AN ABSTRACT OF THE THESIS OF

Soren J. Glaser for the degree of Master of Science in Chemistry presented on June 2, 1998. Title: The Determination of Free Chlorine Utilizing Chemiluminescence with Continuous Flow and Flow Injection Analysis.

Abstract approved: Redacted for privacy

James D. Ingle, Jr.

Two chemiluminescence (CL) reactions were investigated for the determination of free chlorine in water samples with continuous flow analysis (CFA) and flow injection analysis (FIA) methods. The first CL reaction investigated was between hydrogen peroxide and chlorine which produces a weak red CL, and the second reaction was between luminol and chlorine which produces a strong blue CL. A unique observation cell with a spiral flow pattern and concentric injector were developed for the flow analysis. Solid phase base (SPB) columns (MgO) were employed which produce a constant pH effluent, and a solid phase luminol (SPL) column was used to produce a constant concentration of luminol.

For the peroxide/chlorine CL system, a boric acid buffer (pH 8.0) and a pre-cell H_2O_2 concentration of 4.4 mM were used. When the SPB columns were employed, the CL signal increased by a factor of over 100, but the enhancement was not reproducible and thought to arise from a contaminant. Dust, Pt, and pH electrode filling solutions

caused similar enhancement of the CL signal. Further studies with this reaction were abandoned because the contaminant could not be identified or controlled.

The CL signal for the luminol/chlorine reaction was highest near pH 11, and the half-life of the reaction increased from 0.12 to 18 s as the pH increased from 9.3 to 11.2, and a pH of about 10 was generally used. The SPL column with an input of DI H_2O produced an output luminol concentration of 0.1 mM. Flow rates of 2 ml/min for the luminol and 0.2 ml/min for the chlorine were used for both FIA and CFA modes. The calibration curve from 0.2 to 1 mg/l OCl^- exhibited a positive deviation. Chlorine down to 0.05 mg/l was detected.

No metal ions tested were found to produce a detectable CL signal. Cu(II) was found to depress chlorine signals. For tap water, the luminol CL method reported a OCl^- concentration within 0.03 mg/L of that determined with the DPD standard method in both CFA and FIA modes. For the FIA mode, 0.1 mM EDTA was required to minimize a signal depression for tap water and also from Cu(II).

The Determination of Free Chlorine Utilizing Chemiluminescence with Continuous Flow and Flow Injection Analysis

by

Soren J. Glaser

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed June 2, 1998
Commencement June, 1999

Master of Science thesis of Soren J. Glaser presented on June 2, 1998

APPROVED:

Redacted for privacy

Major Professor, representing Chemistry

Redacted for privacy

Chair of Department Chemistry

Redacted for privacy

Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Redacted for privacy

Soren J. Glaser, Author

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF TABLES

The Determination of Free Chlorine Utilizing Chemiluminescence with Continuous Flow and Flow Injection

CHAPTER 1
Introduction

Chlorination has been the method of choice for the disinfection of the public water supply in the United States. Other major uses of chlorine include the production of plastics and paper, treatment of waste water, and reduction of biofouling in cooling water.[1] While chlorination assures the public of water free from microbial activity, over chlorination can lead to the formation of carcinogenic chlorinated hydrocarbons such as trihalomethanes.[2,3] Total maximum trihalomethane concentrations in drinking water are currently regulated at 100 μg/l by the EPA[4], and this level may be lowered to 80 μg/l in the near future.

Reducing the free chlorine concentration in the water to the minimum required for disinfection is the most feasible method for reducing these chlorination by-products. In order to accomplish this reduction and control the chlorine level, water treatment plants must be equipped with a chlorine analyzer which is continuous, accurate, and easy to operate. The currently available procedures for chlorine determination have been shown to suffer from interferences and instability of reagents and to require a moderate level of training.[5-7]

In this thesis, two chemiluminescence reactions are examined for their analytical usefulness in free chlorine detection. Chapter 2 discusses the reaction between hydrogen peroxide and chlorine, which produces a weak red chemiluminescence, and chapter 3

discusses the reaction between luminol and chlorine which produces a strong blue chemiluminescence.

Chlorine is introduced into water by adding sodium hypochlorite salt or by bubbling Cl_2 gas into water. Chlorine gas hydrolyzes immediately in water and reaches equilibrium in seconds according to the following reactions.[8]

$$Cl_2 + H_2O \rightleftharpoons HOCl + H^+ + Cl^- \qquad K_1 = 4.2 \times 10^{-4} \qquad (1.1)$$

$$HOCl \rightleftharpoons H^+ + OCl^- \qquad K_2 = 3.4 \times 10^{-8} \qquad (1.2)$$

In the presence of ammonia chloramines are formed.

$$HOCl + NH_3 \rightleftharpoons NH_2Cl + H_2O \qquad (1.3)$$

$$HOCl + NH_2Cl \rightleftharpoons NHCl_2 + H_2O \qquad (1.4)$$

$$HOCl + NHCl_2 \rightleftharpoons NCl_3 + H_2O \qquad (1.5)$$

Free chlorine is defined as the chlorine compounds interchangeable with Cl_2 in an aqueous medium, and in general is the sum of $Cl_2 + HOCl + OCl^-$. At the neutral pH values associated with drinking water (6-8), free chlorine can be considered to be the sum of OCl^- and HOCl.[8] Hypochlorous acid has up to 25 times the disinfecting power of OCl^- so a method of analysis which can distinguish between the two would be ideal.[8]

Methylene chloride, chloroform, carbon tetrachloride, bromodichloromethane, chlorodibromomethane, and bromoform have all been found to be byproducts of water chlorination.[2,3] These compounds are probably formed due to reactions between free chlorine and humic material and bromine in the water supply. It has been shown that these

chlorinated compounds are normally not initially present in the water before chlorination. There is also concern that many organic compounds which do not normally react with chlorine under conditions found in drinking water (such as n-butanol and benzoic acid) may do so if exposed to UV light, which could occur in open holding tanks.[9] Ozonation, an alternative to chlorination, has not been shown to produce these byproducts, but cost of ozonation is probably responsible for chlorine's continued popularity.[10] ClO_2 is also an alternative to chlorination. However, it is an explosive gas not normally employed in water treatment facilities in the U.S.

Current methods of chlorine analysis reported in *Standard Methods*[11] include the DPD colorimetric, iodometric titration, and amperometric titration methods. No ideal standard method exists, because all of the current popular methods suffer from problems such as unstable reagents, common interferants, and the need for moderate operator skill levels.[2,3]

The experience of this researcher with the DPD colorimetric method has shown it to be difficult to use. The color due to the product formed with the DPD reagent changes continuously during experiments making timing a crucial component in the analysis. High concentrations of monochloroamine interfere positively with the DPD method.[2,3]

The amperometric titration is the standard laboratory test for combined chlorine, but the operator has to be very careful and skilled to assure accuracy.[2,3] This method is more lab and time intensive.

The iodimetric titration method relies on the oxidation of I^- to I_2 by chlorine. This method has been reported to be responsive to any strong oxidant and works well for only high concentrations of chlorine (i.e., above 1 mg/l).[2,3]

Utilizing a chemiluminescence reaction for analytical purposes is attractive because the instrumentation is simple, and it can be adapted for flow analysis allowing continuous monitoring. Compared with fluorescence or absorption detection, there is no need for an outside light source or a monochromator or filter. A flow apparatus for a CL analyzer consists of a sample and reagent delivery system (e.g., pumps, tubing), a mixing chamber, an observation cell, and a PMT photodetector.

There are two chemiluminescence reactions known to produce chemiluminescence with chlorine; the first is the reaction between hydrogen peroxide and chlorine, and the second between luminol and chlorine. Chapter 2 details the design of a unique flow apparatus/detection scheme for the peroxide reaction. Chapter 3 discusses improvements on the apparatus and use with the luminol system. Previous work on both the H_2O_2 reaction[12] and the luminol reaction[13] with chlorine have shown analytical promise, and this thesis deals with the optimizations and practical usefulness of such an analyzer.

Most notably Seitz[14] and Marino and Ingle[13] have investigated the analytical utility of the luminol/chlorine reaction without the presence of H_2O_2. Isacsson and Wettermark developed a flow system for use with the luminol-H_2O_2 system.[15] By eliminating the H_2O_2 from the reaction, the number of species that affect the CL are reduced, and any loss in CL efficiency is made up for by the increased selectivity for chlorine. In the presence of H_2O_2 a wide variety of metal ions such as Fe(II), Cu(II), Co(II), and Cr(III) have been shown to

enhance th CL.[16,17,18] This research focuses on continuous flow analysis (CFA) and flow injection analysis (FIA) utilizing the luminol/chlorine reaction without the presence of H_2O_2 and with novel solid state luminol and pH control.

The CL reaction between H_2O_2 and OCl^- has been investigated extensively because of the unique role singlet oxygen plays in its chemiluminescence. Use of this reaction as an analytical tool has been demonstrated by Marino and Ingle[12] in a discrete sampling system. This research focuses on the use of this reaction in a continuous flow apparatus for potential on-line continuous monitoring of chlorine in a water supply.

Previously, work in our laboratory has been directed at developing flow instrumentation for CL detection. The initial designs for a concentric reactant injector, observation cell and PMT housing were completed by Gander,[18] and the research in this thesis involves significant improvements in the design of these components. The Umpqua Research Center (URC) in Myrtle Creek, OR was a key collaborator in this research. URC was responsible for the development of solid phase buffer columns (SPB) and solid phase luminol reagent columns (SPL) which eliminate the need for much of the normal preparation of reagent solutions. URC also developed a solid state iodine column which could possibly be used as a solid phase standard which would eliminate the need to make solution calibration standards for chlorine.

REFERENCES

1) White, C. G. *The Handbook of Chlorination Second Edition,* Van Nostrand Reinhold Company Inc.: New York, New York, 1986, pp 1-60.

2) Kuo, P. P. K.; Chian, E. S. K.; Chang, B. J. *Environ. Sci. Technol.* **1977**, 11, 1177-118.

3) Luong, T.; Peters, C. J.;Young, R. J.; Perry, R. *Environ. Technol. Let.* **1980,** 1, 299-310.

4) EPA. *Federal Register. Rules and Regulations* Vol. 44, No. 231, Thursday Nov 29, **1979**, 68624.

5) Gordon, G.; Cooper, W. J.; Rice, R. G.; Pacey, G. E. J. Am. Water Works Assoc. **1988,** September, 94-122.

6) White, C. G. *The Handbook of Chlorination Second Edition.* Van Nostrand Reinhold Company Inc.: New York, New York, 1986; pp 214-250.

7) Jolley, R. L.; Lyman, C. W.; Johnson, D. J.; et al. *Water Chlorination Chemistry Environmental Impact and Health Effects.* Lewis Publishers: Chelsea, Michigan, 1990; vol. 6, 90-124.

8) White, C. G. *The Handbook of Chlorination Second Edition,* Van Nostrand Reinhold Company Inc.: New York, New York, 1986; pp 150-200.

9) Eilbeck, W. J.; Mattock, G., *Chemical Processes in Waste Water Treatment.* Elis Horwood Limited: Chichester, England, 1987; pp 119-125.

10) Oliver, B. G.; Carey, J. H. *Environ. Sci. Technol.* **1977,** 11, 893-895.

11) Clesceri, L.A.; Greenberg, A.E.; Trussell, R.R. eds. *Standard Methods for the Examination of Water and Wastewater,* 17th ed. American Public Health Association; Port City Press: Washington D.C., 1989, pp 4.45-4.66.

12) Marino, D. F.; Ingle, J. D., Jr. *Anal. Chim. Acta.* **1981**, 123, 247-253.

13) Marino, D. F.; Ingle, J. D., Jr. *Anal. Chem.* **1981**, 53, 455-458.

14) Seitz, W. R. *J. Phys. Chem.* **1975**, 79, 101-106.

REFERENCES (continued)

15) Isacsson, U.; Wettermark, G. *Anal. Chim. Acta.* **1976**, 83, 227-2239.

16) Seitz, W. R.; Hercules, D. M. *Anal. Chem.* **1972,** 44, 2143-2149.

17) Neary, P. M.; Seitz, R.; Hercules, D. M. *Anal. Lett.* **1974**, 7(8&9), 583-590.

18) Hoyt, S. D.; Ingle, J. D., Jr. *Anal. Chim. Acta.* **1976**, 87, 163-175.

19) Gander, S. Master's Thesis, Oregon State University, **1990**.

Chapter 2

The Determination of Free Chlorine by Chemiluminescence Reaction with Hydrogen Peroxide

Soren J. Glaser and James D. Ingle, Jr.

Department of Chemistry
Oregon State University
Corvallis, OR 97331

INTRODUCTION

Overview. Chlorination of the public water supplies for disinfection purposes results in the formation of unwanted by-products, especially the trihalomethanes such as chloroform.[1,2] These carcinogenic by-products are regulated by the EPA at 100 μg/l.[3] Because no reasonable alternatives to chlorination are widely accepted, chlorination is likely to be employed in the foreseeable future. To limit the concentration of the trihalomethanes, the minimum amount of chlorine should be added to the water to maintain antimicrobial activity. A continuous chlorine analyzer that is accurate and easy to operate is crucial to optimize chlorine use in water treatment plants. A chlorine analyzer could also be used to monitor chlorine levels in industrial applications which use chlorine for paper and plastic production and the prevention of biofouling in cooling water.[4]

Current popular methods available for chlorine detection include colorimetric and amperometric procedures.[5] Unfortunately these methods suffer from either unstable reagents or interferences and require moderate operator skills.[6,7]

This chapter is concerned with the chemiluminescence (CL) reaction between hydrogen peroxide and chlorine as a useful analysis tool. A flow system was developed for the analysis including a unique spiral flow cell and concentric reactant injector. Buffers were eliminated in some experiments by the implementation of solid phase base (SPB). SPB's are columns packed with proprietary forms of MgO from the Umpqua Research Center (URC) in Myrtle Creek, OR. The MgO dissolves in a H_2O inlet stream causing OH^- formation which increases the pH.

Reaction Chemistry. Originally discovered in 1927 by Mallet,[8] the reaction between hypochlorite and hydrogen peroxide produces weak red chemiluminescence at 636 and 703 nm.[9] The reaction mechanism[10] is outlined below.

$$H_2O_2 + OCl^- \rightarrow H_2O + OOCl^- \qquad k_1 = 2.8 \times 10^3 \text{ l mol}^{-1} \text{ s}^{-1} \qquad (2.1)$$

$$2OOCl^- + H_2O \rightarrow 2O_2(^1\Delta_g) + 2\,Cl^- \qquad (2.2)$$

The O_2 gas released from the reaction is in the excited singlet state. And the formation of the chloroperoxy ion is rate limiting.

$$2O_2(^1\Delta_g) \rightarrow 2O_2(^3\Sigma_g) + h\nu\ (633, 703\ nm) \qquad (2.3)$$

Two singlet O_2 molecules combine in the gas phase to relax to the ground (triplet) state producing one photon. This reaction occurs under basic conditions, and the light has been reported to come from the bubbles formed by the reaction.[11] It has been proposed that the reaction is first-order in OCl^- because step 1 is rate limiting.[12]

Most of the reported research concerned with this reaction is mechanistic in nature, but Marino and Ingle[13] have investigated its analytical usefulness in a discrete sampling apparatus. The parameters of the final reaction mixture were pH 8.0 (borate buffer) and 0.05 mM H_2O_2, which yielded a detection limit of 4 µg/l. The resulting OCl^- calibration curve was non-linear (positive deviation) indicating that reaction (3), the deactivation of $O_2(^1\Delta_g)$, may be partially rate limiting. Marino and Ingle provided interference data for over 35 species, of which only ClO_2 would be a possible interferant in tap water analysis.

INSTRUMENTATION

Overview. The apparatus for the chemiluminescence (CL) determination of hypochlorite by reaction with H_2O_2 consists of a reaction observation flow cell, a PMT detector, a concentric reagent injector, two pumps for pumping the reagents, a light tight enclosure (PVC cylinder), a solid phase bed (SPB) column for pH control, an effluent pH meter, and basic signal processing equipment. A schematic of the integrated system is shown in Figure 2.1. Two basic versions of the equipment were employed, and the first version used in this thesis was adapted from previous CL work done by Stuart Gander at Oregon State University.[14]

An H_2O_2 solution and a free chlorine sample or standard are pumped via separate pumps to the concentric injector inside the PVC cylinder. The injector directs and mixes the reactants in the reaction observation cell producing CL within view of a PMT detector. The waste reagents are carried to a flow through pH probe and then to waste.

Concentric Reagent Injector. Two injectors described by Gander were used in these experiments.[14] The first injector is a Beckman burner assembly (No. 4020) normally used as an atomizer for atomic absorption or atomic emission spectrometry. The injector consists of a brass body with two input ports, a tapered nozzle, and a platinum capillary tube used to carry the analyte solution while keeping it separate from the reagent solution, which travels around the outside of this tube. The second injector is similar to the first

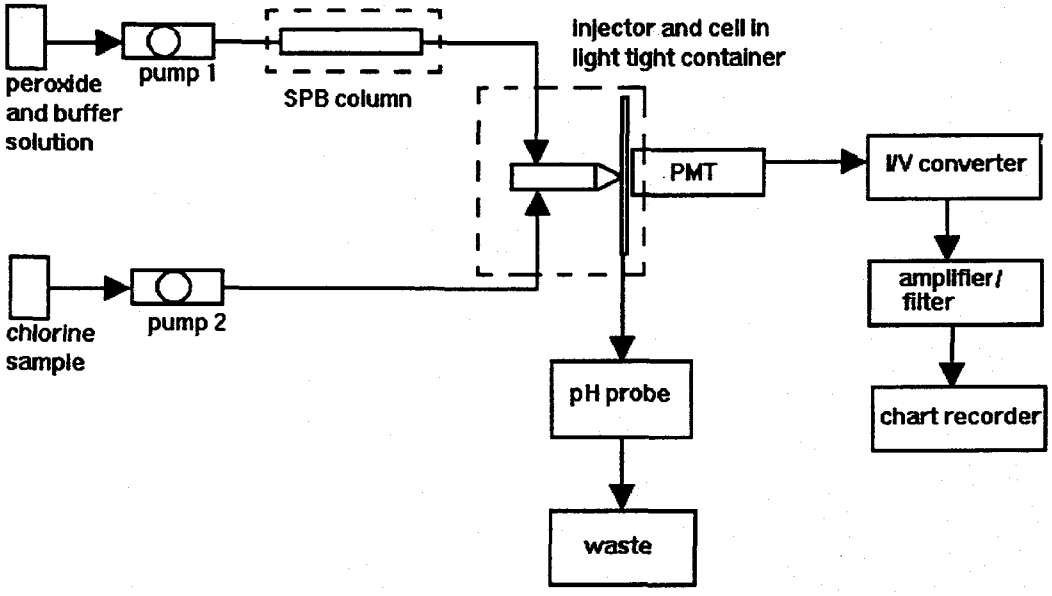


Figure 2.1. Schematic of the H_2O_2/OCl^- Analyzer. This apparatus was used for most of the experimentation. The SPB column in-line with the peroxide stream was sometimes used instead of a buffer solution. In continuous flow mode, both pumps run continually pumping the reagent and sample into the observational cell.

injector but was made in the OSU machine shop by John Archibald, primarily from Delrin (see figure 2.2).[15]

These injectors are both of concentric design, meaning that one reagent is carried through the 0.010-in inner diameter platinum tube with a wall thickness of 0.007 in. The tube ends flush with the end of the nozzle, and the other reagent exits through the annular orifice of the nozzle which surrounds the platinum pipe. The inner and outer diameters of the nozzle are 0.030 and 0.065 in, respectively. Set screws in the nozzle help align the platinum tube to be concentric within the nozzle opening.

The injector screws into the observation cell via a Plexiglas adapter bonded to the bottom plate of the cell, as shown in Figure 2.3. The openings of the injector nozzle are flush with the bottom of the reaction chamber when it is fully threaded into the cell.

Observation Cell. The first generation flow cell developed by Gander consisted of two 1/4-in thick transparent polycarbonate disks separated by metal washers (spacers) and an "O"-ring seal (see figure 2.3). The eight 0.41-mm thick washers, spaced evenly by 4.52 cm in 45 degree arcs, separate the two plates of the observation cell. An "O"-ring seal fits in a groove 1.06 in from the center of the bottom plate. As described above, the reagent and analyte solutions are injected via the orifices of the injector in the center of the bottom plate. The flowing sample and reagent streams hit the upper plate of the cell resulting turbulent mixing. The mixture then flows toward the perimeter of the cell. There are 12 equally spaced drain holes in a groove cut into the bottom plate of the cell at a radius of 1.0 in from the injection port. The total volume defined by the plates separated by the

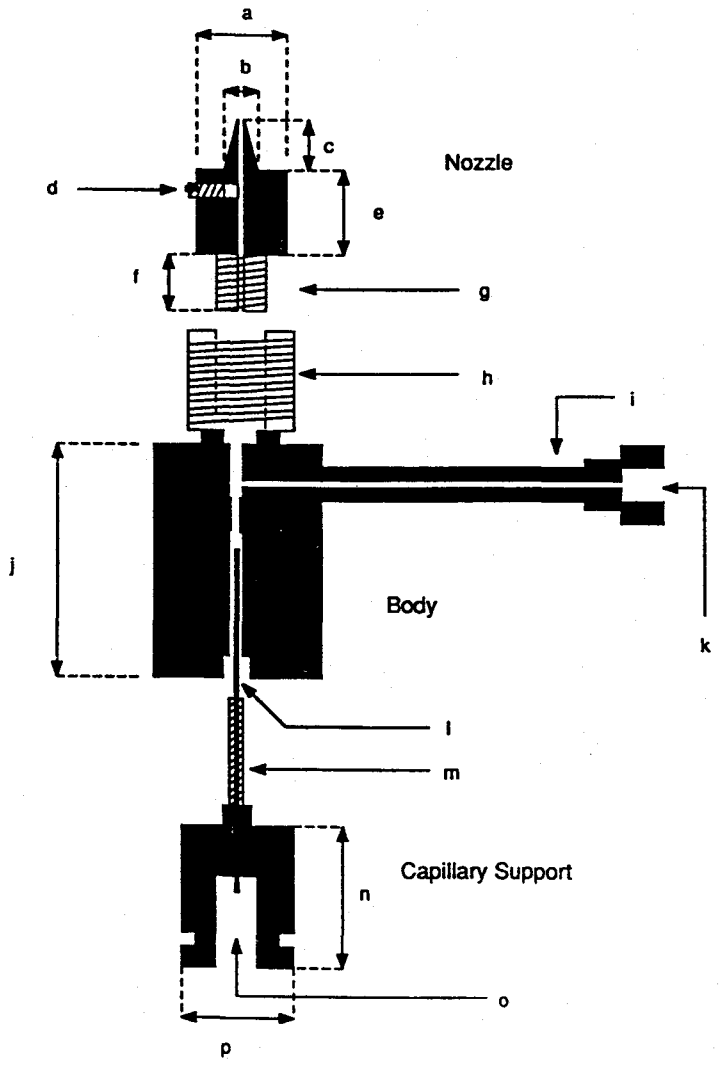


Figure 2.2. Injector Schematic. The injector is made from three sections: a nozzle, a body, and a capillary support. The nozzle is Teflon with a 0.060 in o.d.; (a) 0.39 in; (b) 0.19 in; (c) 0.29 in; (d) one of three 4-40 nylon collimating set screw; (e) 0.49 in; (f) 0.38 in; (g) 1/4-28 thread. The body is Teflon; (h) 1/2-32 thread to screw into the bottom plate of the cell; (i) Delrin side arm which carries the reactant which travels through the annular opening of the injector; (j) body length was 0.88 in with a 0.75 in diameter; (k) outer side arm port is tapped to accept 1/4-28 low pressure end fitting. The capillary support is Nylon; (l) inner conduit of platinum tubing has a 0.016 in i.d. and a 0.028 in o.d. (0.8-mm i.d. Teflon tubing slides over the capillary shaft); (m) 6-32 thread on shaft which is 0.55 in long; (n) 0.48 in; (o) the capillary support is tapped to accept a 1/4-28 low pressure end fitting; (p) 0.45 in. Figure taken with permission from the thesis of Gander.[14]

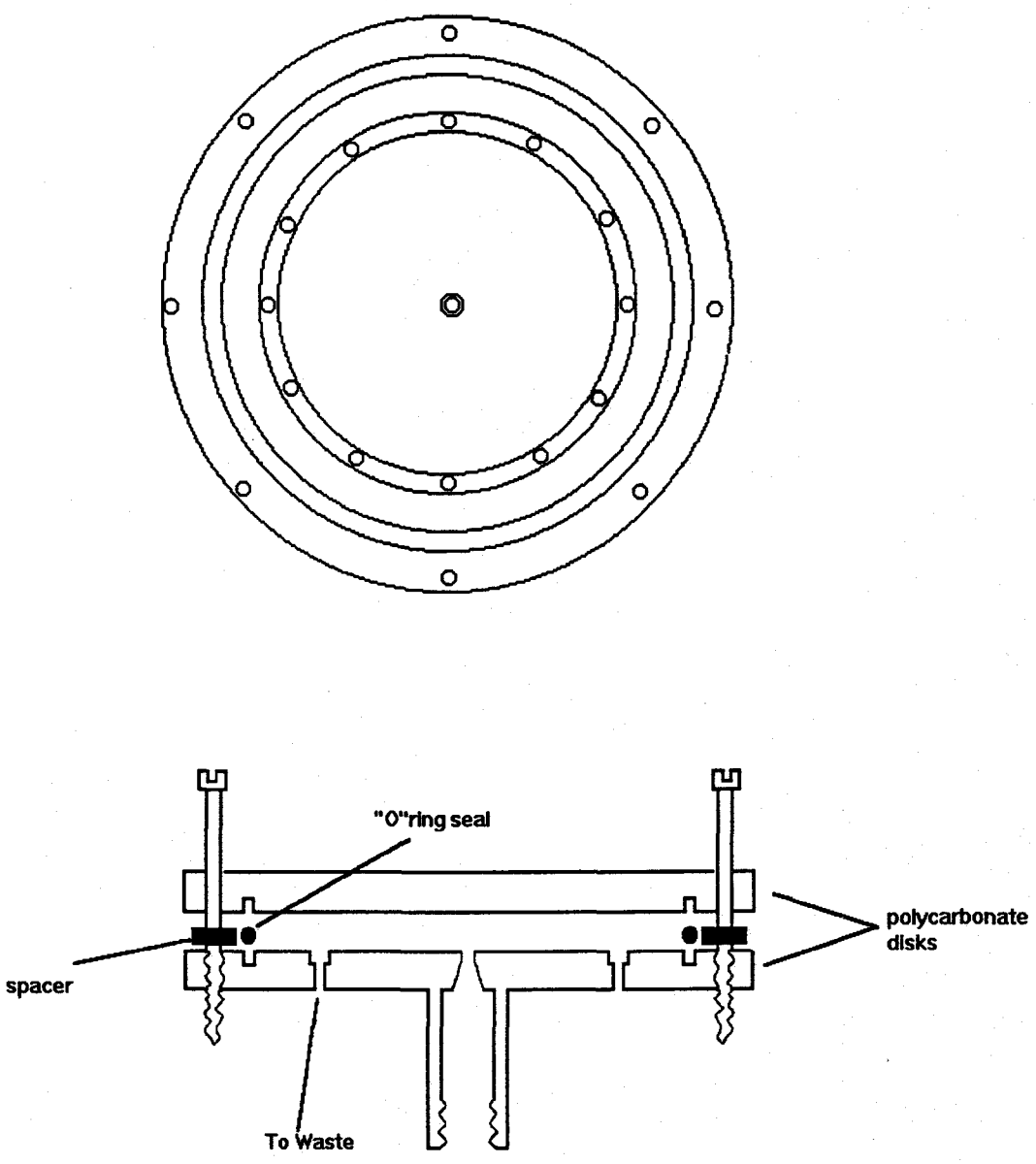


Figure 2.3. Top and Side Views of the Original Observation Cell (concentric injection ports shown in top view). The two polycarbonate plates of the cell are separated by the metal spacers resulting in a total volume of 0.83 cm^3 from the center to the drain grooves. There are 12 drain holes drilled into the bottom plate 1 inch radially from the concentric injection port.

turbulent mixing. The mixture then flows toward the perimeter of the cell. There are 12 equally spaced drain holes in a groove cut into the bottom plate of the cell at a radius of 1.0 in from the injection port. The total volume defined by the plates separated by the washers plus the drain grooves is 0.83 cm^3.

The observation cell used for most experimentation was a considerable refinement of the previous design (Figure 2.3). The top plate of the cell is similar to the previous cell, with an extra set of eight inner holes drilled at 1 in from the center of the plates in a circular pattern, which were used to keep the two plates flush (see Figure 2.4). The bottom plate was manufactured at the OSU machine shop[15] out of Plexiglas by carving a 1-mm by 1-mm spiral groove emanating from a central circular depression, which measures 1-mm deep by 5-mm diameter. When the top plate is fastened to the bottom plate with the sixteen peripheral 4-40 screws, there is a central circular mixing chamber and a 1 x 1 mm spiral pathway in which the mixed reagents and sample travel until they reach a 1-mm diameter exit hole which is located 1 inch radially from the injection port. The total volume of the mixing chamber and spiral pathway was calculated to be 0.99 cm^3 from the cell dimensions. There is approximately 0.5 cm^3 of active cell volume, which is directly within view of the 30-mm diameter head on PMT. There are several extra spirals outside of the PMT viewing area.

The bottom plate is fitted with a polycarbonate 1/4-28 female adapter bonded below the exit port, which is connected with tubing to carry the waste reagents to a flow through pH probe and to waste. The injector screws into the bottom plate via a polycarbonate adapter fitted with 1/2-32 threads to match the threads on the injector.

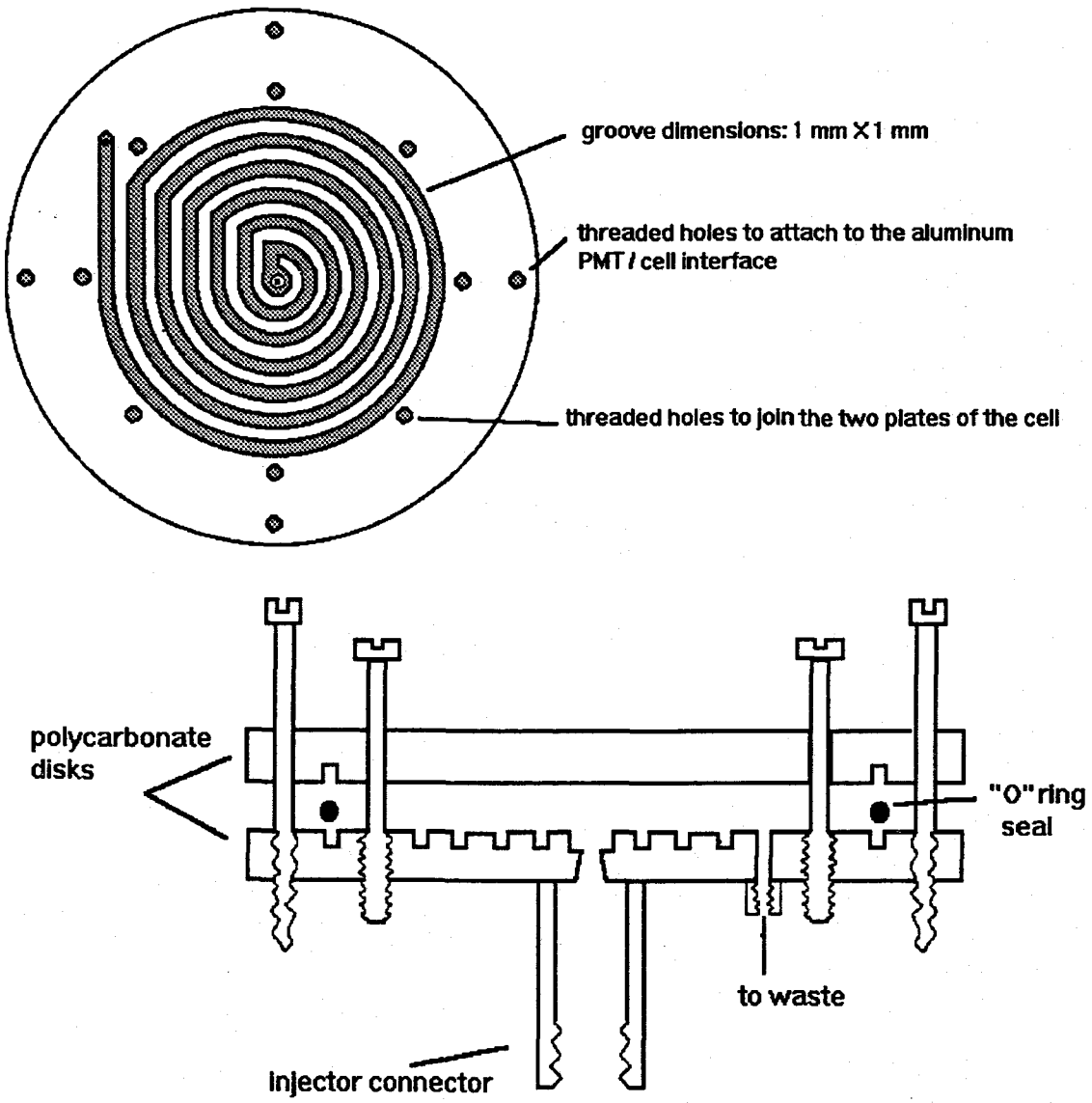


Figure 2.4. Top and Side Views of the Modified Observation Cell. The bottom polycarbonate plate of the observation cell has a 1 mm by 1 mm groove emanating from a central reaction chamber measuring 5 mm in diameter by 1 mm deep. Screws and threaded holes just outside the rings help keep the two plates flush. The "O"-ring was eventually eliminated.

Cell/PMT Interface and Housing. The observation cell, injector, and tubing are held within a 5-in diameter black PVC cylinder by 8 screws that also pass through an aluminum connector plate. The aluminum plate also keeps the end of the PMT flush against the top plate of the cell (see Figure 2.5). The reagent and sample Teflon tubing enters the PVC cylinder through any of four light-tight 1/4-28 feed-throughs located on the sides of the cylinder. The opening of the cylinder opposite the cell is fitted with a plastic cap to make it light tight. The plastic cap is fitted with a removal knob as well as 4 wing-nut fasteners for easy access in to the cylinder. A valve is fitted into the bottom of the cylinder which can be opened to drain solution from any accidental leaks. Figure 2.6 shows how the apparatus is positioned on an aluminum stand parallel to the table top.

Solid Phase Buffer Columns and Other Flow Components. The SPB's consisted of 4 to 8 in long TFE tubes with an id of 0.31 in. The tubes were packed with 75-106 μm $CaCO_3$ or MgO particles, which were retained in the columns by glass wool plugs at either end. Polypropylene compression fittings from Cole Palmer served as end fittings for connection to the reagent tubing.

A Sentron model 1001 ion selective field effect transistor pH system was used to measure the pH of the observation cell effluent. The pH probe was placed in line, to measure the effluent pH from the cell, in a flow through electrode holder made out of Delrin at the OSU machine shop.[15] The in-line probe holder was also fitted with 1/4 -28 threads to attach to the reactant tubing. The reagent and sample pumps were made by FMI (model, Lab Pump Junior). Normal operating flow rates were between 5 and 10

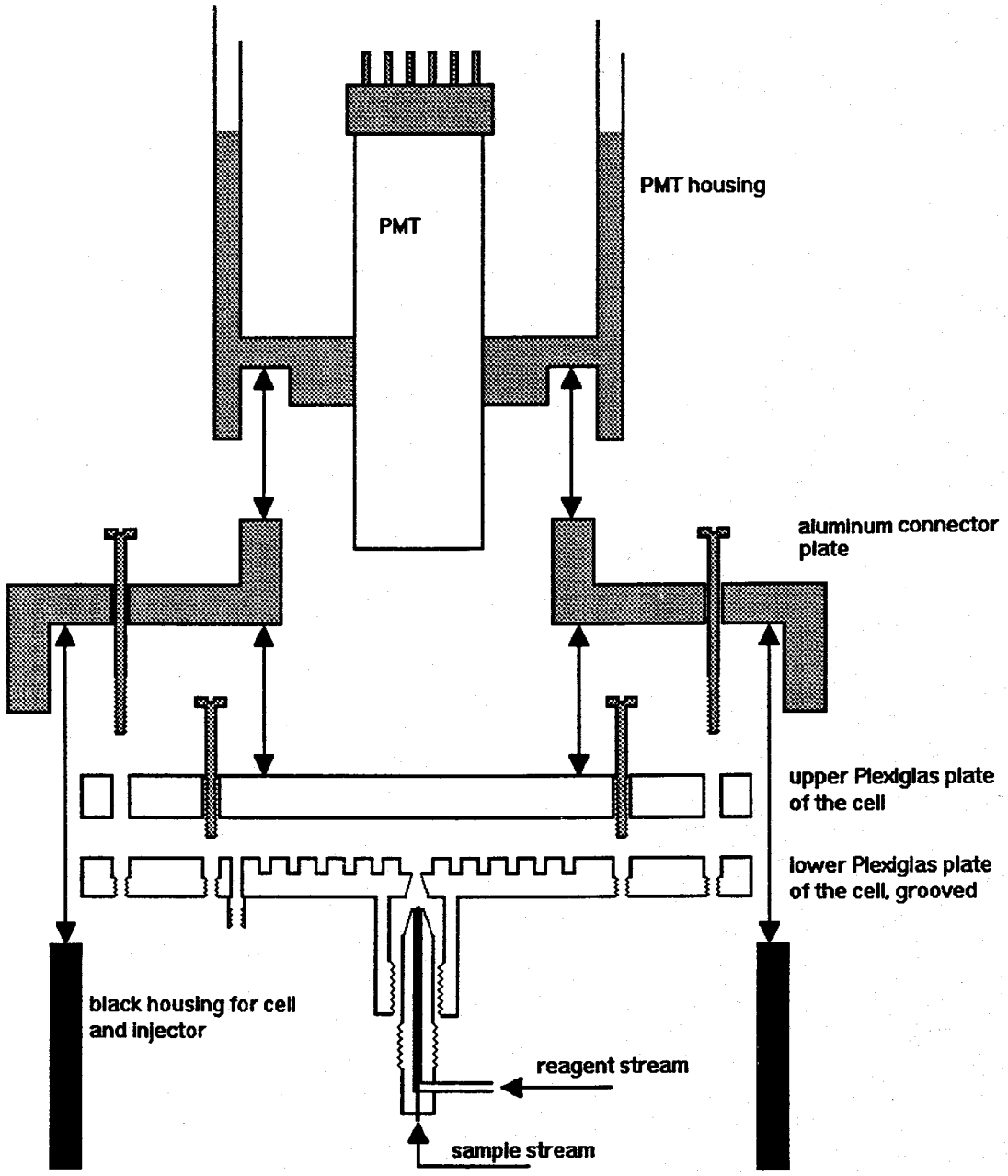


Figure 2.5. Schematic of the PMT/Housing/Observation Cell. The PMT housing slides on to the aluminum connector plate which is fastened to the cell via the outer set of screws. The whole assembly is then fitted onto the rim of the black PVC cylinder to keep out light. When everything is in place, the PMT head is flush with the top plate of the cell.

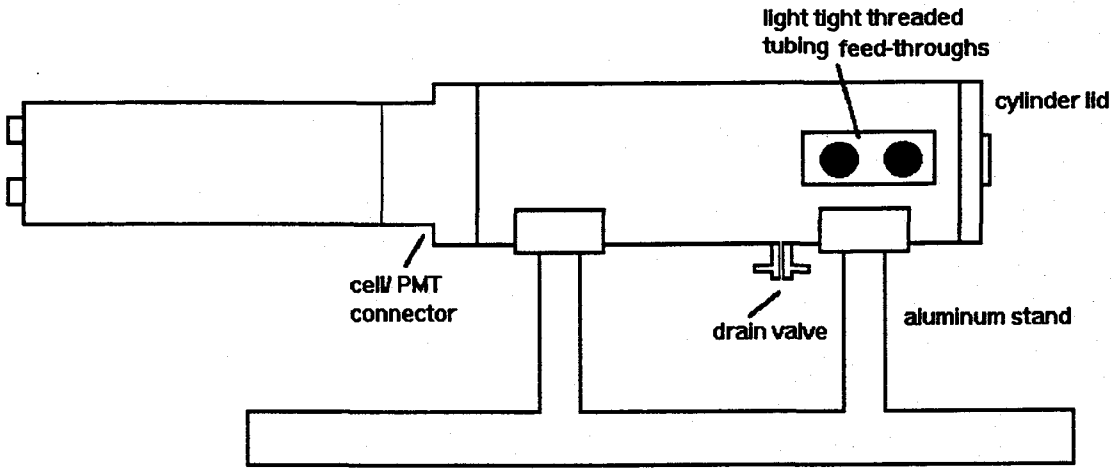


Figure 2.6. Schematic of the Apparatus Stand and Mounting Position. The PVC cylinder is held to the aluminum holder with plastic straps parallel to the ground. Some experiments were performed with a vertical orientation.

ml/min for each stream. All tubing was Teflon (1/16-in od, 0.8-mm id) obtained from Altex and was terminated with flangeless Tefzel 1/4-28 fittings obtained from Upchurch.

Detection, Signal Processing, and Readout Systems. The PMT used for the majority of the experimentation is an RCA type C31059, and a Hamamatsu type R374 was used in one experiment because of its extended sensitivity to near IR light. These head-on PMT's were contained in a Pacific Precision model 62 housing. The PMT was biased by a Keithley 244 High Voltage Supply, which supplied from 600-900 V.

The photocurrent from the PMT was converted to a voltage and amplified by a Keithley 427 current amplifier, with a transfer function from $1x10^6$ to $1x10^8$ V/A. The voltage signal was conditioned by a Spectrum 1021 amplifier and filter to provide further gain if needed. The cutoff frequency was generally set at 0.1 Hz. The readout was performed by a Heath SR-205 single pen chart recorder.

EXPERIMENTAL PROCEDURES

Overview. Normally an OCl^- standard is pumped between 5 and 10 ml/min through the outer port of the concentric injector, and a peroxide solution made by adding peroxide to a boric acid buffer solution, is pumped through the Pt tube of the injector at an equal flow rate. During continuous flow analysis, both solutions were pumped into the observation cell continually. This steady state signal was measured on the chart recorder as the amplitude above the signal from a DI H_2O blank. In some experiments the peroxide solution or a DI H_2O solution was connected to the pump inlet by a three-way valve, so that the reagent could be injected into the observation cell alternating with the DI H_2O stream. These signals were measured as the peak height above the baseline signal.

Reagents and Solution Preparation. All reagents were prepared with DI H_2O from a Millipore Milli-Q system fed with house DI H_2O. The 30% by wt H_2O_2 (analytical reagent grade, stabilized) stock solution was obtained from Mallinckrodt. Working solutions of H_2O_2 were made fresh daily by adding small volumes of the 30% stock solution to the buffer. For example, to make the 4.4 mM H_2O_2/buffer solution, 100 µl of the 30% stock solution were added to 200 ml of the 0.2 M boric acid buffer, usually pH 8. The 100 µl was added using an Eppendorf EDP2 automatic pipet.

A stock sodium hypochlorite solution greater than or equal to 4 % available chlorine (Cl_2) by wt was obtained from Aldrich (chemical number 23930-5) and used to make all chlorine standards. A more dilute OCl^- stock solution was made weekly in DI H_2O, and OCl^- standards were made by diluting this stock solution. Typically 1 ml of the

5% stock chlorine solution was diluted to 250 ml to prepare a 200 ppm available chlorine stock solution, and then this solution was further diluted in 100-ml flasks to make calibration standards in the ppm range. The concentrations of all hypochlorite solutions are specified in terms of available chlorine (Cl_2). Initially the NaOCl solution from Aldrich was assumed to be 5% or 50,000 ppm; later testing of the stock solution, with a standard method, proved this to be a very good estimate (see appendix 1).

Boric acid (analytical reagent grade) obtained from Mallinckrodt was used to prepare most buffer solutions. About 12.4 g of the acid was added to 1 l of DI H_2O for a 0.2 M buffer, and then 0.1 M NaOH was added while the pH was being measured. When the desired pH, usually 8, was obtained, the addition of NaOH was stopped. Sodium phosphate monobasic (analytical reagent grade) obtained from Mallinckrodt was used to prepare buffers in the 8 to 9 pH range, in a manner similar to the boric acid buffers. In some experiments the Clark and Lubbs buffer preparation scheme was used so that the pH would not have to be read.[16]

Proprietary forms of $CaCO_3$ and MgO (75-106 μm) were obtained from the Umpqua Research Co. (URC) in Myrtle Creek, OR where they were packed into TFE columns for use as SPB. Fused MgO (40-mesh, 99+%) was obtained from Aldrich.

Observation Cell Testing. The spiral observation cell, illustrated in Figure 2.4, was developed to solve bubble trapping and unpredictable flow pattern problems encountered with the original cell. To confirm the mixing of reagents within the cell, 10 mM NaOH was pumped through one opening of the injector and 10 mM HCl, with enough

phenolphthalein indicator to visually turn from clear to red upon neutralization, was pumped through the other injector opening. Good mixing was indicated by the mixture changing from clear to red in the cell.

To test where the chemiluminescence was occurring in the spiral cell, the CL signal was monitored with different parts of the top plate blocked with duct tape, which altered the view of the PMT. With a combined flow rate of 20 ml/min, a solution of 2.5 ppm OCl^- was mixed with a 4.4 mM H_2O_2 in a pH 8 boric acid buffer. The viewing area was varied to include the center chamber only, the center and first ring, the center to the second ring, and the center to the third ring which is the the total viewing area of the PMT.

In another experiment the PMT was moved from the center of the cell outward over the outer rings to evaluate if light was being emitted outside of the PMT viewing area. To maintain a light-tight connection between the cell and the PMT, the aluminum collar was removed and duct tape was wrapped around the PMT and the cell housings.

Solid Phase Buffer Columns. In some experiments the pH was controlled by running a non-buffered H_2O_2 solution, made in DI H_2O, through a SPB. The dissolution of the particles in the SPB's creates OH^- ions, which increases the pH of the peroxide solution. The pH of the column eluent was controlled by the contact time of the H_2O_2 solution with the particles. The contact time was altered by varying the bed size of the SPB column or the solution flow rate.

Effect of pH and Reagent Concentrations. The key reaction parameters that were optimized in separate experiments were pH, flow rates, H_2O_2 concentration, and SPB column size and bed content. URC performed most of the studies on the SPB's.

The H_2O_2 concentration was optimized by mixing a 2.5 ppm OCl^- solution with a boric acid buffer (pH 8) containing varying concentrations of peroxide (2.2, 4.4, 8.8 and 17.6 mM). Each of the reactants was pumped at approximately the same flow rate between 7 and 10 ml/min.

To optimize the pH of the reaction mixture, two buffers were needed: a boric acid buffer for pH 8-9, and a phosphoric acid buffer for pH < 8. A solution of 2.5 ppm OCl^- was mixed with a 4.4 mM H_2O_2 solution in buffers with pH values from 7 to 9.3. Flow rates were similar to those used in the H_2O_2 study.

Reaction Half Life. The half-life of the reaction was investigated with a boric acid buffer (pH 8) and a 2.5 ppm OCl^- solution, the peroxide solution and the chlorine standard were pumped into the cell until a steady state signal was obtained. Next, the pumps were shut off and the decay of the signal was recorded. The half-life of the reaction was defined as the time for the signal to decay to half the original signal.

Tests for Interferences. It was observed that the CL signals for a given OCl^- standard were different for different peroxide solutions even if the peroxide solutions were made identically. Signals for a given set of solutions were reproducible. A contaminant was thought to be responsible for this lack of reproducibility, so many potential contaminants

were added to the peroxide solution and mixed with OCl^- standards in the observation cell. The signals with various amounts of suspected contaminants were compared to those with the same solutions before the interferant was added.

To test for dust interferences, dust was collected from shelves and other surfaces in the lab and added to the buffered H_2O_2 solutions. The ratio was approximately 50 mg of dust per 100 ml of H_2O_2/buffer solution.

To test if the pH electrode, used to measure the pH of the buffers, was a contaminant source because of leaking reference filling solution, H_2O_2/buffer solutions were prepared and portions were dispensed into a small beaker, where their pH values were measured, and then discarded. The signal obtained with a buffered H_2O_2 solution and a 2.5 ppm OCl^- solution, which had no contact with the pH electrode, was compared to that of the same solution after contact with the pH electrode. Because of the effects of the pH electrode, common electrode filling solutions were added in the ratio of a few drops to 100 ml of the buffer/H_2O_2 solution. The Orion Green Filling Solution (number 90-00-02) and the clear solutions (numbers 90-00-11 and 81-00-07) were investigated.

Because the filling solution acted as a contaminant, a solution similar to it, but without the green dye, was prepared based on the material safety data sheet (MSDS) for the solution, and this new solution was tested for interferant effects. The MSDS indicated that the following substances were present in the green filling solution; KNO_3 (17.1%), AgCl (trace), Triton X-100 (trace), naphthol green B (0.5%), KCl (5%), NaCl (0.4%), DI H_2O (77%). NaCl, KCl, $AgNO_3$, $FeSO_4$, $CrCl_3$, $CuSO_4$, and $CoCl_2$ at concentrations between 1 and 100 μM, were also added to the H_2O_2/buffer solution in attempts to track

down the contamination. PtO powder obtained from Goldsmith Bros. was added to the buffered H_2O_2 solution to test for possible Pt contamination effects. The ratio used was about 0.1 g PtO to 100 ml of H_2O_2 solution.

To test for possible contamination from skin contact, 100 ml of a 4.4 mM H_2O_2, 0.2 M, boric acid buffer (pH 8) was shaken with my finger as the cap of the flask. The signal obtained before the finger was used was recorded, and compared to the signal observed after the finger was used. Shaking periods were as short as 10 s. Fingers of two other lab mates were also used in similar experiments.

Wavelength Characterization. In an attempt to improve the detection limit, the Hamamatsu PMT was used, which has more sensitivity to the near IR end of the spectrum where singlet O_2 emits, than the original PMT.[9] Unfortunately this change did not improve the detection limit. Because of this result and the many interferences encountered, an experiment was designed to determine if the observed chemiluminescence wavelength from the peroxide/hypochlorite reaction corresponded to that expected from singlet O_2.

Different cut-on filters (a filter that passes light above its assigned wavelength) were placed between the PMT and the cell. If a filter with a 600-nm cut-off passes light from the reaction, the CL could be originating from singlet oxygen which emits at 633 and 709 nm. Filters with cut on wavelengths of 600, 530, 475, 455, 435, 420, 400, and 395 nm were used to determine the wavelength of the emitted light. The effect on the filter cut-on wavelength was investigated with and without the addition of some of the suspected interferents.

RESULTS AND DISCUSSION

Reaction Cell Behavior. Mixing in the original version of the flow cell (Figure 2.3) was visually confirmed from a change in color of the phenolphthalein indicator when solutions of 10 mM HCl/indicator and 10 mM NaOH were pumped into the cell. The red color was noticeable immediately at the point of first contact of the two reagent streams.

Ideally the two reagent streams would mix upon entering the cell and flow out wards evenly towards the peripheral exit ports. Unfortunately, as soon as any air bubbles were introduced to the cell (e.g., when the reagent line was switched between different solutions), some bubbles became trapped in view of the PMT viewing area. Ethanol could be pumped into the observation cell through one of the injection ports, which would lower the viscosity of the solution in the cell, and allow bubbles to be flushed out by tapping on the top of the cell with the end of a screw driver. This technique was deemed very impractical because it involves removing the PMT and up to 5 min of tapping for each bubble that gets trapped.

The improved observation cell based on a spiral design (Figure 2.4) eliminated most of the bubble trapping. Occasionally bubbles became trapped in the central reaction chamber or in the outer rings of the cell, but these could be removed easily by tapping or rotating the cell and aluminum collar. Machining the two plates of the cell to be completely flat and adding the extra set of inner screws to the cell helped keep the cell plates flush and almost completely eliminated the problem.

The mixing efficiency of the improved observation cell was also tested with acid and base solutions as described previously. Virtually instant mixing of the reactant

streams was indicated by the red color from the phenolphthalein solution in the central reaction chamber of the observation cell.

Pumping the stock 30% H_2O_2 solution and the stock 5% OCl^- solution into the new cell produced a visible orange glow in the central mixing chamber if the room was completely dark. When the same H_2O_2 solution was rapidly injected into a beaker of NaOCl in a completely dark room, the same visible reddish glow was observed. These visual studies confirmed that the mixing efficiency of the cell was good, although it was not evaluated quantitatively.

Relative to the CL signal without masking any part of the observation cell from view of the PMT, the CL signal was reduced 17% by blocking the 0.5 cm-diameter mixing chamber, 25% from blocking the center chamber and the first ring of the observation cell, and 67% from blocking the center out to the third ring of the observation cell. Blocking the entire area in front of the 1-in diameter PMT eliminated the signal. Clearly a significant portion of the CL occurs in the outer rings due to the lifetime of the reaction or incomplete mixing of the reactants.

When the PMT was moved to a section of the outermost spirals which are not normally in view of the PMT, the CL signal was about 20% of the signal with the PMT in its normal position. This behavior is consistent with the previous observation.

Effect of H_2O_2 Concentration. The dependence of the CL signal on the H_2O_2 concentration is shown in figure 2.7. A H_2O_2 concentration of 4.4 mM yielded the highest signal, and it was used for most future experiments (see Figure 2.7). With this H_2O_2

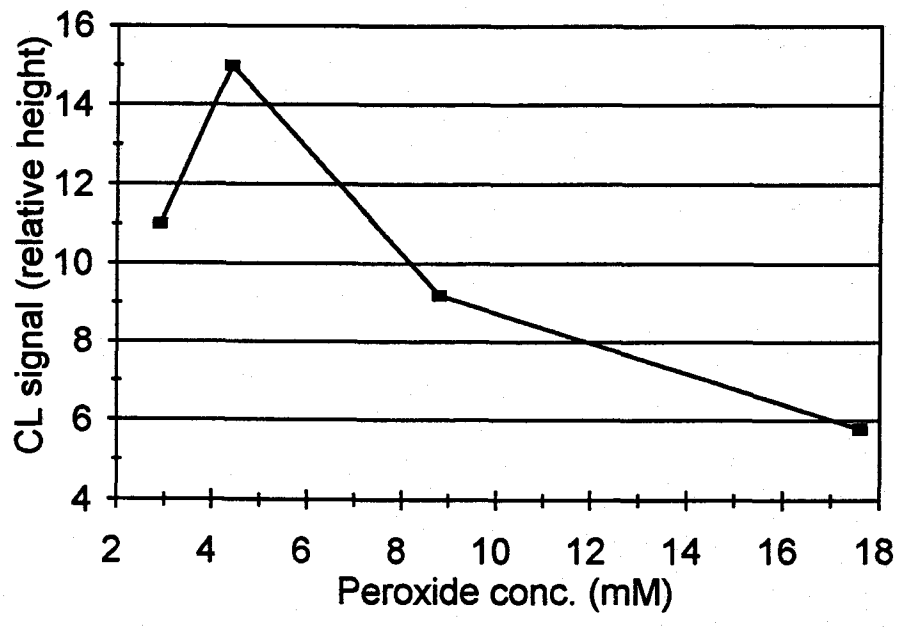


Figure 2.7. Effect of H_2O_2 Concentration on the CL Signal. For these data 2.5 ppm OCl^- was mixed with the peroxide solution, which was adjusted to pH 8, at equal flow rates of approximately 5 ml/min. The points are the average of 2 10-s pulses of H_2O_2 into the OCl^- stream. In all figures, one unit of signal height corresponds to 2 mV on the recorder.

concentration and equal flow rates, the molar ratio of H_2O_2 to a 2.5 ppm OCl^- solution is about 100/1.

Further experiments showed no significant change in the CL signal with peroxide concentrations from 4 to 50 mM. This work was done before contamination problems were realized. Because of the irreproducibility of the signal later encountered between similar solutions, the optimal H_2O_2 concentration is uncertain.

Effect of pH. The effect of pH on the CL signal in the observation cell is shown in Figure 2.8. A pH of 8 for the peroxide/buffer solution gave the highest CL signal, which is in agreement with Marino and Ingle's published results.[13] From this point most peroxide/buffer solutions were made to pH 8 with the Clark and Lubbs preparation scheme. The dependence of the signal on pH appeared significant, but further attempts to reproduce these data were unsuccessful due to contamination problems. The optimum pH appears to be 8 or above.

When an SPB was first employed to replace the buffer solutions, the pH resulting in the maximum CL signal shifted upwards to about pH 10. Because of reproducibility problems, the pH dependence was not determined.

When solutions of OCl^- and H_2O_2 were separately adjusted to pH 8 by the addition of NaOH and mixed in the observation cell, no detectable CL signal was observed for μg/ml levels of OCl^-. Perhaps the CL observed normally was dependent on some contaminants in the buffers or the SPB's.

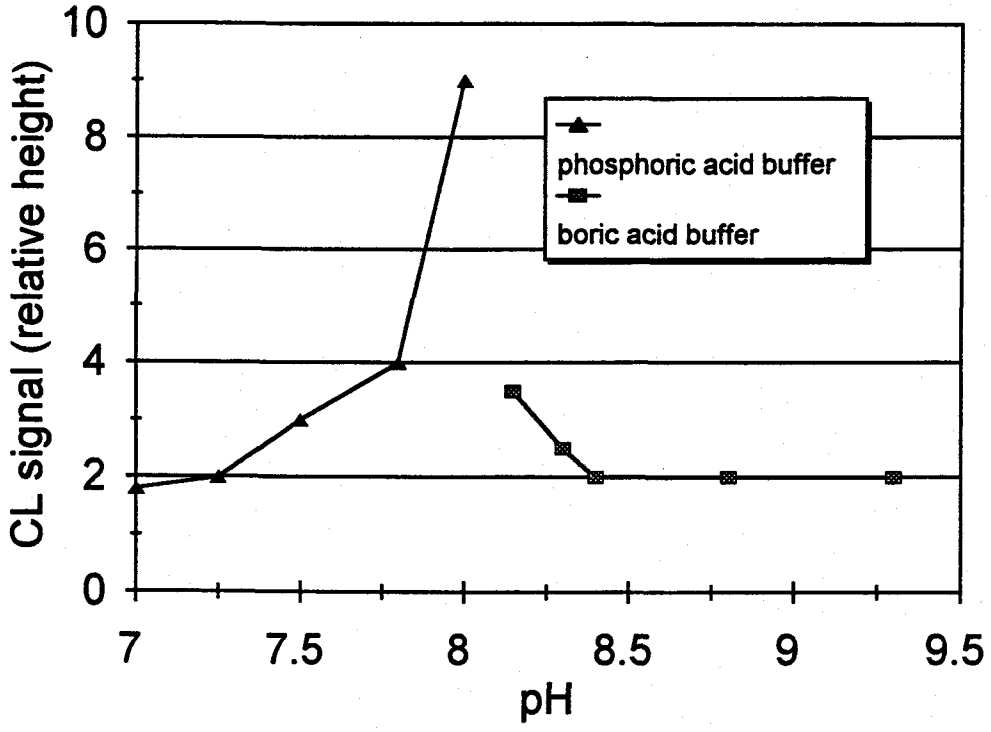


Figure 2.8. pH Dependence of the CL Signal. Two buffers were used to examine the pH dependence of the H_2O_2/OCl^- CL reaction. These data are for a 2.5 mg/l OCl^- sample and 4.4 mM H_2O_2 pre-cell concentration, with approximately equal flow rates of the reactants between 5 and 10 ml/min.

Effect of Flow Rates. The effect of the flow rates was never completely characterized because the largest signal was obtained at the highest obtainable flow rate (10 ml/min for each reactant pump). A fairly high flow rate, about 5 ml/min for each reactant, was necessary to produce detectable CL in the μg/ml range when using the buffers to control the reaction pH. This behavior may be due to better mixing of the reagents at higher flow rates or more OCl^- entering the observation cell per unit time to produce more excited O_2 molecules.

When SPB's were used to alter the pH of the H_2O_2 solutions, reasonable signals were obtained with flow rates as low as 1-2 ml/min per reactant. At these flow rates, a column packed with 5 cm^3 of MgO SPB particles produced an effluent pH between 10.5 and 11. Lower pH values were not examined because the CL signal was already high at pH 10.5.

It has been hypothesized that the CL of this reaction originates from the O_2 gas bubbles generated in the reaction.[16] When high reagent concentrations of 30% H_2O_2 and 5% OCl^- are used, the bubble formation and CL are visually noticeable. With the OCl^- concentration in the μg/ml range, bubble formation may not be high enough to detect the CL until the flow rate becomes high enough. Without enough bubbles, the majority of the excited O_2 molecules produced by the reaction may deactivate via a collisional process in the liquid reagent streams. The bubble theory is supported by the observation that an occasional bubble introduced via one of the reactant streams was often accompanied by a rapid and momentary increase in the CL signal when the solutions were introduced continually. The effect was not reproducible.

Calibration Curve. A typical calibration curve is shown in Figure 2.9. This calibration was obtained by allowing 10-s sample pulses of the H_2O_2/buffer solution into the constant stream of OCl^-. Each point is the average maximum signal height of 5 sample pulses. It was linear and reproducible if run again with the same solutions and flow rates. The calibration was not consistent when different but "equivalent" buffer/H_2O_2 solutions were made. The calibration difference in slopes between "equivalent" buffer/H_2O_2 solutions, prepared on a given day often varied by up to a factor of 2. From one day to the next, the signal for a given OCl^- standard could vary up to even a factor of 100.

Measurable signals were obtained from tap water samples corresponding to 0.1 to 1 ppm Cl_2. However, near the end of this phase of the research, no signal could ever be obtained for an OCl^- standard or sample in the µg/ml range without the addition of one of the known contaminants.

Reaction Half-Life. The average half-life for the peroxide chlorine reaction was determined to be 1.5 s. This half-life is similar to that reported earlier for this reaction by Marino and Ingle.[13] With a volume of about 0.5 ml within view of the PMT and a combined flow rate for the two reagent streams of 20 ml/min, the residence time is about 1.5 s. Based on the estimated residence time and half-life, about 50% of the total CL was collected within the viewed observation volume. This estimate is consistent with the observations in the previous experiment involving masking of parts of the observation cell.

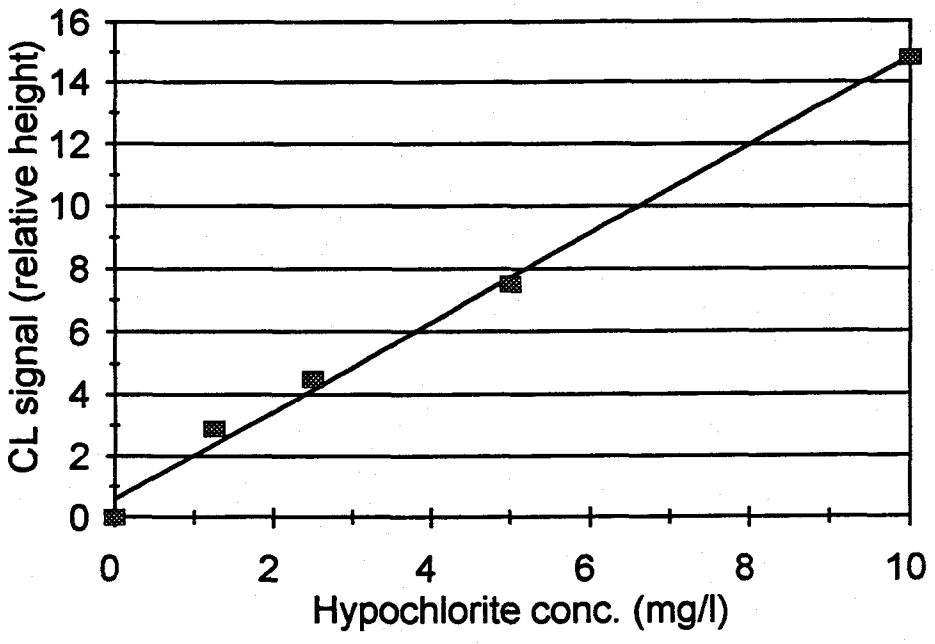


Figure 2.9. Hypochlorite Calibration Curve for CL Reaction with H_2O_2. These data were taken with a 4.4 mM H_2O_2 solution in a boric acid buffer (pH 8) with equal flow rates of the reactants between 5 and 10 ml/min. From these data the DL, defined as twice the background noise, was 0.6 mg/l.

Control of pH with SPB's. The SPB columns, developed at URC, have been shown to produce a constant pH effluent when challenged with DI H_2O. Because the peroxide/chlorine reaction has a strong pH dependence, the pH of the column effluent has to be stable. Figure 2.10 shows the long term stability of this pH for a 5 cm^3 bed of fused MgO challenged with 1 ml/min of DI H_2O for 40 days.

Figure 2.11 illustrates how the effluent pH from an SPB depends on the size of the MgO particles and the solution contact time, which is defined as the ratio of the internal volume of an empty column to the flow rate. Typically the flow rate of the peroxide solution through the SPB's was 1 ml/min for the CL analyzer. Particle sizes from 75-1000 μm and contact times from 1 to 14 min were investigated. The effluent pH increases as the particle size decreases, which is attributed to the increased surface area of the particles allowing more contact with the DI H_2O stream. In this thesis a particle size of 150-300 μm was chosen for its ability to adjust the pH of the peroxide solution to pH 10 with relatively short columns with flow rates near 5 ml/min. Particle sizes smaller than 150-300 μm create pressure problems.

The pH of the SPB effluent is dependent on the small amount of the bed material dissolved in the H_2O stream. Because the SPB's do not perform as a buffer or provide much buffer capacity, challenging the columns with anything other than DI H_2O may cause problems due to naturally occurring buffering species.

For work in this thesis, an unbuffered DI H_2O_2 stream was passed through the columns. The pH of the mixed reactant solution in the observation cell depends on the pH and any buffering capacity of the SPB effluent and the unmodified OCl^- sample stream.

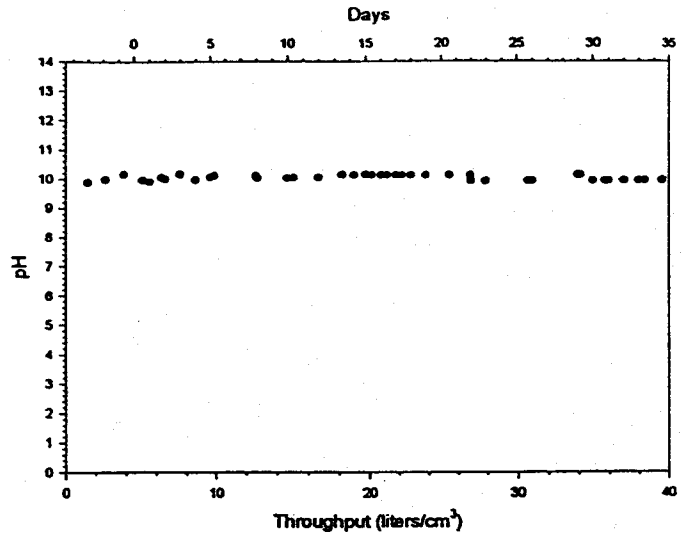


Figure 2.10. SPB Bed Performance with Pure Water Challenge. The pH of the MgO column effluent was very stable at about pH 10 for over a month, with 1 ml/min DI H_2O through a 5 cm^3 bed of fused 75-106 μm MgO particles. Taken with permission from URC.[4]

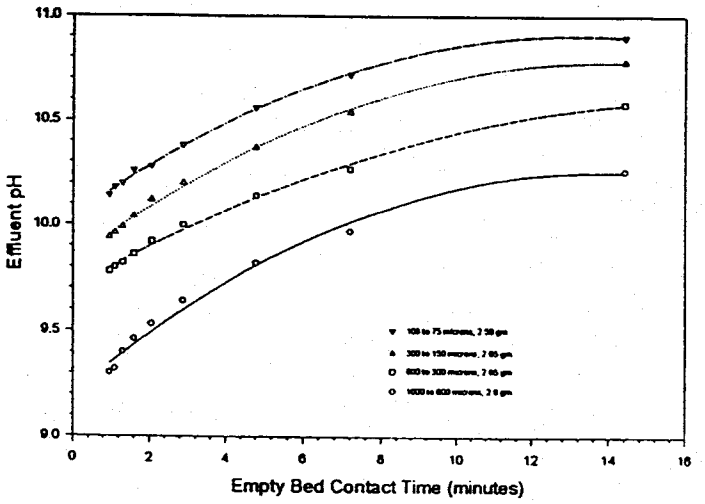


Figure 2.11. Dependence of pH on Contact Time and Particle Size for a MgO SPB Column. The pH of the column effluent can be varied by the flow rate. The longer the H_2O was in contact with the MgO crystals the higher the pH up to a 14 min contact time. The smaller particles produce the highest pH effluent because of their increased surface area. MgO particles between 150 and 300 μm were chosen for the OCl^- analyzer because smaller particles caused significant pressure build up. Taken with permission from URC.[4]

The pH of the column effluent could be adjusted from 8 to almost 11 by varying the flow rates and column sizes. An empty bed volume of 5 cm^3 was large enough to achieve a pH near 11 at flow rates under 5 ml/min. Typically the pH value used with the CL analyzer was adjusted between 10 and 10.5. This is a much higher pH than used with the boric acid buffered H_2O_2. In general, larger CL signals were obtained with a higher effluent pH.

When an OCl^- standard was run through an SPB column and mixed with a H_2O_2 solution in the observation cell, no signal was detected. When the same column was challenged with a H_2O_2 solution and mixed with an OCl^- sample in the cell, still no signal was observed. Apparently, the OCl^- deactivated the column in some way. However, the column did retain its ability to adjust the pH of a given solution from pH 10-10.5.

Investigation of the Interferents. From the observation that two buffer solutions prepared identically usually produced signals differing by up to a factor of 2, it was discovered that the longer the pH electrode was in contact with the solution, the higher the CL signal resulting from that solution. The pH electrode was an Analytical Sensors gel-filled combination electrode (model PH10107B-03-B). Also, when only 2 drops of the saturated KCl solution in which the electrode was stored were added to 200 ml of the H_2O_2/buffer solution, a similar increase in the CL signal was observed. This enhancement effect was observed with OCl^- standards and tap water.

Adding a few drops of the Orion Green Electrode Filling Solution to the H_2O_2/buffer solution, also had similar effects. The "in-house" reference filling solution

prepared according to the MSDS, minus the green dye, did not increase the CL signal. There appears to have been a contaminant in some of the KCl reagent bottles because when KCl from these bottles was added to H_2O_2/buffer solutions (a few mg per 100 ml), the CL signal was enhanced. Contaminated glassware was suspected, but acid washing the glassware overnight did not eliminate the enhancement effect.

Dust could have gotten into some of the reagent bottles and beakers and enhanced the CL signal. It was easily shown that a few milligrams of dust from the laboratory added to the H_2O_2/buffer solution had an effect similar to that of the filling solution. It was suspected that the enhancement was due to a surface catalytic effect. Other contaminants that enhanced the signal included material from this researcher's fingers and PtO powder.

When the SPB columns ($CaCO_3$ and MgO particles) were first used to adjust the pH of the H_2O_2 solutions, the CL signals were larger by about a factor of 100 than those from buffered H_2O_2 solutions. For example, signals on the recorder (5-200 mV) for 1-5 ppm chlorine were obtained with gain settings of $1x10^7$ V/A at a PMT bias voltage of 800 V, for H_2O_2 solutions buffered with boric acid. With H_2O_2 solutions run through a MgO SPB column, the same magnitude signal was produced at a gain of $1x10^6$ V/A and a PMT bias voltage of 500 V. This difference in gain corresponds to a factor of about 400.

The enhanced CL signals obtained with the SPB column were not stable in the continuous flow mode of operation. The steady state signal resulting from the mixture of H_2O_2 and OCl^- slowly decreased as the particles were used. Within 10 h of continuous flow, a 5 cm^3 column of MgO produced signals comparable to signals obtained with

buffered H_2O_2 solutions. The SPB's still adjusted the pH of the H_2O_2 solution even after the CL signal became non-detectable.

Sometimes the SPB particles (MgO or $CaCO_3$) were added directly to a 0.1 mM H_2O_2 solution (approximately 1 g of particles to 1 l of solution), and after the pH had risen to about 10.5, the solution was decanted and mixed with OCl^- in the CL analyzer. This solution produced enhanced signals of the same magnitude observed with fresh SPB columns. The CL signal observed with this decanted solution was stable for at least a day. When the same experiment was repeated with the same SPB particles but a fresh H_2O_2 solution, the CL signal was decreased by about 50%. After 3 or 4 such transfers, the decanted solutions did not produce detectable CL at OCl^- levels between 1 and 10 mg/l.

This behavior suggests that a contaminant washed off the surface of the particles during the process and could explain the observed decrease in the CL signal over time when the SPB columns were employed. It was hypothesized that the signal enhancement was related to small SPB particles that became suspended in the solution and carried to the observation cell, but subsequent crushing of used particles with a mortar and pestle did not result in their re-activation.

MgO particles from Aldrich (40 mesh) were used to adjust the pH of H_2O_2 solutions by the batch addition of 1 g of particles to 1 l of solution, but the resulting decanted solutions did not produce detectable CL signals when mixed with 1-10 mg/l OCl^- in the observation cell. Because the enhancement is dependent on the source of the particles, some specific contaminant is indicated. Contamination could have occurred during the SPB production process performed at URC.

Spectral Characteristics of the Reaction. Different cut-on filters were placed between the observation cell and the PMT and a 0.5 ppm OCl^- solution was mixed with a 0.1 mM peroxide solution that had been in contact with some $CaCO_3$ or MgO from URC. No signal was observed with a 600-nm filter. The 475-nm filter passed 1/6 of the signal and the 395-nm filter passed almost all of the signal.

These results suggest that the primary CL signal observed when using the SPB particles is not from singlet oxygen because the CL from the reaction of H_2O_2 and free chlorine due to excited singlet oxygen occurs at 709 and 633 nm.[9] A different CL reaction involving some contaminant on the SPB particles may be occurring. The emission observed is curiously in the same wavelength range as the CL from the reaction of luminol with chlorine whose emission maximum is at 434 nm. Perhaps these particles contain trace amounts of luminol picked up at Umpqua Research Center. The luminol/chlorine CL reaction is considered in depth in chapter 3.

When dust was added to a boric acid/peroxide solution to enhance the CL, the 600-nm filter also absorbed all of the CL. The 395-nm filter passed this light. Even without the addition of known contaminants to the buffer/H_2O_2 solution, the 600-nm cut-on filter blocked the emission signal from the reaction. However, when a 30% solution of peroxide and 5% sodium hypochlorite solution were mixed in the observation cell, the emission, seen as a visible red glow, was passed by the 600-nm cut-on filter.

In later work after luminol solutions (about 0.1 mM) had been pumped through the system for weeks, further experiments with buffered H_2O_2 solutions were conducted. The

CL signals in these studies were very high, suggesting that luminol can be a contaminant. Further studies are required to support this hypothesis.

CONCLUSIONS

The work on the CL reaction between H_2O_2 and free chlorine was abandoned in favor of investigating the CL reaction between free chlorine and luminol, mainly because of reproducibility problems and occasional lack of the CL signal. Even though a calibration curve could be obtained for free chlorine in the mg/l range, this curve was not reproducible between "equivalent solutions". Contamination effects were encountered which increased CL signals by up to a factor of 400, but the source of the contamination was not discovered. The enhancement effects from the SPB particles, dust, and filling solutions may be due to different contaminants.

Because filter studies suggested that the CL observed was not from singlet oxygen emission, a different CL reaction may be responsible for the CL signals observed in these experiments. If the contaminant responsible for the high signals observed could be identified, and its level controlled, this CL system should be investigated further for its analytical usefulness. Because singlet oxygen emission may occur in the gas phase,[11] singlet oxygen emission may not be observed because few bubbles are produced.

The use of SPB's to adjust the pH of the H_2O_2 solutions for CL was successful. There were no problems with the consumption of SPB particles or the loss of pH adjustment capability. Initially large CL signals from fresh SPB particles were observed; however, the CL signal decreases with the use of the SPB's. Eventually no CL was detected from H_2O_2 solutions whose pH was adjusted with the used particles, even though the SPB's were still capable of adjusting the pH of the solution. Even fresh SPB particles could not be used to adjust the pH of samples or OCl^- standards as they totally eliminated

the CL signal. The SPB columns were employed in the next phase of experimentation, with good results (chapter 3).

The observation cell and injector also worked well and were employed in the next phase of experimentation discussed in chapter 3. Mixing of reagent streams in the central reaction chamber of the observation cell was visually confirmed with acid and base/indicator solutions.

REFERENCES

1) Kuo, P. P. K.; Chian, E. S. K.; Chang, B. J. *Environ. Sci. Technol.* **1977**, 11, 1177-1181.

2) Luong, T.; Peters, C. J.;Young, R. J.; Perry, R. *Environ. Technol. Let.* **1980,** 1, 299-310.

3) EPA. *Federal Register. Rules and Regulations* Vol. 44, No. 231, Thursday Nov 29, **1979**, 68624.

4) White, C. G. *The Handbook of Chlorination Second Edition,* Van Nostrand Reinhold Company Inc.: New York, New York, 1986, pp 1-60.

5) Clesceri, L.A.; Greenberg, A.E.; Trussell, R.R. eds. *Standard Methods for the Examination of Water and Wastewater,* 17th ed, American Public Health Association; Port City Press: Washington D.C., 1989, pp 4.45-4.66.

6) Gordon, G.; Cooper, W. J.; Rice, R. G.; Pacey, G. E. J. Am. Water Works Assoc. **1988,** September, 94-122.

7) Jolley, R. L.; Lyman, C. W.; Johnson, D. J.; et al. *Water Chlorination Chemistry Environmental Impact and Health Effects.* Lewis Publishers: Chelsea, Michigan, 1990; vol. 6, 90-124.

8) Mallet, L. *C.R. Acad. Sci., Paris,* **1927** 185, 352.

9) Marino, D. F.; Ingle, J. D., Jr. *Anal. Chem.* **1981**, 53, 455-458.

10) Khan, A. U.; Kasha, M. *J. Amer. Chem. Soc.* **1970**, 92, 3293-3300.

11) Schaap, A. P., Ed. *Singlet Molecular Oxygen.* Halsted Press: Stroudsburg, PA. 1976; 133-134.

12) Shakhashiri, B. Z.; Williams, L. G. *J. Chem. Ed.* **1976**, 53, 358

13) Marino, D. F.; Ingle, J. D., Jr. *Anal. Chim. Acta.* **1981**, 123, 247-253.

14) Gander, S. Master's Thesis, Oregon State University, **1990**.

REFERENCES (continued)

15) Archibald, J. fabrication performed, Oregon State University Machine Shop, **1995**.

Chapter 3

The Determination of Free Chlorine by Chemiluminescence Reaction with Luminol

Soren J. Glaser and James D. Ingle, Jr.

Department of Chemistry
Oregon State University
Corvallis, OR 97331

INTRODUCTION

Overview. Chlorination of the public water supplies for disinfection purposes results in the formation of unwanted by-products, especially the trihalomethanes such as chloroform.[1,2] These carcinogenic by-products are regulated by the EPA at 100 μg/l.[3] Because no reasonable alternatives to chlorination are widely accepted, chlorination is likely to be employed in the foreseeable future. To limit the concentration of the trihalomethanes, the minimum amount of chlorine should be added to the water to maintain antimicrobial activity. A continuous chlorine analyzer that is accurate and easy to operate is crucial to optimize chlorine use in water treatment plants. A chlorine analyzer could also be used to monitor chlorine levels in industry, which use chlorine for paper and plastic production and reduction of biofouling in cooling water.[4]

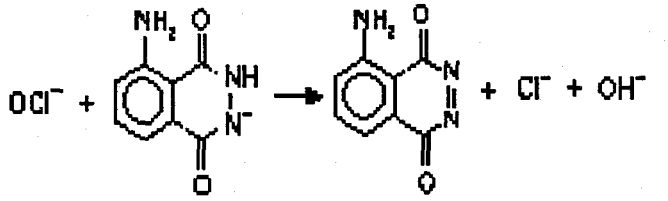
Current popular methods available for chlorine detection include colorimetric and amperometric procedures.[5] Unfortunately these methods suffer from either unstable reagents or interferences.[6,7]

This chapter is concerned with the investigation of the chemiluminescence reaction between luminol and chlorine as a useful analysis tool. This reaction is investigated with both continuous flow analysis (CFA) and flow injection analysis (FIA). This flow system employs solid phase pH control and solid phase luminol reagent delivery.

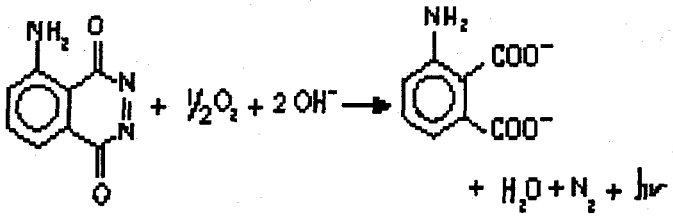
Reaction Chemistry. Luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) is one of the most efficient synthetic chemiluminescence agents known.[8] First discovered by Albrecht[9] in 1928, the oxidation of luminol has been shown to produce a strong blue

chemiluminescence (~ 476 nm) and be useful analytically for the determination of the halogens, hydrogen peroxide, trace metals including Cu, Co, Fe and Cr.[10-12]

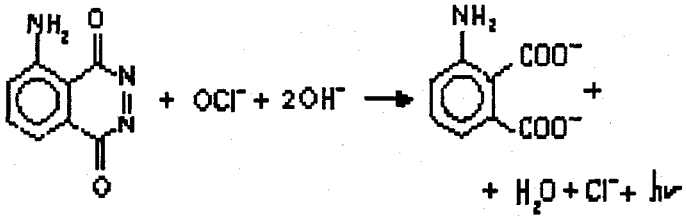
Although luminol is one of the most studied chemiluminescence agents, the mechanism by which CL is produced is still not fully understood. The most probable reaction with hypochlorite is given by Seitz[13] and is shown below.



(3.1)



(3.2)



(3.3)

The first step is the reaction between hypochlorite and luminol to form azaquinone which in turn reacts with an O_2 molecule or another OCl^-. The reaction and CL signal is first-order in OCl^- if the reaction with O_2 is dominant and is second-order in OCl^- if the reaction of azaquinone with OCl^- is the limiting step. Seitz states that no direct evidence

of the azaquinone intermediate exists, but that azaquinones can produce the same chemiluminescence as seen with luminol.

There are several different ways in which luminol can be analytically useful: the analyte reacts directly with luminol to produce chemiluminescence in proportion to the analyte concentration, the analyte acts as a catalyst or activator that enhances the chemiluminescence with luminol and an oxidant, and the analyte inhibits the oxidation of luminol. Most analytical work examining luminol's reaction with chlorine or trace metals has thus far concentrated on the ability of the analyte to enhance the oxidation of luminol in the presence of hydrogen peroxide.

The work described in this chapter, based on the luminol/chlorine chemiluminescence reaction, is unique because H_2O_2 is not used as the oxidant. By eliminating the peroxide, one less reagent is needed, and the potential for interferences from trace metals may also be reduced. Also unique in this chapter is the "reagentless" approach investigated. By "reagentless" it is meant that reagent solutions need not be prepared and that the pH is adjusted via solid phase beds (SPB's) and that the luminol reagent is delivered from a similar solid state luminol bed (SPL). The solid phase reagents that are packed in columns were developed by the Umpqua Research Center (URC) in Myrtle Creek, OR. The SPB columns employed consisted of proprietary forms of MgO which when challenged with a H_2O source release OH^- and Mg^{2+} ions, thus increasing the pH of the resulting column effluent. The SPL column consists of solid phase luminol crystals which dissolve in the input stream of H_2O. The reagent concentration and pH value are controlled by the size of the beds and DI H_2O flow rates, virtually eliminating the

needs for reagent preparation. Also, a solid state iodine column was investigated for its potential use as a calibration standard.

The flow instrumentation used in this chapter are refinements of the equipment used in chapter 2. The unique spiral observation cell was employed and a new design for the concentric reactant injector was tested. The injector allows the reactants to mix within view of the PMT detector. The benefit of chemiluminescence detection is realized in the simplicity of the detection and flow apparatus.

Previous work. The primary papers about the CL determination of free chlorine with luminol (without H_2O_2) are by Seitz[13] and Marino and Ingle.[14] Marino and Ingle investigated the luminol/chlorine reaction in a discrete sampling apparatus and Seitz employed a flow system.

Seitz[13] has proposed the reaction mechanism presented earlier in the chapter between luminol and hypochlorite, which accounts for the first-and second-order nature of the CL signal with respect to hypochlorite. Seitz has also shown that the CL signal is greatest near pH 10 and that as the pH is increased from 9.2 to 11.8, the reaction kinetics slow. The steady-state CL signal from his flow system was measured.

Marino and Ingle[14] observed a proportional CL signal response from the reaction with respect to OCl^- concentration. This is in contrast to this second-order dependence described by Seitz.[13] However, Marino used a higher luminol concentration facilitated by using MeOH as a solvent. The optimum pH was shown to be between 9 and 11. Tap water was analyzed by the CL method and the DPD standard method, and good

agreement was obtained. The CL response for 60 species other than OCl^- was evaluated. Those species most likely to cause an interference in a typical water sample were Fe(II), Fe(III), MnO_4^-, NH_2Cl, and ClO_2, which were found to cause a CL signal, and Mg(II) and Ca(II), which were found to cause a negative interference in the OCl^- CL signal itself. Of these interferants, Ca(II), Mg(II), and Fe(III) were thought to be most important in drinking water and their levels could usually be brought below the interference level by dilution of the sample. The detection limit given by Marino was 0.2 μg/l, which is about two orders of magnitude lower than that of the accepted standard methods.

INSTRUMENTATION

Overview. The apparatus for the chemiluminescence (CL) determination of hypochlorite by reaction with luminol consists of a reaction/observation flow cell, a PMT detector, a concentric reactant injector, two or three pumps for pumping the reactants, a light-tight enclosure (PVC cylinder), an integrated solid phase bed (SPB) column for pH control and solid phase luminol column (SPL) for luminol production, an effluent pH meter, and basic signal processing equipment. A schematic of the integrated system is shown in Figure 3.1.

During continuous flow analysis (CFA), a DI H_2O source is pumped through the SPL/SPB combination producing a constant concentration of luminol at a high pH; another pump controls the flow of a chlorine standard or sample (Figure 3.1A). Both the luminol and the chlorine solutions flow continually into the concentric reactant injector which directs and mixes the reactants in the observation cell producing CL within view of a PMT detector. The resulting CL signal is a steady-state signal recorded as the height above the signal obtained from a DI H_2O blank.

For the flow injection analysis (FIA) mode of operation, the luminol stream is the same as that for CFA mode. A third pump directs a carrier stream (pH adjusted DI H_2O) through a sample injection valve and then to the cell (Figure 3.1B). Another pump alternately fills the sample loop with sample or chlorine standard or directs the sample or standard to waste, depending on the position of the sample injection valve. After injection, a peak-shaped CL signal is observed and is due to a plug of chlorine being flushed into the observation cell where it mixes with the luminol stream to produce CL.

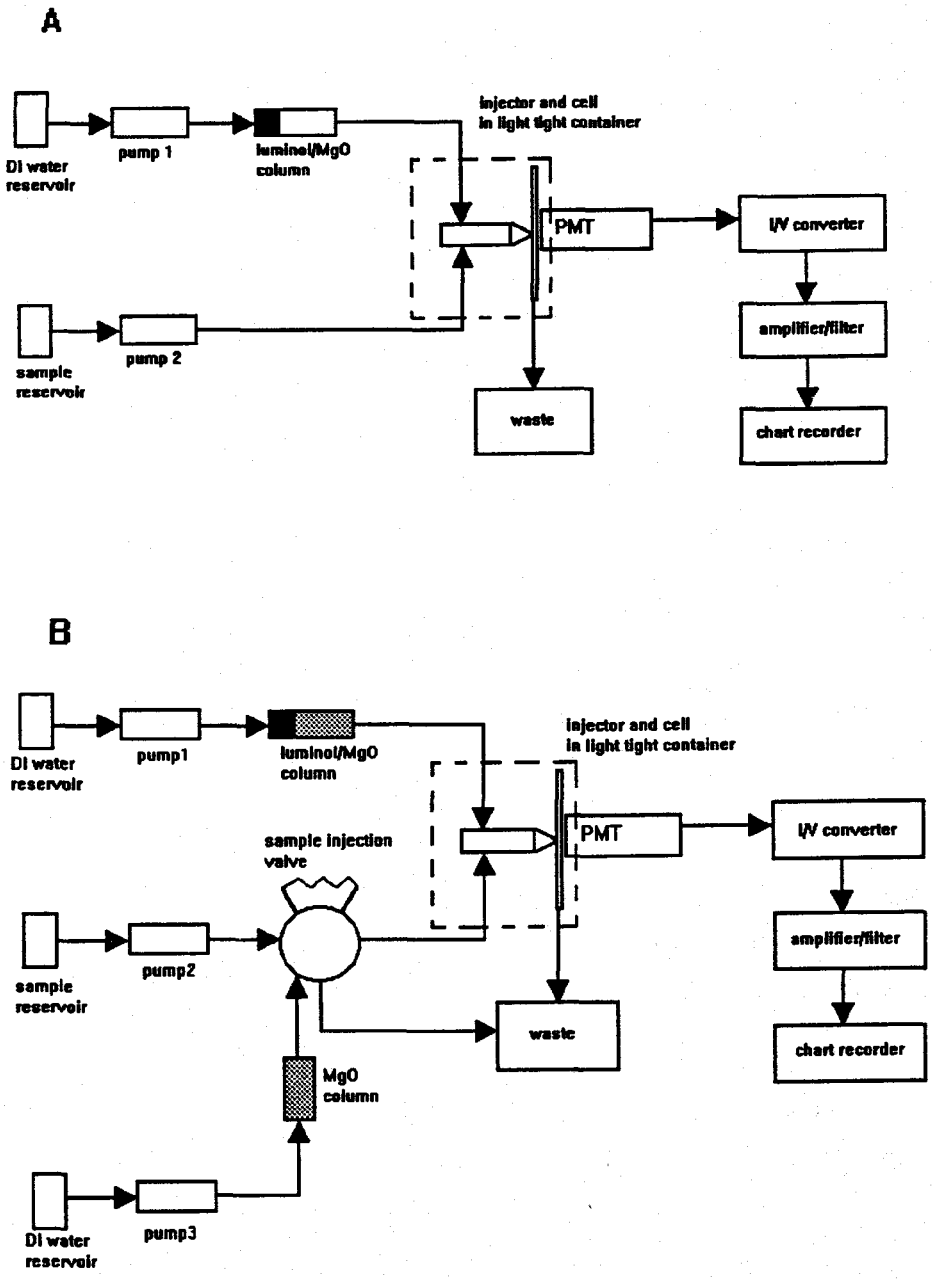


Figure 3.1. Schematic for two versions of the chemiluminescence luminol/chlorine analyzer. Figure A depicts the analyzer in continuous flow mode. Figure B depicts the analyzer in flow injection mode.

For both modes the mixed reactants from the observation cell are carried to a flow-through pH probe and then to waste. In some cases with FIA or CFA, the luminol/MgO column was removed and a luminol solution adjusted to the desired pH was pumped by pump 1.

Concentric Reagent Injector. Three versions of the concentric injector were used in these experiments. The first injector, used by Gander[15], is a Beckman burner assembly (No. 4020) normally used as an atomizer for atomic absorption or atomic emission spectrometry. The injector consists of a brass body with two input ports, a tapered nozzle, and a platinum capillary tube used to carry the analyte solution while keeping it separate from the reagent solution, which travels around the outside of this tube. The second injector, also used by Gander,[15] is similar in proportions to the first injector but was made in the OSU machine shop by John Archibald[16], primarily from Delrin (Figure 3.2). The third injector represents a further and significant refinement of the first Delrin version (Figure 3.3).

These injectors are all of concentric design, meaning that one reactant is carried through the central tube (Pt or stainless steel (ss)) which ends flush with the end of the nozzle, and the other reactant exits through the annular orifice of the nozzle which surrounds the central tube. Set screws in the nozzle help align the central tube so that it is concentric with the annular opening. With this injector design, the reactants do not mix until they are within view of the PMT.

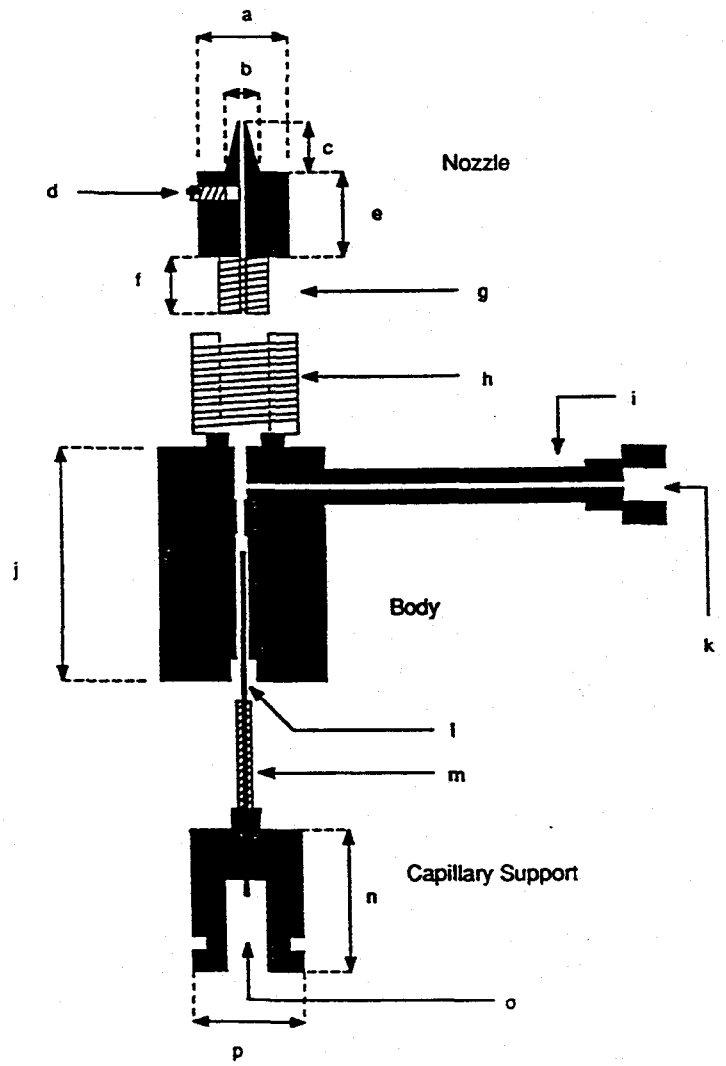


Figure 3.2. Schematic for the First Delrin Injector. The injector is made from three sections: a nozzle, a body, and a capillary support. The nozzle is Teflon with a 0.060 in o.d.; (a) 0.39 in; (b) 0.19 in; (c) 0.29 in; (d) one of three 4-40 nylon collimating set screw; (e) 0.49 in; (f) 0.38 in; (g) 1/4-28 thread. The body is Teflon; (h) 1/2-32 thread to screw into the bottom plate of the cell; (i) Delrin side arm which carries the reactant which travels through the annular opening of the injector; (j) body length was 0.88 in with a 0.75 in diameter; (k) outer side arm port is tapped to accept 1/4-28 low pressure end fitting. The capillary support is Nylon; (l) inner conduit of platinum tubing has a 0.016 in i.d. and a 0.028 in o.d. (0.8-mm i.d. Teflon tubing slides over the capillary shaft); (m) 6-32 thread on shaft which is 0.55 in long; (n) 0.48 in; (o) the capillary support is tapped to accept a 1/4-28 low pressure end fitting; (p) 0.45 in. Figure taken with permission from the thesis of Gander.[15]

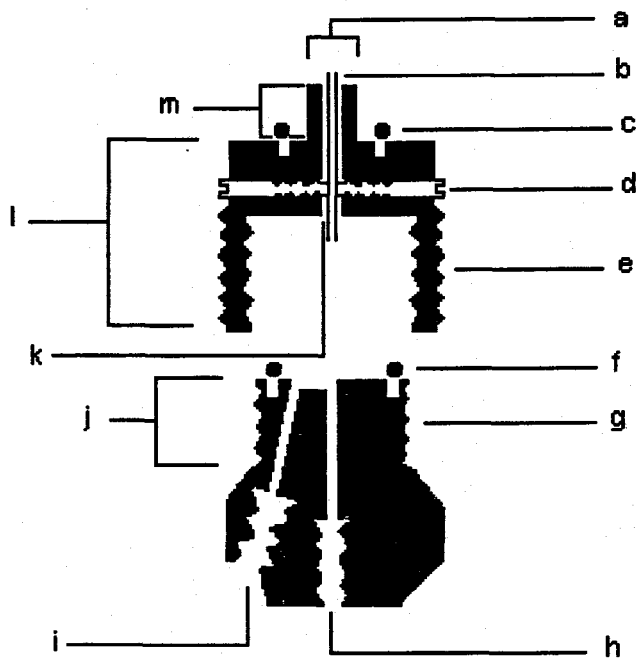


Figure 3.3. Schematic of the Second Delrin Injector. For this final version of the reactant injector, there are three sections, top and bottom Delrin section and a 22-gauge stainless steel tube for the inner conduit of the concentric injection port. The key measurements are: (a) nozzle head diameter, 0.202-in; (b) 22-gauge stainless steel tube, 1-in long, 0.016-in od, 0.028 in od; (c) "O" ring seal; (d) alignment screws; (e) 1-32 threads; (f) "O" ring seal; (g) 3/4-32 threads; (h) 1/4-28 threads for tubing connection for inner conduit of the injector; (i) 1/4-28 threads for tubing connection for annular conduit of the injector; (j) 0.318 in; (k) 0.033-in diameter pathway/opening for the annular conduit of the injector; (l) 0.598 in; (m) 0.20 in nozzle height.

The first two injectors screw into the observation cell via a Plexiglas adapter bonded to the bottom plate of the cell, as shown in Figure 3.4. The openings of the tapered injector nozzles are flush with the bottom of the reaction chamber when it is fully threaded into the cell. The side arm port of these injectors was shortened to about 1 in and tapped with 1/4-28 threads for easy connection to the reactant tubing, which was fitted with 1/4-28 male adapters.

The third injector screws into the cell itself and its nozzle becomes the bottom of the reaction chamber in the observation cell (Figure 3.5). The third injector is a simplification of the other injector designs. This injector has only two main body parts, both made of black Delrin (compare Figures 3.2 and 3.3). The primary objectives for implementing the design changes were to eliminate leaks at the injector/cell interface, to simplify the production processes for the injector and observation cell, and to prevent the conical nozzle from being crushed as it was screwed into the observation cell. The "O"-ring seal below the nozzle eliminated leaking at the cell/injector interface, and the flat nozzle head of the injector kept it from being deformed. The third injector was also designed to minimize any dead volume within the injector by shortening the injector length as well as using passages of smaller diameter.

Observation Cell. Two versions of the observation cell were used. These cells are considerable refinements of the observation cell developed by Gander.[15] The first cell shown in figure 3.4 consists of two 1/4-in thick polycarbonate disks machined in the OSU Machine Shop[16]. The top plate of the cell has 2 sets of 8 attachment screw holes drilled at

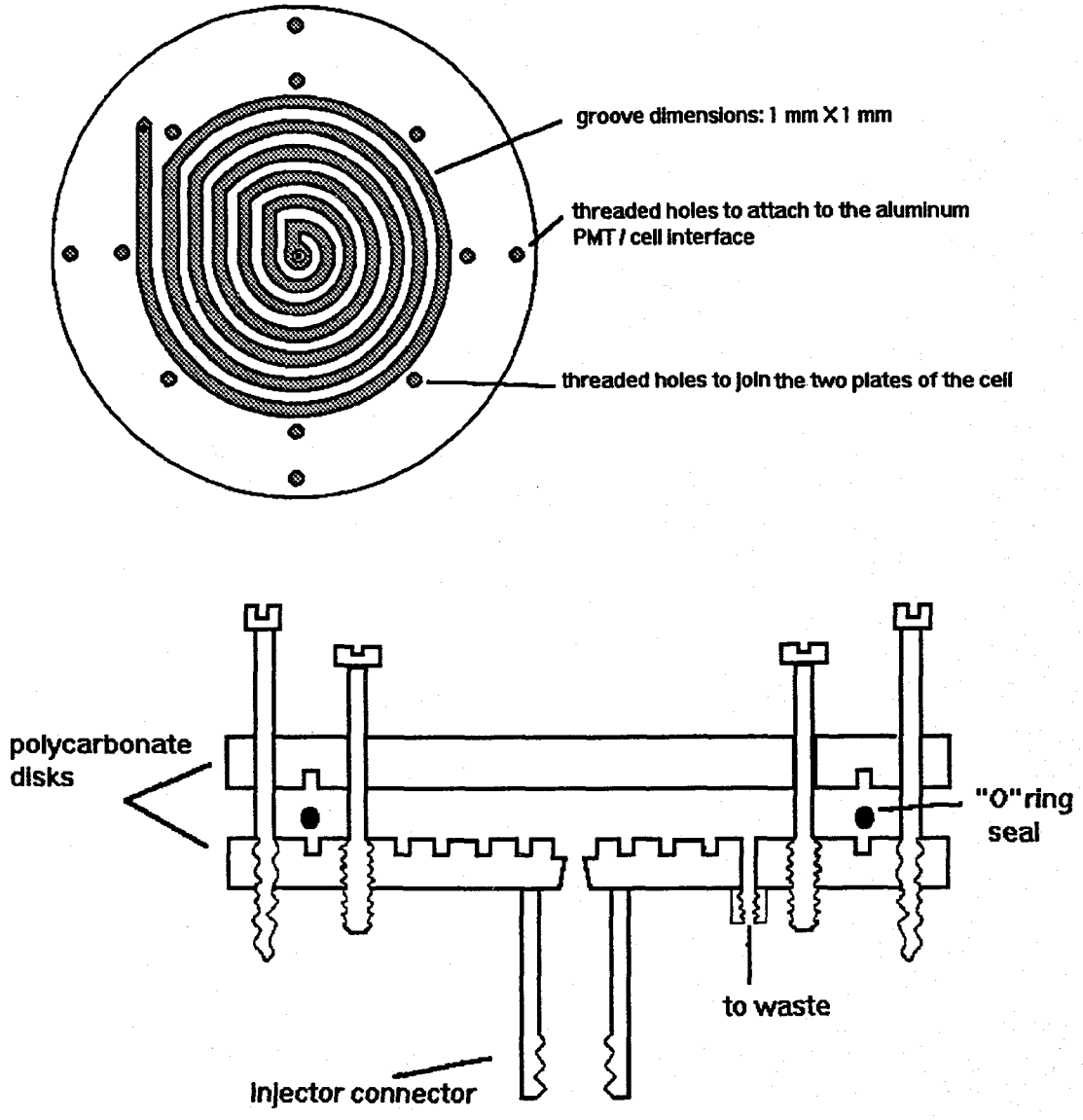


Figure 3.4. Top and Side Views of the Plexiglas Observation Cell. The bottom polycarbonate plate of the observation cell has a 1 mm by 1 mm groove emanating from a central reaction chamber measuring 5 mm in diameter by 1-mm deep. Screws and threaded holes just outside the rings help keep the two plates flush. The "O"-ring was eventually eliminated.

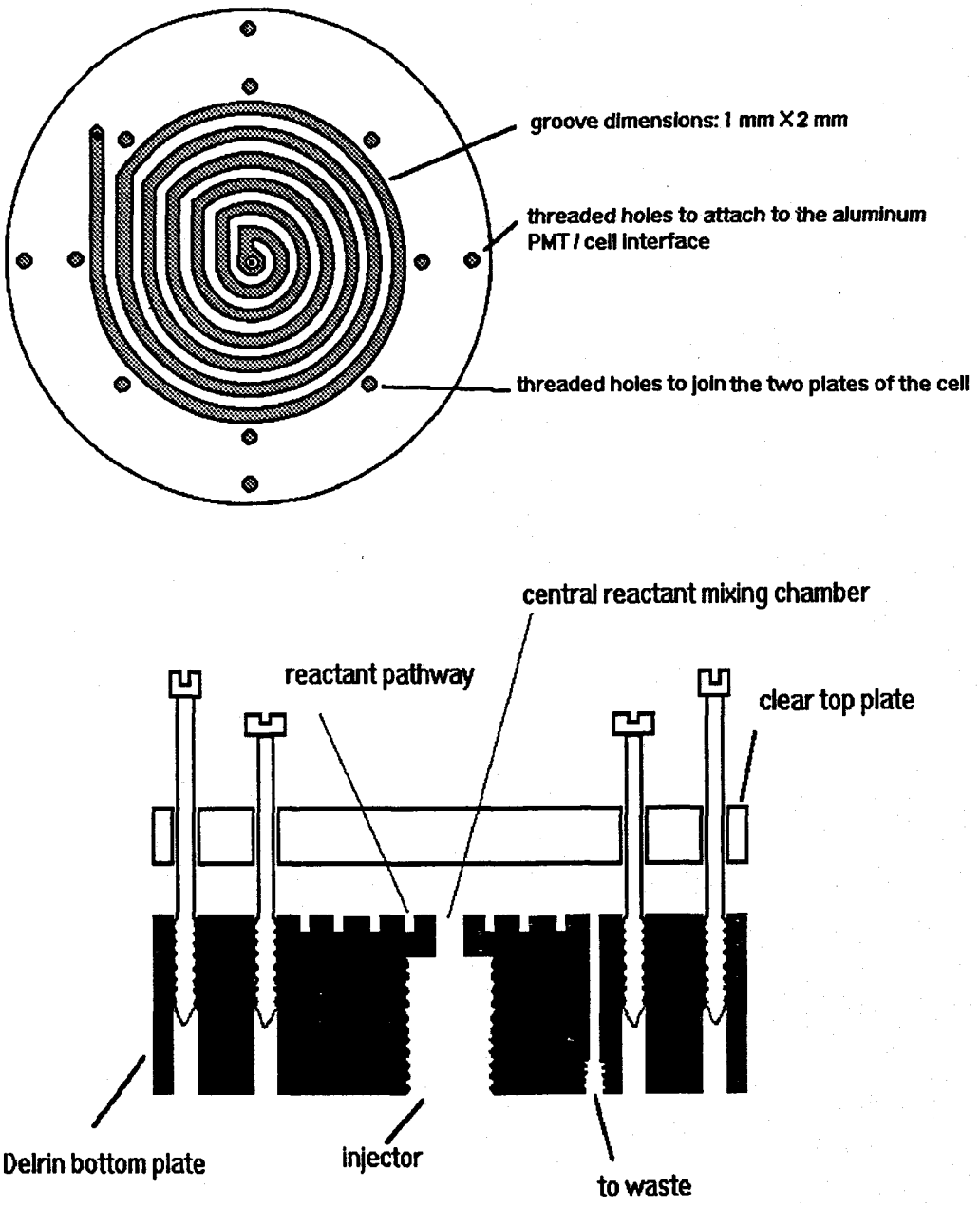


Figure 3.5. Schematic of Delrin Observation Cell. This is the final version of the observation cell (top and side views). The injector screws into the bottom plate of the cell and becomes the floor of the central reaction chamber. The top and bottom disks of the observation cell are 4-in diameter disks. The top plate is 1/4-in thick clear PVC. The bottom plate is 0.886-in thick black Delrin. The spiral groove measures 1-mm wide by 2-mm deep, and the central reaction chamber has a 5-mm diameter and 2-mm depth. The plates are held together by 8 4-40 screws drilled in a circular pattern 1 inch radially from the center. There are 4 4-40 outer screws for attachment to the cell/PMT interface.

1 in and 1.25 in from the center of the plate in a circular pattern. The inner set of screw holes is used to attach the upper and lower plates of the cell, and the outer set is used to attach the cell to an aluminum PMT/housing collar. The bottom plate was manufactured out of Plexiglas by carving a 1-mm by 2-mm deep spiral groove emanating from a central circular depression, which measures 1-mm deep by 5-mm diameter. This plate also has the attachment holes at 1 and 1.25 in from the center. When the top plate is fastened to the bottom plate with the sixteen peripheral 4-40 screws, there is a central circular mixing chamber and a 1 x 2 mm spiral pathway in which the mixed reagents and sample travel until they reach a 1-mm diameter exit hole which is located 1 in radially from the injection port. The total volume of the mixing chamber and spiral pathway was calculated to be 1 cm^3 from the cell dimensions. There is approximately 0.5 cm^3 of active cell volume, which is directly within view of the 30-mm diameter head on PMT. There are several extra spirals outside of the PMT viewing area.

The bottom plate is fitted with a polycarbonate 1/4-28 female adapter bonded below the exit port, which is connected with tubing to carry the waste reagents to a flow through pH probe and to waste. The injector screws into the bottom plate via a polycarbonate adapter fitted with ½-32 threads to match the threads on the injector. Mixing in the central reaction chamber occurs when the reactants are injected into the observation cell and hit the under side of the top plate which initiates turbulent mixing.

The second observation cell used in these experiments was crafted out of black Delrin and clear PVC in the Machine Shop at OSU[16] (Figure 3.5). This observation cell is similar to the polycarbonate cell, but it was designed to be used specifically with the final

injector. The major differences in cell design are that the reactant and the effluent tubing end fitting (1/4-28) screw into the base of the 0.5-in thick bottom plate instead of into a bonded adapter (compare Figures 3.5 and 3.4) and the 0.5-cm diameter flat nozzle head forms the bottom floor of the central reaction chamber. The Delrin material for the cell bottom and the clear PVC for the cell top were chosen as alternatives to Plexiglas which warped over time creating reactant leaks. The spiral pathway in this cell measures 1 x 2 mm deep and creates a ~ 1 cm^3 total cell volume and ~ 0.7 cm^3 active cell volume directly in view of the 30-mm diameter head on PMT. The top plate of this cell is 1/4-in thick clear PVC and both plates have the same pattern of attachment holes as the polycarbonate plates.

The Plexiglas cell was used for the majority of the experiments (Figure 3.4). Good mixing of reactants in this cell was confirmed in previous experiments (Chapter 2). The black Delrin cell (Figure 3.5) was observed to behave similarly to the Plexiglas cell with respect to CL signals, so its mixing efficiency was not experimentally examined.

Cell/PMT Interface and Housing. The observation cell, injector, and tubing are held within a 5-in diameter black PVC cylinder by 8 screws that also pass through an aluminum connector plate. The aluminum plate also keeps the end of the PMT flush against the top plate of the cell (see Figure 3.6). The reagent and sample Teflon tubing enters the PVC cylinder through any of four light-tight 1/4-28 feed-throughs located on the sides of the cylinder. The opening of the cylinder opposite the cell is fitted with a plastic cap to make it light tight. The plastic cap is fitted with a removal knob as well as 4 wing-nut

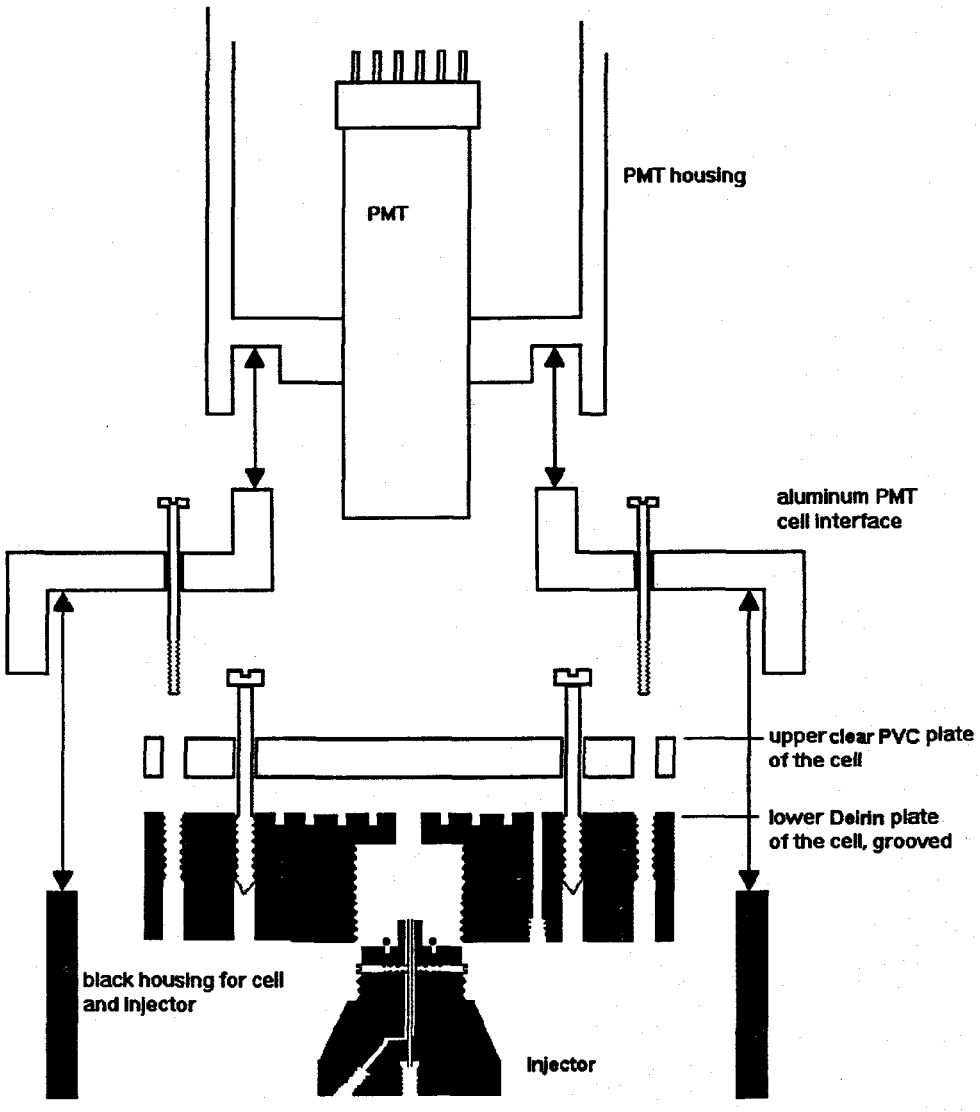


Figure 3.6. Schematic of the PMT/Housing/Observation Cell. The PMT housing slides on the aluminum connector plate which is fastened to the cell via the outer set of screws. The whole assembly is then fitted onto the rim of the black PVC cylinder to keep out light. When everything is in place, the PMT head is flush with the top plate of the cell.

fasteners for easy access in to the cylinder. A valve is fitted into the bottom of the cylinder which can be opened to drain solution from any accidental leaks. Figure 3.7 shows how the apparatus is positioned on an aluminum stand parallel to the table top.

Detection, Signal Processing, and Readout Systems. The PMT used for all of the experimentation was an RCA type C31059. This head-on PMT was contained in a Pacific Precision model 62 housing. The PMT was biased by a Keithley 244 High Voltage Supply, which supplied from 600 to 900 V.

The photocurrent from the PMT is converted to a voltage and amplified by a Keithley 427 current amplifier, with a transfer function from $1x10^6$ to $1x10^8$ V/A. The voltage signal is conditioned by a Spectrum 1021 amplifier and filter to provide further gain (up to 10) if needed. The cutoff frequency was generally set at 0.1 Hz. The readout is performed by a Heath SR-205 single pen chart recorder. All absorbance measurements were taken with a Hewlet Packard (model 8452A) diode array spectrophotometer.

Solid Phase Luminol Column. The Solid Phase Luminol (SPL) column consists of a 0.4-cm id TFE tube packed with a bed of luminol crystals (150-300 μm) obtained from the Umpqua Research Center (URC) in Myrtle Creek, OR. The total volume of the luminol bed was between 1 and 2 cm^3. A bed of 5-10 cm^3 MgO particles (150-300 μm) was packed into the same TFE column, after the luminol bed, so that the resulting column eluent was adjusted to about pH 10.5. Compression end fittings obtained from Cole

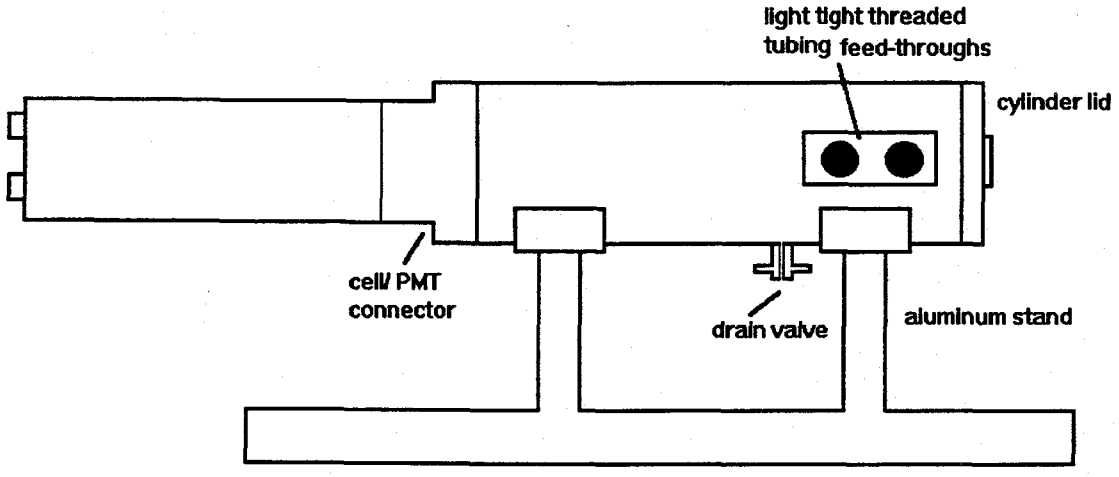


Figure 3.7. Schematic of the Apparatus Stand and Mounting Position. The PVC cylinder is held to the aluminum holder with plastic straps parallel to the table top.

Palmer were used to connect the column to the Teflon reactant tubing. To retain the luminol crystals in the column, glass wool was inserted on either side of the crystal bed. It is recommended that the luminol stream be directed to flow through the outer opening of the concentric injector to avoid dislodged crystal particles from being trapped inside the central tube of the injector.

Solid Phase Buffer Columns and Other Flow Components. The SPB columns consisted of 40- to 80-cm long TFE tubes (0.80-cm id) containing MgO particles (150-300 μm), which were retained in the columns by glass wool plugs at either end. Polypropylene compression fittings from Cole Palmer served as end fittings for connection to the reagent tubing.

A Sentron model 1001 ion selective field effect transistor pH system was used to measure the pH of the observation cell effluent. The pH probe was placed in line, to measure the effluent pH from the cell, in a flow through electrode holder made out of Delrin at the OSU machine shop[16]. The ports to the in-line probe holder were tapped with 1/4 -28 threads so that they could be easily attached to the reactant tubing.

The reagent and sample (or carrier stream) pumps were made by FMI (model RHV). Normal operating flow rates were 1 ml/min for each stream unless otherwise noted. All tubing was Teflon (1/16-in od, 0.8-mm id) obtained from Altex and was terminated with flangeless Tefzel 1/4-28 fittings obtained from Upchurch.

In the FIA mode of operation, a sample injection valve was placed between the chlorine sample/standard reservoir and the observation cell (Figure 3.1B). A pair of

Dionex 4-way valves (Cat. No. 030520) were arranged to construct a sample loop injection valve (Figure 3.8). In load mode, a pump pumps the carrier stream (DI H_2O pH adjusted by an SPB) through a bypass loop which then leads directly to the observation cell, while the sample loop, with a volume between 30 and 200 µl, is filled with analyte solution by another pump. In the injection mode, the carrier stream is directed through the sample loop which carries the sample plug to the observation cell.

The sample valve is switched pneumatically between load and inject modes by sliding a rectangular bar with bored channels to connect different ports and direct the flow. Air pressure for the pneumatic action of the valves is provided by a pair of 3-way solenoid-driven valves, each controlled by a solid-state relay (SSR). The switching between modes is done manually with a switch that directs the output (+5 V) from a Heath transistorized power supply (model, EUW-17) to one of the two SSRs. Hence, for each switch position one SRR is activated and pressure is applied to one side of the slider.

The carrier stream provides a constant blank signal before the sample is injected. When a sample is introduced into the carrier stream, some spreading of the plug occurs in the tubing causing a Gaussian-shaped peak on the chart recorder. It is important to wait long enough (typically 1 min or more) between samples so that the sample loop and reactant tubing are flushed with several loop volumes of sample. During the injection mode, the sample stream is diverted through the bypass loop, so that when the load mode is initiated the carrier stream carries the contents of the bypass loop to the cell resulting in a second peak. The size of the second peak was minimized by reducing the bypass loop volume as much as possible (30 µl).

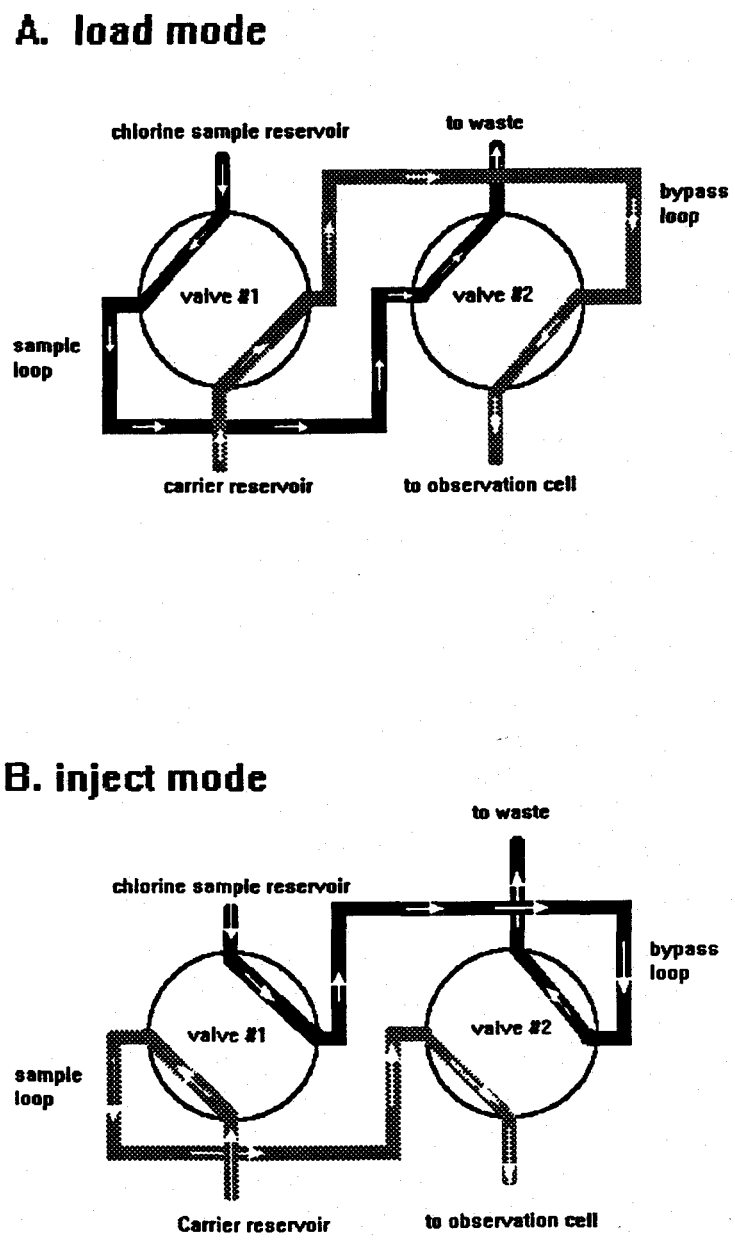


Figure 3.8. Schematic of the Sample Injection Valve. The two 4-way valves are arranged as shown above to create a sample injection valve. Figure A shows the valves in the load mode of operation where the sample loop is filled with sample, and Figure B shows the valves in inject mode where a carrier stream flushes the sample out of the loop towards the observation cell.

An iodine column developed by URC was obtained for investigating the CL produced by the reaction of luminol and iodine. The column consisted of a bed of MCV® resin (3-4 cm^3) which was packed into a TFE column similar to the one used for the luminol column. With a constant flow rate, this column should produce a constant concentration of iodine.

In some experiments the temperature of the standards and DI H_2O solutions were held constant by placing them in a 5-gallon aquarium heated by a B. Braun Thermomix heater (model 1419).

EXPERIMENTAL PROCEDURES

Overview. Normally the OCl^- standard, sample, or H_2O carrier stream was pumped at 1 ml/min through the inner orifice of the concentric injector, and a luminol solution, made either by running H_2O through a SPL or by dissolving luminol powder in flasks of H_2O adjusted to about pH 12 with NaOH, was pumped through the outer orifice of the injector at an equal or greater flow rate. For CFA, the sample injection valve was skipped and both reactants were pumped to the observation cell continually causing a steady-state signal on the chart recorder, whose amplitude above the signal from a DI H_2O blank was measured. In FIA, the luminol stream was continually pumped into the observation cell and the sample injection valve normally directed a DI H_2O carrier stream into the observation cell with occasional plugs of sample being introduced into the carrier stream. These signals were measured as the peak height above the carrier stream signal.

Reagents and Solution Preparation. All reagents were prepared with DI H_2O from a Millipore Milli-Q system fed with house DI H_2O. A stock sodium hypochlorite solution greater than or equal to 4 % available chlorine (Cl_2) by wt was obtained from Aldrich (chemical number 23930-5) and used to make all chlorine standards. A more dilute OCl^- stock solution was made weekly in DI H_2O, and OCl^- standards were made by diluting this stock solution. Typically 2 ml of the 5% stock chlorine solution was diluted to 1 l to prepare a 100 mg/l available chlorine stock solution, and then this solution was further diluted in 1 l flasks to make calibration standards in the mg/l range. The concentrations of all hypochlorite solutions are specified in terms of available chlorine (Cl_2). The 100 mg/l

standard solution was analyzed by the standard method described in appendix 1. Stock luminol solutions (between 0.01 and 0.5 mM) were prepared by dissolving an appropriate amount of luminol powder from Aldrich (97% 12,307-2) in 1 l of DI H_2O adjusted to about pH 12 with NaOH. Luminol is practically insoluble at pH values less than 11.

Proprietary forms of $CaCO_3$ (75-106 μm), luminol, MCV® resin, and MgO were obtained from URC where they were packed into TFE columns for use as solid phase reagent columns. Fused MgO (40-mesh 99+%) was also obtained from Aldrich. Analytical reagent grade $FeSO_4$, $HClO_4$, $FeCl_2 \cdot 4H_2O$, $FeCl_3$, $CuSO_4$, $NiCl_2$, $CrCl_3$, and NaOH were aquired from Mallinkrodt. Reagent grade $CoCl_2$ was obtained from J. T. Baker. Certified ACS plus HCl was obtained from Fischer Scientific. EDTA disodium salt and NH_4Cl were obtained from EM Science. A Fe^{2+} solution as $Fe(ClO_4)_2$ was made by preparing 0.5 M $FeCl_2 \cdot 4H_2O$ in 25 ml of N_2 purged 0.1 M $HClO_4$.

Effect of pH and Temperature. The pH dependence of the CL signal was determined in CFA mode, with solutions of 0.5 and 0.1 mM luminol (between pH 8 and 12) adjusted by the addition of NaOH and HCl. The pH of the mixture of the luminol and a 1 mg/l chlorine standard exiting the observation cell was measured and reported as the mixture pH. CL calibration curves, in CFA mode, were run with chlorine standards of 0.2, 1, 3, and 5 mg/l and three 0.1 mM luminol solutions adjusted to pH values of 10.1, 9.7 and 9.4.

The effect of pH on the half-life of the luminol/chlorine CL reaction was examined with 0.1 mM luminol solutions adjusted to pH values between 9 and 12. Each luminol solution was mixed in the cell with a 1 mg/l chlorine standard until a steady-state signal

was obtained. Then the pumps were simultaneously turned off and the decay was recorded. The half-life was determined as the time for the signal to reach half of the steady-state signal.

On some days the laboratory temperature varied by over 10 °C. To examine temperature effects on the CL reaction, all solutions of DI H_2O and OCl^- were placed in the constant temperature water bath. The water bath was only used when the signals for a given chlorine standard drifted over time.

Effect of Luminol Concentration and Flow Rates. Batch solutions of 0.01, 0.1, 0.25 and 0.5 mM luminol were prepared and adjusted to about pH 10.5 and calibration data were obtained for each luminol concentration with chlorine standards between 0.2 and 5 mg/l in CFA mode. Flow rates were approximately 1 ml/min for each reactant.

A high flow rate of 5.5 ml/min and a low flow rate of 1.3 ml/min were chosen to examine the effect of the flow rate on the CL signal in a CFA experiment. With a 0.1 mM batch solution of luminol adjusted to pH 10 with NaOH, calibration data were obtained with chlorine concentrations from 0.2 to 5 mg/l and luminol/chlorine flow rates of 5.5/1.3, 5.5/5.5, 1.3/1.3, and 1.3/5.5 ml/min.

Testing for Interferences. To test the luminol/chlorine CL reaction for susceptibility to signal enhancement interferences from common metal ions, solutions of $CoCl_2$, $CuSO_4$, $Fe(ClO_4)_2$, $FeCl_3$, and $CrCl_3$ at concentrations of ~ 0.1 mM were mixed in the observation

cell (in CFA mode) with ~ 0.1 mM luminol (pH 10.5) from the SPL column. Flow rates were approximately 1 ml/min for each reactant.

To test the reaction for some species that potentially depress the CL signal, solutions of 0.1 mM $FeCl_3$ and $CuSO_4$ were made by diluting in 1 mg/l chlorine standards. These solutions were mixed in the observation cell (in CFA mode) with ~ 0.1 mM luminol (pH 10.5) from the SPL column. Flow rates were approximately 1 ml/min for each reactant. The CL signals resulting from the contaminant containing chlorine solutions were compared to those from chlorine standards without the added interferents.

In an attempt to eliminate the signal suppressing contamination, EDTA was added to the DI H_2O source (adjusted to pH 10.5 with NaOH) for the carrier stream in some FIA experiments. In general the concentration of EDTA in the carrier stream was 0.1 mM. To test for monochloramine interference, NH_4Cl was added to the hypochlorite standards (up to an equal molar concentration with OCl^-) which were adjusted to pH 9 with NaOH.

Water Analysis. The colorimetric DPD method of chlorine determination[5] was used to check the accuracy of the luminol/chlorine CL method. Standards and tap water samples or waste water samples were run with the luminol/chlorine CL reaction in both the CFA or FIA modes of operation. A DPD calibration curve was run concurrently with the same chlorine standards. The DPD method is described in appendix 2.

Tap water samples were collected from the tap in the laboratory (Gilbert Hall 254), sometimes directly after the tap was turned on and sometimes after the water was run for over 10 min. Waste water samples were collected from the waste water treatment

facilities in Corvallis, OR in December 1995 and Myrtle Creek, OR on 10/11/95. The waste water was sampled from final stage holding tanks just prior to being discharged from the facility.

For FIA and CFA, the luminol concentration was ~ 0.1 mM (pH 10.5) from the SPL column. The flow rates used were typically 2 ml/min for the luminol stream and 0.2 ml/min for the chlorine sample/standard stream (CFA) or the carrier stream (FIA). The typical calibration range was between 0.2 and 1 mg/l chlorine.

Iodine Standard Calibration with MCV Resin. To examine the CL reaction between iodine and luminol, an iodine MCV® resin column was placed in line with a DI H_2O stream (the normal sample stream), and the effluent was mixed with ~ 0.1 mM luminol (pH 10.5) from the SPL in CFA mode. The iodine column was determined by URC to produce approximately 2 mg/l (7.88 μM) iodine with a challenge stream of 1 ml/min DI H_2O.

RESULTS AND DISCUSSION

Effect of pH. As shown in Figure 3.9, the maximum CL intensity occurs near pH 10.7 for a 0.1 mM luminol solution and near 11 for a 0.5 mM luminol solution. A luminol concentration of 0.1 mM is near what is obtainable by running DI H_2O through the SPL column at approximately 1 ml/min and was the concentration used for all of the experiments involving the SPL column. The reason for the down turn in CL intensity at higher pH values may be that the half-life becomes long enough that some of the CL takes place out side of the observation cell. Seitz[13] reports a maximum CL signal at pH 10.1 although he states that the pH maximum may be higher because some of the CL could be taking place after the reactants have left his flow cell. Marino and Ingle[14] observed a broad CL maximum between 9 and 11 with a discrete sampling system.

The maximum pH attainable with the use of the MgO SPB columns was observed to be about 10.7. Given a flow rate of 2 ml/min or less, a column 5-cm in length can easily adjust the effluent pH to 10.5 which was the pH used for a majority of the experimentation. This pH is close to the pH observed to yield the maximum CL signal for the 0.1 mM luminol solution.

The half-life of the luminol/hypochlorite reaction is strongly dependent on the pH. Figure 3.10 shows that the half life of the reaction increases from 0.12 to 18 s as the pH rises from 9.3 to 11.2. The half-life becomes an important factor when the combined reactant flow rate is high enough such that a significant portion of the CL occurs outside of the viewing area of the PMT. In a majority of the luminol experiments, the combined total reactant flow rate was near 2 ml/min and the pH was adjusted to 10.5 (half-life 5.3

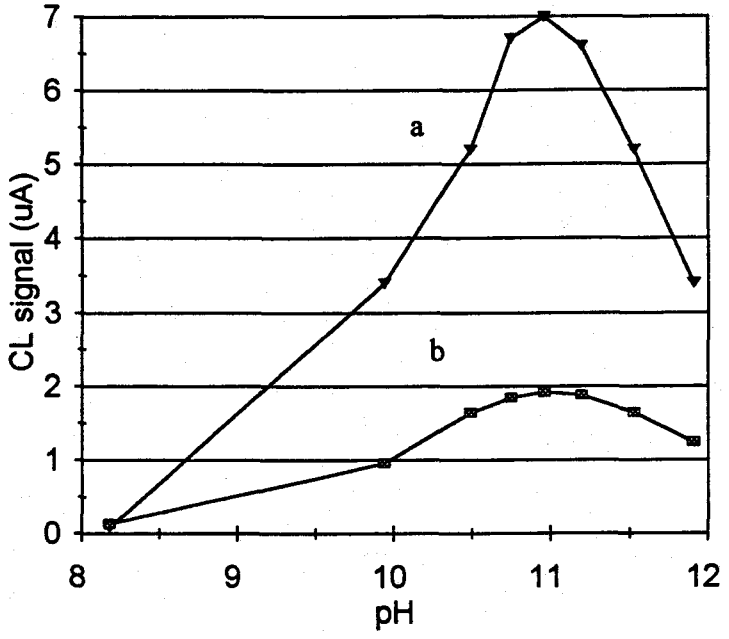


Figure 3.9. Dependence of Signal Intensity on pH and Luminol Concentration. Concentrations of luminol, 0.5 mM (a) and 0.1 mM (b), of different pH values were mixed with a 1 mg/l chlorine solution in the CFA mode.

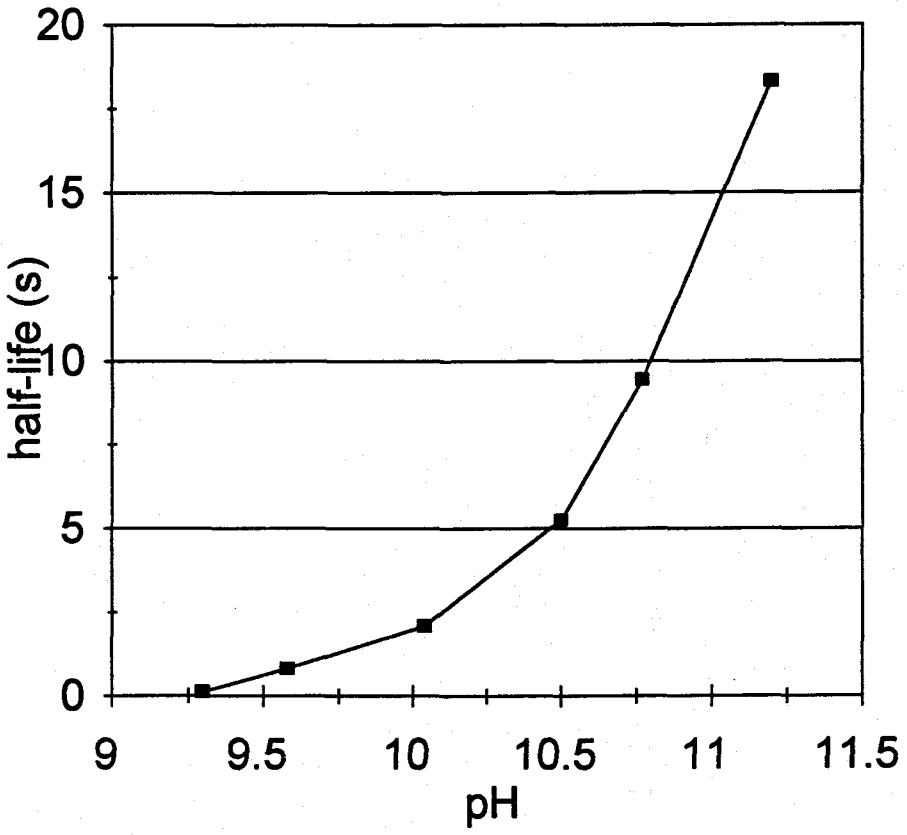


Figure 3.10. Dependence of Half-Life on pH. The time for the steady state signal from 0.1 mM luminol and 1 mg/l chlorine solution to reach ½ its original height was measured with different pH values.

s). For the Plexiglas cell with an observation volume of 0.5 cm^3 and the 2 ml/min combined flow rate, the residence time for the reaction mixture is 15 s. The increase in half-life with increased pH is also supported by Seitz[13] who observed that the rate constant for the reaction decrease with increased pH up to pH 11.8, but then increased again above pH 12.

Calibration curves for different pH values are shown in Figure 3.11. Both positive and negative deviations from linearity were observed, and a higher pH yields a higher CL signal, which is in agreement with the data in Figure 3.9. This is further evidence of the need for a stable pH for the reaction mixture. Seitz[13] has shown previously that the reaction between luminol and OCl^- generally has a positive deviation from linearity (second order nature). This is based on the second-order reaction of the aziquinone intermediate (first oxidation step) and OCl^- (the steady-state concentration of the intermediate is proportional to the OCl^- concentration). The oxidation of the intermediate by OCl^- is proposed to be more efficient (larger rate constant) than the oxidation by O_2. Later when using the SPL for luminol production, calibration curves with a second-order nature were normally observed.

Marino and Ingle[14] reported that the reaction between luminol and OCl^- with a discrete sampling (batch) system produces a linear calibration curve from 0.2 to 500 µg/l, after which a negative deviation from linearity occurs and was attributed to luminol becoming the limiting reactant which seems unlikely. Marino and Ingle did employ MeOH as a luminol solvent and a high luminol concentration of 1.8 mM. The large concentration ratio of luminol to OCl^- may have been responsible for the observed first-order nature of

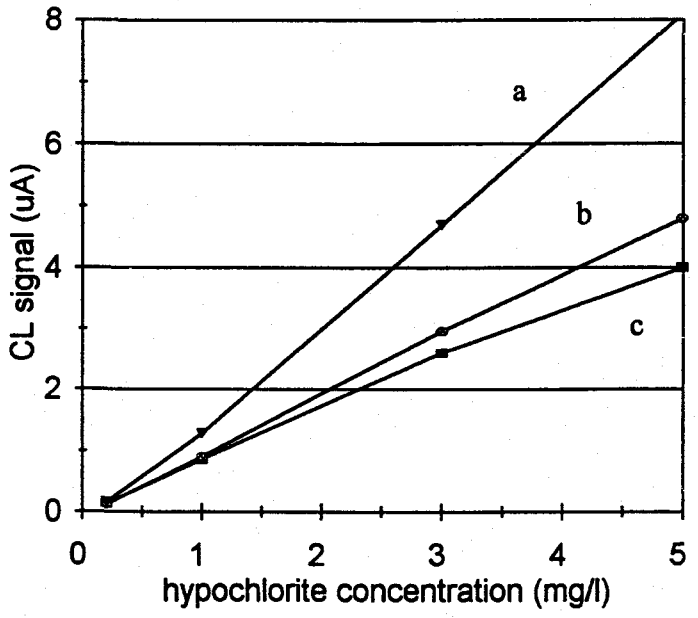


Figure 3.11. Calibration Curves for Luminol/Chlorine at Different pH Values. The luminol concentration was 0.1 mM for each calibration curve and the CFA mode was used. Curve (a) was run at pH 10.1. Curve (b) was run at pH 9.7. Curve (c) was run at pH 9.4.

the reaction. Also the shape of the calibration curve may be affected by the type of sampling system. With a flow system an integrated system is obtained, while with a discrete sampling system, the peak response is measured.

Control of pH and Luminol with SPB's and SPL's. The SPB column, developed at URC, has been shown to produce a constant pH effluent when challenged with DI H_2O as, discussed in chapter 2. Because the luminol/chlorine reaction has a strong pH dependence, the pH of the column effluent has to be stable.

The SPL column employed (1-2 cm^3 bed volume) was challenged with air equilibrated DI H_2O and produced approximately 0.1 mM luminol, which was the level used in all experiments involving an SPL. The luminol concentration of the SPL column effluent was determined by absorbance measurements. A typical absorbance of a 0.1 mM luminol solution at 348 nm was 0.688 AU. URC[17] reports that the concentration of luminol from an SPL can be varied from 0.1 mM luminol with a DI H_2O source to about 0.3 mM with a source adjusted to pH 10. The stability of the SPL concentration with the DI H_2O challenge was not examined.

Effect of Luminol Concentration. As the luminol concentration was increased from 0.01 to 0.5 mM, the initial slope of the calibration curve increased from 0.39 to 6.5 μA/(mg/ml), as shown in Figure 3.12. The CL signal is not proportional to the luminol concentration. The 0.01-mM luminol solution was the only solution investigated which resulted in a serious negative deviation from linearity. This deviation is attributed to

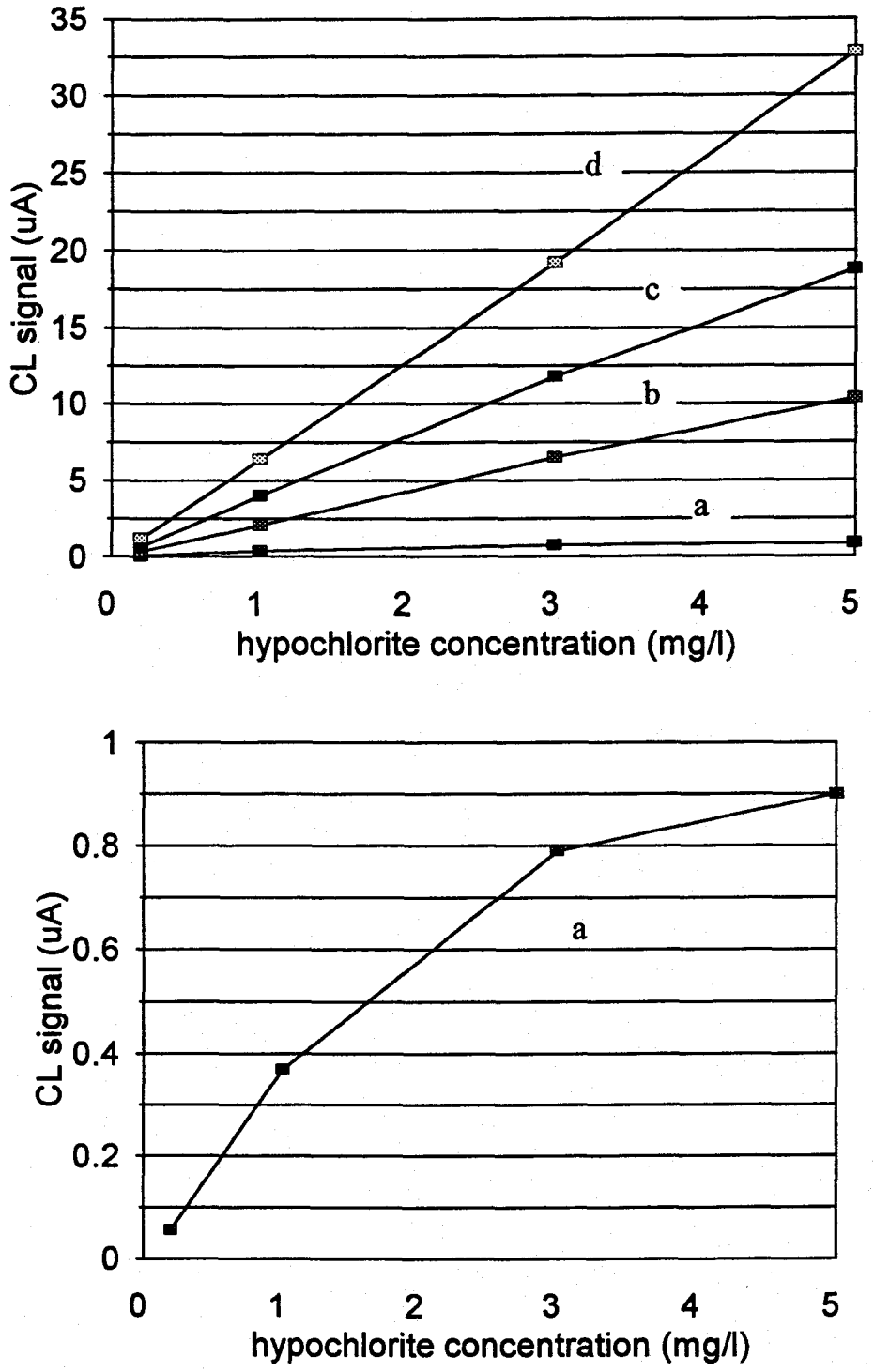


Figure 3.12. The Effect of Luminol Concentration on the Calibration Curve. The calibration curves were run a pH 10 in the CFA mode, and represent luminol concentrations of; (a) 0.01 mM, (b) 0.1 mM, (c) 0.25 mM, (d) 0.5 mM. Curve (a) is shown twice to illustrate its non-linearity. The initial slopes are (a) 0.39, (b) 2.2, (c) 4.2, and (d) 6.5 μA/(mg/l) .

luminol becoming the limiting reactant at chlorine concentrations higher than 1 mg/l which corresponds to 0.014 mM Cl_2. Even with the 0.1 mM luminol, 0.2 mg/l could easily be detected.

A 0.1-mM luminol concentration was used for further of the experiments because this was the concentration naturally produced by the SPL with a DI H_2O challenge. When a SPL column was employed, the calibration curve was more non-linear in the 0.2 to 5 mg/l chlorine range, in general, it had significant second-order nature as discussed later. However, the calibration curves were closer to linear with a new SPL. The second-order character emerged after several hours of operation. The cause for the difference between these calibration curves was not determined.

Marino and Ingle[14] used an in-cell luminol concentration of 1.8 mM which is over 10 times greater than the 0.1 mM pre-cell concentration used in these experiments. Marino achieved this high luminol concentration by using MeOH as the luminol solvent. Seitz[13] used a concentration of 0.4 mM luminol which is near the maximum luminol concentration possible using H_2O as the solvent.

Effect of Flow Rates. Flow rate combinations of 1.3 and 5.3 ml/min were chosen to test the effect of flow rates on the calibration curves (Figure 3.13). When both reactant flow rates were 5.3 ml/min (curve a), the CL signal for the 1 mg/l chlorine solution was 4.0 times greater than that when the flow rates were both 1.3 ml/min (curve c). For the 5 mg/l chlorine solution, the ratio was 3.9. As a first approximation a factor of 4 signal increase is predicted due to 4 times as much chlorine entering the observation cell per unit time.

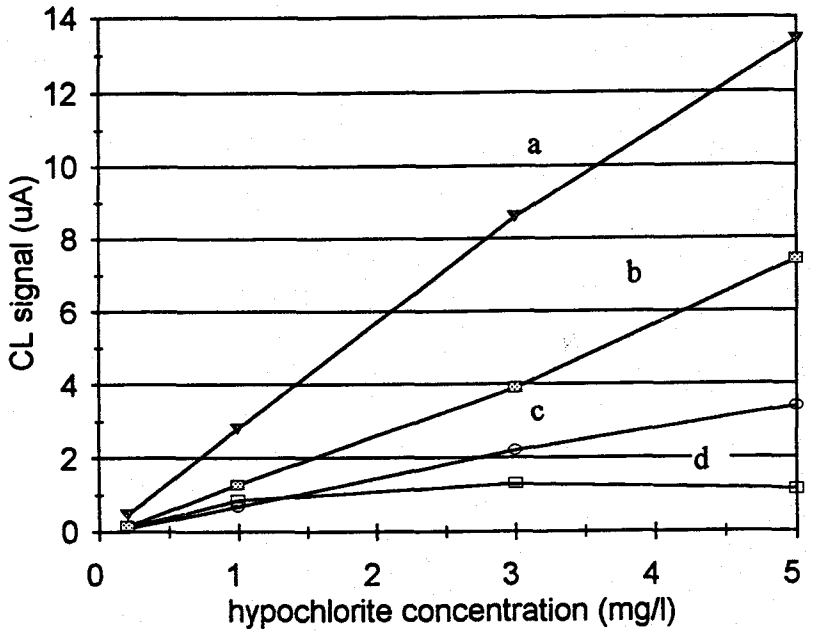


Figure 3.13. Effect of Flow Rate on the Calibration Curve. Calibration curves were run in the CFA mode with 0.1 mM luminol (pH 10) with different flow rate combinations. The luminol/chlorine flow rates in ml/min were (a) 5.3/5.3, (b) 5.3/1.3, (c) 1.3/1.3, (d) 1.3/5.3.

These results suggest that, at pH 10, a combined reactant flow rate of 10.6 ml/min does not cause a significant portion of the CL to occur outside of the PMT viewing area. This agrees with previous half-life data (Figure 3.11). With a 2.1 s half-life and a 10.6 ml/min flow rate, half of the CL should occur within 0.37 cm^3 which is about 70% of the 0.53 cm^3 observation volume. However, at pH 10.5 the half-life increases to 5 s, which would result in half of the CL occurring within 0.88 cm^3. In this case a lower flow rate would be desired to detect most of the CL.

A curve with a slight positive deviation was obtained when the luminol flow rate was 4 times that of the chlorine flow rate (curve b). Compared to curve a, the signals are less. The signal would be expected to decrease due to a smaller delivery rate for chlorine but increase due to a larger concentration ratio of luminol to chlorine. However, when the chlorine flow rate was 4 times that of the luminol (curve d), a negative deviation from linearity occurred (Figure 3.13). This negative deviation may be attributed to luminol becoming the limiting reagent under these conditions. With a flow rate ratio of chlorine to luminol of 4, 2 mg/l chlorine (0.028 mM), and 0.1 mM luminol, the in-cell concentration ratio of luminol to chlorine is 0.89.

Interferences. None of the metal ions tested (Co(II), Cu(II), Fe(III), Fe(II), Cr(III)) produced a detectable CL signal when mixed with luminol (0.1 mM, pH 10.5) in the observation cell. It has been reported that each of these metals can produce a signal with luminol in the presence of H_2O_2.[10-12] Also no signal was observed from tap water that had been boiled to remove free chlorine.

When running tap water through the system as a chlorine sample, with either the CFA or FIA mode, the CL signal decreased over time by up to 50%. When a standard chlorine solution was run immediately following a tap water sample, its signal was depressed (relative to its signal before the tap water sample) for several minutes in the CFA mode and for up to 5 injections in the FIA mode. Even if the sample volume of tap water was reduced to 30 µl in FIA, this attenuation in subsequent signals occurred. If the tap water source was run for 5-10 min before sampling, this depression of the CL signal was eliminated in both FIA and CFA modes.

Changing the input stream to the SPL column from DI H_2O to 0.1 to 1.0 mM EDTA eliminated the signal depression from tap water samples and subsequent standards in FIA mode. EDTA addition was not investigated with the CFA mode.

It was shown that the addition of 0.1 mM $Cu(NO_3)_2$ to chlorine standards produced CL signal suppression similar to that of tap water, and the addition of EDTA to these standards (0.1 mM solution concentration) prevented the suppression of the signal. No signal suppression was observed when Fe(III) or Ni(II) were added to chlorine standards (0.01 mM solution concentration). EDTA forms a strong complex with Cu(II) and apparently masks its interference effect. It is known that the water in the laboratory can contain relatively high concentrations of copper (up to 1 µg/ml or 16 µM) and that running the tap reduces the amount of copper in the water. Hence it was suspected that Cu(II) was the interferant in tap water that suppressed the CL signal and EDTA was used in some future experiments when comparing the FIA CL method to the DPD standard method.

If a molar equivalent of NH_4Cl relative to OCl^- (up to 10 mg/l or 0.19 mM of OCl^-) was added to the chlorine standards (pH 9.2), no CL signal was observed. Monochloroamine is reportedly the dominant chlorine species under these conditions.[18] If the molar ratio of NH_4^+/OCl^- was adjusted to less than 1, the CL signal was about the size expected for the remaining free chlorine in the standard. Monochloroamine is a reported interferant in the DPD colorimetric method of chlorine determination because it can oxidize the DPD reagent and yield a signal (positive interference).[5]

Flow Injection Vs. Continuous Flow. The standard DPD method and CFA CL method were compared for tap water analysis with the Delrin cell or the Plexiglas cell. Figure 3.14 shows traces of typical signals for a 0.5 mg/l chlorine standard run in both CFA and FIA modes. Typically a CFA signal was about 10 times the size of a FIA signal for the same chlorine standard.

For the CFA CL method the following conditions were used: flow rate of 2 ml/min for the luminol stream (~ 0.1 mM) adjusted to pH 10.5 with an MgO SPB, flow rate of 0.2 ml/min for the OCl^- standard/sample, no EDTA. Figure 3.15 shows the non-linear calibration curves for each method that were used to determine the Cl_2 concentrations in three tap water samples.

The analysis results are reported in Table 3.1. Each sample was collected from the tap at a different time. Agreement between methods was within 0.03 mg/l Cl_2.

When tap water samples were analyzed with the luminol CFA method with equal reactant flow rates of 1 ml/min, the chlorine concentrations determined were consistently

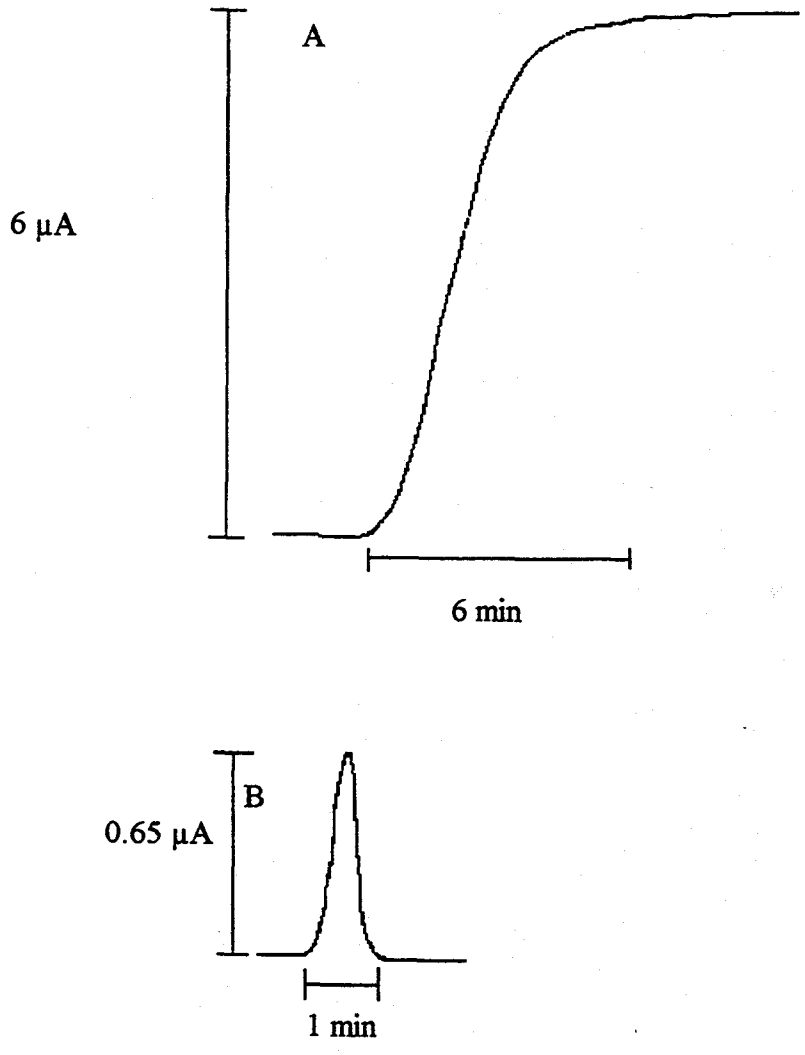


Figure 3.14. Traces of CL Signals in CFA and FIA Modes. Trace A is a CL signal from a 0.5 mg/l chlorine standard in CFA. Trace B is a CL signal from a 0.5 mg/l chlorine standard in FIA mode with a 30 µl injection volume.

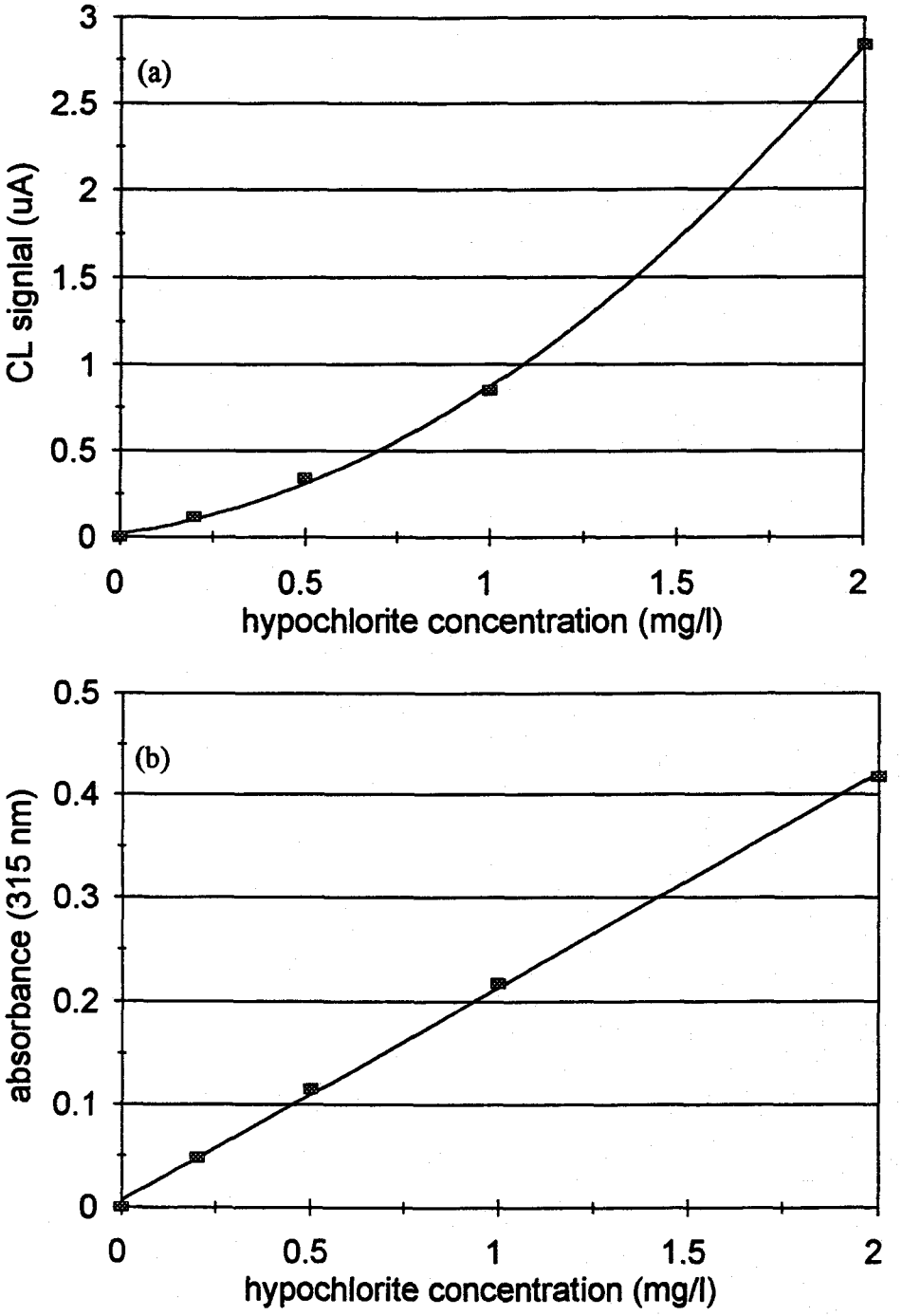


Figure 3.15. Typical Calibration Curves Used to Compare the DPD and CFA CL Methods. Curve (a) luminol/OCl^- calibration curve, with a regression of the form $S = a + b[OCl^-] + c[OCl^-]^2$ where $a = 0.0202$, $b = 0.305$, and $c = 0.551$. Curve (b) DPD/OCl^- absorbance calibration curve ($A = m[OCl^-] + b$ where $m = 0.207$ and $b = 0.00644$).

Table 3.1. Analysis of Tap Water with the DPD and CFA CL Methods.

tap water sample	[OCl^-] DPD (mg/l)	[OCl^-] CL/CFA (mg/l)
# 1	0.74	0.71
# 2	0.46	0.48
# 3	0.84	0.86

lower (by up to 50%) than the value determined by the standard DPD method at the same time. It is recommended that the flow rate of the OCl^- solution be 10 times less than that of the luminol as was the case for the results reported in Table 3.1. This ratio is critical because the luminol stream provides the primary control for the pH of the reaction mixture and the SPB columns do not produce solutions with buffer capacity. The pH of the reaction mixture could be lower than expected if the sample (normally pH $<<$10) had appreciable natural buffer capacity. For the suggested ratio of flow rates of 1 to 10, no difference was observed in the reactant effluent pH of a luminol/tap water mixture and a luminol/OCl^- standard solution mixture. This flow rate ratio also reduces the in-cell concentration of any contaminants that may be introduced with real samples, but also degrades the detection limit for chlorine. Under the conditions used to obtain the data in Table 3.1, the detection limit is approximately 0.05 mg/l.

Tap water samples were analyzed by the DPD method and the FIA CL method with the following conditions: flow rate of 2 ml/min for the luminol stream (~ 0.1 mM)

adjusted to pH 10.5 with an MgO SPB, flow rate of 0.2 ml/min for the OCl^- standard/sample, no EDTA, a sample loop volume of 30 µl. Calibration data are shown in Figure 3.16.

A contamination, believed to be from Cu(II) in tap water samples, was encountered which yielded lower concentrations of chlorine by the CL/FIA method relative to that reported by the DPD standard method as shown in Table 3.2 for the first four samples. The calibration curves in Figure 3.16 apply to these samples. The addition of EDTA (0.1 mM) to the reaction mixture (sample 5) eliminated this interference and the agreement between the two methods was brought within 0.03 mg/l for 0.32 mg/l Cl_2. Sample number 5 was analyzed on a separate day than samples 1-4 and with new calibration curves. The CL calibration curves in Figures 3.15 and 3.16 cannot be compared because they were run over one month apart and with different observation cells.

Table 3.2. Analysis of Tap Water with the DPD and FIA CL Methods.

tap water sample	$[OCl^-]$ DPD (mg/l)	$[OCl^-]$ CL/FIA (mg/l)
# 1	0.68	0.53
# 2	0.86	0.79
# 3	0.86	0.72
# 4	0.74	0.67
#5[a]	0.33	0.32

[a] Sample 5 was spiked to yield 0.1 mM EDTA

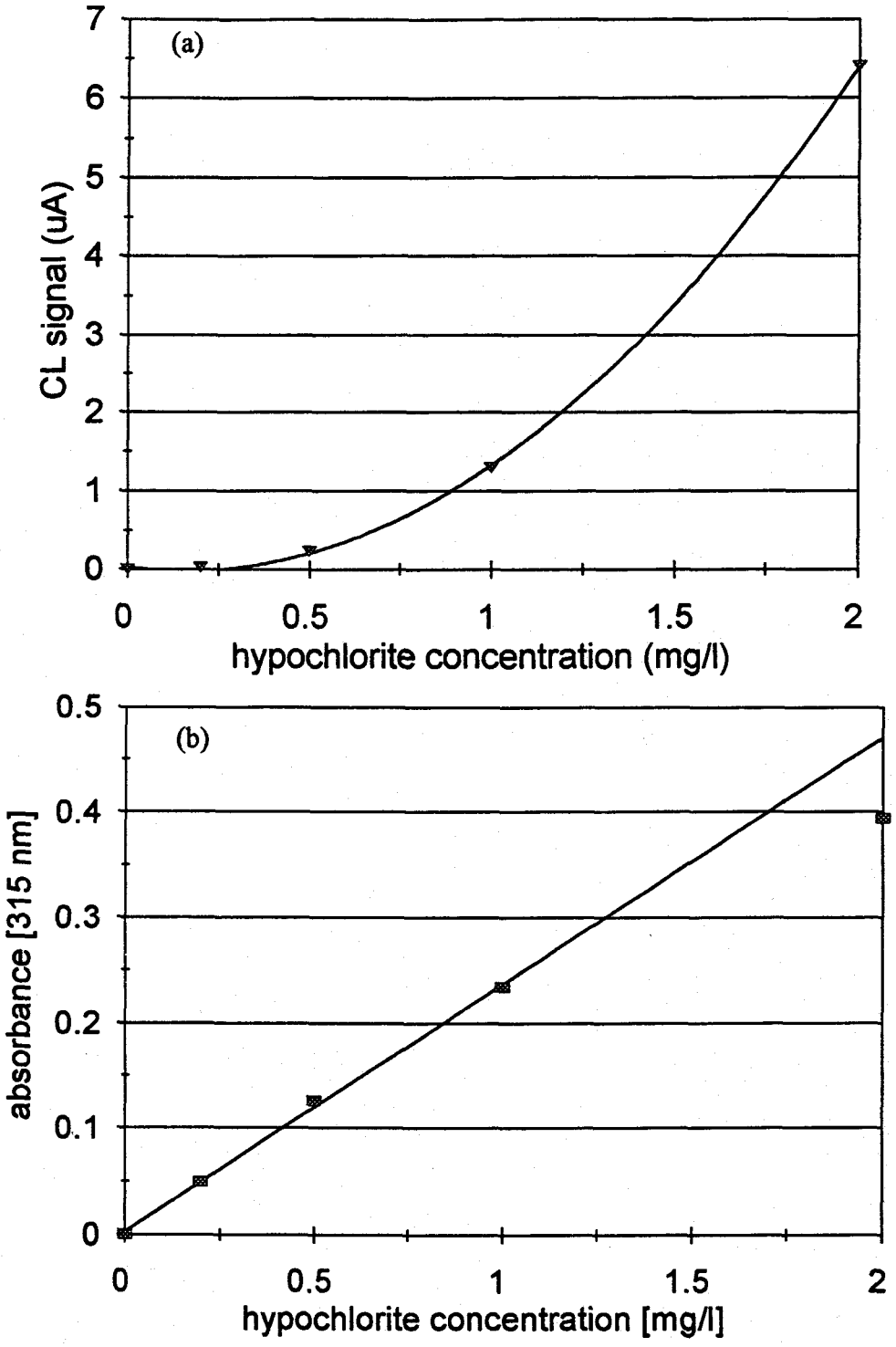


Figure 3.16. Typical Calibration Curves Used to Compare the DPD and FIA CL Methods. Curve (a) luminol/OCl^- calibration, with a regression of the form $S = a + b[OCl^-] + c[OCl^-]^2$ where a = 0.0341, b = -0.592, and c = 1.89. Curve (b) DPD/OCl^- absorbance calibration with the linear regression fit through only the first 4 points ($A = m[OCl^-] + b$ where m = 0.234 and b = 0.00279).

Flow injection analysis is this investigator's preference for the mode of operation of the CL chlorine analyzer. The primary advantage of FIA over CFA is the speed of analysis. When the analyzer is used in CFA mode, it takes up to 10 min between samples and a blank has to be run separately between samples. The long equilibration time is most likely due to the large volume of sample that must be flushed through the observation cell and reactant tubing to achieve constant concentrations in the observation cell. When the analyzer is used in FIA mode, the turnover time per sample injection is be reduced to approximately 2 min. Further time reduction is expected with shorter lengths of reactant tubing. With FIA, the CL signal from the sample or standard is measured closer in time relative to the blank (baseline) signal. Also with FIA, the sample volume is less which conserves standard solutions, reduces contamination of flow components by species in samples, and minimizes pH changes caused by the composition of the sample.

Waste Water Analyses. Waste water samples from the waste water treatment plant in Myrtle Creek, OR gave no detectable signal when they were run through an SPB column and mixed with 0.1 mM luminol (from an SPL) in the observation cell with the CFA mode. Chlorine concentrations between 0.3 and 0.5 mg/l were determined when portions of the same samples were analyzed by the analytical lab at URC with the DPD standard method. After the waste water was run through the SPB, standards had to be run through the column for up to an hour in order to produce signals equal to pre-waste water levels. Apparently some residual contamination from the waste water sample was left in the SPB

which either depressed the CL signal for subsequent samples and then slowly eluted or which consumed chlorine in subsequent samples (i.e., a chlorine demand in the SPB).

Waste water from the Corvallis facility was shown to contain chlorine in the 0.2-0.5 mg/l range with the DPD method, but did not produce a detectable CL signal in the CFA mode. Without the waste water running through an SPB, the signal recovered more quickly after a sample was run, but the CL signal depression lasted for up to several minutes. The contaminant appears to have adhered to the observation cell or Teflon reactant tubing. For these waste water samples, EDTA was never added to the system to test if it eliminated the depression effect, and the FIA mode was used.

Iodine Calibration with MCV Resin. The iodine column supplied from URC produced approximately 8 μM iodine. The CL signal produced from the 8 μM iodine and 0.1 mM luminol in the observation cell was half of the intensity from a 14 μM (1 mg/l) chlorine standard. Based on the assumption a linear calibration curve the iodine produced a signal equivalent to a 7 μM chlorine solution. Further investigation is required because neither the actual I_2 concentration or the iodine speciation was determined at the time of the experiment.

This investigation has shown that it may be possible to use solid state iodine columns as calibration devices for the luminol chlorine analyzer. If this were possible, the need to prepare solution calibration standards could be eliminated. The iodine columns must be examined for long term stability of the iodine concentration and consistency of the response factor relative to chlorine.

CONCLUSIONS

Both FIA and CFA instrumentation and methodology were successfully developed to determine free chlorine in water by the chemiluminescence reaction with luminol. Novel aspects of the instrumentation include a concentric injector, a spiral observation cell, and solid-state control of base and luminol concentrations. In general, calibration curves are non-linear with a positive deviation. The CL methods yield results for chlorine concentration in tap water samples within 0.03 mg/l of that determined with the DPD colorimetric standard method.

The solid-state reagents were successfully employed to produce an effluent stream of luminol at 0.1 mM and pH 10.5 with an input flow rate of DI H_2O at 2 ml/min. The bed eliminates the need for preparing any reagent solutions. This luminol concentration provided CL signals of sufficient intensity to measure residual chlorine concentrations between 0.2 and 2 mg/l with good precision for drinking water. Chlorine concentrations of 0.05 mg/l could be detected under these conditions. Higher luminol concentrations could be achieved by also placing SPB material before the luminol crystals in the SPL column to increase the pH of the input stream. Difficulties were encountered when the SPB beds were used to adjust the pH of tap water samples. Apparently the beds absorbed organic material that then acted as a chlorine demand for subsequent samples or standards. The SPB and SPL beds could be used over a period of 2 months without any degradation of performance. Eventually the volume of the luminol bed decreased to half the original value at which time it was replaced.

The effects of reaction pH, luminol concentration, and flow rate ratios were examined. The following final reaction conditions for CFA were chosen: pH 10.5 controlled by a SPB on the luminol stream, 0.1 mM luminol from a SPL with a DI H_2O input and a flow rate of 2 ml/min, and a chlorine stream flow rate of 0.2 ml/min. The following final reaction conditions were chosen for FIA: pH 10.5 controlled by a SPB on both carrier and luminol streams, 0.1 mM luminol from a SPL with a DI H_2O input and a flow rate of 2 ml/min, a carrier stream flow rate of 0.2 ml/min, and a sample loop volume of 30 µl. At higher pH values (above 11), the half-life of the reaction increases to the point that a significant portion of the CL signal is not observed because the reaction mixture exits the observation cell before the reaction is complete.

Some samples of tap water analyzed with the FIA luminol method yielded lower concentrations than those reported with the DPD method. This interference was attributed to Cu(II) in the tap water because Cu(II) caused a similar depression of the signal when mixed with a chlorine standard. The addition of 0.1 mM EDTA to the luminol stream reduced this depressive interference so that correlation between the two methods was within 0.03 mg/l when analyzing samples in the mg/l range. Iodine from a MCV column was shown to produce a detectable signal when mixed with 0.1 mM luminol in the observation cell. This iodated column has potential to be useful as a solid state-standard for chlorine.

REFERENCES

1) Kuo, P. P. K.; Chian, E. S. K.; Chang, B. J. *Environ. Sci. Technol.* **1977**, 11, 1177-1181.

2) Luong, T.; Peters, C. J.;Young, R. J.; Perry, R. *Environ. Technol. Let.* **1980,** 1, 299-310.

3) EPA. *Federal Register. Rules and Regulations* Vol. 44, No. 231, Thursday Nov 29, **1979**, 68624.

4) White, C. G. *The Handbook of Chlorination Second Edition.* Van Nostrand Reinhold Company Inc.: New York, New York, 1986, pp 1-60.

5) Clesceri, L.A.; Greenberg, A.E.; Trussell, R.R. eds. *Standard Methods for the Examination of Water and Wastewater,* 17th ed, American Public Health Association; Port City Press: Washington D.C., 1989, pp 4.45-4.66.

6) Gordon, G.; Cooper, W. J.; Rice, R. G.; Pacey, G. E. J. Am. Water works Assoc. **1988,** September, 94-122.

7) Jolley, R. L.; Lyman, C. W.; Johnson, D. J.; et al. *Water Chlorination Chemistry Environmental Impact and Health Effects.* Lewis Publishers: Chelsea, Michigan, 1990; vol. 6, 90-124.

8) Seliger, H. H. *Light and Life*. McElroy, W.D.; Gloss, B., Eds.; John Hopkins Press: Baltimore, MD, 1961; 200-205.

9) Albrecht, H. O. *Z. Physik. Chem.* **1928**, 136, 321.

10) Kok, G. L.; Holler, T. P.; Lopez, M. B.; Nachtrieb, H. A.; Yuan, M. *Environ. Sci.Technol.* **1978**, 1072-1076.

11) Hardy, W. M.; Seitz, W. R.; Hercules, D. M. *Talanta*, **1977**, 24, 297-302.

12) Neary, P. M.; Seitz, R.; Hercules, D. M. *Anal. Lett.* **1974**, 7(8&9), 583-590.

13) Seitz, W. R. *J. Phys. Chem.* **1975**, 79, 101-106.

14) Marino, D. F.; Ingle, J. D., Jr. *Anal. Chem.* **1981**, 53, 455-458.

REFERENCES (continued)

15) Gander, S. Master's Thesis, Oregon State University, **1990**.

16) Archibald, J. fabrication performed, Oregon State University Machine Shop, **1995**.

17) Akse, J. Personal communication from Umpqua Research Center, Myrtle Creek, OR. 1996.

18) Valentine, R. L.; Jafvert, C. T. *Environ. Sci. Technol.* **1988,** 22, 691-696.

CHAPTER 4
CONCLUSIONS

Two chemiluminescence methods for the determination of chlorine in water samples have been investigated. The first method is based on the reaction between hydrogen peroxide and chlorine (discussed in Chapter 2) while the second method revolves around the reaction between luminol and chlorine (discussed in chapter 3). Novel flow instrumentation was developed and refined for the investigations which include a spiral flow cell, a concentric reactant injector, solid-phase base (SPB) pH control, and solid-phase luminol (SPL) reagent control. The hydrogen peroxide/chlorine method suffered from contamination problems so the luminol/chlorine reaction was chosen as having more analytical promise.

The flow instrumentation developed can be used in two different modes: continuous flow analysis (CFA) or flow injection analysis (FIA). In particular the flow cell and injector were improved to simplify the design, eliminate leaks, and minimize the dead volume. For the luminol method, the FIA mode is preferred because of the increased speed of analysis, and the combined SPL/SPB column performed well. A luminol concentration of 0.1 mM and a pH of 10.5 was easily achieved with this column, and these levels yielded good precision for the determination of chlorine in the range expected in drinking water (0.2 to 2 mg/l).

Further refinement of the apparatus would be needed before a commercial prototype could be realized. Suggested improvements include shortening the length of reactant tubing to increase sample throughput, optimizing the bed volume and particle size

of the solid-phase columns, using pumps which provide constant flow rates through the columns, tubing, and cell, and controlling the temperature of feed solutions, the columns, and observation cell.

For the studies with the hydrogen peroxide/chlorine reaction, the slope and shape of the calibration curves for chlorine varied by over a factor of 100 between different "equivalent" H_2O_2/buffer solutions. This variation was attributed to a contaminant. Addition of dust, material from skin contact, Pt, or electrode filling solutions to the H_2O_2 solution was found to dramatically increase the CL signal. Contact of the H_2O_2 solutions with the SPB particles also enhanced the CL signal, but the degree of enhancement decreased with contact time. Spectral studies with glass filters showed that the "enhanced" CL observed from the reaction was not in the red portion (~ 700 nm) of the spectrum as has been reported (i.e., due to singlet O_2), but instead the emission occurred at about 400 nm. Because of the contamination problems, lack of reproducibility, and the unexpected wavelength of the emitted CL, this reaction was abandoned in favor of the reaction between luminol and chlorine. If the contaminant was known and could be controlled, the H_2O_2/OCl^- CL reaction could be analytically useful. Further studies should be directed to understanding the nature and mechanism of the "enhanced" H_2O_2 CL reaction.

For the luminol/chlorine system, the calibration curve was non-linear, usually exhibiting a positive deviation. Preliminary results suggest that the calibration curves are more linear with luminol solutions prepared previously rather than on-line with the SPL.

The detection limit is sufficiently low for tap water analysis and could be improved by using a higher luminol concentration or a larger injection volume in the FIA mode.

For tap water analysis, the luminol CL method provided accurate results, in either the FIA or CFA mode, as evidenced by agreement with the results from the standard DPD method of chlorine analysis. For the FIA mode, 0.1 mM EDTA was added to the carrier stream to eliminate a depression in the CL signal which was attributed to Cu(II). To control the reaction mixture pH, it was necessary to adjust the flow rate of the luminol stream (pH ~ 10.5) to about 10 times the flow rate of the sample.

No chlorine was detected in waste water samples. Further work is needed to determine if the chlorine was actually consumed or interferences depressed the signal.

Several future studies are suggested to enhance the potential for commercialization of this apparatus. First, the factors controlling the shape of the calibration curve, such as O_2 concentration, flow rates, pH, and luminol source needs to be better understood. Reduction of the non-linearity of the curve would be advantageous. Second, interference effects need to be characterized more fully. Third, the I_2 column requires further investigation because it has the potential to eliminate the need for preparing solutions of chlorine standards.

The SPB and SPL columns worked well for at least 2 months of operation without replenishing the column materials. However, the concentrations of luminol and the pH of the column effluents need to be monitored more closely for run-to-run consistency.

More evidence is needed to support the hypothesis that Cu(II) is an interferent in tap water samples, and other metal ions should be investigated for signal suppressing

effects as well. The exact level of Cu(II) interference should be determined and compared to the level of Cu(II) in tap water for which an interference is observed. Perhaps an ion exchange column could be employed to remove potential ionic contaminants.

The masking agent EDTA should be investigated for waste water samples. Possibly a solid-phase bed of EDTA crystals could be prepared to replace the EDTA solutions that were used. The mechanism of the Cu(II) interference should also be investigated. Cu(II) may be complexed by luminol which could prevent the luminol from reacting with chlorine. Experiments should be conducted in which luminol concentration is varied with and without Cu(II) present to determine if the signal depression is related to the concentration ratio of luminol to Cu(II).

Temperature effects need to be investigated thoroughly. It was observed that the signal increased slightly with increasing laboratory temperatures. This change in signal with temperature could be due to greater solubility of the luminol crystals in the SPL column. Temperature control may be needed for a commercial instrument, or a mathematical algorithm could be developed to make a correction based on the measured temperature of the reaction mixture.

Preliminary studies indicate that monochloroamine does not cause a CL response (up to a concentration of 10 mg/l). This result should be confirmed more carefully. It would be useful if a CL method could be developed which could differentiate between free chlorine and the chloroamines.

BIBLIOGRAPHY

Akse, J. Personal communication from Umpqua Research Center, Myrtle Creek, OR. 1996.

Albrecht, H. O. *Z. Physik. Chem.* **1928**, 136, 321.

Archibald, J. fabrication performed, Oregon State University Machine Shop, **1995**.

Clesceri, L.A.; Greenberg, A.E.; Trussell, R.R. eds. *Standard Methods for the Examination of Water and Wastewater,* 17th ed. American Public Health Association; Port City Press: Washington D.C., 1989, pp 4.45-4.66.

Eilbeck, W. J.; mattock, G., *Chemical Processes in Waste Water Treatment.* Elis Horwood Limited: Chichester, England, 1987; pp 119-125.

EPA. *Federal Register. Rules and Regulations* Vol. 44, No. 231, Thursday Nov 29, **1979**, 68624.

Gander, S. Master's Thesis, Oregon State University, **1990**.

Gordon, G.; Cooper, W. J.; Rice, R. G.; Pacey, G. E. J. Am. Water Works Assoc. **1988,** September, 94-122.

Hardy, W. M.; Seitz, W. R.; Hercules, D. M. *Talanta,* **1977**, 24, 297-302.

Hoyt, S. D.; Ingle, J. D., Jr. *Anal. Chim. Acta.* **1976**, 87, 163-175.

Isacsson, U.; Wettermark, G. *Anal. Chim. Acta.* **1976**, 83, 227-2239.

Jolley, R. L.; Lyman, C. W.; Johnson, D. J.; et al. *Water Chlorination Chemistry Environmental Impact and Health Effects.* Lewis Publishers: Chelsea, Michigan, 1990; vol. 6, 90-124.

Khan, A. U.; Kasha, M. *J. Amer. Chem. Soc.* **1970**, 92, 3293-3300.

Kok, G. L.; Holler, T. P.; Lopez, M. B.; Nachtrieb, H. A.; Yuan, M. *Environ. Sci.Technol.* **1978**, 1072-1076.

Kuo, P. P. K.; Chian, E. S. K.; Chang, B. J. *Environ. Sci. Technol.* **1977**, 11, 1177-1181.

Luong, T.; Peters, C. J.;Young, R. J.; Perry, R. *Environ. Technol. Let.* **1980,** 1, 299-310.

BIBLIOGRAPHY (continued)

Mallet, L. *C.R. Acad. Sci., Paris*, **1927** 185, 352.

Marino, D. F.; Ingle, J. D., Jr. *Anal. Chem.* **1981**, 53, 455-458..

Marino, D. F.; Ingle, J. D., Jr. *Anal. Chim. Acta.* **1981**, 123, 247-253.

Neary, P. M.; Seitz, R.; Hercules, D. M. *Anal. Lett.* **1974**, 7(8&9), 583-590.

Oliver, B. G.; Carey, J. H. *Environ. Sci. Technol.* **1977**, 11, 893-895.

Schaap, A. P., Ed. *Singlet Molecular Oxygen*. Halsted Press: Stroudsburg, PA. 1976; 133-134.

Seitz, W. R. *J. Phys. Chem.* **1975**, 79, 101-106.

Seitz, W. R.; Hercules, D. M. *Anal. Chem.* **1972**, 44, 2143-2149.

Seliger, H. H. *Light and Life*. McElroy, W.D.; Gloss, B., Eds.; John Hopkins Press: Baltimore, MD, 1961; 200-205.

Shakhashiri, B. Z.; Williams, L. G. *J. Chem. Ed.* **1976**, 53, 358

Valentine, R. L.; Jafvert, C. T. *Environ. Sci. Technol.* **1988**, 22, 691-696.

White, C. G. *The Handbook of Chlorination Second Edition.* Van Nostrand Reinhold Company Inc.: New York, New York, 1986, pp 1-60.

White, C. G. *The Handbook of Chlorination Second Edition,* Van Nostrand Reinhold Company Inc.: New York, New York, 1986; pp 150-200.

White, C. G. *The Handbook of Chlorination Second Edition.* Van Nostrand Reinhold Company Inc.: New York, New York, 1986; pp 214-250.

Appendix

Appendix 1. Standard Methods for the Determination of Free Chlorine in Water Samples

INTRODUCTION

The following procedures were used as the standard methods for determining the chlorine concentration in water samples. Ying Yang originally adapted this method from *Standard Methods for the Examination of Water and Wastewater*[1]. In this colorimetric method, N,N-diethyl-*p*-phenylenediamine (DPD) is used to form a pink colored product upon reacting with chlorine. The absorbance of this pink product is measured at 515 nm and the chlorine concentration can be quantitatively determined. These standard methods can be used to determine free chlorine, combined chlorine and total chlorine in water samples. In these simplified versions of the methods, only the free chlorine and the monochloroamine concentration are measured.

APPARATUS

HP 8452A Diode Array Spectrophotometer.

REAGENTS

Primary standard quality $K_2Cr_2O_7$, $Na_2S_2O_3 \cdot 5H_2O$, starch, sodium borate, mercuric iodide, sodium hypochlorite (NaOCl) solution (approximately 5 %), N,N,-Diethyl-p-phenylenediamine sulfate (DPD), disodium EDTA, Na_2HPO_4, KH_2PO_4, concentrated H_2SO_4, potassium iodide,

mercuric chloride, ferrous ammonium sulfate (FAS), $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, salicylic acid (optional), zinc chloride (optional).

EXPERIMENT

Step 1. Standardizing the $Na_2S_2O_3$ titrant.

Prepare a standard solution of potassium dichromate (0.1000 N). This standard solution is used to standardize a sodium thiosulfate ($Na_2S_2O_3$) solution. Dissolve 4.904 g anhydrous potassium dichromate $K_2Cr_2O_7$ of primary standard quality in distilled water and dilute to 1000 ml in a volumetric flask to yield a 0.1 N solution. Store in a flask or a glass-stoppered bottle.

Prepare a standard sodium thiosulfate titrant (0.025 N). This solution is used to standardize a 100 µg/ml chlorine solution. Dissolve 6.2 g $Na_2S_2O_3 \cdot 5H_2O$ in 800 ml of DI H_2O, add 4 g sodium borate and 10 mg mercuric iodide to improve the stability of the solution. Add DI water to bring the total volume to 1 l.

Prepare a starch indicator solution by pouring 5 g starch into 1 l of boiling DI water, stir and let settle overnight. Use the clear supernate only. Preserve with 1.25 g salicylic acid, 4 g zinc chloride, or a combination of 4 g sodium propionate and 2 g sodium azide per l of starch solution. Check the bottom layer of solution before using, discard the solution if suspended precipitates are observed. This solution should last 3 weeks.

Add, with constant stirring 1 ml of concentrated H_2SO_4, 10.0 ml of 0.10 N potassium dichromate standard solution, and 1 g KI to 80 ml deionized water. Titrate immediately with 0.025 N sodium thiosulfate titrant until the yellow color of the liberated iodine is almost discharged. Add 1 ml of starch indicator solution and continue titrating until the blue color disappears.

$$Normality \quad of \ Na_2S_2O_3 = \frac{1}{ml \ Na_2S_2O_3 \ consumed}$$

For accurate work, standardize this solution daily in accordance with the directions given above, using a 0.01 N or 0.025 N dichromate solution. Use sufficient volumes of these standard solutions so that their final dilution is not greater than 1 + 4. The standard titrant (0.0250 N sodium thiosulfate) is equivalent to 886.3 µg chlorine as Cl_2/1.0 ml.

Step 2. Standardize 100 ppm (µg/ml) Chlorine Solution and Make a Series of Chlorine Standards In the Range of 0.05 to 4 ppm (µg/ml).

Dilute 2 ml of 4% sodium hypochlorite solution with 1 l deionized water to obtain a chlorine solution of about 100 ppm. Standardize this chlorine solution as follows:

Place 2 ml acetic acid and 10 to 25 ml DI water in a flask. Add about 1 g KI. Measure into the flask a suitable volume of chlorine solution (100 to 200 ml). In choosing a convenient volume, note that 1 ml of 0.025 N $Na_2S_2O_3$ titrant is equivalent to about 0.9 mg chlorine. Titrate with standardized 0.025 N $Na_2S_2O_3$ titrant until the yellow iodine

color almost disappears. Add 1 to 2 ml of starch indicator solution and continue titrating until the blue color disappears. Record the amount titrated as titration result *a*.

Determine the blank by adding identical quantities of acid, KI and starch indicator to a volume of deionized water corresponding to the sample used for titration. Perform blank titration A or B as follows.

A: if a blue color develops, titrate with 0.025 N $Na_2S_2O_3$ until disappearance of blue color and record amount as result *b*, *b* is negative here.

B: if no blue color occurs, titrate with 0.0282 N iodine solution until a blue color appears. Back titrate with 0.025 N $Na_2S_2O_3$ and record the difference *as b*, *b* is positive here.

Calculate the chlorine concentration according to :

$$mg\ Cl\ as\ Cl_2/ml = \frac{(a + b)\ x\ N\ x\ 35.45}{ml\ sample}$$

Once the free chlorine concentration of the 100 ppm solution is obtained, make a series of chlorine standards in the range of 0.05 to 4 mg/l, such as 0.2, 1, 2, 3 mg/l respectively.

Step 3. DPD Colorimetric Method: Instrument Calibration and Sample Measurement.

This the method is very sensitive to pH, temperature, and reaction time, it is essential to keep the reaction conditions and working procedures the same while performing all of the measurements.

Make a phosphate buffer solution by dissolving 24 g anhydrous Na_2HPO_4 and 46 g anhydrous KH_2PO_4 in DI water. Combine this solution with 100 ml of DI water in which

800 mg EDTA has been dissolved. Dilute to 1 l with distilled water and add 20 mg $HgCl_2$ to prevent mold growth and interference in the free chlorine test caused by any trace amounts of iodide in the reagents. The buffer solution made here is of pH 7.2.

Make a N,N,-Diethyl-p-phenylenediamine (DPD) indicator solution by dissolving 1 g anhydrous DPD sulfate in DI water containing 8 ml of 1 + 3 H_2SO_4 (made by mixing water with concentrated H_2SO_4 by a volume ratio of 1 : 3) and 200 mg disodium EDTA. Make up to 1 l, store in a brown glass-stoppered bottle in the dark, and discard when discolored. Periodically check the solution blank for absorbance and discard when absorbance at 515 nm exceeds 0.002/cm.

This method is only suitable for chlorine concentration up to 4 mg/l. When the chlorine concentration exceeds 4 mg/l, dilution is required before measurement.

Spike 100 µl each of phosphate buffer solution and DPD indicator reagent into a spectrophotometric cell. Rapidly inject 2.0 ml of DI water from two1 ml Eppendorf pipets while the sample cell is in the spectrometer. Take a blank reading on this mixture at 515 nm.

Repeat the procedures described above, substituting 2.0 ml of a chlorine standard or unknown sample for the 2.0 ml of DI water. Read the absorbance 6-10 consecutive times on the spectrophotometer at 515 nm and record the maximum absorbance observed. The consecutive readings are necessary because the DPD reaction mixture continues to gain color for a few seconds after injection and then rapidly begins to lose this color. Create a

calibration curve from the data and determine the concentration of chlorine in the sample. Record this result as A. The timing is critical here because the color fades over time, and the higher the chlorine concentration, the faster the color fades, so be consistent. A typical absorbance for a 1 µg/ml Cl_2 solution is between 1.9 and 2.1 AU.

Determine the monochloroamine concentration by adding a small crystal of KI (about 0.1 mg) and mix. If dichloramine concentration is expected to be high, instead of a small crystal add 0.1 ml (2 drops) freshly prepared KI solution (0.1 g/100 ml). Read the color immediately. Record this as result B. The NH_2Cl concentration is calculated from (B - A).

Step 4. Check the Chlorine Concentration with the Standard FAS Method

This is a titrimetric version of the DPD method. DPD is used as an indicator in the titration procedure with ferrous ammonium sulfate (FAS). As this method shares reagents used with the DPD colorimetric method, control of the temperature, timing, and pH is required.

Make a standard ferrous ammonium sulfate (FAS) titrant by dissolving 1.106 g $Fe(NH_4)_2(SO_4)_2.6H_2O$ in distilled water containing 1 ml of 1 + 3 H_2SO_4 and make up to 1.0 l with freshly collected deionized water. This standard may be used for one month. The FAS titrant is equivalent to 100 µg Cl as Cl_2/1.0 ml.

Determine the free chlorine concentration by titration with FAS. This method is only suitable for concentrations of total chlorine up to 5 mg/l. If total chlorine exceeds 5 mg/l, use a smaller sample volume and dilute to a total volume of 100 ml. Mix usual volumes of buffer reagent and DPD indicator solution with distilled water ***before*** adding sufficient sample to bring total volume to 100 ml. If sample is added before buffer, the test does not work.

Place 5 ml each of phosphate buffer solutions and DPD indicator solution in titration flask and mix. Add 100.0 ml sample, or diluted sample and mix. Titrate rapidly with standard FAS titrant until red color is discharged. Record this as result A.

Determine the monochloroamine concentration with the FAS titration method. Add one very small crystal of KI (about 0.5 mg) or 0.1 ml (2 drops) KI solution and mix. Continue titrating until the red color is discharged again. Record this as result B.

Again, as in the DPD colorimetric method, calculate the free chlorine concentration according to reading A and calculate the monochloroamine concentration with (B - A).

1. Clesceri, L.A.; Greenberg, A.E.; Trussell, R.R. eds. *Standard Methods for the Examination of Water and Wastewater,* 17th ed. American Public Health Association; Port City Press: Washington D.C., 1989, pp 4.45-4.66.