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Determination of Iron(II) Concentrations in Seawater Using Flow Injection Analysis and Chemiluminescence

Mark Keller Kinnan
University of North Florida

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Faculty Sponsor: Dr. Stuart Chalk, Assistant Professor of Chemistry and Physics

Introduction

The ocean is a fragile ecosystem that can easily be damaged. If one component of the ocean such as crabs, fish, or nutrients is altered, the entire ecosystem is put in jeopardy. Each aspect of the ocean depends on the functions of the organisms found throughout the marine environment. For example, crabs are scavengers that keep the ocean floors clean of dead fish and other organisms. Without crabs, the bottom of the coastal ocean zones would become a decaying wasteland.

The oceans are delicate because important nutrients such as iron play a key role in the stability of ocean life. Even though there is an abundance of iron in the earth's crust, only low levels of iron are found in seawater. Without iron, oceanic organisms would become extinct. Plants use iron for photosynthetic and respiratory electron transport, nitrate reduction, chlorophyll synthesis, and detoxification of reactive oxygen species.[16]

Phytoplankton are located at the base of the food chain. All heterotrophic marine organisms, defined as acquiring nutrients and fuel by consuming other living organisms, depend on the phytoplankton either directly or indirectly. Heterotrophs that directly eat the phytoplankton are called primary consumers. Any heterotroph that feeds on primary consumers is called a secondary consumer. The organisms that feed on the secondary consumers are called tertiary consumers.[18] By destroying the phytoplankton the rest of the food chain either comes to extinction or finds new ways to adapt for survival. Not only would marine organisms be affected, but terrestrial animals would be put in danger as well. Many species of birds and reptiles feed on the marine organisms. Even humans would be affected by an extinction of marine organisms.

Low concentrations of iron in seawater affect both biotic and abiotic systems. Research has shown that the amount of algae in seawater may play a role in air-sea exchange of carbon dioxide as well as climate change.[19] Since iron is an important nutrient to algae, it is possible that having more iron(II) in seawater will yield more algae.

Phytoplankton are a major consumer of carbon dioxide. In theory, if phytoplankton are limited in number, more carbon dioxide will be present in the atmosphere and contribute to the greenhouse effect. An increased concentration of iron would, therefore, promote phytoplankton blooms that would consume carbon dioxide. There would then be less carbon dioxide in the atmosphere to prevent heat from radiating away from earth. In 1996, an iron seeding experiment was performed in the open ocean to determine whether phytoplankton density would be increased. Phytoplankton rapidly increased in number. The research project results suggested that iron seeding the polar oceans would cause atmospheric carbon dioxide to decrease by as much as 10%.[19] Decreasing atmospheric carbon dioxide is beneficial because over abundance of this substance in the atmosphere causes the Green House Effect. Carbon dioxide prevents radiant heat from leaving earth, thereby increasing the overall temperature.

Iron can be present in ion form or metal form. The ion forms come in two ways: either iron(II) or iron(III). It is the iron(II) that is the important nutrient for phytoplankton and the iron being assessed. Iron(III), however, forms an organic complex that is used by protozoan and zooplankton as an uptake nutrient[1].

The development of special iron(II) detecting systems would allow a better understanding of the relationship between iron(II) concentrations and phytoplankton growth. Currently, the relationship is poorly understood among scientists. Knowing the iron(II) concentrations will, therefore, enhance our knowledge of the biochemical processes in ocean waters.

Background

Chemiluminescence, in simple terms, is the emission of photons from a chemical reaction. It changes chemical energy into light energy.[5] The chemiluminescent signal is described by:[1]

$$I_{CL}(t)=F_{CL}\frac{dC(t)}{dt}$$

$$F_{CL}(+)=F_{EX}F_{L}$$

$I_{CL}(t)$	The chemiluminescent intensity measured in photons/second
F_{CL}	The chemiluminescent efficiency or Quantum yield (photons/molecule reacted)
F_{EX}	The fraction of molecules which reach an excited state
F_{L}	The fraction of molecules in the excited state which luminescence
$\frac{dC(t)}{dt}$	The rate of reaction for the chemiluminescence reaction

Two compounds, an analyte (A) and chemiluminescent reagent (B), react to form a new compound (C*) in an excited state. The excited state compound returns to a lower energy state (C) upon the release of a photon (hv).[2] A generalized sequence of events is:[5,11]

$$A + B \rightarrow C^* \rightarrow C + hv$$

A diagram showing the generalized sequence is:[13]

In addition to the analyte and chemiluminescent reagent, oxygen and alkaline solutions are required to achieve maximum quantum yield of photon release.[2] Experimentation performed by Alwarthan et. al. indicated that molecular oxygen is not *the* sole oxidant responsible for the chemiluminescent reaction.[3] The chemiluminescent reagent used for testing the concentration of iron(II) in seawater is 5-amino-2,3-dihydro-1,4-phthalazinedione, also know as luminol.

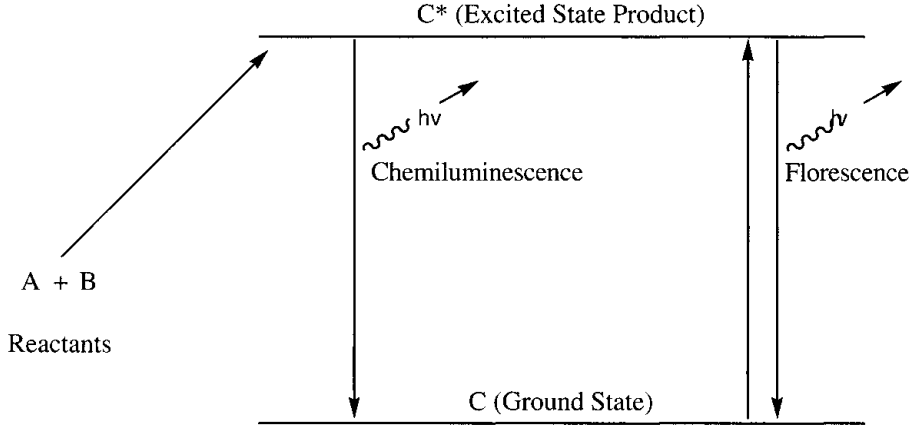


Figure 1. Chemiluminescence vs. Florescence.[14]

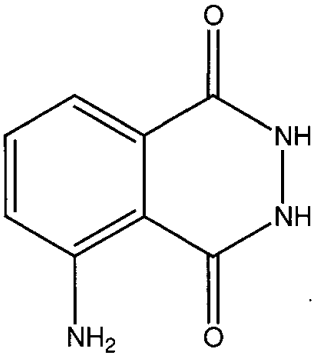


Figure 2. Structure of Luminol.

Luminol is a cyclic hydrazide and one of the more common reagents used for chemiluminescence. The chemiluminescence of cyclic hydrazides involves an oxidative reaction that produces an electronically excited state of the corresponding carboxylate.[4] Even though the chemiluminescent reactions of cyclic hydrazides are not completely know,[10] the reactions occur at millisecond time scales at pH above 10.[9] There are, however, a few observations that are know. Molecular oxygen is consumed and nitrogen is evolved in nearly equal molar amounts.[5,6] A generalized reaction mechanism found throughout the literature for luminol is:

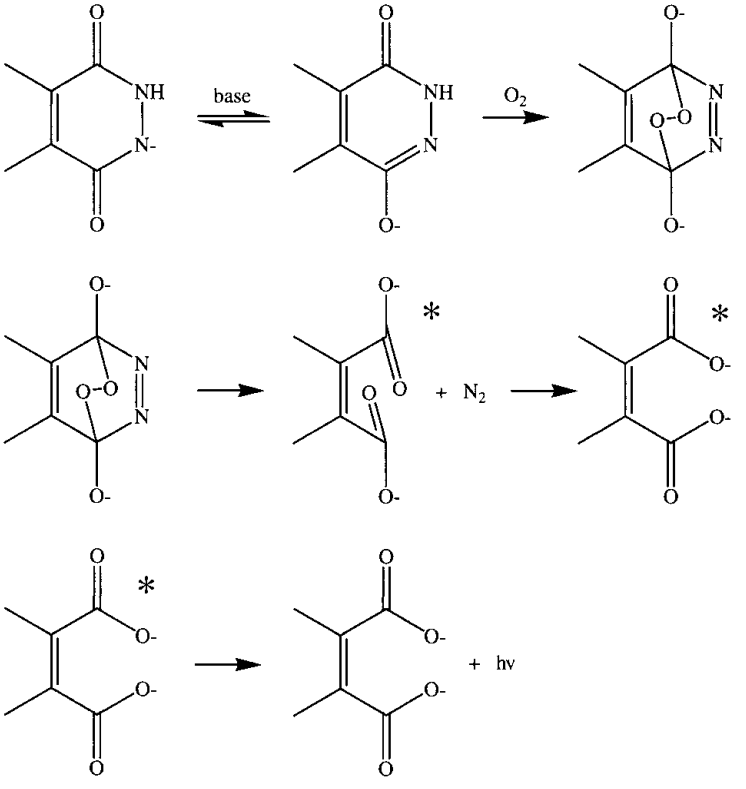


Figure 3. Possible reaction mechanism for luminol.[5,7,14]

Protic and aprotic solvents are capable of being used for chemiluminescence.[5] The system in study is a protic system, which requires a base, hydrogen peroxide, and an oxidizing agent for chemiluminescence to occur.[5] The base used throughout the experimentation is sodium hydroxide (NaOH).

First Methodology and Design

Chemicals Used

All the chemicals that were used each had their own key role. There are several different solutions that are used to determine the concentration of iron(II). Plastic flasks and bottles are preferred over glass because there is a lesser chance to break the plastic. Glass can also trap metals such as iron in its chemical structure. Plastics, on the other hand, are less likely to trap metals and it is easier to remove any metals that may be trapped. The Q-H_2O used throughout the entire experimentation is made from a combined Elix and Millipore water purification system. The "Q" in Q-H_2O is a symbol representing the water has been purified. The different solutions made up are as follows:

1) carrier acid solution: The acid used for making this solution is hydrochloric acid (HCl) and is purchased from Fisher Scientific in TraceMetal grade. It has a pH of 12.1. Before the acid is used, it is sub-boiling distilled three times to remove as many contaminants, especially iron, as possible.[8] The end result is a change in pH from 12.1 (stock) to 9.42 (triple distilled). The pH of the triple distilled HCl may vary, but is roughly 9. The carrier acid solution is then made by taking roughly 1.06 mL of the distilled HCl and diluting it in a 1-liter volumetric flask using Q-H_2O to achieve a concentration of 0.0100M.[8] The carrier acid stream continuously moves through the system to transport the reagent and sample through the system.

2) carbonate buffer solution: Sodium Carbonate was manufactured by Acros and was of hygroscopic grade. The carbonate solution is used to buffer the solution when it mixes with the carrier acid in the chamber. The solution is made by dissolving 10.599g of Na_2CO_3 using Q-H_2O in a 500mL volumetric flask.[3,8]

3) reagent solution: The reagent used was luminol and manufactured by Alexis and Fluka. The solution is made by dissolving 0.8858 g of luminol using the carbonate buffer solution in a 500 mL volumetric flask.[3,8] The pH of the luminol solution is then adjusted to 12.470.05 using 2M NaOH. The reagent solution continuously flows through the system to transport and react with the iron(II) containing sample.

4) iron standard solution. Ferrous ammonium sulfate was manufactured by Thorn Smith and of standard grade. The solution was made up once a month to a concentration of $1.00e^{-2}$M by dissolving 0.7101 g into a 250 mL volumetric flask.[8] Q-H_2O was used to dilute the ferrous ammonium sulfate in the flask.

5) iron sample solutions. The iron sample solutions varied from flask to flask and with the experiment. Six volumetric flasks of size 50.00mL and 100.00mL were used to contain the samples. Samples are made by taking an aliquot of the iron standard and diluting it using Q-H_2O. The size of the aliquot is varied in making the iron sample solutions at different concentrations. The purpose of making six iron sample solutions is to make a calibration curve.

Design of the Prototype Apparatus

In order to begin mixing the different chemicals used for the experiment, a sophisticated pump and chamber system has to be devised. The basis for the design comes from Li et. al.[12] The system uses the principle of flow injection analysis (analyzing a sample as it continuously moves through the system). The light that is produced from the mixing needs to be trapped because the amount of light given

off is proportional to the concentration of iron(II). A completely dark chamber is needed to prevent external light from reaching the photoelectric sensor.

A reaction cell is attached to the photoelectric cell and is the location where the luminol and iron(II) mix. The sensor is wrapped in foam, to minimize outside vibrations, and placed into a PVC chamber capped at both ends. The chamber is made out of black four-inch diameter PVC pipe. Two screw cap fittings are used to close off the thirteen-inch piece of black PVC pipe. A small hole was drilled into the top of the chamber to allow the power wires and tubing to reach the PMT and reaction cell. The hole was sealed with black electrical tape and black foam to prevent any light from entering the chamber. The color black was chosen for all the parts because it absorbs light. Variable speed water pumps are then used to transport the different solutions into and out of the reaction cell located inside the PVC pipe.

The pumps that were used include a peristaltic pump and a milligat pump. The peristaltic pump could push two solutions through the system and was used with the sample and carrier acid solutions. Since the peristaltic pump pulsates, the reagent could not be used with the pump because the flow rate of reagent would vary with every pulsation from the pump. Instead, a milligat pump was used to push the reagent into the system. The milligat pump uses a different kind of technique to pump liquids. A generalized schematic diagram of the layout of the system is:[14]

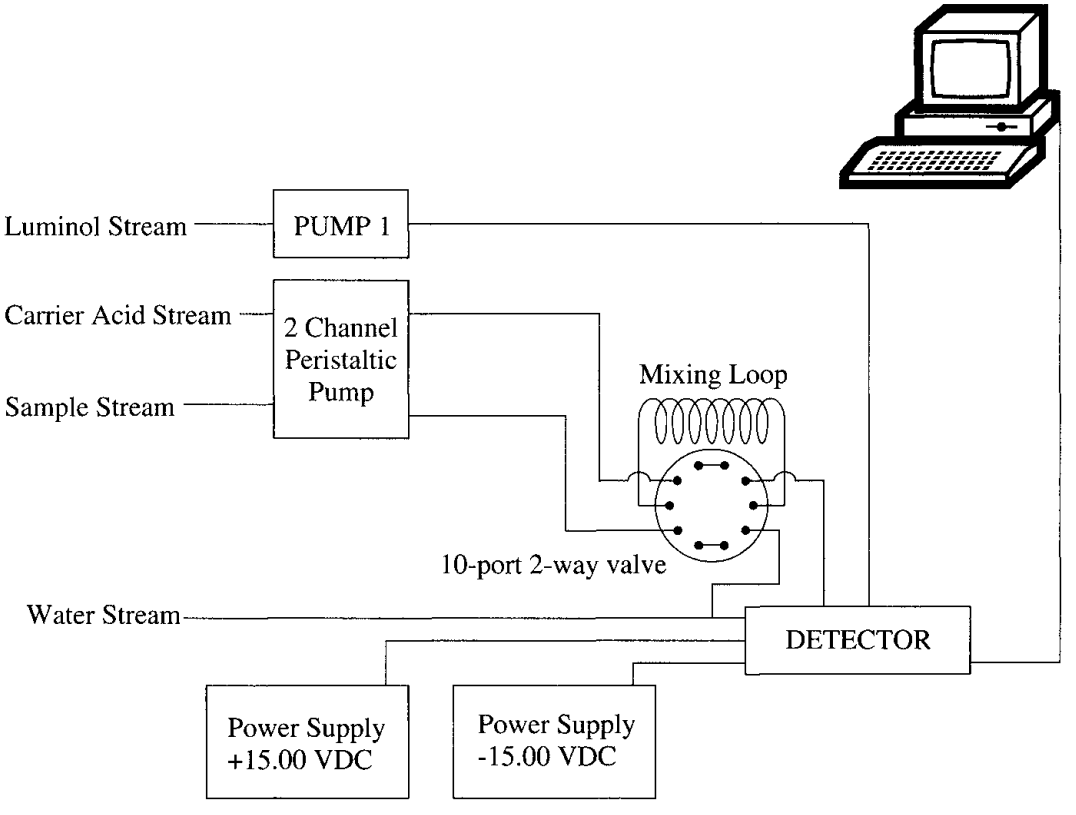


Figure 4. Schematic Diagram of the Layout of the System.

The reaction cell is a complex array of tubing. All the pieces are made of plastic, Teflon, and silica to ensure extraneous metal ions are not introduced into system. The photomultiplier tube that was chosen was a Hamamatsu H5784 series. It has a wavelength recognition range from 185nm to 650nm. The light that is produced from the chemiluminescent reaction has a maximum wavelength peak height at 426nm.

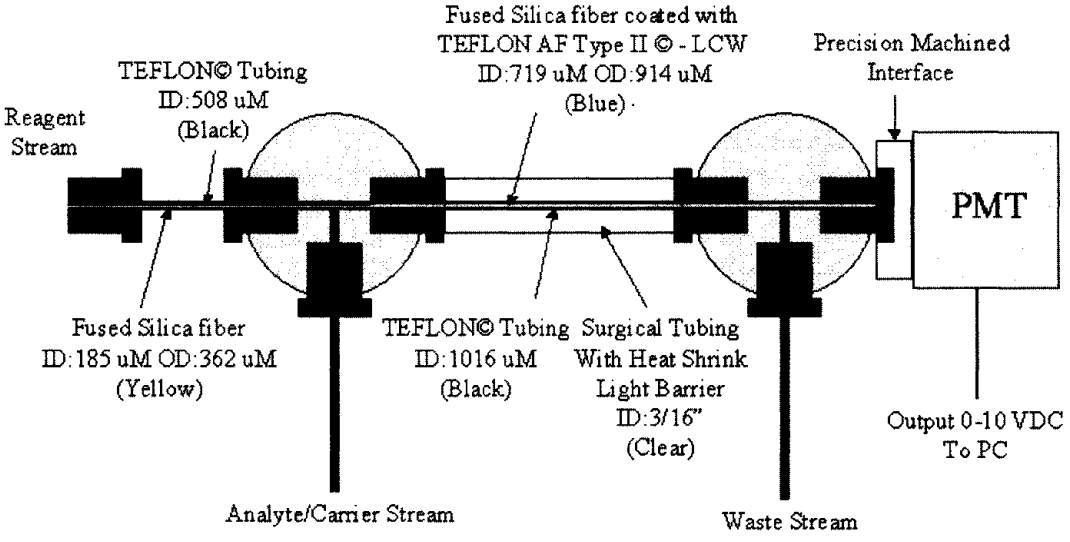


Figure 5. Schematic of the Reaction Cell.

Methodology

The method for determining iron concentration in seawater involves many different components working in conjunction to analyze the amount of iron(II) in a sample. Flow injection analysis, chemiluminescence, and standard additions are the three essential components in analyzing the iron concentration in a sample.

The luminol reacts with the iron in the sample solution to produce a flash of light. The reaction occurs in a fiber optic tube and travels to the ends of the tube. The light is picked up at one end by a sensor called a photomultiplier tube (PMT). The reaction has

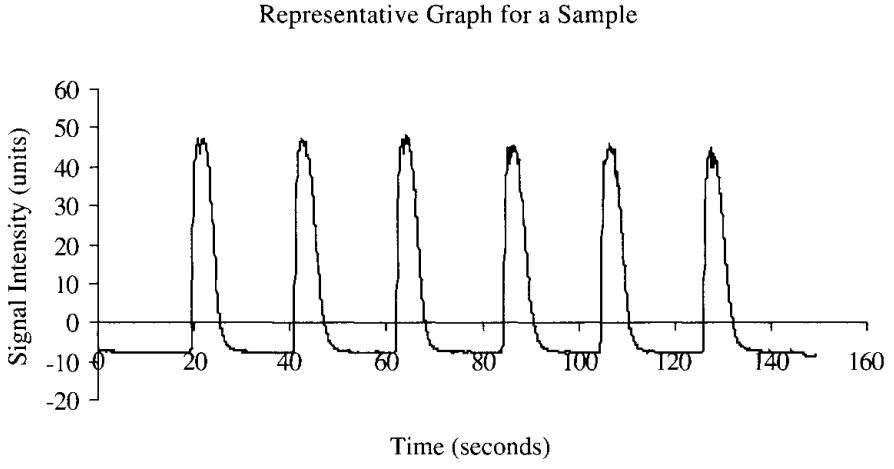


Figure 6. A typical set of peaks after analyzing a sample solution.

to occur in a completely dark chamber to prevent outside light from reaching the PMT. A signal from the PMT is then sent to a computer where a signal peak is recorded. The height of the peak is related to the amount of light that is emitted which is proportional to the concentration of iron in the sample.

After the data has been collected the mathematical technique of standard additions is used to calculate the concentration of iron(II).[15] An x-y graph is plotted with the signal intensity on the y-axis. The x-axis contains the milliliters (mL) of iron added to the sample solutions. For example, 0.05 mL of an iron(II) standard of known concentration are added to sample solution #1 that already contains an unknown amount of iron. This sample is then analyzed and a signal peak is obtained. Then 0.25 mL of the iron(II) standard are added to sample solution #2. The sample is analyzed and a signal peak is obtained. When all six sample solutions are analyzed, a graph is plotted. A linear trend line is added to the graph to obtain the slope and y-intercept. The slope is represented by *m* and the y-intercept is represented as *b*. The ratio of *b* to *m* yields the concentration of sample solution.

Optimization of the System

In order to achieve the maximum height for a signal peak, many variables were manipulated using the same iron standard sample. The effects of pH on the chemiluminescent intensity from mixing is important in acquiring maximum signal.[12] One variable that can easily be altered is the effluent pH. The effluent is the waste that is generated after the chemical reaction has occurred. The pH of the effluent can easily be measured by using a digital pH meter.

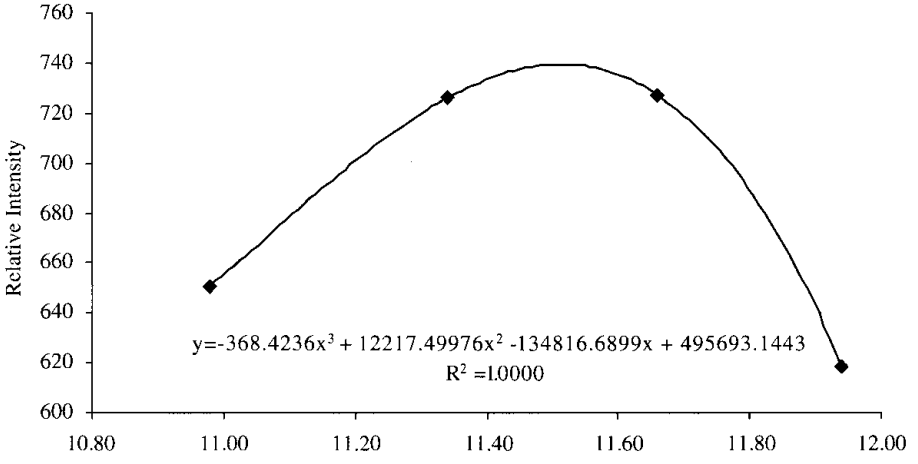


Figure 7. Peak Intensity as a Function of pH Effluent.

The largest signal occurred at a pH of 11.510.05.

The second variable that was modified was the flow rate of the peristaltic pump. The peristaltic pump is responsible for flowing the carrier acid and analyte sample through the system. The flow rate was found to yield the largest signal at a relatively fast flow rate.

The literature showed a variety of concentrations of luminol that were used for FIA. The concentration of the luminol was experimentally determined to function best at 1 x 10^{-2} M for the way it was used.

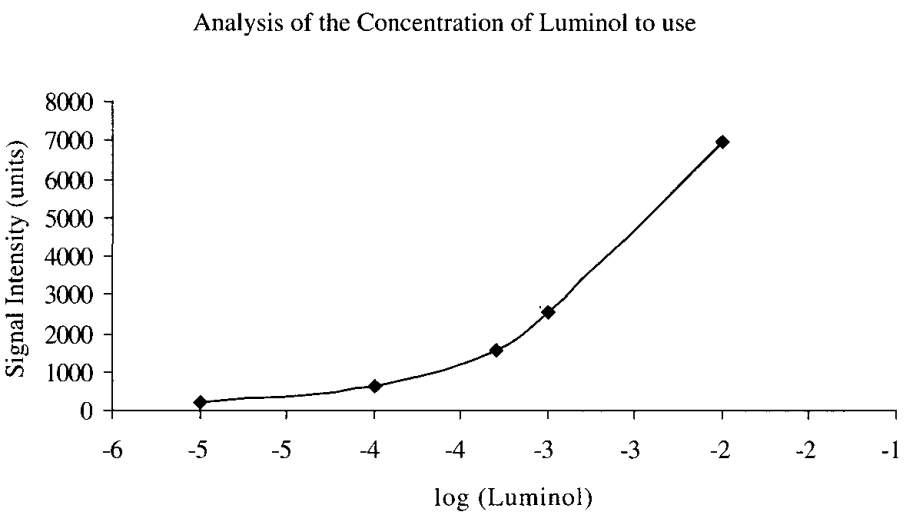
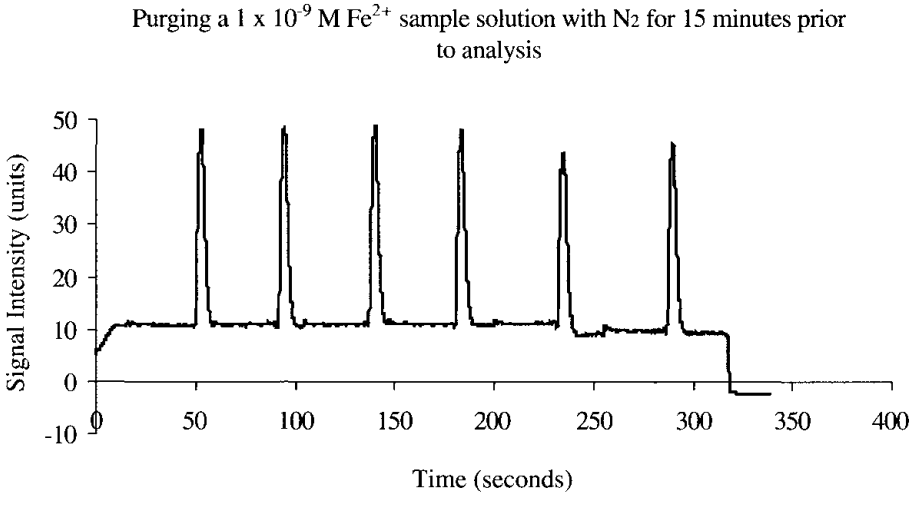


Figure 8. Concentration of luminol.

For the carrier acid, only HCl could be used. Through experimentation and reviews of the literature, HCl gave the largest signal intensity. That is due to the chloride ion. The chloride ion helps to preserve the Fe^{2+} ions in the solutions.

The intensity of chemiluminescence is greater when the solutions were deoxygenated by a stream of nitrogen.[3] The nitrogen purging was tested and experimentation showed a minimum of 5 minutes were needed for a significant difference to be seen. Purging more than 5 minutes was unnecessary. When initial tests were performed using nitrogen, a purge time of 15 minutes was used.



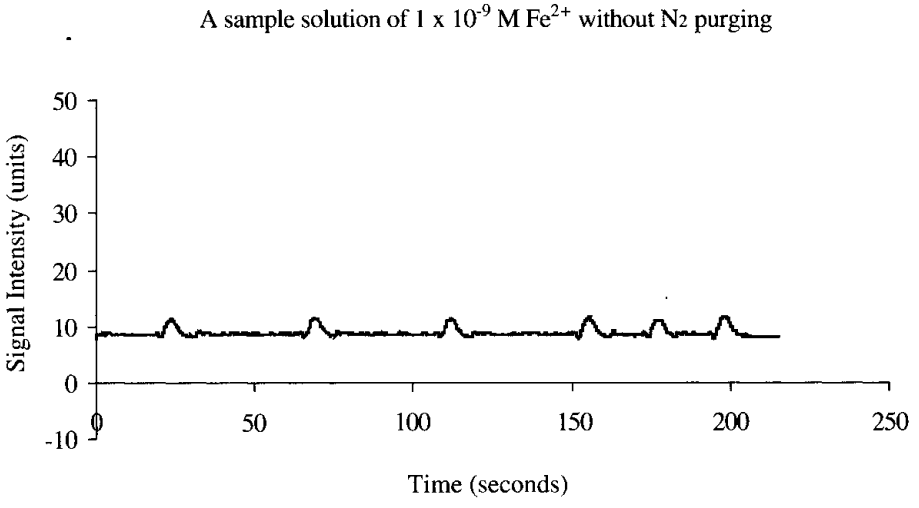


Figure 9. Purging versus No Purging of Sample Solutions.

Change in Methodology

Using a Manual Micro Control Valve:

One of the problems associated with the first methodology is the amount of sample preparation time. It takes approximately two to three hours to prepare all the samples for running. Analysis of the samples takes another thirty to sixty minutes. If sample preparation and analysis go correctly the first time, then the experiment time will a minimum of three hours.

Due to the intensive amount of time associated with the experiment, a quicker method was resolved. Instead of making six sample solutions containing various known concentrations of iron, two solutions are made. One of the two solutions contained a known amount of iron in an acidic solution of pure water. The second solution contained a known amount of iron in an acidic solution of seawater. A milligat pump was used to transfer both of the solutions into the reaction chamber. The flow rates of each solution were controlled by a manually operated micro-control valve. A diagram of the setup can be seen in Figure 10 below.

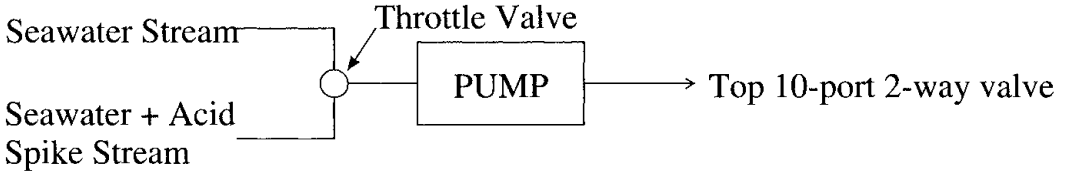


Figure 10. Diagram of throttle location.

The use of the throttle valve eventually led to the evolution of a second methodology and design.

Second Methodology and Design

Chemicals Used

Some of the solutions used for the first methodology and design were used for the second methodology and design. The carrier acid solution, carbonate buffer solution, reagent solution, and the iron standard solutions were the same. The solutions are made up to the same concentrations. The iron sample solutions are not used for the second methodology.

Design of the Prototype Apparatus

The design discussed in the "First Methodology and Design" is similar to the second design, see Figure 11. The only drastic change is the number of Milligat pumps used in the system. The peristaltic pump was removed and replaced with three Milligat pumps so that now there are four Milligat pumps. The reaction cell remains unchanged from the first design.

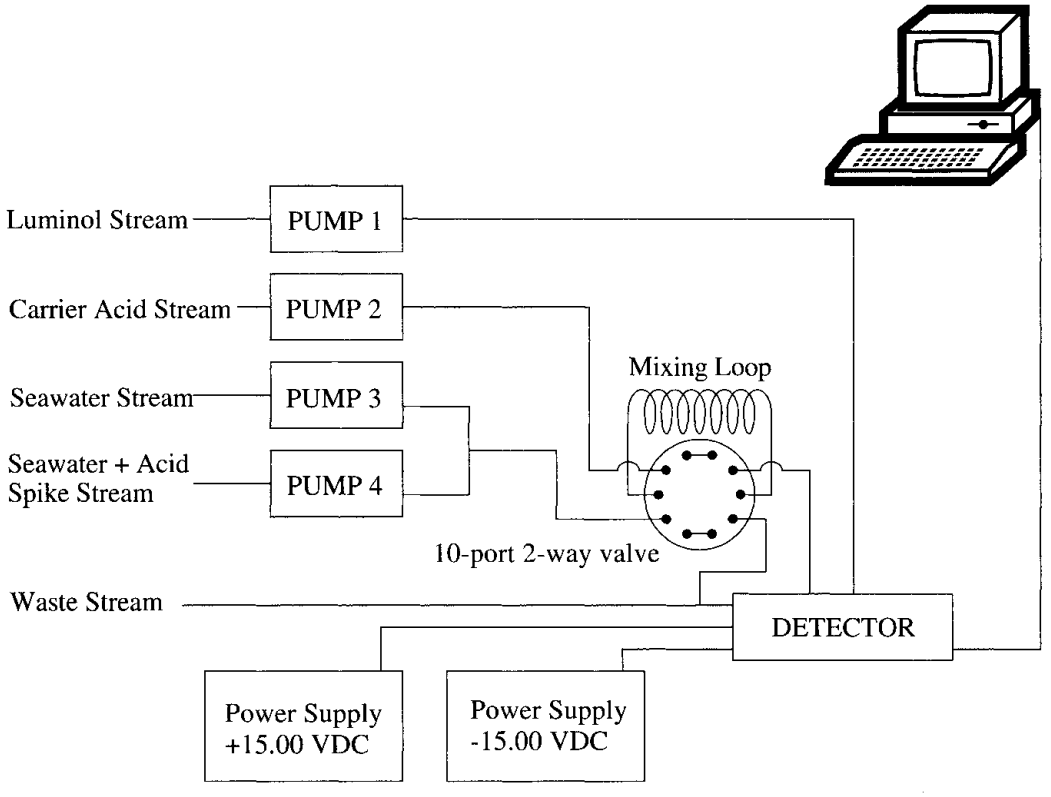


Figure 11. Second instrument design.

Methodology

Currently, the method of analysis has changed to find a more efficient analysis procedure. Instead of using ten solutions to analyze the concentration of seawater, four solutions are used. Only one iron solution is made up in place of the six initially used. The pump speed is varied instead of using different sample solutions. Flow rates and backpressure have been experimentally checked to provide correct analysis for the concentrations. Backpressure was measured qualitatively (visually) by operating one pump and observing the liquid in the tubing connected to the other three (turned off) pumps. Air bubbles were used as indicators of liquid flow. There was little to no back flow observed in the tubing. The analysis of the data is easier because only one graph is produced instead of six.

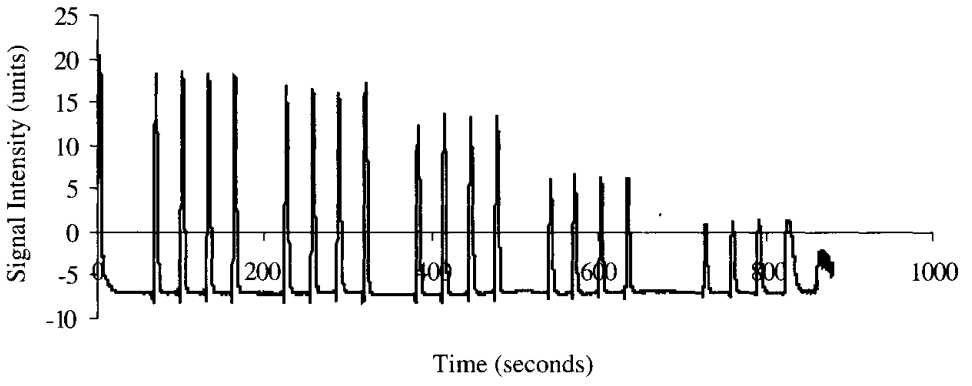


Figure 12. Representative graph of the current data collection method.

Optimization

At this time no re-optimizations of the system have been performed. Since the system setup changed, all the different variables (pH effluent, flow rates, luminol concentrations) have to be retested.

Amount of Light Collected

The setup of the reaction chamber allows for approximately 50% light collection. This is justified because the light has only two directions of travel in the Teflon coated silica fiber; the light travels to the ends. Since only one PMT is used at one end of the silica tubing, roughly 50% of the light evolved during the reaction is collected.

Since light travels spherically outward from its point of origin, most of the light evolved will hit the silica fiber tubing, see Figure 12 below.

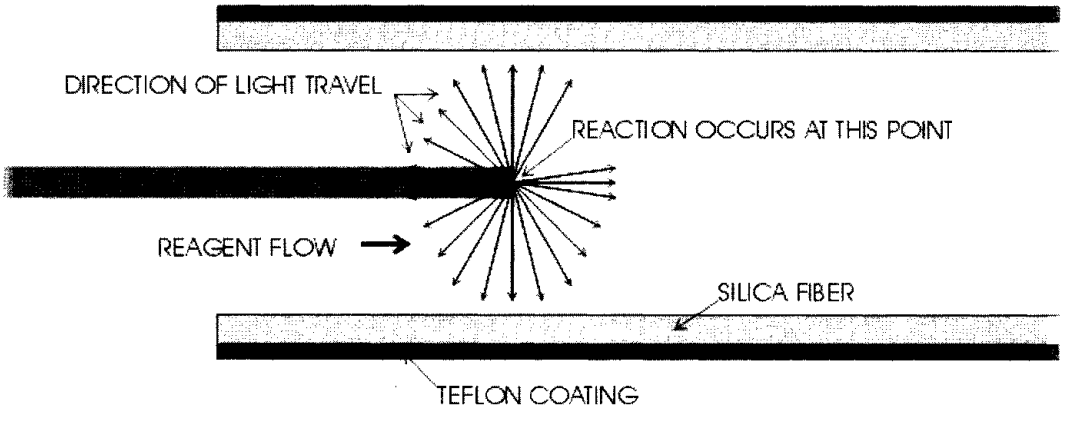


Figure 12. Direction of travel of light.

The ends of the silica fiber light up and the light is recorded on one end by the PMT. The light that is lost results from two phenomenon. First, the light that reaches the silica fiber travels to both ends of the tube. Only half of the light will reach the end of tubing with the PMT. Second, light from the reaction point will travel backwards against the reagent flow and be lost.

A crude theoretical amount of light collected is calculated using surface areas. It is assumed that exactly fifty percent of the light that hits the silica fiber tubing will be collected by the PMT. All the light that travels straight towards the PMT will be collected. All the light that travels out the other end of the tube will be lost. Adding the surface area of the inside of the silica tubing and the ends is the total surface. Subtracting half the area of the cylindrical part of the tubing will take into account half the light that is lost due to the light traveling to the ends of the tubing.

Assuming the reaction takes place at the end of the Sample Flow tubing, the distance between the ends of the Sample Flow tubing and the end of the silica fiber tubing is 1.0 cm. The radius of the silica fiber tubing is 0.03595 cm. The angle ? can be calculated by:

$$\theta = tan^{-1}\left(\frac{r}{1.0cm}\right) = tan^{-1}\left(\frac{0.03595cm}{1.0cm}\right) = tan^{-1}(0.03595) = 2.1°$$

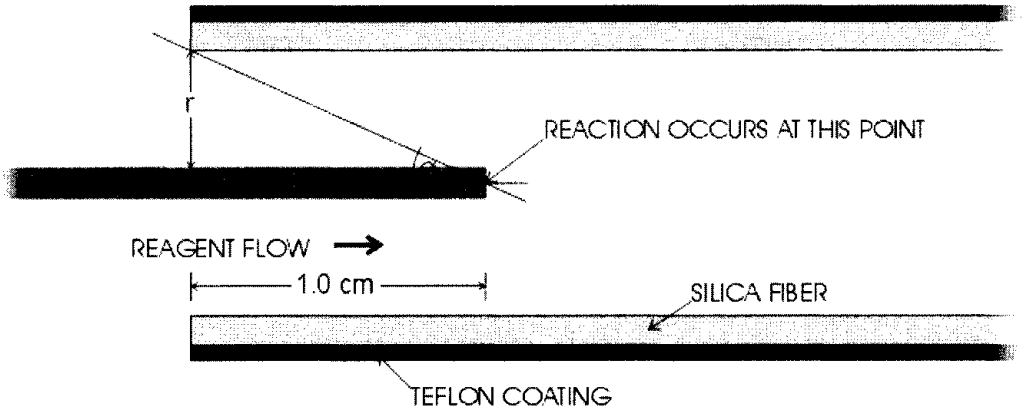


Figure 13. Angle between silica fiber and tubing.

Discussions

Although the reaction occurs on a millisecond time frame, some possible conclusions can be drawn from the data. Looking back at the reaction mechanism and the fact that nitrogen appears not to play a role, it is curious what effect purging the sample solutions with nitrogen has. Nitrogen does not play a role in the reaction because there is no equilibrium reaction involving nitrogen. Nitrogen is only produced and not consumed.

Purging the solutions with nitrogen removes gases, such as oxygen. However, oxygen is used in the reaction by forming a bridging dioxygen bond. It would appear that removing oxygen would hinder the reaction, but the signal generated from removing the oxygen is larger than when

oxygen is present in larger quantities in solution.

Contrary to our findings, Jianxiu et. al. found purging with nitrogen decreased their signal, and purging with oxygen increased their signal. Jianxiu et. al. used thiol-

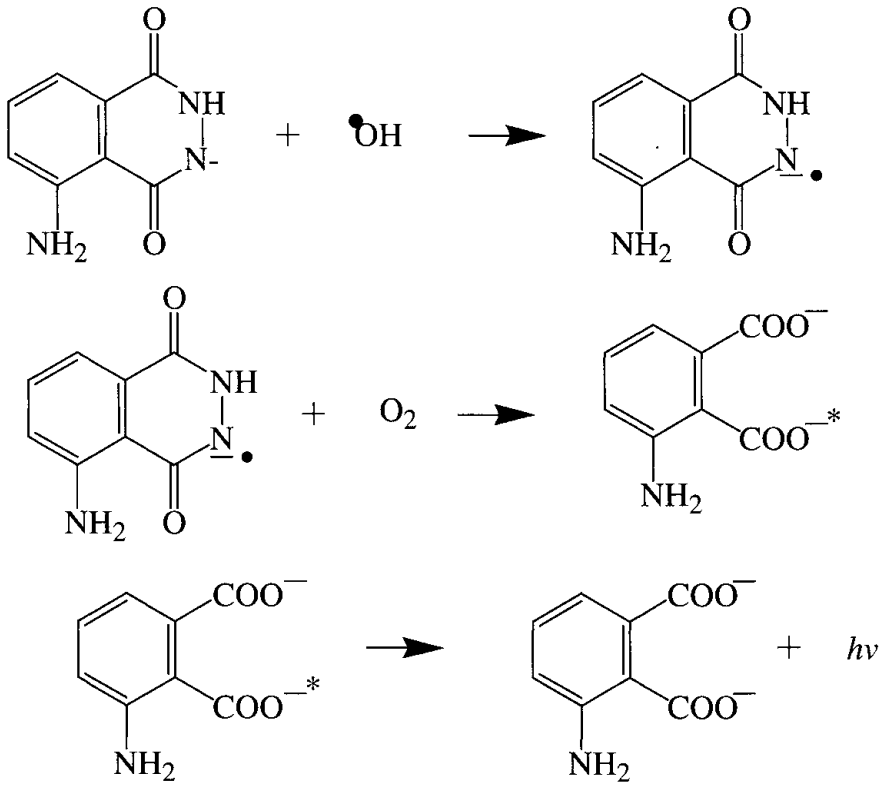


Figure 14. Possible reaction mechanism of luminol.[20]

containing compounds to reduce dissolved oxygen to hydroxyl radicals.

In addition to the reaction mechanism of luminol, there is also a reaction mechanism for iron with hydrogen peroxide and hydrogen peroxide with hydroxyl radical.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + HO^{-}$$

$$Fe^{2+} + HO^{\bullet} \rightarrow Fe^{3+} + HO^{-}$$

$$HO^{\bullet} + H_2O_2 \rightarrow HO_2 + H_2O$$

$$HO_2^{\bullet} + Fe^{2+} \rightarrow Fe^{3+} + HO_2^{-}$$

$$HO_2^{\bullet} + Fe^{3+} \rightarrow Fe^{2+} + O_2 + H^{+}$$

Figure 15. Fenton chemistry.

Current and Future Changes

Many different forms of luminol exist that are possible candidates as substitutes for the 5-amino-2,3-dihydro-1,4-phthalazinedione form currently being used. A chemically and structurally different form of luminol may have higher chemiluminescent quantum yield than the current form of luminol. A new form of luminol will be used in conjunction with the flow injection analysis system currently in use. The reasoning for wanting to find a better form of luminol is to detect lower levels of iron(II) with better accuracy and precision. This application will then be used to test ocean water as well as pond, stream, well, and tap water. The future changes that are in the planning stages are:

a) Optimization of the reaction conditions of iron(II) with a luminol derivative for the following variables: pH, solvent matrix, concentration of the luminol derivative, optimum instrument settings to produce the largest signal to noise ratio, flow rates, and repeatability of method.

b) Removal of flaws in the new method for determining iron(II) concentrations. Four solutions are made with the new method versus ten with the old method. This will provide faster data collection and analysis.

c) Implementation of the reaction chemistry for iron(II) with a luminol derivative in an FIA system using the liquid core waveguide detector. Calibration, background noise, detection limit estimation, reproducibility, and linear dynamic range will all be evaluated.

d) Analysis of other sources of water such as tap, well, and UNF pond water for iron(II). Reproducibility, repeatability, spike recovery and interferences will be investigated in order to evaluate the ruggedness of the methodology for low concentration levels of iron(II).

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