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Study of the effects of CO₂(g) on the Fe(II)-luminol chemiluminescence system: application to ultra-trace analysis of Fe(II)

Sara Beth Lovitz
Colby College

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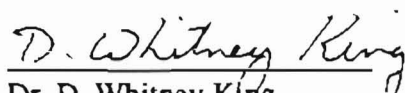
Sara Beth Lovitz

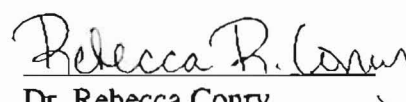
Department of Chemistry
Colby College
May 22, 2001

**Study of the effects of $\text{CO}_2(\text{g})$ on the $\text{Fe}(\text{II})$ –luminol
chemiluminescence system: Application to ultra-trace
analysis of $\text{Fe}(\text{II})$**

Sara Beth Lovitz

Submitted in partial fulfillment of the
Requirements for Honors in Chemistry


Dr. D. Whitney King
Associate Professor, Mentor


Dr. Rebecca Conry
Assistant Professor, Reader

Department of Chemistry
Colby College
May 22, 2001

Abstract

The Fe(II)-catalyzed oxidation of luminol has been a well-accepted chemiluminescence method for the trace and ultra-trace analysis of Fe(II) and Fe(III) in natural waters. Investigation into this chemiluminescence system has shown that CO₂(g) is essential to Fe-catalyzed luminol chemiluminescence. In absence of CO₂(g) there is no chemiluminescent signal. These reactions worked well in the past because CO₂ is present in most laboratory H₂O supplies. Intentional saturation of CO₂ results in a 5-fold increase for this conventional system.

We propose a mechanism for CO₂(g) enhancement that includes formation of a CO₂-superoxide intermediate, which serves as the oxidant of luminol to initiate formation of the luminol radical and subsequent chemiluminescent reactions. Given the new mechanistic understanding of the Fe-luminol system we have optimized a FIA technique in terms of pH and reagent flow rates. The pH dependence of the system was modeled in terms of, and is in good agreement with, observed experimental results. With the incorporation of CO₂(g), detection limits can be improved to below 100 picomolar.

Acknowledgements

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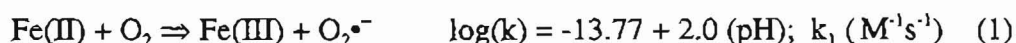
1. Introduction

The redox cycling of iron plays a critical role in the speciation and solubility of Fe, which in turn controls the geochemistry and bioavailability of Fe in marine and fresh water systems. Iron plays a central role in many biological processes as an essential micronutrient, in surface waters it may be the limiting micronutrient, and it may regulate the ability of certain oceanic regions to absorb CO_2 [1]. Iron is needed more than all other nutrient metals, in the greatest quantity by phytoplankton and heterotrophic bacteria. The composition of phytoplankton species and species diversity is influenced by the abundance of Fe [2]. The study of iron species in natural water systems poses a challenge because dissolved iron is found in two oxidation states at extremely low concentrations (often below 1 nM) [3].

There are a number of instrumental methods that overcome these difficulties and are routinely used to measure iron concentrations in natural waters. Of recent interest is analysis of Fe(II)/Fe(III) by competitive ligand equilibration/catalytic cathodic stripping voltammetry (CLE-CSV) [3-4]. The method is based on complexation and preconcentration of Fe(III) by 1-nitroso-2-naphthol (NN)[4] or salicylaldoxime [3] followed with CSV. Iron(III) concentration can be measured selectively by masking iron(II) with 2,2-dipyridyl (Dp) [4]. The iron(II) concentration can then be calculated from the difference of iron measured in absence and presence of Dp. Detection limits for this method are 0.08 nM, 0.08 nM, and 0.1 nM for reactive iron, iron(III), and iron(II) respectively [4]. Disadvantages are that the method is slow, tedious, and requires considerable expertise on the part of the analyst. The methods are not suitable for kinetic studies when Fe(II) concentrations are changing rapidly.

Conventional methods for Fe analysis in lake and seawater have been done by preconcentration followed by graphite furnace atomic absorption spectroscopy. The method is well established with excellent detection limits of 0.05 nM using a 300 mL sample. Disadvantages to the system are a large sample volume, a long analysis time, the method is tedious, and difficult to use at sea [5].

A number of Flow Injection Analysis (FIA) methods have been proposed to help automate Fe analysis. Standard colourimetric techniques don't measure Fe(III) sufficiently but can be used to measure the redox speciation of iron [4]. Another alternative to colourimetric detection involves flow injection analysis using in-line preconcentration onto a column of resin-immobilised 8-hydroxyquinoline (8-HQ) and spectrophotometric detection at 514 nm [5]. The precision of this method for a 0.35 nM Fe sample is 2.5% and the detection limit is 0.025 nM [5]. A widely accepted FIA chemiluminescence technique involves oxidation of Fe(II) species to the Fe(III) ion by oxygen to produce a steady state concentration of superoxide (Rxn 1) followed by subsequent reactions with luminol (5-amino-2,3-dihydro-1,4-phthalazinedione).



As reported by King et al [6], the method can be used for Fe(II) and total iron measurements in natural water systems, and recently has been used in extensive studies on the Fe(II) oxidation kinetics in Swiss lakes [1]. The FIA chemiluminescence system has many advantages over the previously mentioned methods: rapid sample analysis, limited sample handling, multiple analyses on one sample, small sample volumes (10-20

mL), good precision and low detection limits, inexpensive, straightforward, and at-sea analysis of iron [7]. The technique measures any Fe(II) species which can be oxidized to Fe(III) within the resonance time of the flow cell. These species include many Fe(II) complexes which can be oxidized such as $\text{Fe}(\text{OH})^+(\text{aq})$, $\text{Fe}(\text{OH})_2^0(\text{aq})$, and $\text{Fe}(\text{CO}_3)^0(\text{aq})$, and most organic-Fe(II) complexes.

In all past studies of Fe, the chemiluminescence signal has been based on Fe-oxidized chemiluminescence of luminol. The mechanism was thought to involve a superoxide intermediate, which subsequently reacted with luminol to produce light. Similarly, luminol has been used for the analysis of Cr and Co where these metals catalyze production of superoxide from H_2O_2 [8]. An interesting observation by Xiao et al was that $\text{CO}_2(\text{g})$ positively impacted the chromium and cobalt chemiluminescence signal [9]. The authors attributed this enhancement of the signal to formation of a superoxide- CO_2 intermediate. Experimentation with a copper(II)-phenanthroline chemiluminescence system shows complete quenching of the signal upon addition of $\text{CO}_2(\text{g})$ [9]. Again, this was attributed to a superoxide- CO_2 complex.

Annadotedly, many investigators have found Fe-luminol reactions produce a significantly improved signal if the reagent has been allowed to "age" for several days in the lab [9]. Previous reports attributed this effect to the base induced precipitation of trace metal impurities in the luminol. An alternative explanation is that the increase in the signal was due to the increased concentration of $\text{CO}_2(\text{g})$ that was entering the solution from the atmosphere over time. In this paper we specifically investigate the role $\text{CO}_2(\text{g})$ may play in the Fe(II)-luminol analytical system.

2. Experimental

2.1 Apparatus

The flow injection analysis system is shown schematically in Fig. 1. All solutions are delivered using an eight-channel peristaltic pump. A Valco Inc., Model E36 automated, ten-port, low pressure injection valve is used for sample injection in the FLA system. The sample is mixed with luminol reagent and the stream passes in a spiral flow cell situated in front of a Hamamatsu He124-06 integrated photomultiplier tube (PMT) where it produces light. The responsivity of the PMT at 1200 volts is $8.48 \times 10^{11} \text{ V/Watt}^{-1}$. The voltage supplied to the PMT is between 700 and 1000 volts. Simple stop-flow experiments show that the reaction rates are extremely fast and have a half-life of ≤ 10 msec. Thus, the reactions are occurring within the 15 second residence time of the flow cell. The data are collected using a DGH1411 15 bit analog to digital converter which also serves to control the valve. National Instruments Lab View software is used for communication, valve sequencing, and data collection.

Carbon dioxide was added to sample stream using a CO₂ exchanger, consisting of 1 meter coil of 5 mm AF Teflon® tubing placed inside a 500 mL high density polyethylene bottle filled with the appropriate gas. This tubing is a highly permeable membrane which allows for rapid gas exchange [10]. The pCO₂ in the exchange apparatus was controlled by mixing pure CO₂ with argon(g).

2.2 Analytical Conditions

All solutions were prepared with 18 uΩ Milli-Q Barnsted Nanopure water using clean techniques and stored in acid-washed bottles. The luminol (5-amino-2,3-dihydro-

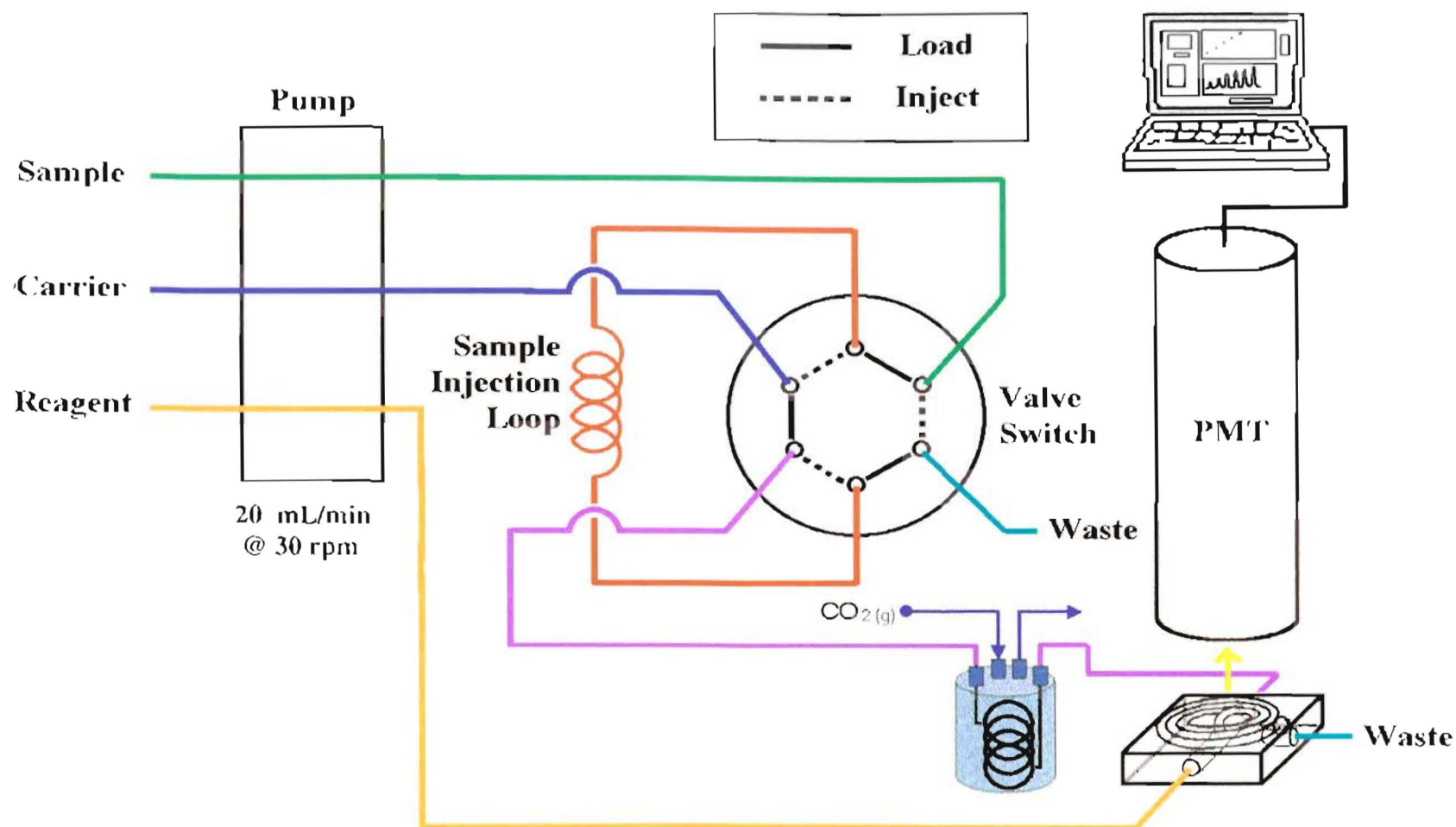


Fig. 1. Diagram of a flow injection system demonstrating the path of sample through the system. The reagent is 0.5 mM luminol in 1 M ammonia adjusted to pH~11. The sample contains varying concentrations of Fe(II) solutions prepared in 0.001 M HCl. The carrier is Milli-Q H₂O. Flow rates are a linear function of pump speed.

1,4-phthalazinedione) reagent was from Sigma (St. Louis, MO, USA), ferrous ammonium sulfate and concentrated HCl (trace metal grade) from Fisher Scientific Company (Fair Lawn, NJ, USA), ammonium hydroxide reagent, ACS grade from ACROS organics (NJ, USA), and ascarite(II) from Arthur H. Thomas Company (Philadelphia, PA, USA). The chemiluminescent reagent was 0.5 mM luminol solution made in 1 M ammonia buffer at pH~11. The Fe(II) stock solutions (1 mM), were prepared in 18 $\mu\Omega$ Milli-Q water and acidified to pH~3 with HCl to prevent oxidation. Working Fe(II) standards (1 μ M) were prepared daily from 1 mM stock solutions. The final pH of the reaction mixture in the flow cell is controlled to 0.1 pH units by the luminol buffer. Superoxide used for mechanistic studies was produced at 100 μ M concentrations by the benzophenone/2-proponal photolysis described by Boland et al [11].

2.3 Removal of CO₂

When necessary CO₂ was removed from the system by sparging the H₂O used for reagent preparation with argon(g) overnight. All subsequent solutions were prepared using the CO₂-free water. The solution bottle tops were fitted with ascarite columns (1.5 cm, 10 cm) to prevent recontamination with atmospheric CO₂.

3. Results and discussion

3.1 Effects of CO_2

Figure 2 shows the signal enhancement of $\text{CO}_2(\text{g})$ on the $\text{Fe}(\text{II})$ -luminol system. A 1 atmosphere partial pressure of carbon dioxide was used to saturate the sample stream with CO_2 and these results were recorded. Versus the conventional FLA system, using solutions that had been fully equilibrated with laboratory air, $\text{CO}_2(\text{g})$ addition produced a 5-fold increase in signal. Carbon dioxide clearly plays a critical role in the chemiluminescence method. To quantify the effect of CO_2 , further studies were undertaken using CO_2 -free reagents.

Figure 3 shows the effect of CO_2 exclusion on chemiluminescence signal. Almost no signal was observed when $\text{CO}_2(\text{g})$ was absent. Argon(g) was used in the gas exchanger to prevent any uptake of CO_2 by the highly permeable membrane. When $\text{CO}_2(\text{g})$ was added in place of the argon(g) an immediate increase in signal was observed reaching an equilibrium signal after several minutes. The effect is reversible. Replacing $\text{CO}_2(\text{g})$ with argon(g) produced a rapid drop in the signal and the signal continued to decrease as the $\text{CO}_2(\text{g})$ was replaced with argon. These results imply that CO_2 not only enhances the signal but is also fundamental to the Fe-luminol system.

The mechanistic implications of CO_2 experiments are interesting as it is unlikely for CO_2 to react with iron or luminol directly. To investigate the nature of the CO_2 effect we measured the influence of CO_2 on the direct superoxide-luminol chemiluminescent method (Fig. 4). In this experiment the $\text{Fe}(\text{II})$ in the system was replaced with a superoxide sample and the chemiluminescent signal was observed. In the absence of CO_2 there was no signal. In the presence of CO_2 chemiluminescence by 10 μM superoxide

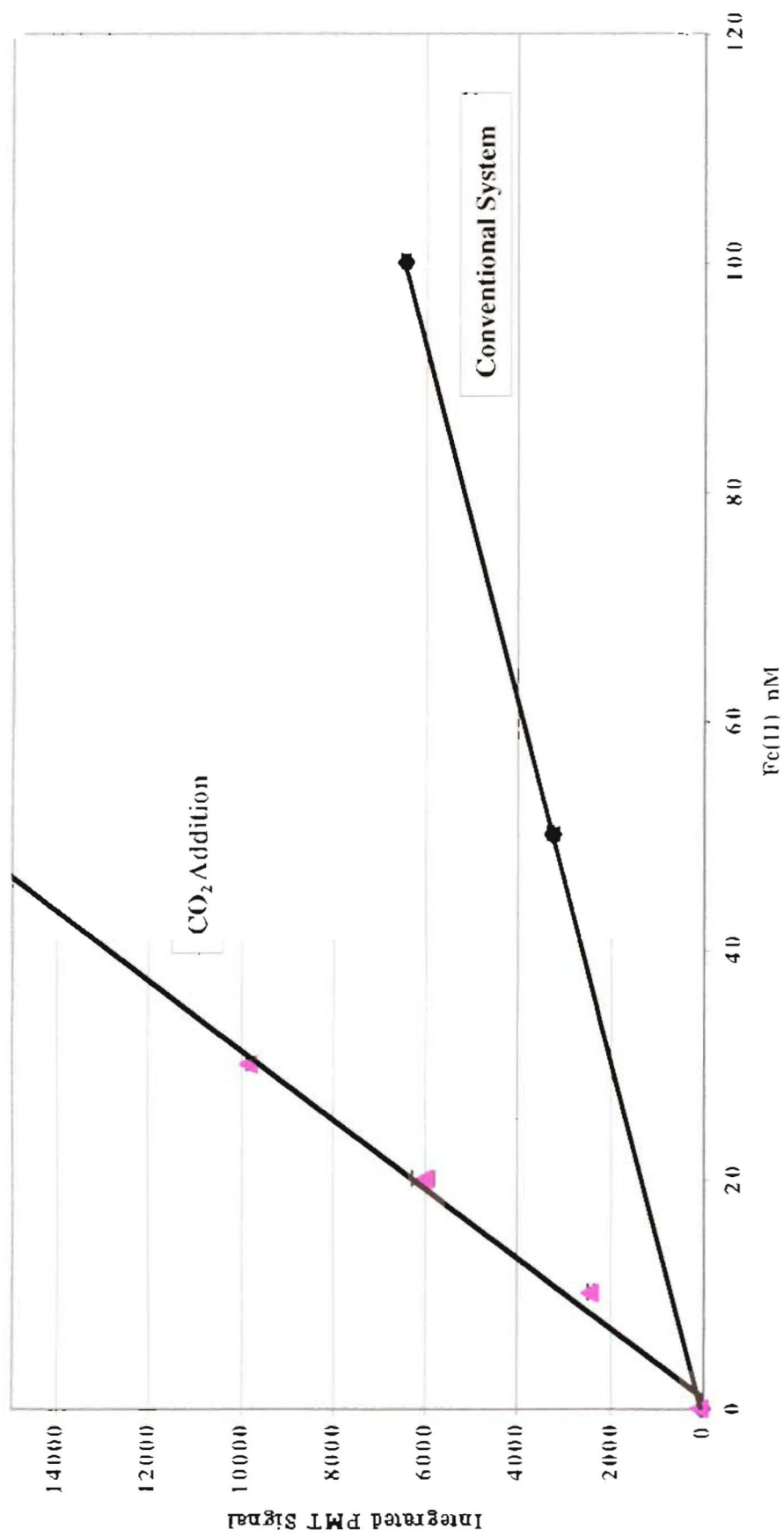


Fig. 2. Plot of signal vs. Fe(II) concentration, with and without CO₂. (PMT at 700 volts)

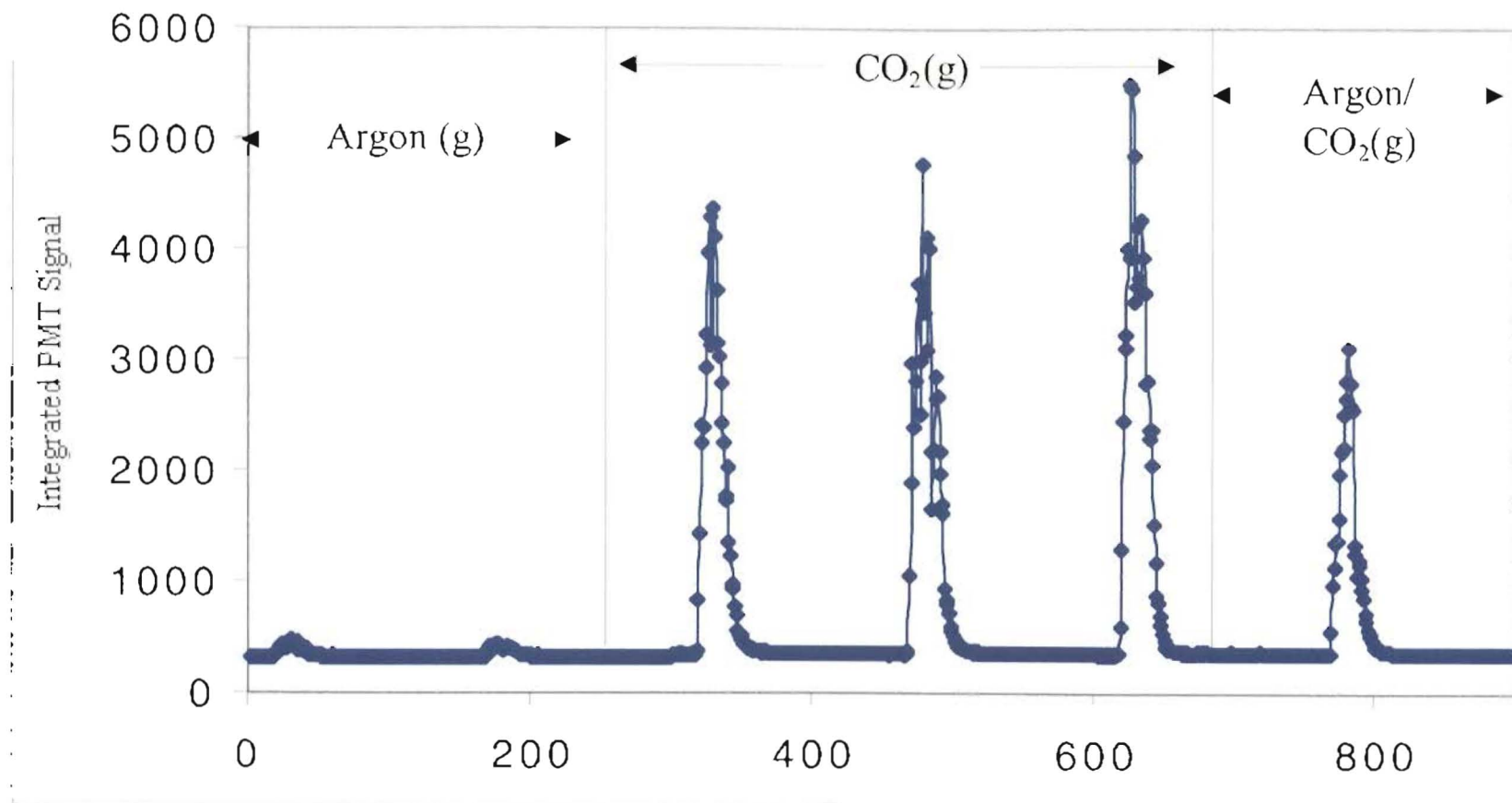


Fig.3. Argon/ $\text{CO}_2(\text{g})$ test demonstrating the signal enhancement by $\text{CO}_2(\text{g})$. Concentrations of the Fe(II) and luminol reagents, pump speed, and the PMT voltage (900 volts) were held constant.

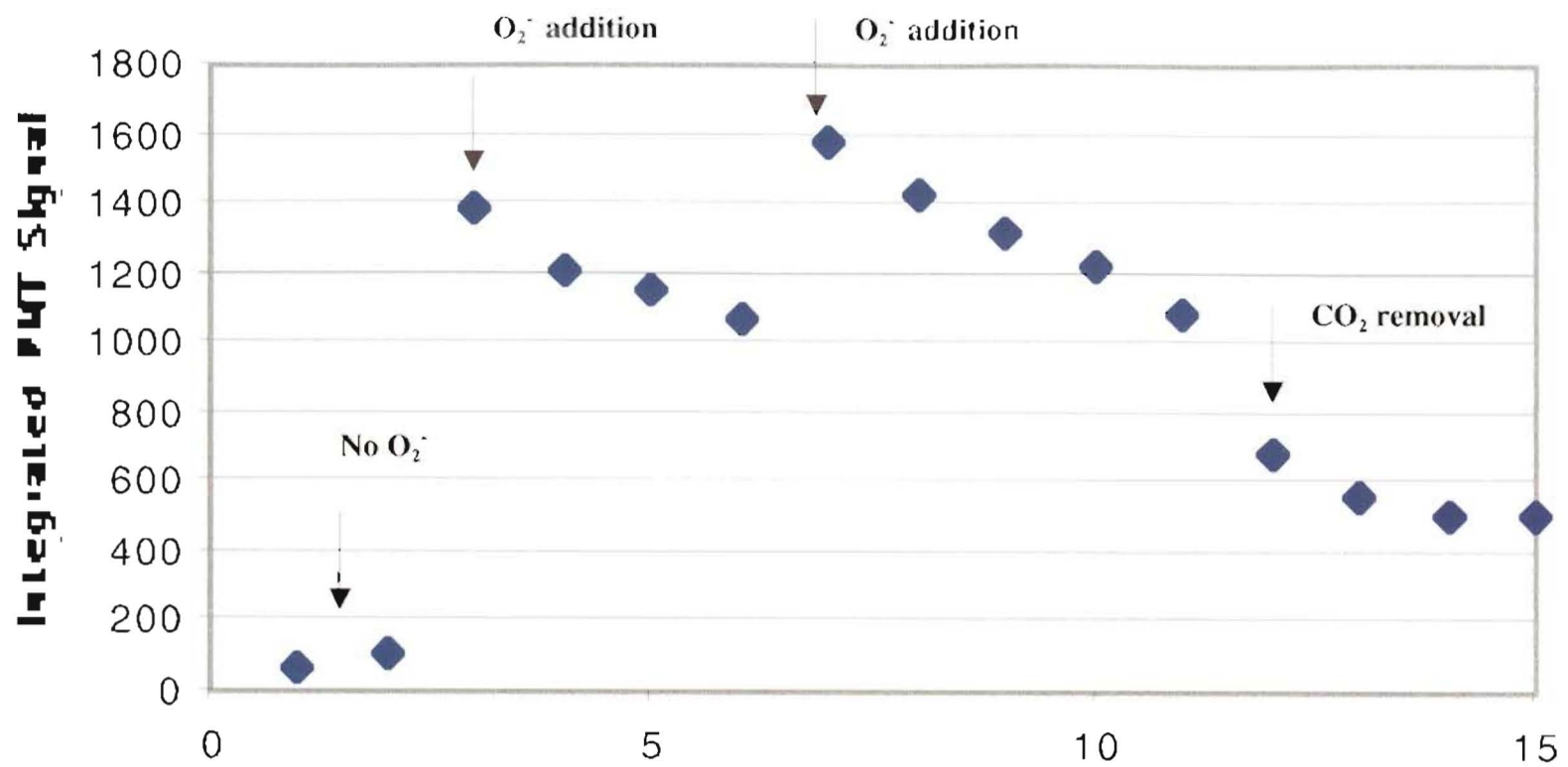


Fig. 4. Plot of superoxide-luminol chemiluminescent signal demonstrating CO_2 enhancement. (PMT at 1000 Volts)

was readily observed and decreases rapidly when CO_2 is removed (Fig. 4). Though smaller than the Fe(II) signal, this experiment also demonstrated that superoxide-mediated luminol chemiluminescence requires CO_2 . It is insightful to put these observations in the context of luminol chemiluminescence mechanism.

Mechanism

The mechanism of luminol chemiluminescence (Fig. 5) has been reported by Xiao et al [8] based on extensive studies by Merényi, Lind, and coworkers [12-15]; however, a $\text{CO}_2(\text{g})$ effect has never been documented. The first step involves the oxidation of luminol to form the luminol radical. Superoxide has been proposed as the oxidizing species. The luminol radical can then react with superoxide to form luminol hydroperoxide. Alternatively, the luminol radical can react with O_2 or itself to form the diazoquinone, which subsequently reacts with HO_2^- , the conjugate base of HOOH , to form luminol hydroperoxide. Hydroperoxide decomposes to form a cyclic intermediate and ultimately 3-aminophthalate in its excited state, which decays to its ground state emitting light. However, the bulk of mechanistic studies have looked at the reactions involving the luminol radical with superoxide to form the hydroperoxide through the formation of 3-aminophthalate [12]. Many species have been shown to oxidize luminol to the luminol radical in the first step of the reaction. These species include: superoxide, iodine, H_2O_2 plus a peroxidase [16], chlorine dioxide, and the hydroxyl radical [17].

In the iron-luminol system, superoxide had been proposed as the oxidizing species for this first step. However, some investigators have been skeptical that superoxide is an effective oxidant of luminol, though it is known to react rapidly with the luminol radical.

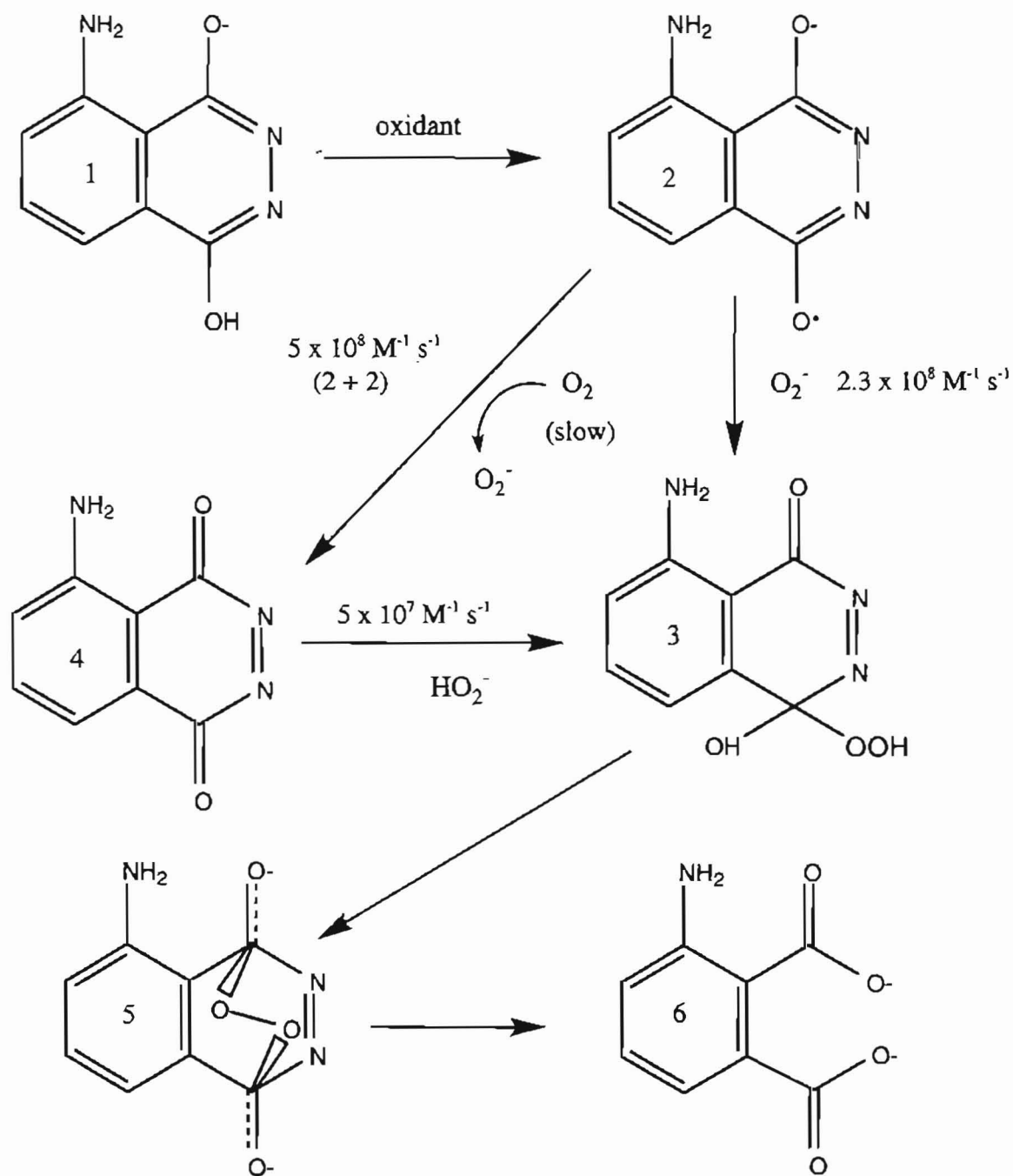


Fig. 5. Structures and mechanism involved in luminol chemiluminescence.

1) luminol 2) luminol radical 3) luminol hydroperoxide 4) diazaquinone
5) cyclic intermediate species 6) 3-aminophthalate

We know superoxide is produced (Rxn 1) by oxidation of Fe(II) with O₂[6]. It was surprising to think CO₂ might react with superoxide. We have not found any reports suggesting a reaction between superoxide and carbon dioxide in aqueous systems; however, these reactions have been reported in aprotic systems. Superoxide radical will undergo nucleophilic addition of CO₂ to form the anion radical, CO₄^{•-}. The CO₄^{•-} radical is also an effective nucleophile toward CO₂ to give an adduct, C₂O₆^{•-}. This species is then rapidly reduced by a second superoxide radical to produce the dianion, C₂O₆²⁻ (Rxns 2-4) [18]. It is possible that these reactions may occur in aqueous solution as well. We



propose that a superoxide-carbon dioxide reaction product (CO₄^{•-}, C₂O₆^{•-}, C₂O₆²⁻) is the oxidant that drives the initial luminol reaction. A superoxide-CO₂ complex is consistent with the enhancement of the Fe system, direct superoxide system, and published Cr and Co systems. Our experiments can not differentiate between the different complexes. However, from simple mass balance calculations we predict that it is the first species, CO₄^{•-}. Our experiments do not provide conclusive evidence for this hypothesis.

3.2 System optimization

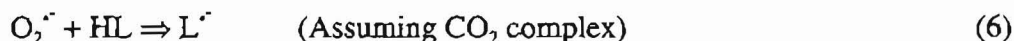
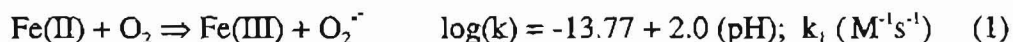
Using these mechanistic insights, we set out to optimize the system. An investigation into the dependence of CO₂ pressure on the signal (Table 1) was conducted. The CO₂ partial pressure was controlled by mixing CO₂ and Ar(g). It was found that analytical signals at pCO₂ of 0.25 Atm. approached 94 % of the signal at pCO₂ of 1.0

Atm, suggesting that the position of the superoxide-CO₂ reactions are shifted in favor of products for the analytical conditions tested.

Table 1. Analytical signal measured as a function of pCO₂ normalized to a pCO₂ of 1 Atm.

pCO ₂	% Signal
0	10
0.07	57
0.12	69
0.25	94
1	100

The system response to the pH of the luminol solution was tested from 8.67 to 11.22. The system conditions with regard to sample, reagent, and carrier were the same as above. At all pHs the reaction rates were fast relative to the resonance time of the sample in the flow cell. The pH dependence of the luminol sample mixture is shown in Fig. 6 with the optimal pH at approximately 9.7. A number of processes were proposed to explain this pH dependence. These include: pH dependent formation of superoxide from Fe(II) oxidation (Rxn 1), further oxidation of Fe(II) (Rxn 5) - assumed pH independent, and superoxide oxidation of luminol to the luminol radical (Rxn 6) where reaction pH is determined the acid-base chemistry of luminol. Clearly, one or more of these reactions require CO₂ as a necessary reactant. However, in the absence of experimental data we will use published superoxide rates, recognizing these will probably change with additional mechanistic studies.



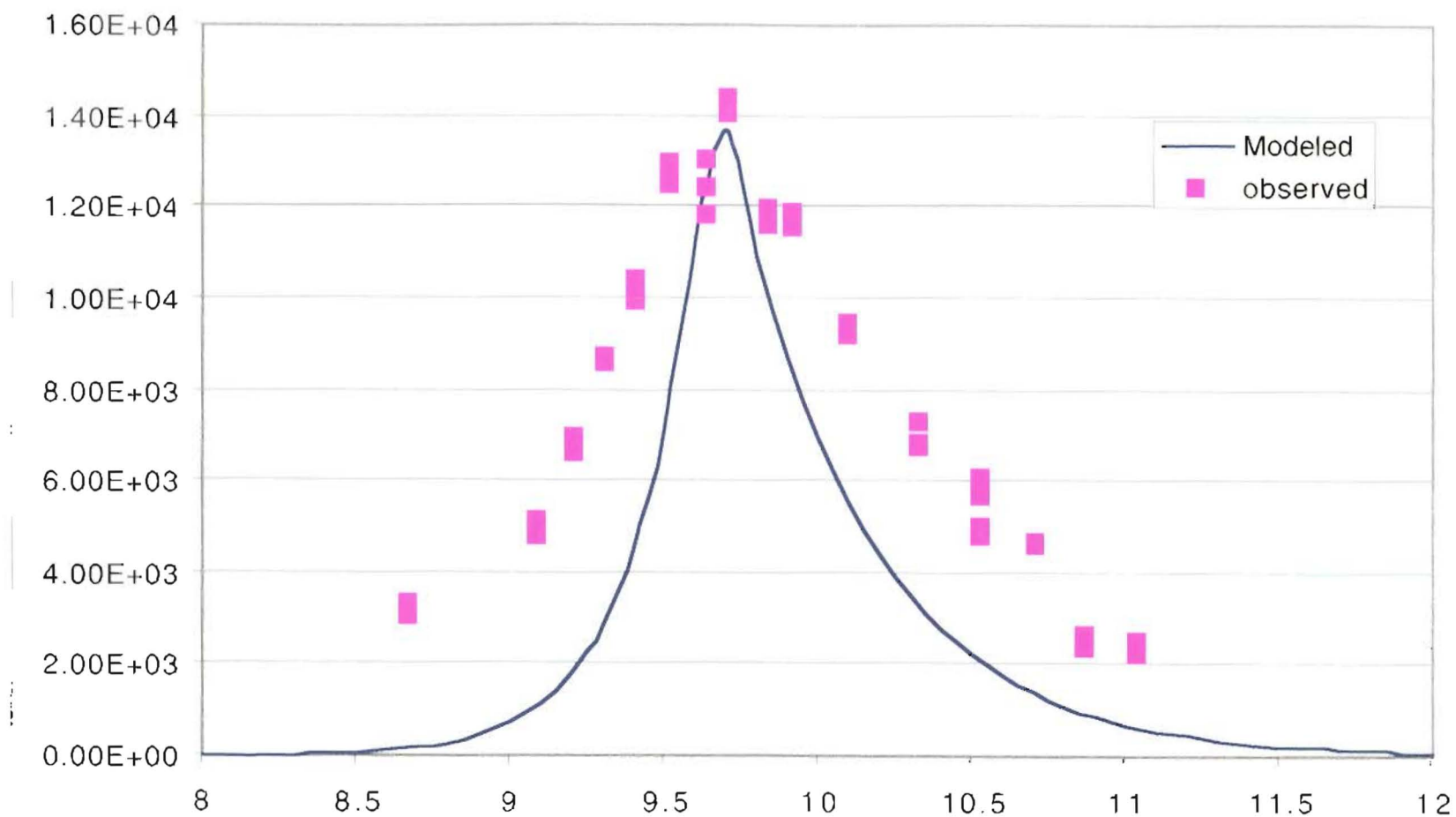


Fig. 6. Signal of the Fe-luminol system pH dependence. Modeled vs. observed dependence. The observed optimal pH was found to be at 9.7. (PMT at 700 volts)

At steady state these processes are equal and can be rearranged to produce the steady state concentration of superoxide. The steady state concentration of superoxide is described by equation 1:

$$SS[O_2^-] = \frac{k_1[Fe(II)][O_2]}{k_L[L] + k_{Fe(II)}[Fe(II)]} \quad (1)$$

The steady state chemiluminescent signal is described by the superoxide-luminol reactions. Photon flux is proportional to equation 2:

$$flux = k_L[L][O_2^-] \quad (2)$$

This function is plotted in figure 6 and agrees well with the data when scaled to a known concentration of Fe(II). We view this model as a diagnostic tool to understand the pH dependence of the signal. The intensity of the curve varies linearly with iron concentration and reproduces the pH dependence correctly. However, the model predicts a shift in the pH maximum with a change in luminol concentration. This has not been specifically investigated and is a priority for future studies.

Further optimization of the system was done as reagent flow rate was specifically investigated. Because the chemical reactions are fast we expect that an increase in flow rates will result in a substantial increase in signal. This was investigated by changing the flow rate at which the FLA system was operated (Fig. 7). The signals were shown to increase with increasing pump speed, reflecting an increase in signal to noise ratio by a factor of 7. Mass balance would suggest that the total photon flux should be independent

of flow rate if all the reactions are occurring in the flow cell. In fact, this value varies by a factor of two, proving again that the reactions are extremely fast. Theoretically, the reagents could be pumped faster, however there is a practical limit over 30 rpm (~20 mL/min) on the tubing and the flow cell. The system is not yet limited by the reactions, but by the flow system integrity.

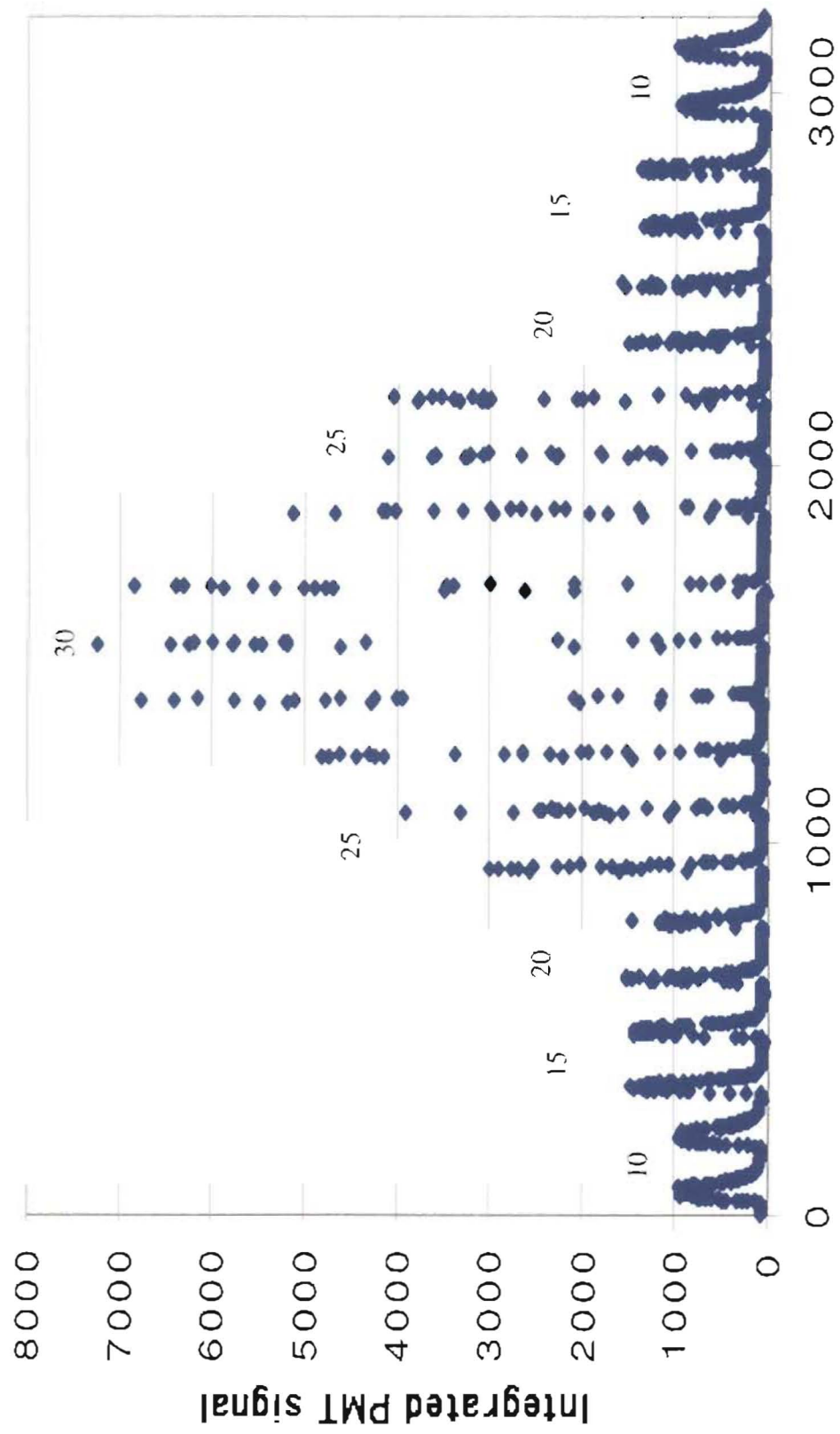


Fig. 7. Plot of varying pump speeds: 10-30 rpm (6.5-20 mL/min). (PMT at 750 volts)

4. Conclusion and Future Work

These experimental results have significant implications for the determination of Fe(II) concentrations. The results of this work demonstrate that $\text{CO}_2(\text{g})$ not only enhances the Fe(II)-luminol chemiluminescent signal, but that it is a necessary component of the system itself. A plausible explanation is the formation of a superoxide- CO_2 intermediate. With this information detection limits improved by a factor of 5 to 10. Extrapolating this enrichment to past system performance, 100 picomolar detection limits for Fe(II) should easily be obtained in both fresh and sea water. It is now also possible to explain some of the empirical observations of luminol aging made in past work, in a quantitative way. Potential interference caused by variation of pCO_2 in natural samples can be avoided by the intentional addition of CO_2 .

Based on the luminol pH experiments, we now have a better sense of the pH dependence of the system. The signal at a $\text{pH} \sim 9.7$ is up to 4-5 times higher than the signal at higher and lower pHs. The model qualitatively fits data and implies that the optimal pH is a function of luminol concentration. This can be tested in the future to further refine the model. The pump speed and stop-flow experiments reaffirmed that the reactions are extremely fast and occur within the residence time of the flow cell. With these new insights, a much better flow system can be developed. The improved mechanistic knowledge of the role of $\text{CO}_2(\text{g})$ on the system allows for use of the system in different natural water types and better understanding of their varying signal responses. However, these results have also raised a number of unresolved questions.

We do not know the exact nature of the CO_2 -superoxide species that we are suggesting is the oxidant involved in luminol chemiluminescence. It can not be ruled out

that the role of $\text{CO}_2(\text{g})$ is in a direct reaction with luminol or any of the luminol based reaction intermediates. The next step is to address these questions experimentally. Further research into the exact mechanism of $\text{CO}_2(\text{g})$ signal enhancement would allow for a better understanding of the system as a whole. The system also needs to be extensively tested in the field for different natural water environments to look into possible interferences, determine detection limits in various water types, and effects of $\text{CO}_2(\text{g})$ on the natural H_2O systems.

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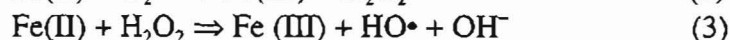
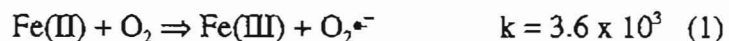
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Appendix:

Fe-Oxidation Reaction Mechanisms [4]:



Solution Preparation:

Luminol - A 0.5 mM luminol solution was made by dissolving 0.0997 g of luminol in a 1 liter 1 M ammonia buffer at pH~11. The buffer was prepared by mixing 132.6 mL of concentrated ammonium hydroxide with 20 mL 1 M HCl in pure H₂O measured by weight to make a 1 L solution. The exact pH measured with a pH probe and recorded.

Iron - 1 mM Fe(II) stock solutions were prepared in pure water and acidified to pH~3 with concentrated HCl to prevent oxidation. The 10⁻⁶ M Fe(II) standards were freshly prepared from this stock Fe(II) solution, in pure water acidified to pH~4 with concentrated HCl.

CO₂(g) Exclusion:

A carboy was filled with pure water and sparged with argon(g). Ascarite columns were placed on the carboy and solution bottles to prevent CO₂(g) contamination from the atmosphere. Solutions were then prepared using this CO₂-free water. Two holes were drilled in the solution bottle tops using a size A drill bit. Fittings were put in the holes, one to attach to the ascarite column and the other to put the tubing through into the bottle.