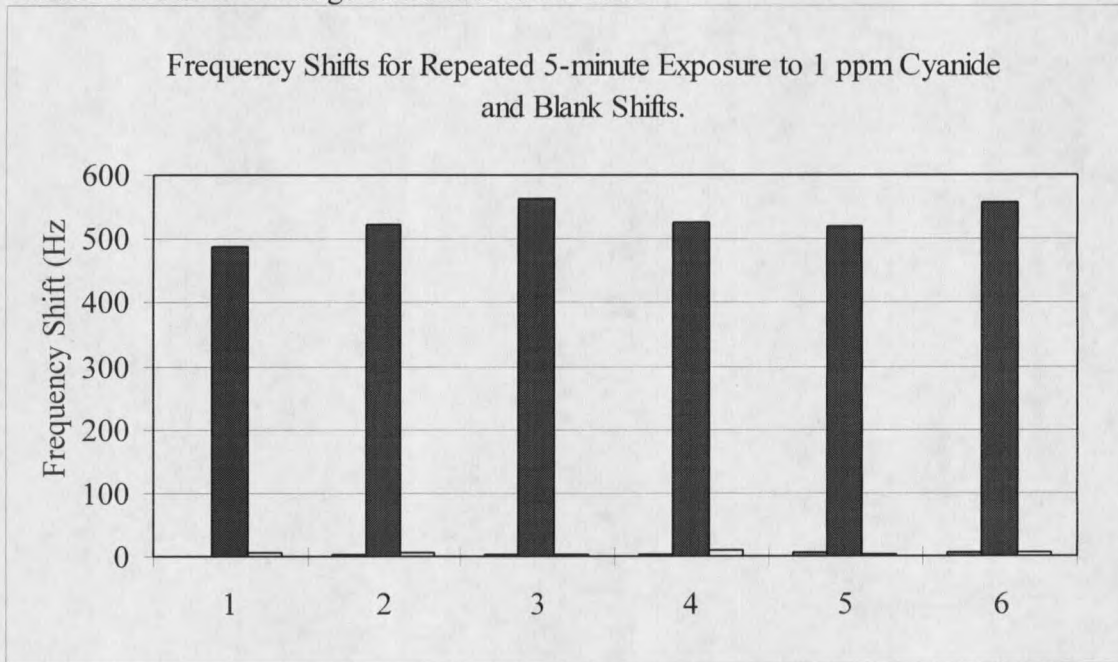
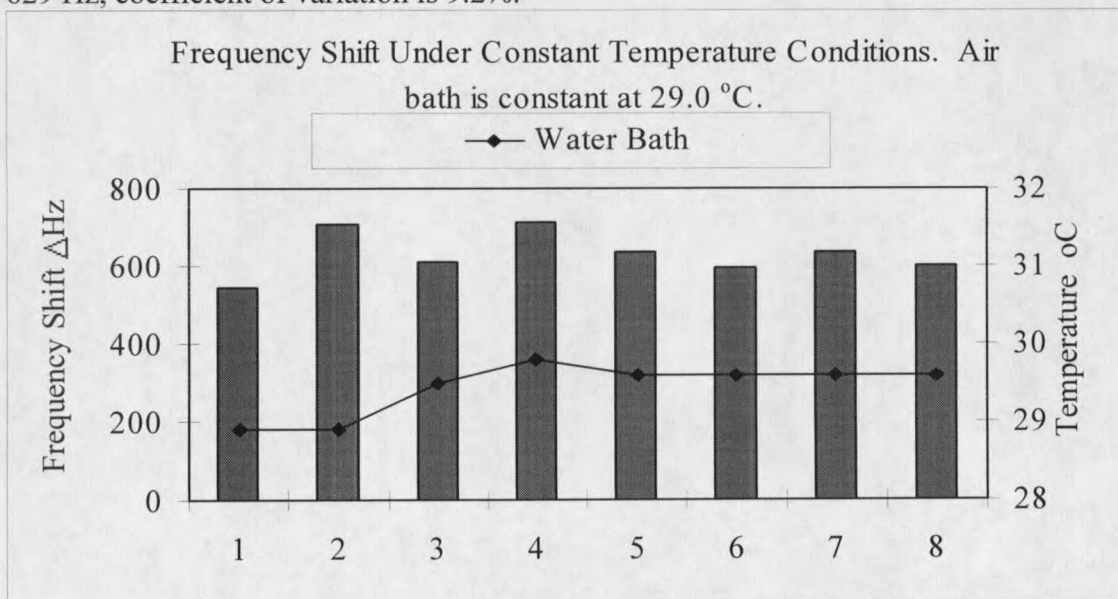


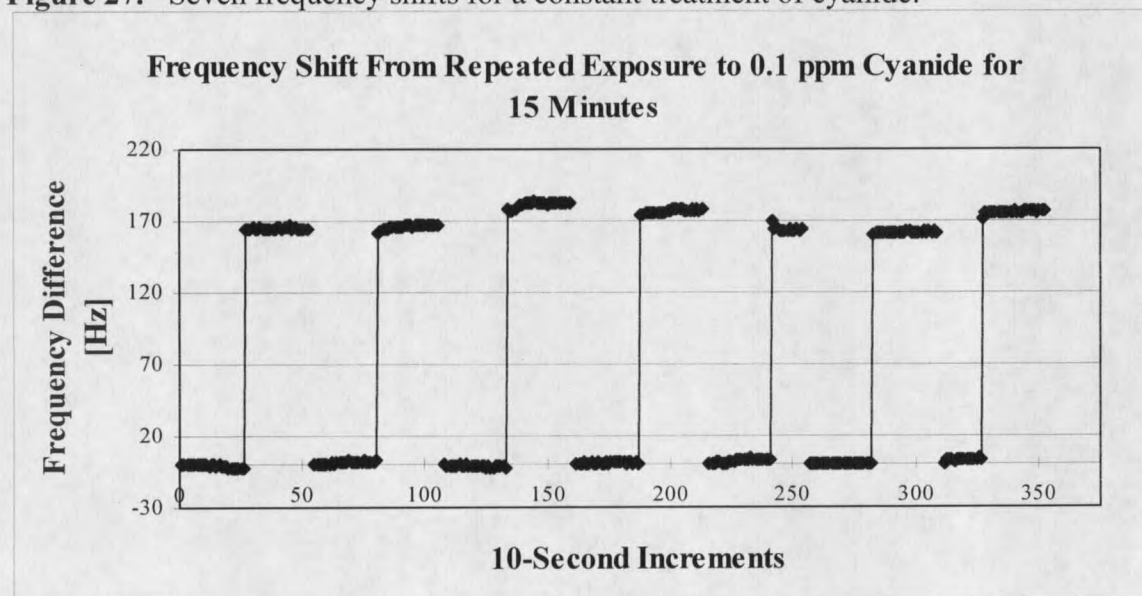
Figure 26. Frequency shifts for repeated treatment at constant temperature. The sample solution was in a water bath, the flow cell in an air bath. The average frequency shift is 629 Hz, coefficient of variation is 9.2%.



The frequency shifts were from 5-minute exposure to 2 ppm cyanide. It is surprising that the precision did not improve for the constant temperature bath; however, the average frequency shift for the same treatment was 629 Hz as compared to 523 Hz for ambient temperature condition (approximately 21-26 degrees Celsius). The greater mean frequency shift for the warmer environment suggests that the extent of reaction is temperature dependent. Also, the precision does not appear to be due to fluctuations in temperature, as a 9.2 % coefficient of variation was achieved for constant temperature conditions compared to 5.2 % when the reaction occurred in ambient temperature.

Repeatability of the instrument for a constant treatment is shown in Figure 27. For 7 treatments of 0.1 ppm cyanide the average shift was 169.1, with a standard deviation of 7.9 Hz and 4.7% coefficient of variation.

Figure 27. Seven frequency shifts for a constant treatment of cyanide.



This run shows the potential for excellent precision at low concentrations of cyanide. There were several factors that contributed to the precision demonstrated here. Care was taken to rotate and exchange the crystal so that a fresh surface of gold was always used. The crystals were pretreated with water. The temperature constant at 29 degrees Celsius.

Calibration. The relationship between cyanide concentration and frequency shift for this instrument is given in the following calibration graphs, Figures 28-30 and Table 6. The frequency shift was not found to be dependent on the crystal when the crystals were made by the same manufacturer and received in the same shipping. When a second order of crystals was ordered six months later from Universal Sensors, the "new batch" responded differently to cyanide, and a new calibration curve was made. Both graphs are included to illustrate this. Linear regression analysis for the first batch of crystals showed a slope of 1898.6 (R^2 of 0.9964). The new batch of crystals showed a slope of 1473.5 (R^2 of 0.9882). The lower response of the new crystals could be due to thickness of gold plated on the crystal or quality of electroplating process.

Figure 28. The relationship between cyanide concentration and frequency shift is linear to 1 ppm.

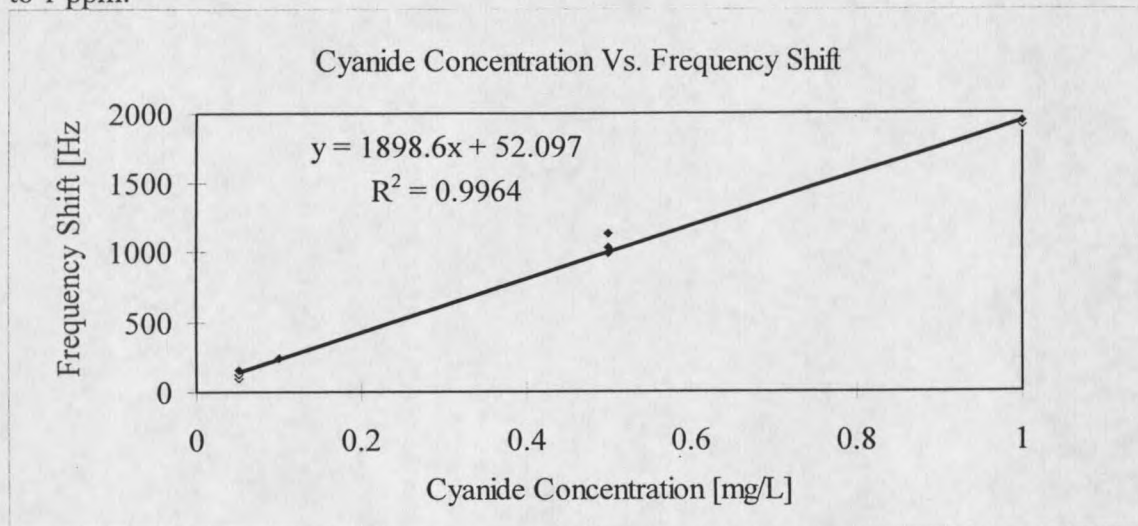


Figure 29. The relationship between cyanide concentration and frequency shift is linear to 1 ppm. A second order of crystals from Universal Sensors responds differently to cyanide treatment.

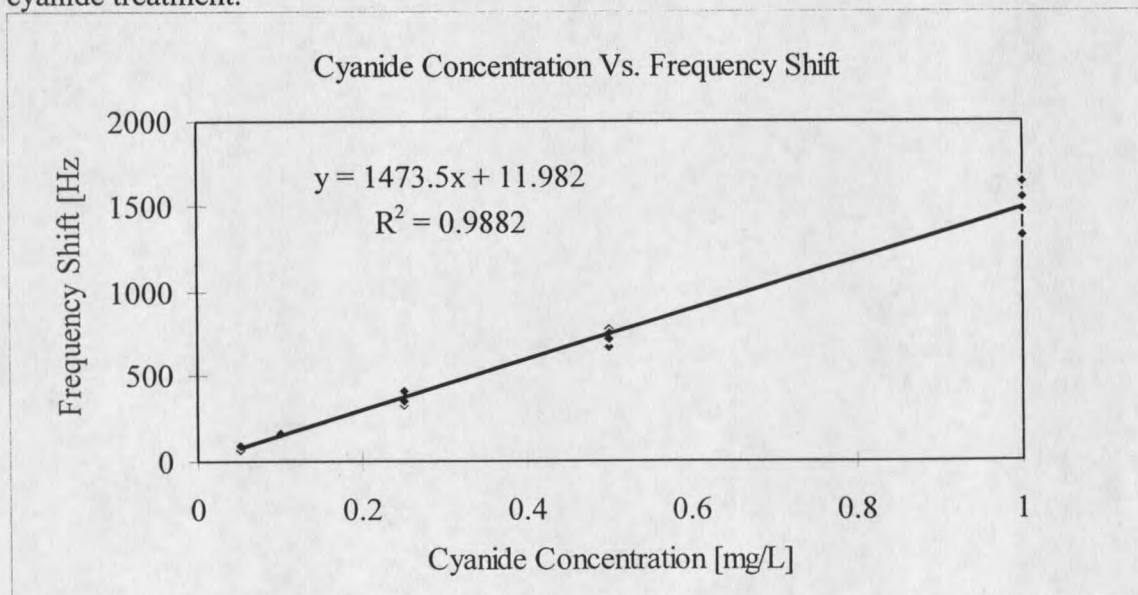
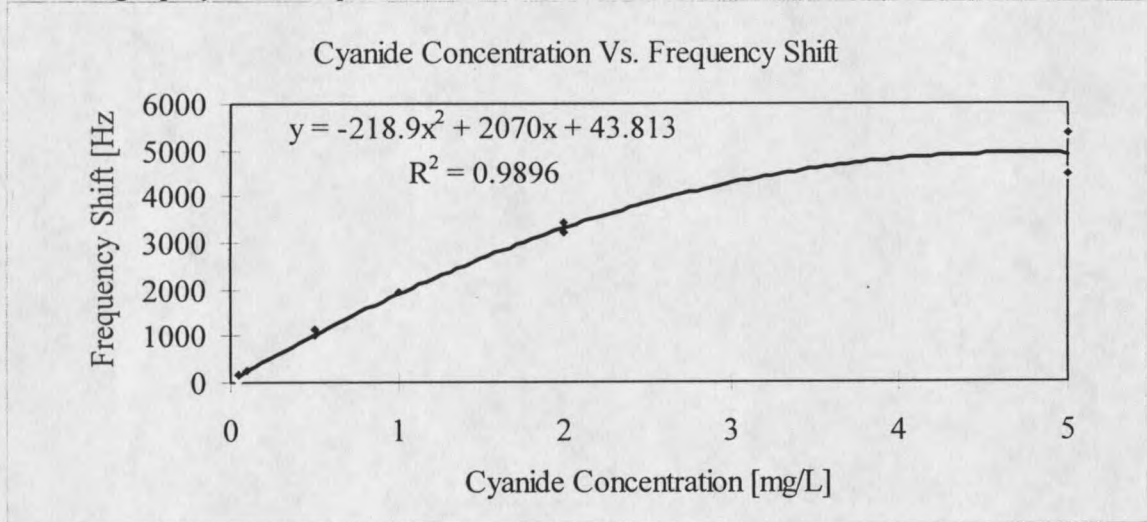


Figure 30. This relationship between cyanide concentration and frequency shift curves off fitting a polynomial equation for concentrations above 1 ppm.



The calibration curves (Figures 28 and 29) demonstrate the dependency of the response on the crystal manufacture and the linear relationship between cyanide concentration and instrument response below 1 ppm. For our sampling method and instrumentation the leaching process is not linearly related to the cyanide concentrations for concentration levels above 1 ppm (Figure 30). According to the equation by Sauerbrey the relationship is linear for all concentrations. Other authors have reported the relationship to be linear within a given range. This suggests the leaching process to be limited by other physical or chemical phenomenon when samples are above a certain concentration (see Chapter 5: Conclusions, p. 63).

Table 6 . Data from calibration curve presented in Figure 29.

<i>Conc. [ppm]</i>	$\Delta H z$	$\Delta H z$	$\Delta H z$	$\Delta H z$	$\Delta H z$	$\Delta H z$	$\Delta H z$
0.05	73	95	88	90			
0.1	179	173	167	160	167	167	159
0.25	326	419	410	352			
0.5	665	777	753	713			
1	1637	1543	1327	1476			

Statistical analysis of the data in Table 6 is presented in Tables 7 and 8 from a method in the literature for determining variability in calibration regression lines (Finney 1978). It is evident that the coefficient of variation drops significantly for increasing number of observations for an unknown sample (Table 7). Variability becomes more significant at lower concentrations as observed in Table 8.

Table 7. Statistical analysis of calibration data showing variation in number of observations.

<i>Shift input in $\Delta H z$</i>	<i>No. of observations</i>	<i>ppm CN</i>	<i>Variation in ppm CN</i>	<i>Coefficient of variation</i>
500 Hz	1	0.33 ppm	0.039 ppm	11.80%
500 Hz	3	0.33 ppm	0.023 ppm	7.10%
500 Hz	5	0.33 ppm	0.019 ppm	5.70%
500 Hz	10	0.33 ppm	0.014 ppm	4.40%

Table 8. Statistical analysis of calibration data showing variations in frequency shift.

<i>Shift input in $\Delta H z$</i>	<i>No. of observations</i>	<i>ppm CN</i>	<i>Variation in ppm CN</i>	<i>Coefficient of variation</i>
100	3	0.060 ppm	0.024 ppm	41%
800	3	0.53 ppm	0.024 ppm	4.50%
1200	3	0.80 ppm	0.026 ppm	3.20%
2000	3	1.3 ppm	0.033 ppm	2.40%
2600	3	1.7 ppm	0.041 ppm	2.30%

Observations Concerning Sampling and Reproducibility:

There were several observations concerning sampling and reproducibility that need to be noted. The data reported by Nomura shows excellent sensitivity (linear calibration graph to 2.6 ppb) and more important, low standard deviations, for example 15.5 Hz standard deviation for 5 determinations of 5×10^{-6} M CN (0.13 ppm) with an average response of 430 Hz. Data reported by Bunde and Rosentreter shows poor reproducibility, in one case, the RSD (relative standard deviation or coefficient of variation) was reported to be 35% for a flow rate of 0.17 ml/min. Bunde and Rosentreter claimed the precision of the system to be dependent on the flow rate, and did get an RSD of 3.7% for a flow rate of 0.34 ml/min. Poor reproducibility for a silver-plated crystal experiment in which the same flow rate was used is shown in table 5, where an RSD of 14 % was achieved.

Reproducibility for our system cannot be traced to noise induced from instrument drift, temperature, or even the presence of air bubbles on the surface of the crystal during sampling. The work done by Bunde and Rosentreter present poor reproducibility for certain cases even after subtracting the frequency of the blank signal. This implies that reproducibility is not being significantly affected by the sampling procedure.

In our experiments, the second batch of crystals seemed to have a gold coating that was less durable than the first batch, and it was observed after several samplings with 1 ppm solution of cyanide that the cyanide was completely etching away the gold directly beneath the flow inlet of the flow cell. The frequency shift after sampling a few times

was very low, and when the flow inlet was rotated to the other side of the crystal the frequency shift was as expected from the first frequency shifts. Soon another hole formed on the crystal, and the frequency shift lost reproducibility.

An additional observation from the data presented in Figures 28 and 30 is that the standard deviation increases with the concentration. In our sample flow cell the reproducibility of the leaching process declines with exposure to higher concentrations of cyanide. Again, at higher concentrations the response is not linear, suggesting mass loss may be defined by another phenomenon besides cyanide concentration. For the data reported in Table 6 care was taken to rotate the crystal and use fresh crystals often in an effort to keep the leaching process as reproducible as possible.

Nomura's sampling method, in which the crystal was immersed in a beaker of constantly stirred cyanide solution, by nature would allow for cyanide to be leached in the most reproducible manner. Her results are in agreement with this hypothesis, as she achieved coefficients of variation of 4%. Rosentreter and Bunde used a flow-through method, in which the gold available for leaching is not reproducible and changes with time and extent of reaction. It is plausible that there is a specific flow rate, as Bunde and Rosentreter suggest, in which enough time is allowed for cyanide to diffuse to the surface of the crystal and reach equilibrium with the gold, leaching the gold in a reproducible manner. This rate may be different for the silver plated crystal; thus, the reproducibility is poor as presented in Table 4.

The immediate leaching of the gold by the cyanide suggests this reaction is diffusion limited. This is in agreement with Bunde and Rosentreter (Bunde 1993) who

compared experimental reaction efficiencies and rate constants for cyanide-gold complexes and cyanide-silver complexes. Reproducible leaching of the gold-plated crystal must be accomplished in order to achieve reproducible mass loss and system response. From the observations presented in this chapter and from the literature, it is possible this can be achieved by allowing a constant flux of cyanides across the entire surface of the crystal.

While our system showed reasonably good reproducibility, this is under special circumstances in which the crystals are from the same batch and the technician is very experienced. The reproducibility and usefulness of this method may depend on development of an improved sampling method which allows the gold to be leached in a reproducible manner as demonstrated by Nomura.

CHAPTER 5: CONCLUSIONS AND FUTURE RECOMMENDATIONS

Conclusions

The system as presented is significantly cost-effective as compared to other detection methods for cyanide and previous work done with piezoelectric cyanide detection. Unlike instruments used previously for this work our instrument is stable without a temperature bath or faraday cage and not susceptible to noise. The sampling method outlined here shows the direct relationship of instrument response to cyanide concentrations in the sub ppm range. Use of a computer interface allows for easy data collection and control of a number of parameters during sampling. The system is partially automated.

Statistical analysis of the regression line was performed, and variability in frequency shifts becomes increasingly significant for concentrations below 0.05 ppm. It is my observation that the physical phenomenon on the surface of the crystal during sampling are limiting the reproducibility. This observation is supported by results in the literature and sampling protocol used by the authors to leach gold from the surface of the crystals with cyanide. It is hypothesized that reproducibility and sensitivity can be dramatically improved with the design of a sampling configuration in which a consistent flux of cyanide ions are introduced to the crystal surface.

Instrument Stability

Upon turning on the instrument and monitoring the frequency difference between a reference and sampling crystal, a warm up trend is observed in which the frequency increases for 15-70 minutes and then stabilizes. This is correlated with temperature inside the instrument which increases due to heat given off from a voltage regulator. After warming up the system as designed with a reference crystal and heterodyne mixer is stable within a few Hz out of 10 million Hz for periods up to 13 hours.

The stability of the instrument with respect to temperature changes of the piezoelectric crystals deteriorates when the crystals are not in the same temperature environment and are not correcting for each other. This implies that the stability of the instrument is due to the use of a reference crystal. It is plausible that other temperature effects and environmental noise are corrected for by the use of a reference crystal.

Response to Sampling Procedure

These experiments were run in an effort to discern where in the sampling procedure any mass gain or loss of the crystal was occurring or frequency shift induced from physical phenomenon associated with the sampling procedure was occurring. There are few conclusive remarks that can be made from the experiments. There is a marked response to a blank treatment. It cannot be narrowed down to one specific step in the sampling procedure but there does appear to be at least two places in the sampling procedure where frequency shifts are possible, drying the crystal and exposing the crystal

to water. The shift from drying implies the frequency changes just from handling or temperature change or other physical phenomenon. The extreme shift from the first wetting of the crystal and the increase in shift from wetting/drying as compared to just drying implies that there is some additional phenomenon occurring associated with exposing the gold to water. The response from wetting and drying the crystal and from performing a blank run are very similar indicating that length of exposure to liquid and exposure to high concentrations of NaOH do not further increase the response to the sampling protocol.

These results show that the crystal frequency does shift from something other than exposure to cyanide. This shift may affect the sensitivity of the system but does not affect the reproducibility of the response to cyanide.

System Performance for Cyanide Determination

The relationship between cyanide concentration and frequency shift due to loss of gold by cyanide for a variety of conditions is documented in this thesis. Mass loss of the piezoelectric crystal induced by exposure to cyanide can be calibrated to cyanide concentration and the response is linear up to 1 ppm. A stable baseline frequency allows for the determination of frequency shift without noise problems experienced with less stable instruments. When the crystal properties change, the response to cyanide remains linear.

The instrument shows good reproducibility for 7 shifts of 0.1 ppm cyanide. Reproducibility becomes better for concentrations of 0.5 ppm and lower. Mass loss and

frequency shift response curves off for 2 and 5 ppm cyanide. Statistical analysis shows that variability decreases for increasing number of observations. These results exemplify the potential for this relationship to be exploited for the commercial development of a cyanide detection method.

Observations

Holes observed in the gold plating on the surface of the crystal directly below the inlet of cyanide into the flow cell implies the flow cell used for sampling allows cyanide to leach the gold in a very small area on the crystal. In turn this observation implies that the reaction between cyanide and gold is limited by diffusion of the cyanide ion to the surface of the crystal. The sampling protocol used in these experiments does not leach cyanide in a reproducible manner after a certain amount of experiments. For low concentrations the reproducibility is better indicating the loss of reproducibility is greater for a greater exposure of the surface to cyanide.

In the literature sensitivity and reproducibility was different for two different sampling protocols, suggesting reproducibility of the instrument response to be dependent on the manner in which the cyanide leaches the gold-plated crystal. When care is taken to always expose a fresh surface of gold to the cyanide the reproducibility is similar to the reproducibility presented by Nomura.

Suggestions For Further Work

- Improvements made to the sampling procedure to allow for even, reproducible leaching of the gold by cyanide may improve sensitivity and reproducibility.
- Determine the effect of flow rate and other sampling parameters on response, precision.
- Determine maximum concentration for sampling procedure which allows for good reproducibility.
- The system could be completely automated. This would make it useful for monitoring waste effluent.
- Analyze environmental samples.
- Apply instrument to other piezoelectric detection schemes

BIBLIOGRAPHY

ASTM, Annual Book of Standards, American Society for Testing and Materials, Philadelphia, Pennsylvania. Designation D-2036 (1991).

Bard, A.J., in Electroanalytical Chemistry, a Series of Advances, Marcel Dekker, Inc., New York, vol. 17 (1991).

Bunde, R.L., and J.J. Rosentreter, "Development of a Flow Injection Analysis System Utilizing Piezoelectric Crystals for the Determination of Cyanide Employing Small Sampling Sizes", Microchem. Journal 47: 148-156 (1993).

Cassinelli, M.E., "Progress Toward Developing a Monitoring Method for Hydrogen Cyanide in Air", National Institute for Occupational Safety and Health, Division of Physical Sciences and Engineering, Method Research Branch, Cincinnati, Ohio. NTIS PB86-236171. (1986).

Clesceri, L.S., A.E. Greenberg, and R.R. Trussel in Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Washington D.C., 17th edition (1989).

Czanderna, L., (ed.) Applications of Piezoelectric Quartz Crystal Microbalances, Elsevier, New York (1984).

Deakin, M.R. and D.A. Buttry, "Electrochemical Applications of the Quartz Crystal Microbalance", Anal. Chem. 61: 1147A-1154A (1989)

Doudoroff, Peter, "Toxicity to Fish of Cyanides and Related Compounds", Environmental Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Duluth, Minnesota, EPA-600/3-76-038 (1976).

Douglas, G., D.H. Mc Daniel and J.J. Alexander in Concepts and Models of Inorganic Chemistry, John Wiley and Sons, Inc., New York, 2nd edition (1983)

EPA (U.S. Environmental Protection Agency), "Ambient Water Quality Criteria for Cyanides", EPA Environmental Criteria and Assessment Office, Office of Water Regulations and Standards, Environmental Research Lab, Duluth, MN EPA-440/5-80-037 (1980).

EPA (U.S. Environmental Protection Agency), "Destruction of Cyanide in Wastewaters: Review and Evaluation", U.S. Environmental Protection Agency, Water Engineering Research Laborator, Cincinnati, Ohio EPA-600/S2-88/031 (1988).

EPA (U.S. Environmental Protection Agency), "Quality Criteria for Water," U.S. Environmental Protection Agency, Washington, D.C. EPA-440/5-86-001 (1986).

El Ghawabi S.H., M.A. Gaafar, A.A. El-Saharti, S.H. Ahmed, K.K. Malash, R. Gares, "Chronic Cyanide Exposure: A Clinical Radioisotope and Laboratory Study", Br J Ind Med 32:215-219 (1975).

Finney, D. J. in Statistical Method in Biological Assay, Macmillan Publishing Co., Inc., New York (1978).

Goulden, P.D., B.K. Afghan and P.B. Brooksbank, "Determination of Nanogram Quantities of Simple and Complexed Cyanides in Water:" Anal. Chem., 44:1845-50 (1972).

Heming, T.A. and R.V. Thurston, "Physiological and Toxic Effects of Cyanides to Fishes: a Review and Recent Advances", D Van Zyl (ed.), Cyanide and the Environment, Proc. of a Conf., Tucson, A.Z., Colorado State Univ., Fort Collins, CO. (1985)

Kimball, G.L., L.L. Smith, Jr., and S.J. Broderius. "Chronic Toxicity of Hydrogen Cyanide to the Bluegill", Trans. Am. Fish. Soc. 107: 341-345 (1978).

King, W.H., Anal. Chem. 36: 1735 (1964.)

McGivney, S. E. and S. P. Shelton, "Detection and Treatment of Ferrocyanide Generated by the Photographic and Electroplating Industries", D Van Zyl (ed.), Cyanide and the Environment, Proc. of a Conf., Tucson, A.Z., Colorado State Univ., Fort Collins, CO. (1985).

Minunni, M., M. Mascini, G.G. Guilbalt and B. Hock, "The Quartz Crystal Microbalance as Biosensor. A Status Report on its Future", Anal. Lett. 28: 749-64 (1995).

Nomura, T. and O. Hattori, "Determination of Micromolar Concentrations of Cyanide in Solution with a Piezoelectric Detector", Anal. Chim. Acta, 115:323-326 (1980).

Neuman, J., Analytical Chemist, personal communication.

Oak Ridge National Laboratory (ed.), "Toxicological Profile for Cyanide", Syracuse Research Corporation, Agency for Toxic Substances and Disease Registry, U.S. Public Health Service in collaboration with U.S. Environmental Protection Agency (1989).

Palladino, D., "Polarography Now Measures nMole Quantities of Cyanide; Computerized Technique is Highly Sensitive, Multimodal, Safe", R&D, 30: 126 (1988).

Parzen, B., in Design of Crystal and Other Harmonic Oscillators, John Wiley and Sons, Inc., New York, NY (1983).

Pohlandt, C., Jones, E.A. and Lee, A.F. "A Critical Evaluation of Methods Applicable to the Determination of Cyanides", Journal of the South African Institute of Mining and Metallurgy, Jan.: 11-17 (1983).

Rechniz, G. and M. Smit, "Toxin Detection Using a Tyrosinase-coupled Oxygen Electrode" Anal. Chem. 65:380-5 (1993).

Roe, F., personal communication.

Rolfes, H. and F. Munshower, Methods of Neutralizing Cyanide in Mining Wastes and Waste Waters, Reclamation Research Unit Publ. No. 8901, Paper presented at Mont. Acad. Sci. Mtg. (1989).

Rosentreter, J.J. and R.K. Skogerbee, "Trace Determination and Speciation of Cyanide Ion by Atomic Absorption Spectroscopy", Anal. Chem. 63: 682-688 (1991).

Rosentreter, J.J., personal communication.

Sauerbrey, G.Z., Z. Phys., 155:206 (1959).

Schumacher, R. "The Quartz Microbalance: A Novel Approach to the In-Situ Investigation of Interfacial Phenomena at the Solid/Liquid Junction", Angew. Chem. Int. Ed. Eng., 29:329-343 (1990).

Shelton, R.D., J.W. Haas III, E.A. Wachter, Applied Spectros 48:1007 (1994).

Smit, M.H. and A.E.G. Cass. "Cyanide Detection Using a Substrate-Regenerating Peroxidase-Based Biosensor", Anal. Chem., 62:2429-36 (1990).

Thomas, J., D. Burge and D. Togami, "Ion Chromatography Uses Only One Column to Get All the Ions; Modern IC Determines Weakly Dissociated Ions in a Wide Variety of Complex Mixtures of Materials", R&D, 26: 135 (1984).

Tomoda, A, and K. Hashimoto, "The Determination of Cyanide in Water and Biological Tissue by Methemoglobin", J. of Haz. Materials 28: 241-249 (1991).

Towill, LE, JS Drury, BL Whitfeild, EB Lewis, EL Galyan and AS Hannons. Reviews of the environmental effects of pollutants: V. Cyanide,. EPA Health Effects Research Lab. Office of Research and Development. p. 190 (1978).

U.S. Department of Health and Human Services, "Cyanide Toxicity", Case Studies in Environmental Medicine, HE 20.7907:15 (1991).

Von Michealis, H., 1985. "Role of cyanide in gold and silver recovery", D Van Zyl (ed.), Cyanide and the Environment. Proc. of a Conf., Tucson, A.Z., Colorado State Univ., Fort Collins, CO. 51-64 (1985).

Ward, M. D. and D. A. Buttry, "In situ Interfacial Mass Detection with Piezoelectric Transducers", Science 249: 1000 (1990).

Way, J.L., "Cyanide Intoxication and its Mechanism of Antagonism", Ann Rev Pharmacol Toxicol, 24:451-81 (1984).

- APPENDIX

PROGRAMS WRITTEN FOR COMPUTER INTERFACE

PROGRAM FOR TEMPERATURE EXPERIMENT

```
1 PAUSE
2 START Timer1 FOR min
3 PRINT "to stop experiment switch x to down (off) and press w at the pause pr
  omp" ON LINE 17
4 PRINT "TEMPERATURE SHOWN BELOW WILL INCREASE EVERY 2 MINUTES FOR 10 MINUTES"
  ON LINE 21
5 RESET COUNTER
6 PRINT INPUT FROM Timer1 ON LINE 19
7 SEND On TO OUT-2
8 DELAY 1 sec
9 SEND Off TO OUT-2
10 DELAY 8 sec
11 SEND INPUT FROM Temp1 TO COL-B
12 SEND INPUT FROM Counter TO COL-A
13 IF INPUT FROM SwX = Off GOTO 78
14 IF INPUT FROM Timer1 <= 5.000000 GOTO 28
15 IF INPUT FROM Timer1 <= 10.000000 GOTO 48
16 IF INPUT FROM Timer1 <= 15.000000 GOTO 68
17 IF INPUT FROM Timer1 >= 15.000000 GOTO 78
18 START Timer2 FOR sec
19 PRINT INPUT FROM Temp1 ON LINE 23
20 PRINT INPUT FROM Timer2 ON LINE 20
21 IF INPUT FROM Temp1 >= 28.000000 GOTO 24
22 SEND On TO OUT-1
23 WAIT UNTIL INPUT FROM Temp1 >= 28.000000
24 IF INPUT FROM Temp1 < 28.100000 GOTO 27
25 SEND Off TO OUT-1
26 IF INPUT FROM Timer2 >= 6.000000 GOTO 5
27 GOTO 19
28 START Timer2 FOR sec
29 PRINT INPUT FROM Timer2 ON LINE 20
30 PRINT INPUT FROM Temp1 ON LINE 23
31 IF INPUT FROM Temp1 >= 30.000000 GOTO 35
32 SEND On TO OUT-1
33 WAIT UNTIL INPUT FROM Temp1 >= 30.000000
34 IF INPUT FROM Temp1 < 30.100000 GOTO 37
35 SEND Off TO OUT-1
36 IF INPUT FROM Timer2 >= 6.000000 GOTO 5
37 GOTO 29
38 START Timer2 FOR sec
39 PRINT INPUT FROM Temp1 ON LINE 22
40 PRINT INPUT FROM Timer2 ON LINE 19
41 IF INPUT FROM Temp1 >= 32.000000 GOTO 45
42 SEND On TO OUT-1
43 WAIT UNTIL INPUT FROM Temp1 >= 32.000000
```

```
44 IF INPUT FROM Temp1 < 32.100000 GOTO 48
45 SEND Off TO OUT-1
46 IF INPUT FROM Timer2 >= 6.000000 GOTO 5
47 GOTO 39
48 START Timer2 FOR sec
49 PRINT INPUT FROM Temp1 ON LINE 23
50 PRINT INPUT FROM Timer2 ON LINE 20
51 IF INPUT FROM Temp1 >= 34.000000 GOTO 55
52 SEND On TO OUT-1
53 WAIT UNTIL INPUT FROM Temp1 >= 34.000000
54 IF INPUT FROM Temp1 < 34.100000 GOTO 57
55 SEND Off TO OUT-1
56 IF INPUT FROM Timer2 >= 6.000000 GOTO 5
57 GOTO 49
58 START Timer2 FOR sec
59 PRINT INPUT FROM Temp1 ON LINE 23
60 PRINT INPUT FROM Timer2 ON LINE 20
61 IF INPUT FROM Temp1 >= 36.000000 GOTO 65
62 SEND On TO OUT-1
63 WAIT UNTIL INPUT FROM Temp1 >= 36.000000
64 IF INPUT FROM Temp1 < 36.100000 GOTO 67
65 SEND Off TO OUT-1
66 IF INPUT FROM Timer2 >= 6.000000 GOTO 5
67 GOTO 59
68 START Timer2 FOR sec
69 PRINT INPUT FROM Temp1 ON LINE 23
70 PRINT INPUT FROM Timer2 ON LINE 20
71 IF INPUT FROM Temp1 >= 38.000000 GOTO 74
72 SEND On TO OUT-1
73 WAIT UNTIL INPUT FROM Temp1 >= 38.000000
74 IF INPUT FROM Temp1 < 38.100000 GOTO 76
75 SEND Off TO OUT-1
76 IF INPUT FROM Timer2 >= 6.000000 GOTO 5
77 GOTO 69
78 PRINT "PRESS W TO STOP OR PRESS RETURN TO START OVER" ON LINE 17
79 PAUSE
80 IF INPUT FROM SwW = Pressed GOTO 82
81 GOTO 1
82 STOP
```

PROGRAM FOR SAMPLING WITH CYANIDE

```
1 PAUSE
2 PRINT "to stop experiment switch x to down (off) and press w at the pause pr
  omp" ON LINE 17
3 SEND 1.000000 TO Aux
4 DELAY 1 sec
5 START Timer1 FOR min
6 PRINT "counting now... to stop switch x off and press w" ON LINE 19
7 PRINT "Ambient temperature below followed by air bath temperature" ON LINE 2
  1
8 SEND On TO OUT-2
9 DELAY 1 sec
10 SEND Off TO OUT-2
11 DELAY 10 sec
12 PRINT INPUT FROM Temp1 ON LINE 23
13 PRINT INPUT FROM Temp2 ON LINE 22
14 PRINT INPUT FROM Timer1 ON LINE 20
15 SEND INPUT FROM Counter TO COL-A
16 RESET COUNTER
17 IF INPUT FROM Timer1 >= 5.000000 GOTO 21
18 IF INPUT FROM SwX = Off GOTO 20
19 GOTO 8
20 PRINT "TURN SWITCH X BACK ON" ON LINE 18
21 PRINT "**Place crystal in cell *Turn on pump *Press return to sample or press
  w" ON LINE 19
22 PAUSE
23 IF INPUT FROM SwW = Pressed GOTO 86
24 CLEAR TEXT SCREEN
25 PRINT "Prewashing system for 2 minutes, or press w to start sampling" ON LIN
  E 21
26 PRINT "Ambient temp below followed by airbath temp" ON LINE 21
27 START Timer2 FOR min
28 PRINT INPUT FROM Timer2 ON LINE 20
29 PRINT INPUT FROM Temp2 ON LINE 22
30 PRINT INPUT FROM Temp1 ON LINE 23
31 IF INPUT FROM Temp1 >= 29.000000 GOTO 34
32 SEND On TO OUT-1
33 WAIT UNTIL INPUT FROM Temp1 >= 29.000000
34 IF INPUT FROM Temp1 < 29.100000 GOTO 36
35 SEND Off TO OUT-1
36 IF INPUT FROM SwW = Pressed GOTO 39
37 IF INPUT FROM Timer2 >= 2.000000 GOTO 39
38 GOTO 28
39 CLEAR TEXT SCREEN
40 PRINT "VALVE SWITCHED TO B: SAMPLING FOR CYANIDE FOR 15 MINUTES OR PRESS W T
  O W" ON LINE 19
41 PRINT "Ambient temp below followed by airbath temp" ON LINE 21
42 SEND 0.000000 TO Aux
43 DELAY 1 sec
```

```
44 START Timer2 FOR min
45 PRINT INPUT FROM Timer2 ON LINE 20
46 PRINT INPUT FROM Temp2 ON LINE 22
47 PRINT INPUT FROM Temp1 ON LINE 23
48 IF INPUT FROM Temp1 >= 29.000000 GOTO 51
49 SEND On TO OUT-1
50 WAIT UNTIL INPUT FROM Temp1 >= 29.000000
51 IF INPUT FROM Temp1 < 29.100000 GOTO 53
52 SEND Off TO OUT-1
53 IF INPUT FROM SwW = Pressed GOTO 56
54 IF INPUT FROM Timer2 >= 15.000000 GOTO 56
55 GOTO 45
56 CLEAR TEXT SCREEN
57 PRINT "5 minutes is up! VALVE SWITCHED TO A FOR 2 MINUTE WASH OR PRESS W" ON
   LINE 21
58 SEND 1.000000 TO Aux
59 DELAY 1 sec
60 START Timer2 FOR min
61 PRINT INPUT FROM Timer2 ON LINE 19
62 PRINT INPUT FROM Temp1 ON LINE 22
63 PRINT INPUT FROM Temp2 ON LINE 23
64 IF INPUT FROM Temp1 >= 29.000000 GOTO 68
65 SEND On TO OUT-1
66 WAIT UNTIL INPUT FROM Temp1 >= 29.000000
67 IF INPUT FROM Temp1 < 29.100000 GOTO 69
68 SEND Off TO OUT-1
69 IF INPUT FROM SwW = Pressed GOTO 72
70 IF INPUT FROM Timer2 >= 2.000000 GOTO 72
71 GOTO 61
72 CLEAR TEXT SCREEN
73 PRINT "*Turn off pump *Switch to air *Turn on compressor *Return for 5 minut
   e dry" ON LINE 21
74 PAUSE
75 START Timer2 FOR min
76 PRINT INPUT FROM Timer2 ON LINE 22
77 IF INPUT FROM SwW = Pressed GOTO 80
78 IF INPUT FROM Timer2 >= 5.000000 GOTO 80
79 GOTO 76
80 CLEAR TEXT SCREEN
81 PRINT "sampling is done: place crystal in system and press return, or press
   w" ON LINE 21
82 PAUSE
83 IF INPUT FROM SwW = Pressed GOTO 85
84 GOTO 5
85 CLEAR TEXT SCREEN
86 PRINT "If you really want to exit switch X to down, otherwise press return"
   ON LINE 21
87 PAUSE
88 IF INPUT FROM SwX = Off GOTO 90
89 GOTO 5
90 STOP
```

MONTANA STATE UNIVERSITY LIBRARIES



3 1762 10293567 1