ENHANCEMENT OF LUMINOL CHEMILUMINESCENCE BY CARBON DIOXIDE IN THE ABSENCE OF ANY ADDED OXIDANT: INSTRUMENTATION AND APPLICATIONS

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CHAPTER I

INTRODUCTION

Importance of Chemiluminescence Determinations

Chemiluminescence is an interesting phenomenon that can be observed in gas-, liquid-, and solid-phase reactions, and all of these reactions have been used in biomedical, toxicological, and environmental analyses as well as related areas [1-4]. In solution, for example, chemiluminescence finds many applications for the determination of metal ions, inorganic anions, biomolecules, carcinogens, and drugs in a variety of environmental and clinical matrices [1-20].

The advantages of chemical analyses based on chemiluminescence include high sensitivity, wide linear dynamic range, and simple instrumentation. The high sensitivities are mainly ascribed to the absence of a need for a radiation source which reduces or eliminates Raman and Rayleigh scattering and noises associated with the source. This allows the detector to be operated at an extreme photomultiplier voltage [21] with a significant improvement in signal-to-noise ratio relative to conventional fluorescence detection [13]. Femtomole detection limits are not uncommon with attomole levels detected for some compounds [22]. As little as 120 molecules have been detected in the case of certain enzymes [16]. Calibration responses in chemiluminescence analysis are often linear over several orders of magnitude (e.g., the log-log calibration graph for morphine is linear over the range 10^{-4}-10^{-10} M [23]). Simple instrumentation is another reason for the popularity of chemiluminescence determinations. Usually, all that is required is some mechanism for reagent delivery and mixing, a reaction cell, and a photon detector.

Objectives of this Research

Of all aqueous chemiluminescent reactions, the luminol reaction is the most widely used reaction in chemiluminescence determinations. Although it has been extensively studied, the mechanism of this reaction is still not fully understood and some intriguing phenomena about the reaction are often reported. In this dissertation a new interesting phenomenon is reported, its mechanism is discussed, and analytical applications based on this phenomenon are developed.

Another goal of this research was to develop a versatile chemiluminescence detection system. Many instrumentation schemes have been designed to meet the specific requirements of each application, but none of them is universal. The instrumentation developed in this research utilized flow-injection technique to achieve good reproducibility in measurements. The uniquely designed reaction cell makes it possible for the instrument to be used for all operational modes in flow injection analysis as well as with immobilized reagents.

Basically, this dissertation is divided into two parts. The first part addresses the development of the novel chemiluminescence detection system. This instrument makes it possible to pursue successfully both fundamental studies and application research. In the second part of this dissertation, an interesting phenomenon based on the carbon dioxide enhancement of luminol chemiluminescence is reported and some applications are proposed. It was found that when some metal ions or metal complexes are used as rate modifiers, carbon dioxide can dramatically increase the chemiluminescence intensity of luminol even in the absence of any added oxidant. Based on the studies on this phenomenon, a sensitive method for the determination of cobalt(II) is proposed. Moreover, a novel sensor for carbon dioxide in the gas phase has been developed and reported.

In Chapter II, a literature review of chemiluminescence of aqueous solutions is presented. This chapter begins with a general discussion of the requirements for

chemiluminescence to occur, including factors which determine the efficiency of emission from a chemiluminescent reaction. Then, the major chemiluminescent reactions in aqueous solution involving luminol, lucigenin, lophine, pyrogallol, gallic acid, acridinium, and peroxyoxalate are discussed along with their analytical applications. The most recent mechanistic work for the luminol chemiluminescent reaction is also summarized. Two types of instrumentation for chemiluminescence analysis are discussed in this chapter, namely the batch method and the flow injection method. The advantages and disadvantages by using these two strategies are compared.

In Chapter III, the design of a multipurpose system for flow injection chemiluminescence determinations and its performance are examined. The major part of this system is the flow cell in which the chemiluminescent reaction takes place and the light emission is detected. Inside the cell is placed a disc in which a magnetic bar is embedded. The rotation of this disc, driven by a magnetic stirrer, rapidly and thoroughly mixes the reactants and therefore produces a much stronger chemiluminescence when compared to other means of mixing. This flow cell can be used in many types of operations encountered in flow injection analysis. The surface of the disc is also an ideal carrier for the immobilization of a reagent, such as the catalyst or promoter of a chemiluminescent reaction. When a reagent is immobilized on the disc, the rotation of the disc helps to achieve a higher chemiluminescence intensity with less immobilized reagent. The electronic parts of this system were carefully selected to increase signal and reduce noise.

Chapter IV describes how carbon dioxide gas enhances the chemiluminescence emission of luminol in the absence of added oxidant. First of all, the metal ions and the metal complexes which can stimulate chemiluminescence emission in the absence of added oxidant are characterized. Then, the effects of several factors, such as pH, the properties of rate modifiers, the age of luminol solution, and the bubbling of nitrogen and oxygen gases, on the enhancement are examined when these ions and complexes are used as rate

modifiers for the luminol chemiluminescent reaction. A possible mechanism for the enhancement is proposed based on all experimental results.

In Chapter V, the results reported in Chapter IV are applied for the determination of cobalt(II), one of the best rate modifiers for luminol chemiluminescent reaction that stimulates very strong light emission in the presence of carbon dioxide. This chapter discusses the analytical characteristics of the cobalt(II)/luminol/carbon dioxide system. Compared to other chemiluminescence systems used for the determination of cobalt(II), this system provides the highest sensitivity, widest linear dynamic range, and less interference from other metal ions.

Chapter VI presents a novel approach for the direct determination of carbon dioxide in the gas phase, based on the enhancement of luminol chemiluminescence by carbon dioxide when cobalt(II) phthalocyanine is present. This chapter also discusses how it can be applied for monitoring carbon dioxide in atmospheric air and in human breath.

In the Appendix A, the computer programs used to draw graphs and extract intensity vs. time data are listed. The kinetic data in the presence of different rate modifiers are given in Appendix B.

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CHAPTER II

ENHANCED CHEMILUMINESCENCE IN AQUEOUS SOLUTION AND ITS ANALYTICAL APPLICATIONS: A LITERATURE REVIEW

Classification of chemiluminescent reactions

Definitions

Chemiluminescence can be defined as the production of electromagnetic radiation (ultraviolet, visible or infrared) by a chemical reaction, while bioluminescence, as a special case, is the visible chemiluminescence produced by living organism or chemical systems derived therefrom [1].

Almost every chemiluminescence process is affected by certain metal ions, inorganic and organic metal complexes, and/or enzymes through their influence on the rate of reaction. Some of them are true catalysts because their chemical states do not change at the end of reaction, while some are only promoters as they cannot be recycled during the reaction. In most cases, however, the role of these species has not been well identified. Especially when a metal ion is used to increase the reaction rate, it is not clear if the metal ion is a true catalyst or a promoter. Since both catalyst and promoter increase the rate of a chemiluminescent reaction, both are properly called *rate modifiers* in this dissertation to avoid the difficulty in distinguishing between catalyst and promoter.

Classification

There are many inorganic and organic chemical reactions that are known to produce light. Chemiluminescent reactions have been classified as direct or indirect (Figure 2.1), depending on the origin of the chemiluminescence [1]. The latter is also described as sensitized or energy-transfer chemiluminescence.

With direct chemiluminescence, the reaction generates the primary excited-state species which is then responsible for light emission. In contrast, in sensitized reactions the excited product is not the light emitter, but rather transfers its energy to a fluorescent acceptor which then emits light. The discrete energy transfer step in sensitized chemiluminescent reactions has distinct advantages in the design of analytical systems, since optimum structural features can be designed and incorporated into separate chemical reactant and fluorophore molecules. For example, unlike in a direct chemiluminescent reaction, the spectrum of the light emitted is not determined by the product but instead by the fluorophore. Hence, the fluorophore in sensitized chemiluminescence is selected to provide the optimum compromise between excitation and emission wavelengths, chemical stability, and quantum yield.

Characteristics of Chemiluminescence

Chemiluminescent emission can be characterized by the four parameters of "color", the intensity, the rate of light emission, and the rate of decay of intensity. Apart from the obvious role of the reactants, reaction conditions such as acidity and medium have a significant impact on the progress of a chemiluminescent reaction and thus affect the above four parameters. Reaction time and duration of chemiluminescent reactions vary from very rapid and/or short-lived (< 1 s) to very slow and/or long-lasting (> 1 day) [1].

From an analytical viewpoint, the intensity of chemiluminescence has the most impact on the application of a chemiluminescent reaction since it is the chemiluminescence

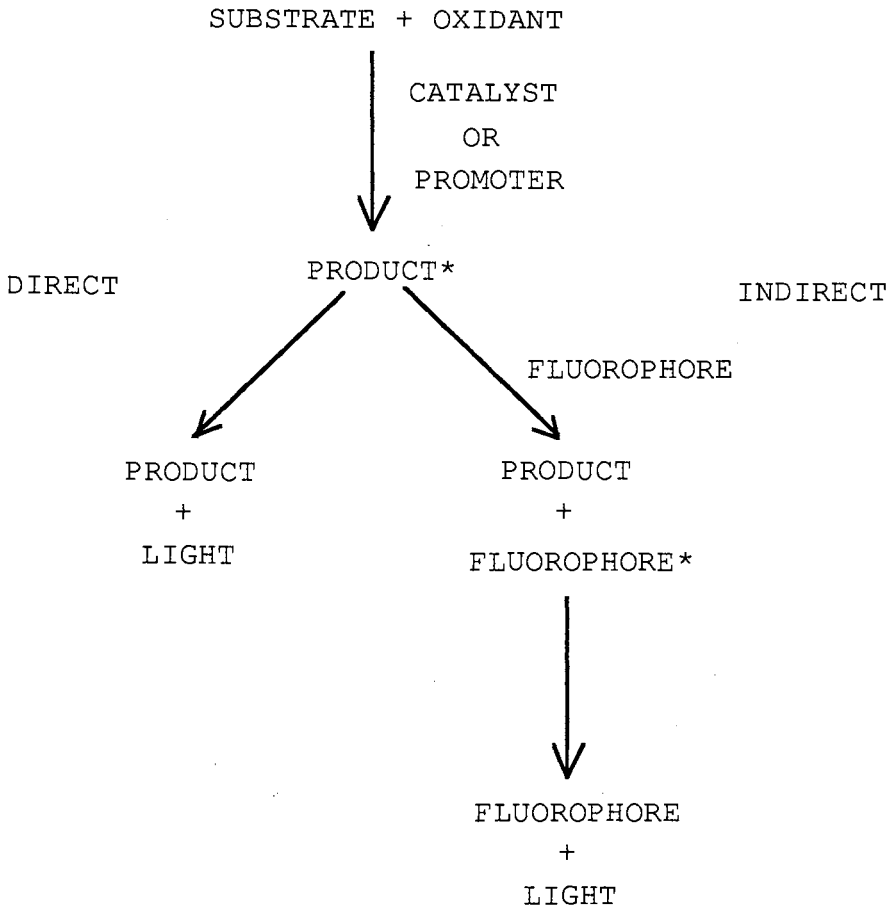


Figure 2.1. Two types of chemiluminescent reactions.

intensity that is measured (either as an integral over a certain time or a transient response) in most applications. The chemiluminescence intensity is dependent on the rate of reaction, its efficiency at generating molecules in an excited state (expressed as the quantum yield) and, in sensitized chemiluminescence, on the fluorophore [1].

The quantum yield of a chemiluminescent reaction (Φ_{CL}) is the product of the efficiencies of the excitation and emission steps. It can be defined as [2]:

$$\Phi_{CL} = \frac{photons\ emitted}{reagent\ molecule\ reacted}$$

The values of Φ_{CL} range from 10^{-15} to nearly 1 [3, 4]. Higher values are usually associated with bioluminescent reactions whereas the chemiluminescent reactions commonly exploited for determinations have typical Φ_{CL} values of 0.001-0.1. However, the almost complete absence of background emission means that even very inefficient systems with much lower Φ_{CL} (<0.001) can be monitored and employed for analysis.

The "color" of chemiluminescent emission, as well as the value of Φ_{CL}, can be affected markedly by the polarity of the solvent, the solution temperature, and, under the influence of secondary physical processes, by the quenching of the excited state. For example, the Φ_{CL} of luminol oxidized in dimethyl sulfoxide (DMSO) is 0.05 compared with 0.01 in water and the respective colors are blue-violet (425 nm) and blue-green (480-502 nm) [3]. The properties of some chemiluminescent reactions are summarized in Table 2.1.

Chemiluminescent Reactions

Requirements for Chemiluminescence

Chemiluminescence is relatively uncommon because most reactions release the energy in the form of heat. For a reaction to be chemiluminescent, an excited state molecule must be produced during the course of the reaction. The observed emission

Table 2.1. Emission wavelength and quantum yield (Φ_{CL}) of some organic chemiluminescent reactions under different conditions

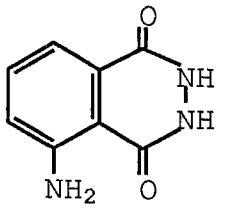
Reaction	Color (λ_{max})	Φ_{CL}	Ref.
Luminol in DMSO	Blue-violet (480-502 nm)	0.05	3
Luminol in aq. alkali	Blue (425 nm)	0.01	3, 5
Lucigenin in aq. alkali	Blue-green (440 nm)	0.016	5
Lophine in alcoholic NaOH	Yellow (525 nm)		4, 5
Pyrogallol in aq. alkali	Reddish pink		3
Peroxyoxalate (TCPO) using 9,10-diphenyl-anthracene as fluorophore	Blue	0.07-0.50	3, 5

stems from the ejection of a photon from this excited state. Three essential features are associated with a chemiluminescent reaction, namely the reaction must be exothermic in order to generate sufficient energy for formation of the electronically excited state; the minimum energy requirement is 180 kJ/mol for emission in the visible region; a pathway must exist for the formation of the electronically excited state and the excited state must be capable of losing its energy as a photon or be capable of energy transfer to a fluorophore.

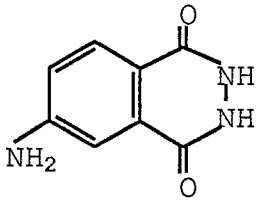
Chemiluminescence Reagents

There are general guidelines for predicting chemiluminescent behavior. For instance, if a compound itself or its oxidation product is fluorescent, then there is the possibility that oxidation of this compound will produce chemiluminescence. However, there are many exceptions to this principle and, in many cases, chemiluminescent reactions cannot be predicted. Nonetheless, many solution-phase chemiluminescent reactions involve the oxidation of an aromatic substance such as one of those given in Figure 2.2. Established systems most commonly used for analysis fall into one of a limited number of types, as follows.

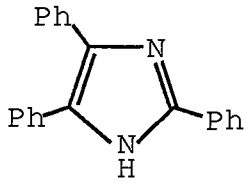
The Luminol Reaction. This is the most important and the most commonly used chemiluminescent reaction in aqueous solution. Chemiluminescence is produced by the oxidation of luminol (5-amino-2,3-dihydrophthalazine-1,4-dione or 5-amino-phthalhydrazide) in basic medium by oxidants including H_2O_2, O_2, OCl^-, I_2, and MnO_4^- [6-8]. The chemiluminescence is usually very weak in the absence of a rate modifier. Rate modifiers for this reaction include metal ions, metal complexes, and heme-containing enzymes and compounds. Many metal ions such as Co(II), Cu(II), Ni(II), Cr(III), Fe(II), Rh(III), and V(IV) [6, 19, 25-35] greatly increase the rate of this reaction (therefore the chemiluminescence intensity), among which Co(II) is reported as the best rate modifier. These metal ions are believed to possess two oxidation states which are separated by one [6]. For example, the oxidation state of cobalt can be +2 or +3 The oxidation state of



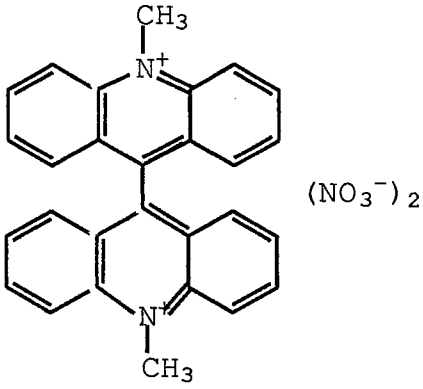
Luminol



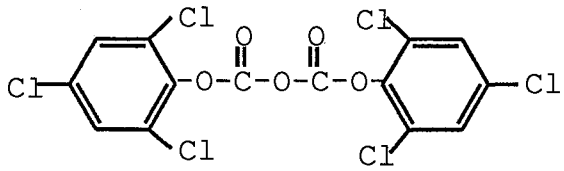
Isoluminol



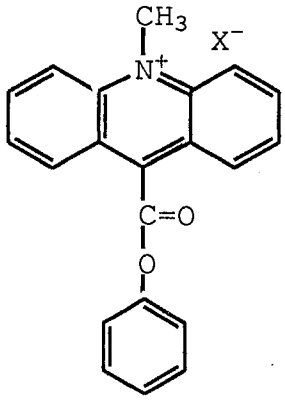
Lophine



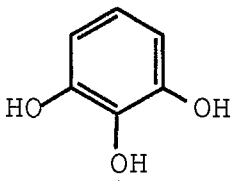
Dimethylbisacridnium
Nitrate (Lucigenin)



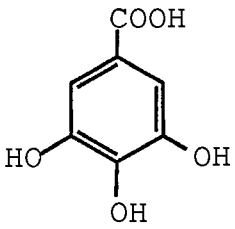
Bis-(2,4,6-Trichlorophenyl)
Oxalate



Acridinium Ester



Pyrogallol



Gallic Acid

Figure 2.2. Selected chemiluminescent compounds.

copper +2 and copper with the oxidation state +3 is believed to exist in aqueous solution When chromium changes its oxidation from +3 to +6, it may have intermediate +4 and +5 states. Manganese(II) only has little, if any, enhancement effect on the chemiluminescence of luminol reaction However, the complexes of Mn(II) with amines [36] and citrate/1,10-phenanthroline [37, 38] stimulate strong chemiluminescence emission. It is believed that the complexing agent stabilizes the intermediate oxidation state of manganese, Mn(III). The inorganic complexes $SbCl_6^-$ [39], Cu(II)-amine [40], and $Fe(CN)_6^{3-}$ [41-44] have been used to enhance the chemiluminescence intensity. Hexacyanoferrate(III) is especially efficient; it acts as co-oxidant as well as promoter in the reaction [1].

The natural heme-containing compounds such as cytochrome c [45], hemin [46, 47], hematogen, and hematin [48] and enzymes have been used to increase the chemiluminescence intensity of luminol. Two types of peroxidases, microperoxidase [49-51] and horseradish peroxidase [52-56], have been used in the chemiluminescent reaction between luminol and H_2O_2 (or hydroperoxides). Although these enzymes are among the most efficient rate modifiers, the efficiency of enzymes is limited because the luminol chemiluminescent reaction produces light only in strongly basic medium (pH > 10) which denatures the enzymes. Therefore, metalloporphyrins have been used as the substitutes for peroxidases [57-60]. Two metalloporphyrins, Mn(II)-$TPPS_4$ [tetra kis(sulfophenyl) porphyrin] and Fe(II)-TMPyP [tetra kis(*N*-methyl-pyridiniumyl) porphyrin], were found to be good rate modifiers in the luminol chemiluminescent reaction with H_2O_2. Compared to peroxidases, Mn(II)-$TPPS_4$ and Fe(II)-TMPyP are even more efficient because they can be used at the optimum pH of the reaction [59, 60].

Many researches have been performed to elucidate the mechanism of the luminol reaction [61-67]. Of particular importance has been the work using pulse radiolysis by Merenyi et al. [64-67] and the work in aprotic solvents by White et al. [61-63]. The light emitting species has been identified as 3-aminophthalate, one of the products of the reaction [62]. The chemiluminescent reaction of luminol with a hydroperoxide or H_2O_2 is thought

to undergo a free radical process as shown in Figure 2.3 [64-67]. The formation of the semiquinone radical is essential for the emission of light [65].

The Lucigenin Reaction. Like luminol, oxidation of lucigenin (bis-*N*-methylacridinium nitrate) occurs in basic solution, producing light that lasts several minutes. While dissolved oxygen can act as oxidant in this reaction, the chemiluminescence of lucigenin is enhanced by hydrogen peroxide but not by other oxidizing agents used in the luminol reaction, such as OCl^- and $Fe(CN)_6^{3-}$ [23]. The lucigenin reaction is also promoted by a range of metal ions similar to those used with the luminol reaction including some (e.g., Bi(III)) that do not stimulate the luminol reaction [9]. The complete list of metal ions that can enhance the chemiluminescence of lucigenin is given in reference [24]. Among those ions, Tm(III), Yb(III), Fe(III), Fe(II), Ru(III), Os(VIII), Co(II), and Ag(I) bring about a dramatic increase in chemiluminescence intensity, and Co(II) is again the best rate modifier. The light-emitting product of the reaction, *N*-methylacridone, is insoluble in water and is therefore deposited on the walls of a flow system and on the window of the detector, which creates a problem in quantitative work. To prevent formation of the precipitate, a low level of surfactant sodium dodecyl sulfate may be added to the reaction mixture [10, 11].

The Gallic Acid and Pyrogallol Reactions. Gallic acid (3,4,5-trihydrobenzoic acid) is oxidized in strongly basic solution by hydrogen peroxide to give out light. Compared to the luminol and lucigenin systems, only a few metal ions [Co(II), Mn(II), Ag(I), Cd(II), and Pb(II)] significantly increase the light intensity of the gallic acid chemiluminescent reaction [19]. Due to the similarity of the two compounds, pyrogallol and gallic acid have similar chemiluminescence behaviors. In addition to Co(II), Mn(II), Ag(I), Cd(II), and Pb(II), other ions such as Mn(VII), Cr(VI), Cr(III), Pt(IV), and Au(III) can stimulate strong chemiluminescence emission form pyrogallol [20]. For both gallic acid and pyrogallol reactions, Co(II) is again the rate modifier that brings about the most

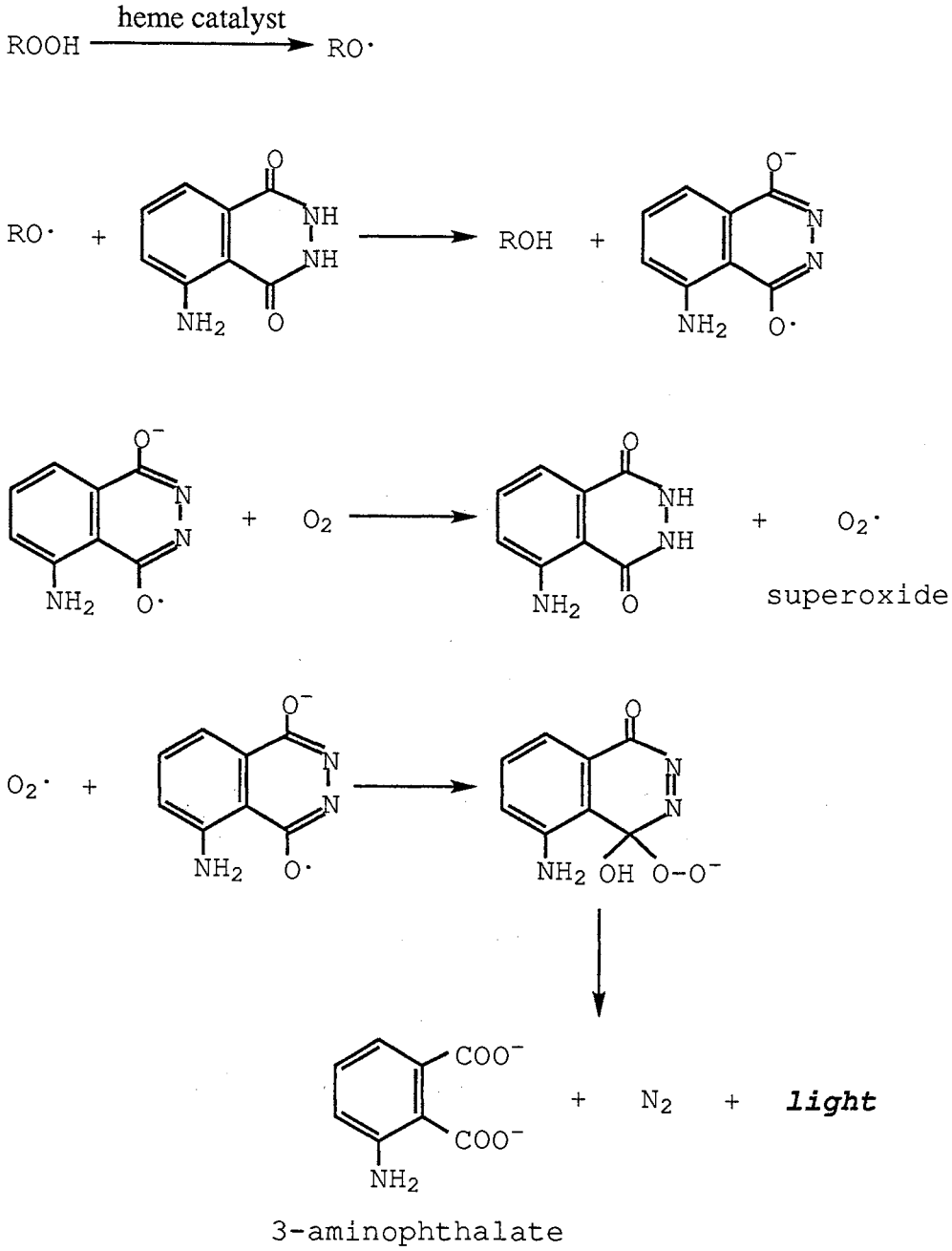


Figure 2.3. Mechanism of luminol chemiluminescence in the presence of a heme-containing rate modifier.

intense chemiluminescence. The chemiluminescent reaction of pyrogallol is also very sensitive to the presence of trace amounts of horseradish peroxidase and hemoglobin [21, 22]

The Lophine Reaction. Lophine (2,4,5-triphenylimidazole) has a limited solubility in water and is generally dissolved in a polar organic solvent. Therefore the lophine chemiluminescent reaction usually takes place in aqueous alkaline DMSO, methanol, or ethanol by using hydrogen peroxide as oxidant. Along with hydrogen peroxide, hypochlorite, $Fe(CN)_6^{3-}$, $AuCl_4^-$, or MnO_4^- can be used to produce strong light emission [15, 16]. The mechanism of this reaction is thought to involve the formation of a dioxetane which decomposes with the emission of light [17, 18]. Although the lophine chemiluminescent reaction is the one which is the least sensitive to metal ions, some metal ions such as Mg(II), Fe(III), Os(IV), Ir(IV), Co(II), Cu(II), and Cr(III) can significantly increase the chemiluminescence intensity [15, 16] and again Co(II) causes the largest enhancement.

The Acridinium Reaction. No rate modifier is required in the acridinium reaction to produce strong chemiluminescence. Intense light emission, in the form of a rapid flash of less than 2 seconds, is triggered just by addition of a suitable base and oxidant (e.g. NaOH and H_2O_2) [68]. The light emitter is the oxidation product of acridinium, *N*-methylacridone. The advantages of this reaction include simplicity of reaction conditions, high chemiluminescence efficiency, and compatibility with aqueous media [69].

The Peroxyoxalate Reaction. This chemiluminescent reaction involves the oxidation, usually with hydrogen peroxide, of various aryl oxalate esters in the presence of a fluorophore. Peroxyoxalate reactions are among the most efficient, and hence the most sensitive non-biological chemiluminescent reactions known, with quantum yields up to 0.5 [3, 12]. Moreover, the peroxyoxalate systems are less affected by experimental variables

than other chemiluminescent systems. The chemiluminescence, however, is quenched by easily oxidized species such as bromide, iodide, sulfite, nitrite, organo-sulfur compounds and substituted anilines [13]. The best known aryl oxalate esters are bis(2,4,6-trichlorophenyl) oxalate (TCPO) and bis(2,4-dinitrophenyl) oxalate (DNPO). Each particular ester has different optimum pH for chemiluminescence [12], whereas the emission wavelength is characteristic of the sensitizer. For example, TCPO can be used from pH 5 to 9 with a maximum light emission at pH 7, while DNPO can be used down to pH 3.5. The insolubility in water of most peroxyoxalate esters, and their susceptibility to hydrolysis are disadvantages in many analytical applications. Thus, organic solvents are a prerequisite for maximum reagent solubility and stability. Mixed solvents including t-butanol/water, dioxane/water, and ethyl acetate/methanol/water have been used successfully as reaction media [1].

Some organic bases such as imidazole [70, 72], pyridine, sodium salicylate, tetra-butylammonium perchlorate, and 4-dimethylamine pyridine [71] have been found to have catalytic effect on the peroxyoxalate chemiluminescent reaction. Imidazole is the most frequently used rate modifier, but the selection of an appropriate base depends on the peroxyoxalate used in the reaction and the reaction conditions.

The organic base-catalyzed chemiluminescent reaction between H_2O_2 and peroxyoxalate is affected by some metal ions [73]. These metal ions may cause delay in the achievement of maximum intensity and a change in the chemiluminescence intensity. The following metal ions show the enhancement effect on the imidazole-catalyzed reaction: Cu(II)>Cr(III)> Pb(II)>Fe(III)>Mn(II)>Zn(II)>Al(III)>Sn(II)>Fe(II)>Ni(II)> Co(II). Cobalt(II), which is the best rate modifier in the chemiluminescent reactions discussed previously in this section, causes the least enhancement. Another commonly used rate modifier in the peroxyoxalate chemiluminescent reaction is cytochrome c [45, 74-77].

A wide variety of fluorescent compounds have been investigated in peroxyoxalate chemiluminescent reactions [14, 78]. Among those screened are fluorescent dyes such as

the rhodamines and fluoresceins, heterocyclic compounds such as benzoxazoles and benzothiazoles, and a number of polycyclic aromatic hydrocarbons such as anthracenes, tetracenes, and perylenes. This latter class of compounds appears to be the best fluorophore, since they are highly fluorescent as well as easy to excite.

Applications

Chemiluminescence is a transient process. The intensity of light emitted vs. time profile is a smooth curve with a maximum. Four parameters extracted from this curve can be analytically useful: maximum intensity, the rate of the buildup or the decay of chemiluminescence, and the area under the curve (i.e., the integrated light output over a fixed fraction of the total reaction time) [82]. These parameters, except the decay of chemiluminescence which is determined by the fluorescence properties of the light emitting species, are proportional to the rate of chemiluminescent reaction. The chemiluminescence intensity is the most commonly used parameter for the quantification of any species whose concentration determines the rate of the reaction. The analytical versatility of chemiluminescent reactions depends on the fact that reaction conditions can be adjusted so that any one of the reaction components (i.e., chemiluminescent substrate, oxidant, catalyst or promoter, and sensitizer), can be made rate determining by adjusting concentrations so that the analyte is the limiting reactant and all other reagents are in excess.

Determination of Rate Modifier

The basis for determination of a rate modifier is that a rate modifier significantly influences the chemiluminescence intensity even at very low concentration, and the higher the concentration, the stronger the intensity. A linear relationship between the concentration of rate modifier and the intensity of chemiluminescence exists over a certain range of rate modifier concentration.

As metal ions, metal complexes, and enzymes can be used as rate modifiers, each of them can be determined with certain chemiluminescent reaction and their quantifications have found applications in analytical chemistry. Table 2.2 summarizes the detection limits of some metal ions by using chemiluminescent reactions of luminol, lucigenin, gallic acid, and lophine. As can be seen, these chemiluminescence systems, especially luminol and lucigenin reactions, are very sensitive for the determination of metal ions; the detection limit for Co(II) can be as low as 0.0007 ppb [6].

Determination of Substrate

The substrates of a chemiluminescent reaction include the chemiluminescence reagent, the oxidant, and the sensitizer. A chemiluminescent reaction is analytically useful in the determination of a substrate as long as it gives out measurable light emission at low concentration of the substrate. Many chemiluminescent reactions can be used for this purpose. For example, the luminol reaction has been used to determine ClO^- [82], H_2O_2, and luminol [83] with detection limits of 7.5 ng/ml, 10^{-6} M, and 10^{-6} M, respectively. The peroxyoxalate reaction has been used to determine H_2O_2 with a detection limit of 3×10^{-9} M [70] as well as polyaromatic hydrocarbons, the sensitizer of this reaction, in the 10^{-12} - 10^{-13} g range [84, 85].

Used as Indicator Reaction

Chemiluminescent reactions are widely used as the indicator reaction in analytical chemistry. For example, because most chemiluminescent reactions use H_2O_2 as oxidant, any reaction which produces H_2O_2 may be indicated by a chemiluminescent reaction and therefore the components of this reaction can be indirectly determined. This is commonly employed in bioanalytical chemistry.

Blood glucose has been determined by using the chemiluminescent reaction of luminol as the indicator reaction [44]. First of all, ß-D-glucose is converted into D-

Table 2.2. Detection limits of some metal ions in four chemiluminescence systems.

metal ion	detection limit[a], ppm			
	luminol	lucigenin	gallic acid	lophine
Co(II)	$7x10^{-7}$[6]	$2x10^{-8}$[24]	$4x10^{-4}$[19]	$1x10^{-7}$[15]
Mn(II)	$5x10^{-4}$[6]	0.6[24]	0.4[19]	15[15]
Ag(I)	30[79]	0.03[24]	0.5[19]	10[15]
Cd(II)	*(b)*[79]	*(c)*	0.8[19]	100[15]
Pb(II)	1.0[79]	0.2[80]	1.0[19]	10[15]
Cr(II)	$5x10^{-5}$[6]	0.2[24]	>5[19]	0.25[15]
Cu(II)	$6x10^{-5}$[6]	0.1[24]	>6[19]	0.17[15]
Fe(II)	$6x10^{-4}$[6]	$6x10^{-6}$[81]	>6[19]	*(c)*

(a) The definition for detection limit in the corresponding reference is followed. It may be different in different references.

(b) Chemiluminescence is suppressed.

(c) Detection limit is not available.

gluconic acid and H_2O_2 under the catalysis of glucose oxidase. The H_2O_2 subsequently reacts with the luminol-$Fe(CN)_6^{3-}$ mixture to produce chemiluminescence, which is proportional to the ß-D-glucose concentration. The method gives a linear dynamic range from 10^{-8} to 10^{-4} M glucose. As little as 10^{-20} moles of ß-D-galactosidase were detected based on its catalytic effect on the decomposition of *o*-nitrophenyl-ß-D-galactoside into D-galactose which then underwent further reaction to produce H_2O_2 determined by the microperoxidase-catalyzed isoluminol chemiluminescent reaction [49].

Chemiluminescence immunoassay is another important area in which chemiluminescent reactions are successfully used as indicator reactions. In chemiluminescence immunoassays, the antibody or the antigen is first coupled with a chemiluminescent reagent or with the rate modifier of a chemiluminescent reaction (either an enzyme or a metal-porphyrin complex). After completion of the immunoreaction, the coupled chemiluminescence component is quantified with the corresponding chemiluminescent reaction, and the interested antigen or antibody can be determined. Acridinium [68, 69, 86-88], lucigenin [103, 104], isoluminol, luminol, peroxidase [86-88], and Mn(II)-tetrakis(sulfophenyl) porphine [89] have been successfully used for this purpose.

Instrumentation

The instrumentation for chemiluminescence measurements is simple. It generally consists of three basic units: a reagent addition device, a sample cuvette, and a light measuring device (Figure 2.4). Electronic accessories are necessary to amplify and process the signal received by the detector. The result is displayed on a readout unit (e.g., recorder, oscilloscope, or plotter). A computer can be used to control the addition of reagent and process the data. Because there is only one emitting species, almost all luminometers do not have a wavelength discrimination device [1].

Figure 2.4. Block diagram of a luminometer.

Depending on the method of reagent addition, the instrumentation for chemiluminescence measurements can be classified as a static or a flow system. All other chemiluminescence detection schemes are derived from these two basic systems.

Static Systems

In a static system, the reagent addition device is a syringe or an automatic injector and the sample is placed in a cuvette which is positioned in front of the detector. The luminescent reagent is added manually with the syringe or mechanically with the automatic injector. Mixing of sample and reagent is usually provided by the forceful addition of reagents without further stirring although some instruments have sophisticated mixing devices [8]. The resulting chemiluminescence intensity is monitored by a detector over a predetermined time interval. Figure 2.5 illustrates a simple static system with a syringe as the reagent addition device. This type of instruments are also called batch luminometers.

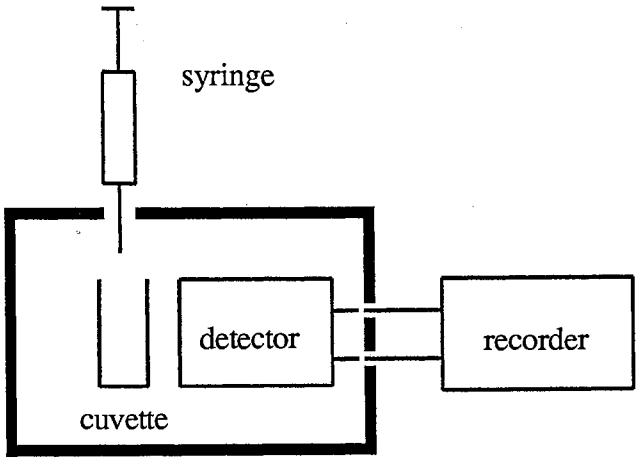


Figure 2.5. Typical static system for chemiluminescence detection.

Batch luminometers are convenient to use but suffer from several limitations. The force of injection and the exact positioning of the injector tip will vary from measurement to measurement especially when the reagent is added manually with a syringe. This will adversely affect the precision if the chemiluminescent reaction is fast enough so that a significant fraction of analyte reacts before the solution is homogeneous. Moreover, bubble formation upon injection causes scattering of light, resulting in unreproducible measurement. Also, the force of injection may not be sufficient to inducing mixing if reagent and sample differ in density [8]. Because of these factors, relative standard deviation (RSD) for static systems is usually about 20% when a manual syringe is used. The RSD can be greatly improved when the reagent is added via automatic injection because the automatic injector can provide reproducible positioning and injection force [8].

Flow Systems

Various flow systems have been devised to permit rapid and reproducible mixing of sample and reagents [8, 91]. Compared to static systems, flow systems offer much smaller RSD. Figure 2.6 shows a schematic diagram of a typical continuous-flow system for chemiluminescence detection.

In a flow system, a pump is usually used as reagent addition device. Reagent(s) and sample are transported through plastic tubing and meet immediately before flowing through a cell placed in front of the detector. The design of the mixing device as well as the means of retaining the emitting solution in view of the detector are important. Mixing is most effective at a T-piece, but a Y-junction can also be used (Figure 2.7) [8]. Other factors to consider in designing a flow system for chemiluminescent measurements are reagent flow rate, length and diameter of tubing, and the order of reagent addition. These parameters affect the peak shape and height as well as the sensitivity of detection [26, 27, 92, 93].

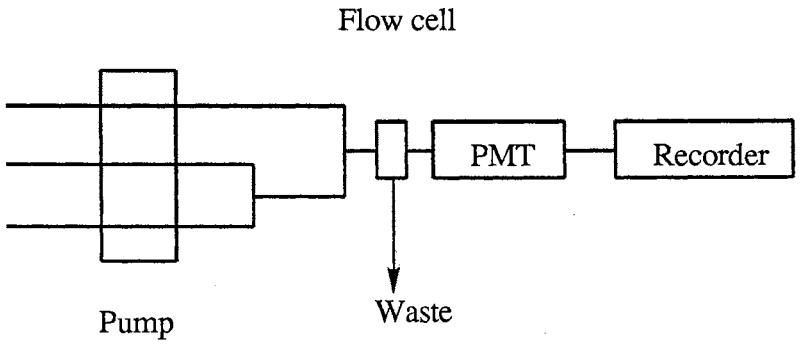


Figure 2.6. Schematic diagram of a typical flow system for chemiluminescence detection.

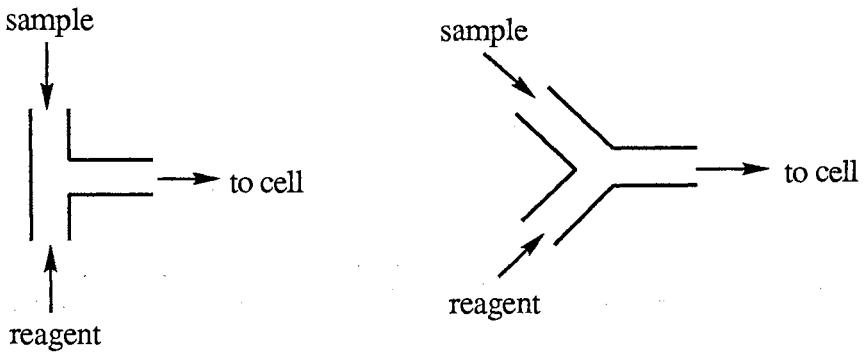


Figure 2.7. Two types of reagent mixing devices.

Modification to Static and Flow Systems

Various modifications on the conventional static and flow systems have been made to meet special requirements for some chemiluminescent reactions, and to improve sensitivity and reproducibility. Two approaches are commonly used: (1) stopped-flow technique, and (2) immobilization of reagent.

The Stopped-flow Technique. Stopped-flow is a mechanical modification which stops the pump for a certain time after the reaction components have been mixed. It possesses all advantages of flow systems and offers a number of additional features. The most attractive feature of the stopped-flow approach is that the light emitting solution may be retained in the cell for whatever time is desired. The variation of chemiluminescence intensity with time can then be monitored, thus allowing kinetic measurements and integration of the signal profile [90].

Use of Immobilized Reagents. This approach has been widely used with enzymes physically or chemically immobilized on solid supports such as micro glass or polymer particles [97], and membranes [98, 99]. It offers many advantages, including the conservation of valuable reagents, increased enzyme stability, and compatibility with automated continuous-flow analyzers [1]. It has been applied to both static [94, 95] and flow [96-98] systems. The intensity of chemiluminescence with immobilized reagent depends on both the kinetics of the chemiluminescent reaction and on the efficiency of the mass transfer processes involved in bringing the reactants into contact [1].

Limitations of Chemiluminescence Determinations

Although chemiluminescence analysis has advantages such as high sensitivity, wide dynamic range, low background, and simple instrumentation over other optical approaches, it has two major limitations: the generally poor understanding of the chemiluminescent

reaction mechanism and the lack of analyte selectivity. The first limitation makes it difficult to predict the chemiluminescence behaviors of a chemical species [1]. Since several potential analytes can be detected by a chemiluminescent reaction, the selective detection of a single analyte is sometimes difficult to accomplish.

In order to improve selectivity, chemical treatment such as complexation can be applied to a sample prior to the chemiluminescence detection. For example, Cr(III) has been determined in the presence of other metal ions by adding excess amount of EDTA to the sample [99]. All of the metal ions, except Cr(III), were quickly complexed with EDTA, so they were no longer available for reaction with the luminol-H_2O_2 system. Since the complexation reaction of Cr(III) with EDTA is very slow, the Cr(III) in a sample can be quantitated with reasonable specificity.

Another approach to alleviate the selectivity problem is to couple the chemiluminescence detection with a chromatographic separation of the analyte from interfering species in a sample. Ion exchange chromatography has been used to separate the mixture of metal ions for subsequent quantitation by a chemiluminescent reaction [27, 79, 100, 101]. Hydrogen peroxide also has been determined by peroxyoxalate chemiluminescent reaction combined with high-performance liquid chromatographic separation [102].

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CHAPTER III

DEVELOPMENT OF INSTRUMENTATION FOR CONTINUOUS-FLOW CHEMILUMINESCENCE DETECTION

Introduction

Since the mid-1970's, the applications of chemiluminescent reactions in analytical chemistry have been attracting increased interest [1]. The relative simplicity in instrumentation has contributed greatly to such increase in popularity. Although most commercial luminometers are based on the static or batch system [2], the use of continuous flow has received increased attention in recent years [3-8] because of its unique advantages over static system (see Chapter II for details). Among the three basic units of an instrument for continuous-flow chemiluminescence detection (i.e., pump, flow cell, and detector), the flow cell is the most important part. It determines the efficiency of the reaction and affects the efficiency of light detection. Correspondingly, two factors must be considered in designing a flow cell: (1) the means to mix reactants, and (2) the geometry of the cell. An ideal flow cell should provide rapid mixing for reactants and favor high luminescence intensity. It should also efficiently retain the emitting medium in front of the detector. A variety of flow cells have been used in continuous-flow systems [9], and in some of them vibration or magnetically activated balls have been incorporated to aid in mixing. At present, the most commonly used cell is the spiral flow cell made of a piece of glass tube and the mixing of reactants is provided with a T-piece or a Y-shaped mixing element [10-14]. Another kind of cell has to be used [15] for the popular continuous flow/stopped-flow/continuous flow operation, and in this case the mixing is provided by forcefully injecting the sample into the flow cell.

This chapter reports the design and evaluation of a novel versatile continuous-flow chemiluminescence detection system comprising a rotating reactor/cell that fulfills the two requirements discussed above. This rotating reactor/cell utilizes small volumes and efficiently makes use of the catalyst or promoter of the chemical reaction leading to luminescence.

Experimental Section

Design of Flow Cell

Figure 3.1 illustrates the details of the flow cell. The cell body was constructed of PTFE and the external surface was painted black to make it light-tight. It is composed of two separate parts and a disk. The upper part accommodates two inlets and one outlet for the passage of solution(s), and a glass window to let light pass through to the detector. The lower part has three grooves on its surface and a round chamber at its center. The PTFE disk (its surface smoothly polished to reduce light scatter and to increase light reflection) contains embedded a miniature stirring bar (PTFE-coated Micro Stir bar from Markson Science, Phoenix, AZ). A PTFE needle is attached to the disk at the center of its bottom. The needle's point rests on a machined inverted conical indentation at the center of the chamber on the lower part that permits a smooth disk rotation driven by a magnetic stirrer. The disk is slightly smaller in diameter than the chamber so that it can be accommodated into the chamber. The upper and lower parts of the flow cell are made fluid-tight by fastening them with six screws and a concentrically placed O-ring 5.85 cm in diameter (Figure 3.1), and they are arranged so that the inlets and outlet on the upper part are connected to the chamber by the grooves on the top of the lower part. The depth of the chamber is greater than the thickness of the disk; therefore the difference between them determines the volume of the flow cell. The volume of the cell used here was 133 μl, but it could be changed simply by changing the thickness of the disk.

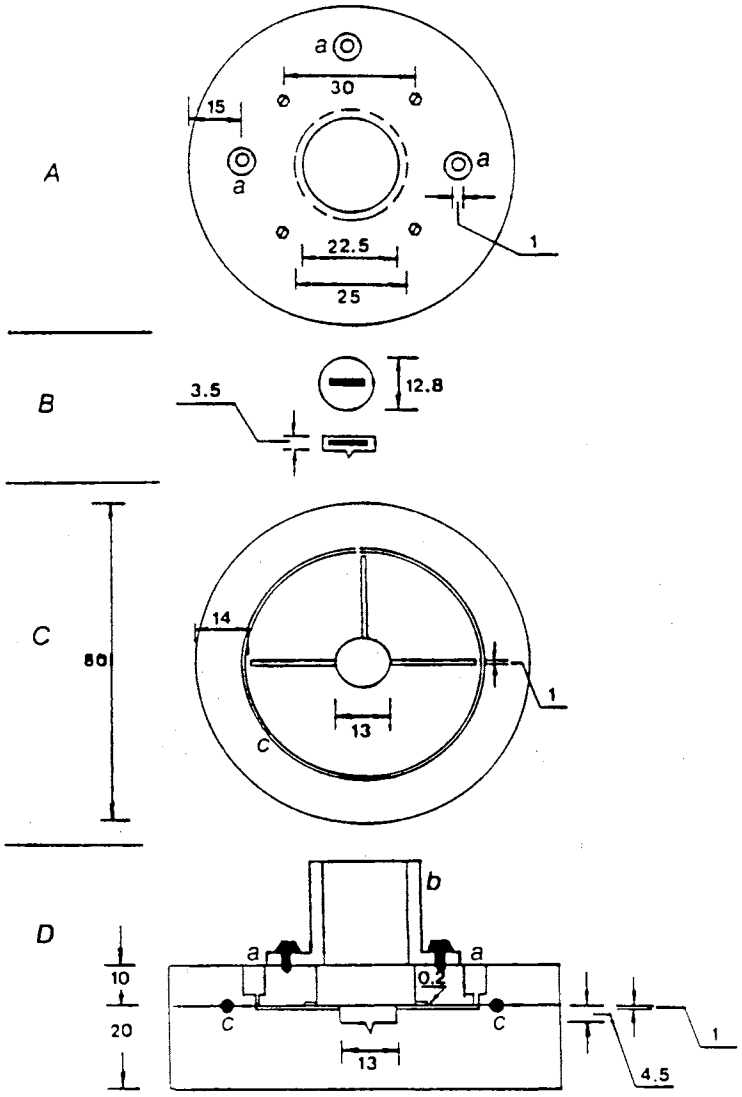

Figure 3.1. Schematic representation of the flow-through cell containing the rotating disk. A: upper cell body; B: rotation disk showing positioning needle point and embedded magnetic stirring bar; C: lower cell body showing solution inlet/outlet channels; D: cross-section view showing the flow path and optical connector. (a) Solution inlet/outlet points; (b) Optical connector to photomultiplier tube; (c) O-ring. All measurements are in mm.

The center part of the upper portion of the cell has a circular opening for a microscope slide circular cover made of chance microsheet glass (Arthur Thomas, Philadelphia, PA), which provides the optical window. Just above this glass window is located the photo-detector (a RCA 1P28A side-on 9-stage photomultiplier tube). The photomultiplier tube housing and the cell are interconnected with Canon-type optical connectors and therefore can be separated from each other very easily.

Components of the Instrumental Setup

Figure 3.2 shows a block diagram of the custom-assembled flow system unit used to detect chemiluminescence in this study. A Minipuls 2 peristaltic pump (*P*) was used as reagent transportation device. A manually operated injection valve (Rheodyne Type 50, 4-way Teflon rotary type) was used as sample injector. The rotation velocity of the disk inside the flow cell (*FC*) was controlled by adjusting the voltage applied to a magnetic stirrer (*MS*) through a variable transformer (*VR*) with an output between 0 and 140 V and 7.5 maximum amperage (The Superior Electric Co., Bristol, CT). The magnetic stirrer is custom-made to rotate a magnet with a Dayton Model 3M247 motor from Dayton Electric, Chicago, IL. It is designed to generate no heat, a problem that is common with most commercially available stirrers and which adversely affects the reproducibility of chemiluminescence measurement. A RCA 1P28A side-on 9-stage photomultiplier tube (*PMT*), powered by a Model 204-10L high-voltage power supply (*PS*) (Pacific Precision Inst., Concord, CA), was used to detect light emission from the flow cell and the voltage applied to it was -1000 volts. The signal from the *PMT* was processed through auxiliary electronics (*AE*) and then displayed as a chemiluminescence intensity (volts) vs. time curve on a digital oscilloscope (*DO*): Nicolet 1090A with 94A plug-in and Model D amplifier (Nicolet, Madison, WI). The maximum intensity was usually directly read out from the oscilloscope. The signal was also printed out on a HP7470A plotter through a HP9825A desktop computer (Hewlett-Packard, Palo Alto, CA).

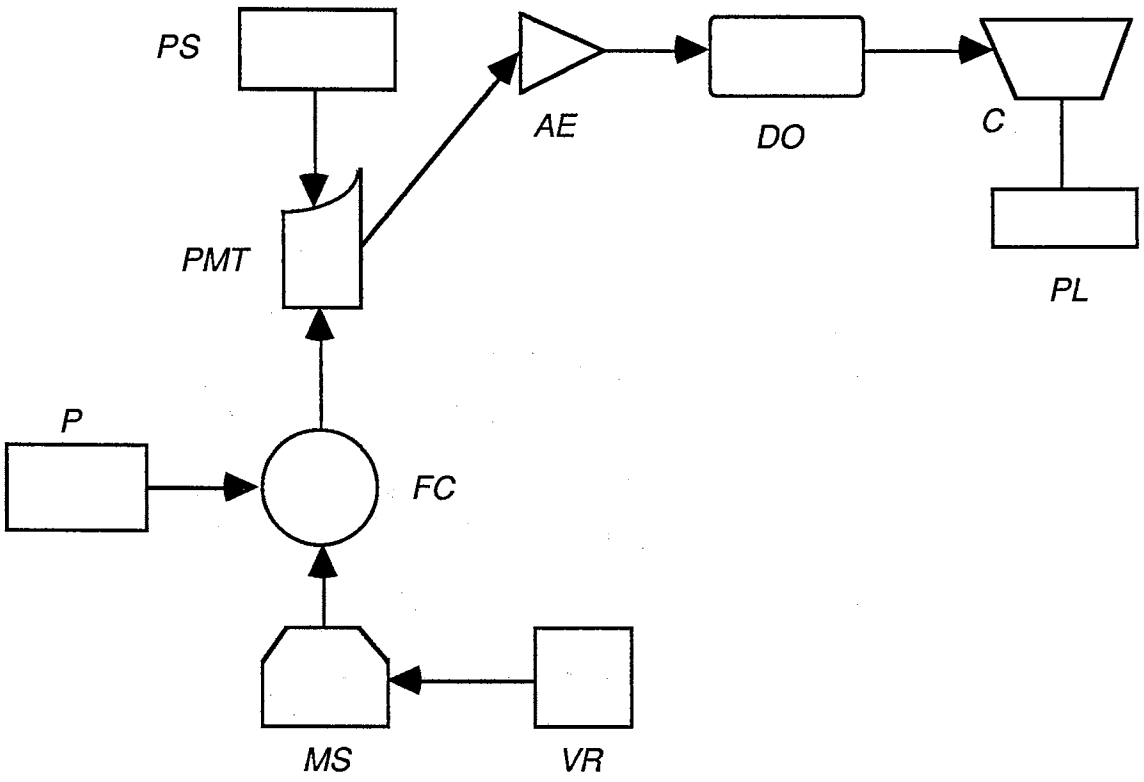

Figure 3.2. Instrumental setup used to measure chemiluminescence. P: pump; PMT: photomultiplier tube; PS: power supply; AE: auxiliary electronics; DO: digital oscilloscope; C: desk top computer; PL: plotter; MS: magnetic stirrer; VR: variable transformer. See text for details.

All tubing used for solution transport was of Tygon (rated at 1.0 ml/min from Alpkem, Clackamas, OR). Whenever a second pump was needed, a FIAtron SHS-200 microprocessor-controlled solution-handling system (FIAtron Systems, Milwaukee, WI) was used (e.g., to implement programmed continuous-flow/stopped flow/continuous-flow operation).

Reagents and Solutions

The water used for solution preparation was deionized and further purified by distillation in an all-borosilicate glass still with a quartz immersion heater. Except as noted, all reagents used were of analytical reagent grade.

When cobalt phthalocyanine (CoPc) was used in solution, it was prepared from CoPc obtained from Pfaltz and Bauer (Flushing, New York) and by shaking excess solid CoPc with a 0.1% v/v ethanol aqueous solution. After standing for several hours, the supernatant liquid was decanted into a clean container and this saturated solution was used as the working solution of CoPc. The 1.0×10^{-2} M aqueous solution of $Fe(CN)_6^{3-}$ solution was prepared from $K_3Fe(CN)_6$ (Fisher Scientific, Fair Lawn, NJ).

Luminol, obtained from Lancaster Synthesis (Windham, NH), was prepared as a 5.0×10^{-3} M solution in 0.10 M phosphate buffer and the pH of the solution was adjusted to 11.4 with sodium hydroxide. This solution was used when CoPc was used as rate modifier. When $Fe(CN)_6^{3-}$ was used as rate modifier, a 2.0×10^{-3} M luminol solution was prepared in 0.10 M sodium carbonate and the pH of the solution was adjusted to 10.5 with sodium hydroxide. All luminol solutions were allowed to stand for at least one day to attain stability before use.

A 8.8×10^{-6} M hydrogen peroxide solution was used throughout this study unless otherwise stated. It was daily prepared by diluting a 3% v/v H_2O_2 stock solution (Mallinckrodt, St. Louis, MO). This solution was not standardized because no quantitative measurements were involved in this study.

Physical immobilization (adsorption) of CoPc was accomplished by first dissolving a small amount of the solid complex in toluene and then equilibrating (by shaking) this solution with the adequate amount of controlled-pore glass, CPG (surface area: 24 m^2/g, mean pore diameter: 1273 Å. Electro-Necleonics, Fairfield, NJ). Prior to equilibration, the CPG was washed with 2.0 M HCl to remove possible metal oxides, washed thoroughly with distilled water, and dried in an oven at 70°C overnight. After equilibration and separation of CPG from excess CoPc solution, the toluene was evaporated, and the CPG-immobilized CoPc was air dried.

Model Chemiluminescent Reaction

The chemical reaction used as model for the evaluation of this chemiluminescence detection system is the most popular one in chemiluminescence measurements: luminol + H_2O_2. The rate of this reaction has been found to be increased by at least 20 metal ions, some metal complexes, and some enzymes, as mentioned in Chapter II. Two rate modifiers were employed in this work: hexacyanoferrate(III) and cobalt(II) complexed with phthanocyanine (CoPc). The hexacyanoferrate(III) rate modification is a typical promotion, but the CoPc seems to behave, under certain conditions, as a true catalyst. The rate enhancement (therefore the chemiluminescence intensity enhancement) by CoPc is significantly more efficient than that of hexacyanoferrate(III). Most important, the rate of the reaction is fast in the presence of CoPc because the strong chemiluminescence lasts only about 1 ~ 2 seconds, while it is relatively slow in the presence of hexacyanoferrate(III) as the bright light emission may last more than ten minutes. By using the chemiluminescent reaction of luminol stimulated by these two rate modifiers, the response of this chemiluminescence detection system for both fast and slow reactions was properly evaluated.

Results and Discussion

The chemiluminescence detection system presented here is versatile, and different flow configurations including flow injection, continuous sample feeding, and stopped-flow operations can be simply achieved by changing the connections of sampling tubing. Because the unique characteristic of this detection system is that a rotating disk is introduced in the flow cell to help mixing of reactants, and to serve as a bed for the use of immobilized reagents, the system has been evaluated mainly with regard to: (1) relevance of disk rotation, and (2) signal-to-noise ratio of acquired chemiluminescence signal. First of all, an auxiliary electronic circuit was carefully designed to increase the signal and to reduce the noise associated with the rotation of the disk.

Optimization of Auxiliary Electronics

As shown in Figure 3.3, the auxiliary electronic circuit is basically composed of two operational amplifiers. This circuit plays a very important role in the detection of chemiluminescence and in the improvement of signal-to-noise ratio.

The light detected by the PMT is output as a current. Since a voltage signal is easier to process than a current signal, a current-to-voltage converter (*OP1*) was employed to convert the output current signal from the PMT into a voltage signal, as well as to perform some amplification. The magnitude of amplification was determined by the impedance of resistor R_3. Larger impedance values give greater amplification. However, a larger impedance slows down the response of the system. For a fast chemiluminescent reaction, a slow response is not desired because the short-life signal associated with a fast reaction cannot be fully acquired by the detection system, resulting in a significant loss in sensitivity. It was found that a 50 kΩ resistor made the system have a response time small enough to detect the chemiluminescence from a fast chemiluminescent reaction like

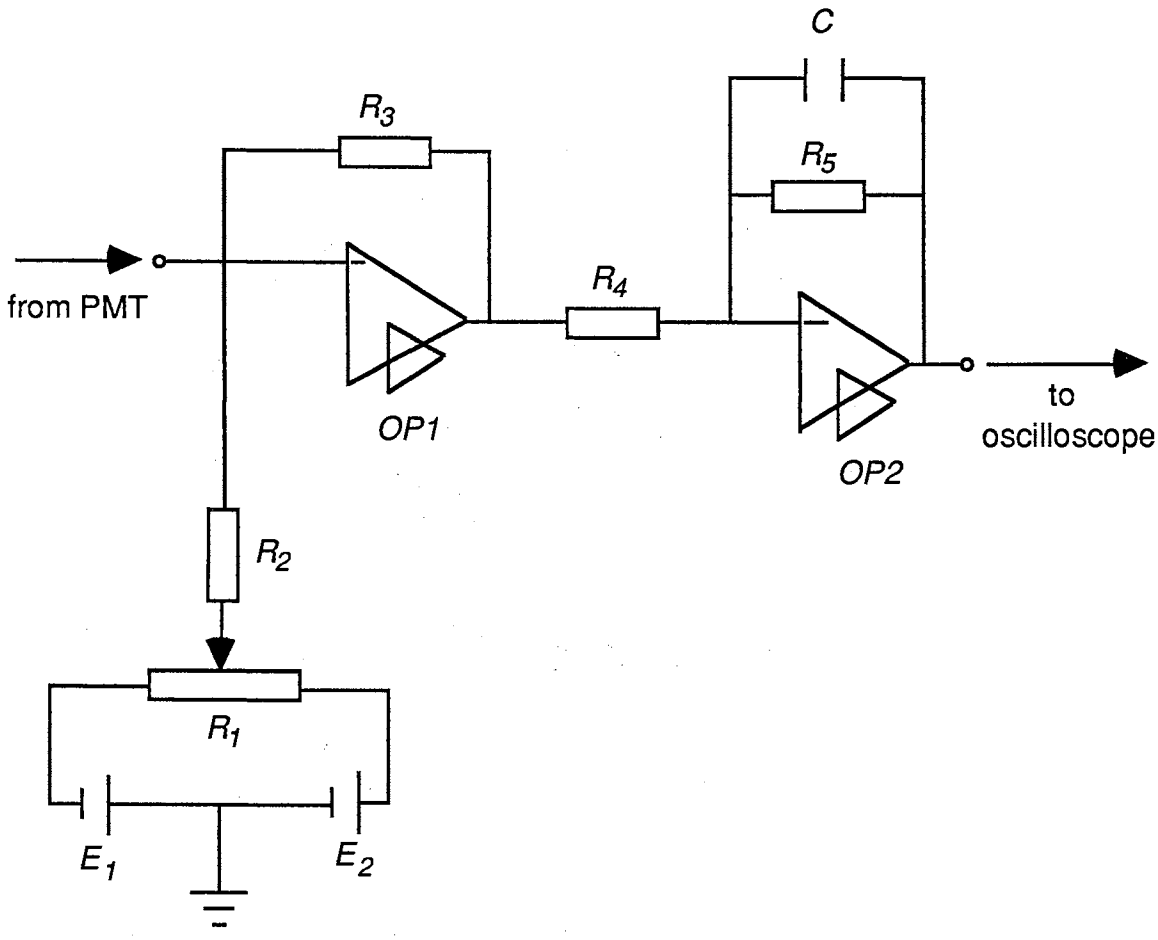

Figure 3.3. Auxiliary electronics used to increase the signal-to-noise ratio and to compensate for background. $E_1 = E_2 = 0.4$ V; $R_1 = 0 \sim 1000$ Ω; $R_2 = 100$ kΩ; $R_3 = 50$ Ω; $R_4 = 120$ Ω; $R_5 = 10$ kΩ; C = 14.7 mF. OP1 and OP2: chopper-stabilized operational amplifiers.

the luminol-H_2O_2 reaction "catalyzed" by CoPc as well as to give a satisfactory amplification.

The second operational amplifier (*OP2*) provides further amplification for the signal from the current-to-voltage converter so that the system is able to detect very weak chemiluminescence. Substituting a set of precision resistors for the feedback impedance R_5 of OP2 allows to select a multiplication factor that is appropriate for different intensity levels of chemiluminescence. By adding a capacitor (C) parallel to R_5, this operational amplifier also serves to reduce the noise arising from the rotation of the disk inside the flow cell.

It was noticed that the rotation of the magnetic disk in the flow cell not only helps in mixing the reactants, but also produces a periodically changed, AC-like noise. The frequency of the noise was affected by the rotation velocity of the disk; the higher the velocity, the higher the noise frequency. The magnitude of noise was different when different rate modifiers were used. Of the two chemiluminescent reactions used in this work, the luminol-H_2O_2-$Fe(CN)_6^{3-}$ reaction gave a much greater noise than the CoPc-"catalyzed" reaction.

Compared to the noise, the chemiluminescence of interest is basically a DC signal because it does not rapidly change with time. Hence, a capacitor C with a small capacitance was used in parallel with the feedback impedance R_5 to form a low-pass filter which allows the passage of the DC signal and block the high frequency AC noise. The cutoff frequency of this low-pass filter is [17]

$$fc = 1/(2\pi R_5 C)$$

and its time constant is [17]

$$t_c = R_5 C$$

As can been seen, a larger capacitance corresponds to a lower cutoff frequency and reduces noise in a larger extent. However, a large capacitance will increase the response time, which is defined as the time for 99.7% of full response and equal to $5t_c$ [17]. A

slow response does not affect the measurement of slow chemiluminescent reactions such as the luminol-H_2O_2-$Fe(CN)_6^{3-}$ reaction, but it will result in much lesser value than the actual intensity for a fast chemiluminescent reaction like the luminol-H_2O_2-CoPc reaction. Therefore, for a slow chemiluminescent reaction, a capacitor with large capacitance can be used to suppress the noise effectively. For a fast chemiluminescent reaction, a compromise between noise reduction and signal acquisition must be made because a large capacitance is desired in order to suppress noise effectively, but a small capacitance is desired in order to have a fast response. For example, if C is 10 μF, the noise level for the luminol-CoPc blank was 8 mV and the response time was 0.5 second when the impedance of R_5 was 10 kΩ and the rotation velocity of the disk was 258 rpm. If C was increased to 14.7 μF, the noise level was reduced to 4 mV and the response time was increased to 0.75 second. In this work, a 14.7 μF capacitor was used along with a 10 kΩ resistor. The cutoff frequency offered by this low-pass filter is 1.06 Hz. This is good enough for noise suppression and fast enough for data acquisition for both the CoPc- and the $Fe(CN)_6^{3-}$-modified luminol chemiluminescent reaction. If a resistor with lower resistance is used when strong chemiluminescence is measured, a capacitor with higher capacitance may be used to maintain an appropriate cutoff frequency.

It is interesting to notice the effect of rotation velocity of the disk on the effectiveness of the low-pass filter, and this is shown in Figure 3.4. As mentioned above, the rotation velocity of the disk influences the frequency of noise. At low rotation velocity, the noise was observed to be sine-shaped and its frequency to increase with the increase in rotation velocity. Because a low-pass filter works better against the noise with high frequency, high rotation velocity is always desired. It was observed that signal and baseline became smoother and smoother as the rotation velocity of the disk was increased. However, the improvement was insignificant after the rotation velocity exceeded 258 rpm.

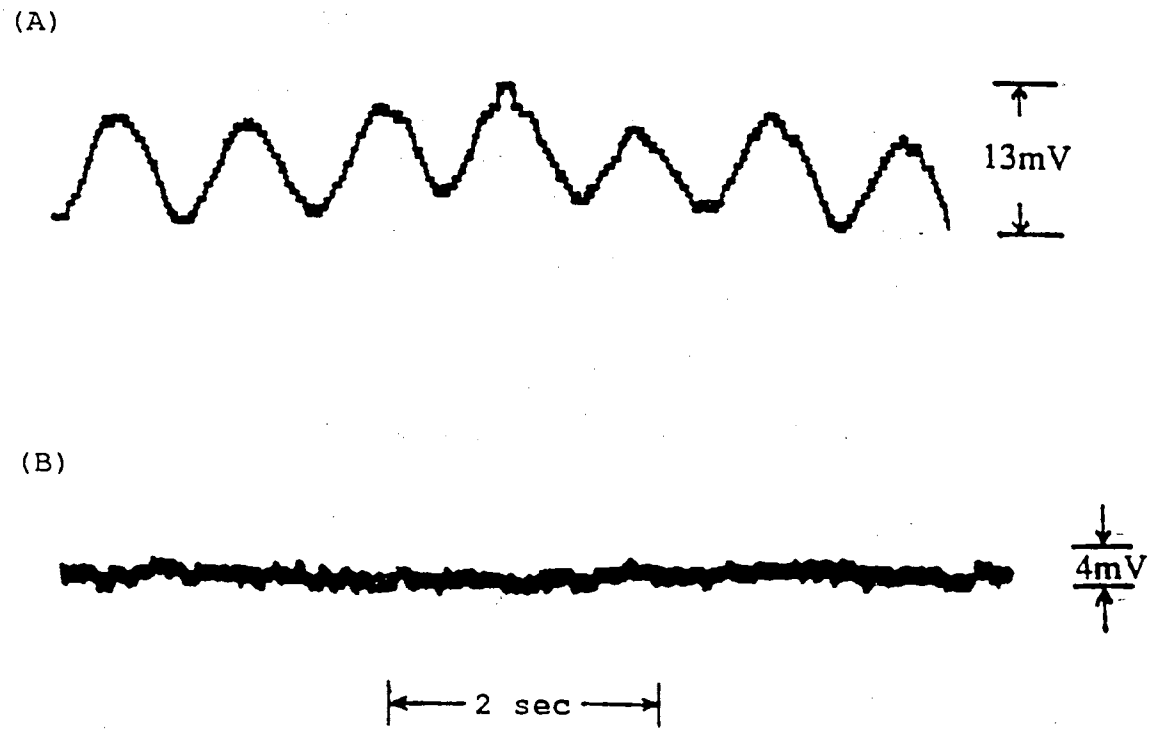

Figure 3.4. Effect of rotation velocity of the disk in the flow cell on the magnitude of noise. Disk rotation: (A) 58 rpm, and (B) 664 rpm. Luminol-CoPc blank. Continuous sample feeding mode (operational mode B in Figure 3.5, but water was used instead of H_2O_2), [Luminol] = 5.0×10^{-3} M, R_5 = 10 kΩ, C = 14.7 μF.

In this circuit, resistors R_1 and R_2 and DC power sources E_1 and E_2, form a unit for the compensation of PMT dark current and background chemiluminescence. It is necessary because the background sometimes could be as high as several hundred millivolts and it would overwhelm even a moderately strong signal. In order to make compensation, the position of the wiper on the variable DC voltage divider, R_1, is adjusted to certain position so that a current equal to the sum of PMT dark current and background current, but of opposite sign, is added to the current-to-voltage converter at the inverting input. In such case, the reading on the digital oscilloscope was zero. If the compensation is done with the blank solution, the PMT dark current and background current are automatically subtracted from the current generated by the analyte solution before it is sent to the operational amplifiers.

Flow Systems

Figure 3.5 shows the basic configurations applicable to this flow system. These are all common strategies found in the literature. In this section, the influence of the rotation of the disk in the flow cell on chemiluminescence emission will be discussed. Moreover, the effects of other factors such as flow rate and sample size on chemiluminescence measurements will also be examined. The advantages and disadvantages of these operational configurations in chemiluminescence analysis are then compared.

Flow Sample-Injection Operational Mode (Figure 3.5A). In this mode, the luminol, rate modifier, and hydrogen peroxide solutions were delivered by a single peristaltic pump using tubing with the same diameter for reagents and sample. Injection of sample (H_2O_2) was accomplished by means of a manually operated Rheodyne Type 50 four-way injection valve. The luminol and rate modifier solutions were pre-mixed with a T-piece mixing element before entering the injection valve. The mixture of luminol and catalyst solutions served as the carrier for the injection of hydrogen peroxide. The length of tubing through which the mixture of luminol and rate modifier traveled before it

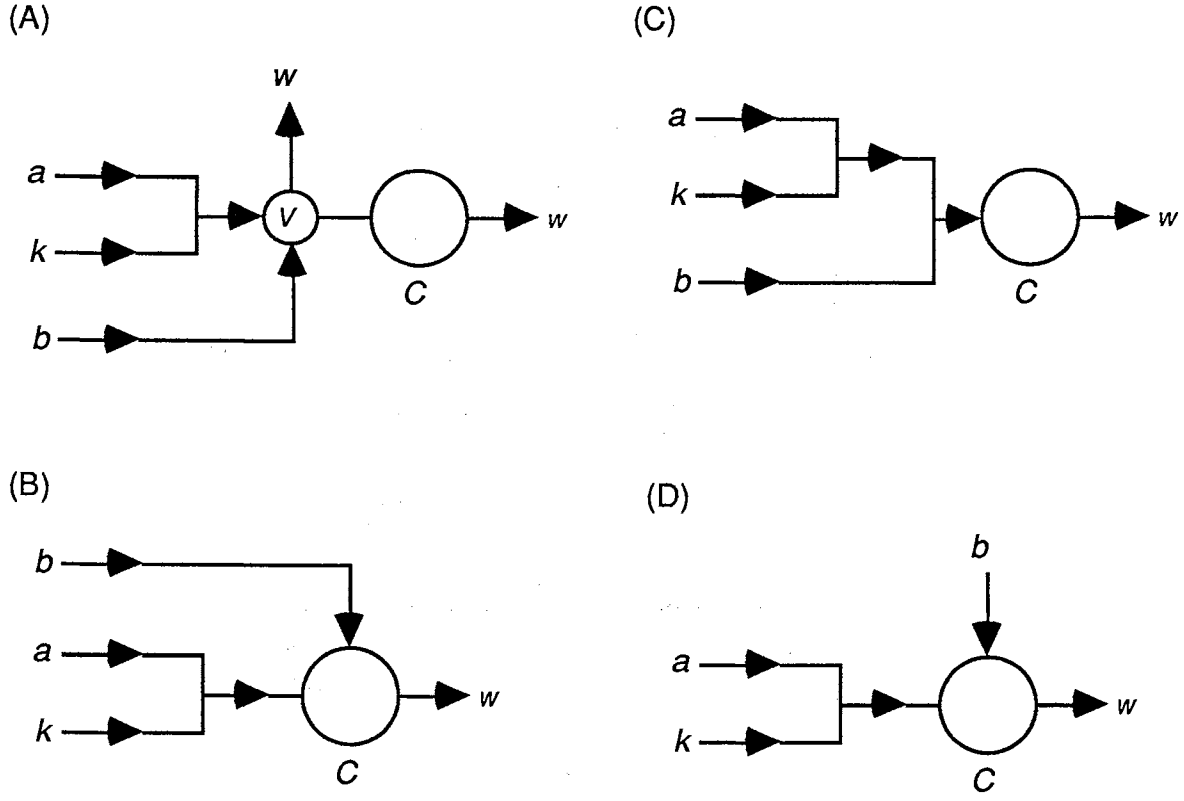

Figure 3.5. Schematic representation of the different flow configurations used to characterize the flow system. A: Configuration with intercalation of a small plug of sample solution. B and C: Configurations for continuous sample feeding operation. D: Configuration for stopped-flow operation. (a) line for introduction of main reagent (luminol), (b) line for introduction of second reactant (H_2O_2), (k) line for introduction of rate modifier, (w) waste line, (V) injection valve, (C) flow cell with rotating disk.

entered the injection valve was 20 cm long. This piece of tubing was used to reduce the background of the chemiluminescence produced in the absence of hydrogen peroxide. The background, especially when $Fe(CN)_6^{3-}$ was used, was quite strong if luminol and rate modifier solutions were not pre-mixed before entering the flow cell. A strong background was also accompanied with high level of noise, resulting in the decrease in detection limit. Other pre-treatment methods used to reduce the background did not give better results. These included (1) mixing luminol and hydrogen peroxide or rate modifier and hydrogen peroxide first and (2) preparing luminol and rate modifier in one solution. The second pre-treatment method even resulted in total quenching of chemiluminescence for both CoPc and $Fe(CN)_6^{3-}$ modified reactions. Hence, the luminol and rate modifier solutions were pre-mixed in a 20-cm long tubing not only in this operational mode but also in all other operational modes shown in Figure 3.5.

When the injection valve is set at the loading position, the mixture of luminol and rate modifier passes through the valve and then enters the flow cell. At the same time, the hydrogen peroxide solution passes into the valve through a loop and is discarded as waste. When the injection valve is set at the injection position, the loop is on the way of the reagent mixture (luminol and rate modifier) to the flow cell; thus, hydrogen peroxide inside the loop is intercalated in the stream of the mixture of reagents and is pushed into the flow cell where it reacts with the reagents. It is very important to make the connecting tubing between injection valve and flow cell as short as possible. The reason is that once hydrogen peroxide is intercalated into the stream of the luminol-rate modifier mixture, the chemiluminescent reaction takes place at the two ends of the hydrogen peroxide plug. Diffusion facilitates the reaction. The extent of this undesired reaction is greater if the hydrogen peroxide plug travels longer within the stream of carrier solution. Obviously, this reaction consumes hydrogen peroxide before it enters the flow cell to produce the chemiluminescence detected by the PMT; therefore, the intensity of chemiluminescence will be greatly reduced if a long connecting tubing is used. The

effect of the length of connecting tubing is more significant if the chemiluminescent reaction is fast. In this work, a 2-cm connecting tubing was used as the shortest possible connection.

Similar to the effect of the length of the connecting tubing, the flow rate of luminol-rate modifier mixture influences the intensity of chemiluminescence. Both connecting tubing and flow rate affect the residence time of the hydrogen peroxide plug in the stream of carrier solution. A longer residence time makes hydrogen peroxide to be consumed more before it reaches the flow cell. Therefore, a fast flow of carrier solution is desired in order to reduce the residence time and thus obtain higher chemiluminescence intensity. For example, the chemiluminescence intensity of luminol-H_2O_2-CoPc reaction increased from 0.707 V at 1.8 ml/min to 3.904 V at 4.5 ml/min of reagent mixture. The intensity is increased almost 6 times, while the flow rate is only increased 3 times. However, a further increase in flow rate resulted in a slight increase in signal but a much more consumption of reagents and sample. The flow rate of the luminol-CoPc mixture giving the best signal was 4.5 mL/min, while the flow rate of hydrogen peroxide was 2.4 mL/min. For the slow luminol-H_2O_2-$Fe(CN)_6^{3-}$ reaction, the flow rate of carrier solution also affected the chemiluminescence intensity, but not as much as for the fast luminol-H_2O_2-CoPc reaction. The noise level was not affected by the flow rate of the reagents.

The sample size, which is determined by the length of the injection valve loop, has different effects on fast and slow chemiluminescent reactions. For the fast luminol-H_2O_2-CoPc chemiluminescent reaction, a 14.7-cm long sampling loop was found to be the best, which gave a chemiluminescence intensity 4 times more than a 6-cm long loop, while a 7.6-cm long sampling loop was the best for the slow luminol-H_2O_2-$Fe(CN)_6^{3-}$ reaction. Although a loop shorter than 7.6 cm gave lesser intense chemiluminescence when $Fe(CN)_6^{3-}$ was used as rate modifier, the shape of the signal peak was almost symmetric. Longer sampling loops did not increase the intensity but decreased it,

because the signal peak was split in double or even triple peaks in such cases. In general, the intensity vs. time profile became wider as the sample size increased.

A regular convection movement is introduced by rotating the disk in the flow cell. It helps with thorough and rapid mixing of reactants, resulting in great increase in reaction rate and therefore a dramatic increase in chemiluminescence emission. Figure 3.6 illustrates the effect of disk rotation on the chemiluminescence of the luminol-H_2O_2-CoPc reaction. When the disk was static, mixing of hydrogen peroxide and reagents was accomplished only by the natural hydrodynamic force caused by pumping. This process is slow and incomplete. In such cases, the signal was only 0.496 V for the injection of 8.8×10^{-6} M H_2O_2 and the shape of the signal peak exhibited the characteristics of a slow chemiluminescent reaction: slowly rising and slowly decaying. After the disk began to rotate, the intensity increased with an increase in the rotation velocity until 664 rpm was reached; at this point, the chemiluminescence intensity was 3.808 V, which is 8 times greater than 0.496 V, and the signal peak was very sharp. A further increase in rotation velocity did not increase the intensity beyond this maximum value. A similar phenomenon was observed for the slow luminol-H_2O_2-$Fe(CN)_6^{3-}$ reaction, but a much slower disk rotation (only 258 rpm) brought about the maximum intensity, which was 3 times stronger than when there was no rotation.

Quantitative measurements made with this operational mode can be based on the height of the signal peak. Under optimum conditions, such as sample size, flow rate, and disk rotation velocity, and when the sample injection was carefully controlled, the relative standard deviation (RSD) for 11 measurements of 8.8×10^{-6} M H_2O_2 was 1.6%. It was found that the reproducibility of measurement, in a great extent, is dependent on the reproducibility of the operation of the sample injection valve. The RSD might be as high as 7.2% if care is not taken to operate the injection valve appropriately. Good reproducibility can be ensured by using an automatic sample injection valve. However,

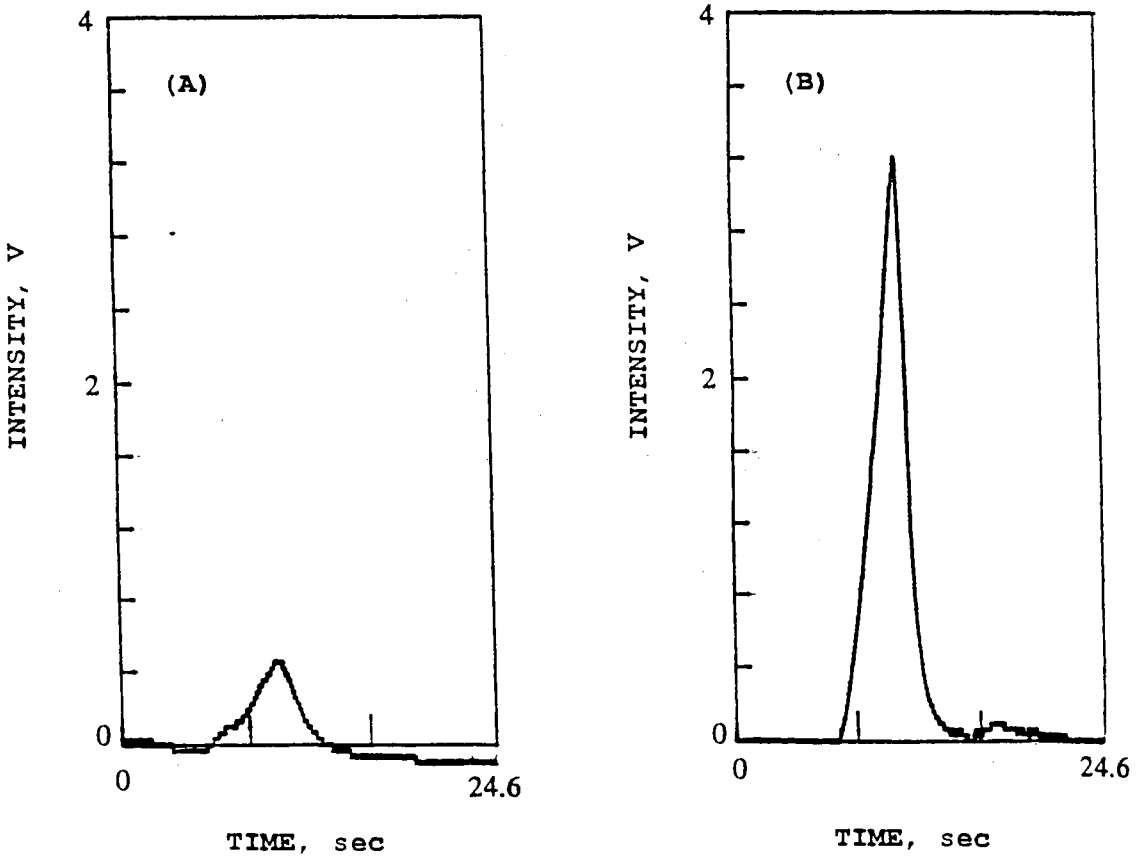

Figure 3.6. Signals illustrating the effect of disk rotation using CoPc as rate modifier and using the flow sample-injection strategy of Figure 3.5A. A: no rotation. B: disk rotating at 664 rpm. Other experimental conditions as described in text.

sensitivity may be lost because the connecting tubing between valve and flow cell cannot be as short as that used with a manually operated sample injection valve.

Continuous Sample feeding Operational Modes (Figure 3.5B and 3.5C). In these modes, one pump is used to deliver both reagents and hydrogen peroxide solutions. The reagents and hydrogen peroxide are continuously sent through the flow cell until a steady state signal is reached. The typical chemiluminescence intensity vs. time profile is shown in Figure 3.7 and Figure 3.8. Before making measurements, the reading on the oscilloscope was adjusted to zero by changing the impedance of resistor R_1 shown in Figure 3.3 when water was introduced into the flow cell instead of hydrogen peroxide.

When the flow cell was operated as illustrated in Figure 3.5B, two separate inlets were used to deliver reagents and hydrogen peroxide, respectively. The mixture of luminol and rate modifier was sent to the flow cell through one inlet and hydrogen peroxide through the other one. This prevents the chemiluminescent reaction from taking place before it gets in front of the detector because hydrogen peroxide and reagents did not meet until they entered the flow cell.

Without the rotation of the disk, the hydrogen peroxide and reagent solutions are only mixed by the hydrodynamic motion caused by the flow. This was found to be quite ineffective as the chemiluminescence intensity for 8.8×10^{-6} M H_2O_2 and CoPc as rate modifier was almost zero even at the highest flow rate the pump could deliver (6.6 ml/min for luminol-CoPc mixture and 3.4 ml/min for hydrogen peroxide). As with any of the configurations used in this work, the effect of rotation was pronounced. As can be seen from Figure 3.7, the intensity of chemiluminescence increases with an increase in the rotation velocity of the disk. The intensity was only 0.80 V at 58 rpm, 2.40 V at 258 rpm, and 11.36 V at 874 rpm.

The flow rate also affects the signal level. As expected, a fast flow will give strong chemiluminescence because it increases the flow per unit time of hydrogen peroxide and reagents into the cell. For example, at 874 rpm, the signal was only 1.76 V

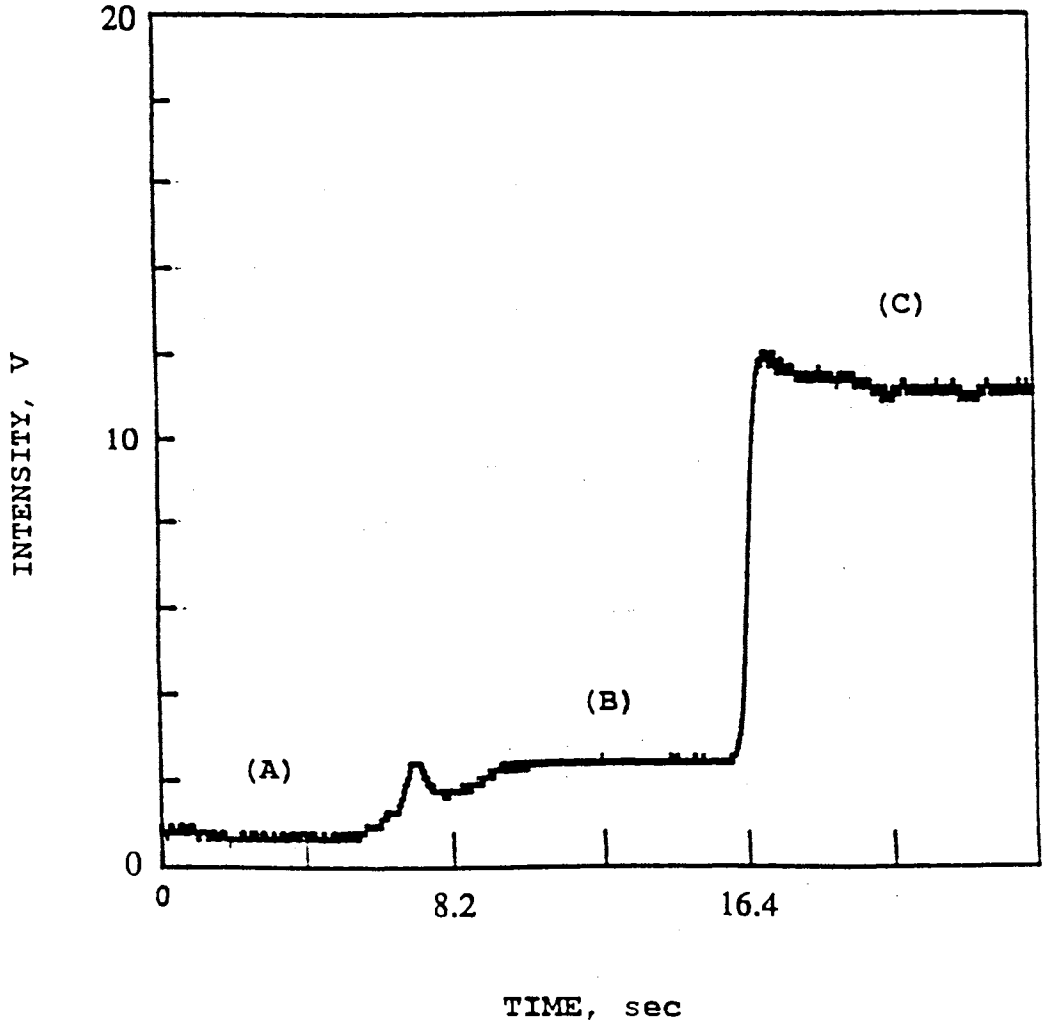

Figure 3.7. Effect of disk rotation velocity using CoPc as rate modifier and using the continuous sample feeding strategy of Figure 3.5B. A: 58 rpm. B: 258 rpm. C: 874 rpm. Other experimental conditions as described in the text.

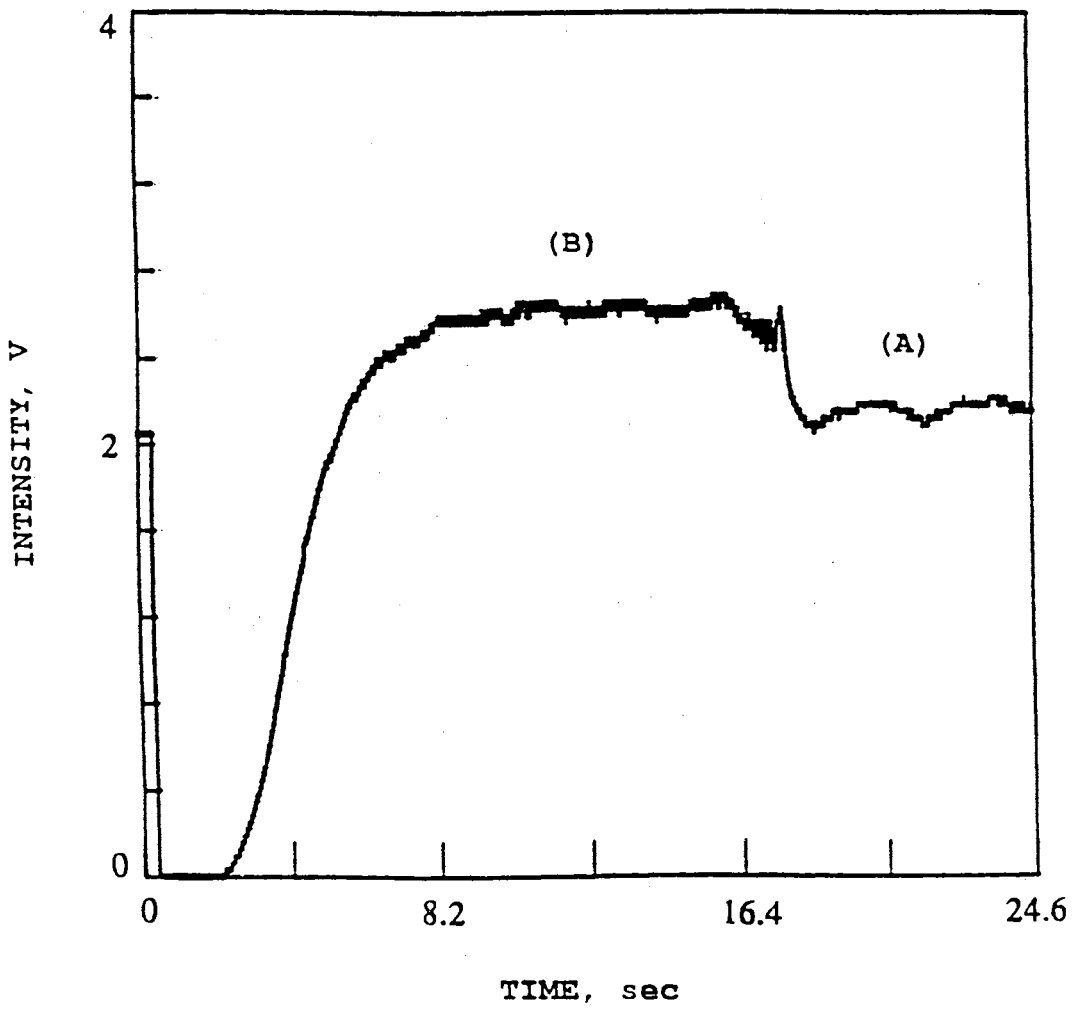

Figure 3.8. Effect of disk rotation using $Fe(CN)_6^{3-}$ as rate modifier and using the continuous sample feeding strategy of Figure 3.5C. A: no rotation. B: 452 rpm. Other experimental conditions as described in the text.

for 8.8 x 10^{-6} M H_2O_2 when the flow rates were 1.2 ml/min for reagents and 0.6 ml/min for hydrogen peroxide. Under the same conditions, the intensity was increased 5 times to 8.32 V when the flow rates of luminol-rate modifier mixture and hydrogen peroxide increased to 3.9 ml/min and 2.0 ml/min, respectively. Moreover, a low flow rate creates noise problem. The noise level was observed to decrease with an increase in the flow rate until 3.9 ml/min of reagent mixture and 2.0 ml/min of hydrogen peroxide were achieved. After that, the noise level did not change appreciably.

In the continuous sample feeding operational mode represented in Figure 3.5C, luminol and rate modifier were mixed together at one T-piece just before mixing with hydrogen peroxide at another T-piece mixing element. Then, the mixture traveled through a short connecting tube before entering the flow cell. The tube connecting the T-piece and the flow cell should be as short as possible because the chemiluminescent reaction takes place immediately after the reagents and hydrogen peroxide are mixed at the second T-piece. This tube was 1-cm long in the experiments described here. Obviously, a fast flow results in a better yield in chemiluminescence detection because it reduces the loss of chemiluminescence outside the view of the detector as well as it helps to thoroughly mix the reactants. In the experiments, the highest flow rates that the pump could deliver (6.6 ml/min for luminol-rate modifier mixture and 3.5 ml/min for hydrogen peroxide solution) provided the best conditions. Disk rotation, however, resulted in different effects with the fast chemiluminescent reaction (CoPc as rate modifier) and the slow reaction ($Fe(CN)_6^{3-}$ as rate modifier). For the fast reaction, rotation caused a decrease rather than an increase in the intensity. For example, without rotation the chemiluminescence intensity was 13.28 V for 8.8 x 10^{-6} M H_2O_2, but when the disk rotated at 664 rpm, the intensity decreased by 10.8% to 11.84 V. The noise level, on the other hand, decreased with the rotation by 11.9%; and, on average, the signal-to-noise ratio was about the same with or without rotation. For the slow luminol-H_2O_2-$Fe(CN)_6^{3-}$ reaction, the rotation of the disk increased the signal some. As shown in Figure 3.8, the

rotation at 452 rpm produced a signal 21% larger than without rotation, increasing the chemiluminescence intensity from 2.166 V to 2.624 V. Fast rotation also reduced the noise level.

The signal-to-noise ratio could be improved by using two pumps to deliver the luminol-rate modifier mixture and the hydrogen peroxide solution separately. This could greatly reduce the noise level because the pulsation caused by the flow, the major cause of the noise, may be reduced. The reproducibility of measurements made with these two operational modes is not as good as that with the operational mode of Figure 3.5A. The RSDs for 11 measurements of 8.8×10^{-6} M H_2O_2 were 2.1% and 2.4% for the operational modes shown in Figure 3.4B and 3.4C, respectively.

Stopped-Flow Operational Mode (Figure 3.5D). Two pumps are required for the stopped-flow operation: one for the hydrogen peroxide solution and the other for the mixture of luminol and rate modifier. The pump for the delivery of hydrogen peroxide must have a time-control device so that the injection time of hydrogen peroxide, i.e., the amount of hydrogen peroxide injected into the flow cell, can be controlled precisely. A microprocessor control unit, the FIAtron SHS-200, was used for such a purpose.

In order to make a measurement, the pump continuously delivering the mixture of luminol and rate modifier was stopped when the flow cell was filled; then, a predetermined volume of hydrogen peroxide solution was introduced in the flow cell via the other pump. The amount of hydrogen peroxide was determined by the time during which the pump was switched on. The rotation of the disk provided the mixing of reactants. Before making another measurement, the pump delivering hydrogen peroxide was turned on for about 20 seconds so that the solution remaining in the tubing was flushed out and fresh hydrogen peroxide was ready to be delivered into the flow cell. Then, the pump delivering the reagent mixture (luminol + hydrogen peroxide) was turned on until the flow cell was again filled with the mixture of luminol and rate modifier. The

typical signal recorded as chemiluminescence intensity vs. time profile is shown in Figure 3.9.

In this operational mode, the flow rate of reagent mixture does not affect the intensity of chemiluminescence, but the flow rate of the hydrogen peroxide solution does. For example, the intensity of chemiluminescence produced by the luminol-H_2O_2-CoPc reaction increased 7 times when the flow rate of hydrogen peroxide was increased from 1.0 ml/min to 5.5 ml/min. This is because the chemiluminescence intensity is proportional to the concentration of hydrogen peroxide in the flow cell. A higher flow rate brings more hydrogen peroxide into the flow cell per unit time, and this results in a higher concentration of hydrogen peroxide. The flow rate can be increased by increasing the pump speed and/or using a sampling tubing with larger diameter. These two strategies can be adopted at the same time in order to obtain the best result. However, pump speed cannot be increased indefinitely and the sampling tubing diameter that can be used is limited by the design of the pump. Thus, the time for the injection of hydrogen peroxide has to be prolonged to deliver enough hydrogen peroxide to react with reagents completely. Experimental results showed that the chemiluminescence intensity increased with the increase in the injection time, and a maximum intensity was reached at certain point which was mainly determined by the concentration of luminol. A further increase in the injection time resulted in excess hydrogen peroxide to be added to the flow cell in a short time, diluted reagent mixture in the flow cell, and therefore lowered the chemiluminescence intensity. The flow rate and injection time of hydrogen peroxide have the same effect on both slow and fast chemiluminescent reactions. For example, the chemiluminescence intensity of luminol-H_2O_2-$Fe(CN)_6^{3-}$ reaction increased 32% when the injection time increased from 1 second to 3 seconds at a flow rate of 5.5 ml/min, while under the same conditions the increase was 28% for luminol-H_2O_2-CoPc reaction.

Just as in most of the other operational modes discussed in this chapter, disk rotation enhanced the chemiluminescence for both fast and slow reactions. For a slow

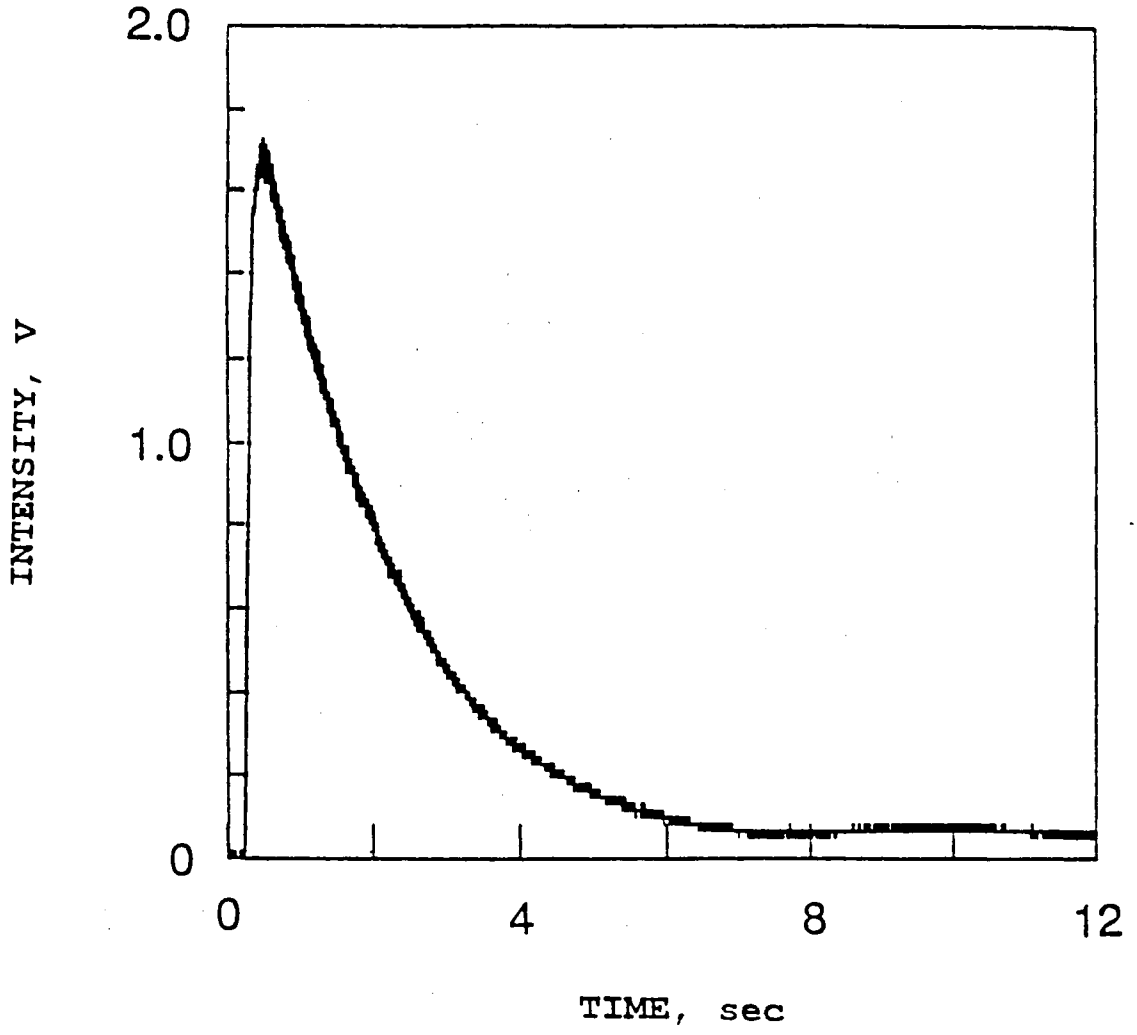

Figure 3.9. Signal profile obtained by using the stopped-flow mode of Figure 3.5D, showing the entire signal profile for the chemiluminescence of the luminol + H_2O_2 reaction using $Fe(CN)_6^{3-}$ as rate modifier. Disk rotation velocity: 874 rpm. Luminol: 5.0 x 10^{-3} M, pH 13.00. H_2O_2: 8.8 x 10^{-3} M. $Fe(CN)_6^{3-}$: 1.0 x 10^{-5} M

chemiluminescent reaction utilizing $Fe(CN)_6^{3-}$ as rate modifier, the rotation velocity should be greater than 258 rpm because slower rotation creates noise problems. At optimum rotation velocity, the enhancement in chemiluminescence caused by disk rotation is greater for the fast reaction than for the slow one.

Quantitative measurements made with the stopped-flow operation can be based on the height of the signal peak. Compared to other operational modes shown in Figure 3.5, the stopped-flow operation has the best measurement reproducibility: the RSD for 11 measurements with a 8.8×10^{-6} M H_2O_2 was 1.4%.

The stopped-flow operation makes it possible to record the intensity vs. time profile for the whole chemiluminescence process (as an example, see Figure 3.9). As a result, the kinetic parameters such as reaction order and rate coefficient can be determined. In order to obtain this type of profile precisely representing the kinetic behavior of the chemiluminescence process, the hydrogen peroxide and the reagent mixture solutions must be mixed instantaneously. Hence, the injection time of hydrogen peroxide, especially for the fast chemiluminescent reaction, should be as short as possible and the speed of the pump for hydrogen peroxide injection needs to be slowed down to prevent hydrogen peroxide from being injected into the flow cell by the inertial movement of the pump after it is stopped. Attention should be paid to the effect of the rotation velocity on the smoothness of the curve. Noise caused by the slow rotation makes the curve not smooth and therefore affects the precision of kinetic measurements. Consequently, the rotation velocity of the disk should be as high as possible.

Determination of Quantum Yield. The intensity vs. time profile obtained by the stopped-flow operation is very useful for the determination of the quantum yield (Φ_{CL}) of a chemiluminescent reaction. As discussed in Chapter II, the total number of photons emitted from the reaction must be known in order to calculate Φ_{CL}. To determine this quantity, integration under the chemiluminescence intensity vs. time profile is required

because the integrated area (A) is proportional to the total number of photons emitted during the reaction (N) [18]. The equation relating these two quantities is:

$$N = K \, x \, A$$

where K is a proportional constant which is determined by the dimension of the flow cell, detector sensitivity, and signal amplification. It can be calculated by measuring the chemiluminescence emission area of a standard emitter solution for which the total number of photons emitted from the reaction is known. Lee and Seliger have reported such a luminol standard [19, 20]. It is the reaction between luminol and hydrogen peroxide at pH 11.6 catalyzed by hemoglobin. Lee and Seliger reported this solution emitted $(9.75 \pm 0.7) \times 10^{14}$ photons/unit-absorbance/ml, but a recent determination by the National Institute of Standards and Technology yielded a value of $(10.1 \pm 0.5) \times 10^{14}$ photons/unit-absorbance/ml [21].

The integration is accomplished by using the electronic circuit shown in Figure 3.10. It consists of a chopper-stabilized operational amplifier (OP), a resistor (R), a capacitor (C), and a two-way switch (SW). The input (V_i) of this circuit was the output signal from the auxiliary electronics of Figure 3.3 and its output (V_o) was sent to the oscilloscope for display. The signal is integrated vs. time only when the switch is set at position (a). The output V_o is zero when it is at position (b). The relationship between the integrated area (A) and V_o is:

$$A = -(RC)V_o$$

The resistance of R needs to be adjusted for different input signals so that the output is kept in an adequate range for recording.

Use of the Rotating Disk as Carrier for an Immobilized Reagent. The rotating disk, besides aiding in mixing reactants, provides an ideal surface for support of immobilized reagents. It is especially useful for the immobilization of a real catalyst which can be used repeatedly. Compared to two commonly used methods for the immobilization of rate modifier, packing the immobilized rate modifier in a piece of glass

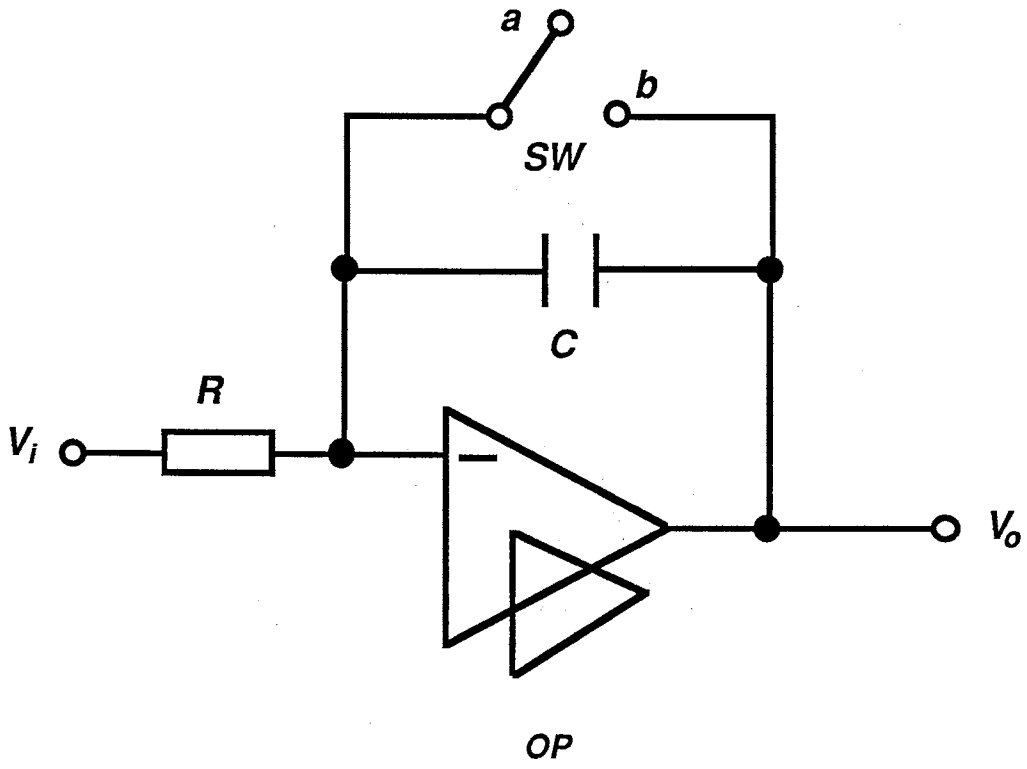

Figure 3.10. Electronic circuit used for integration. OP: operational amplifier (MeKee Pederson, Danville, CA, Model MP-1031). R: resistor, 100 ~ 1000 Ω. C: capacitor, 1.0 μF. SW: two-way switch; position (a), integration; position (b), reset. V_i: input. V_o: output.

tube or immobilizing rate modifier on a membrane [22-24], immobilization of the rate modifier on the rotating disk provides a higher detection efficiency because it permits the reaction to take place only inside the cell and only until the sample and reagents are in contact with the immobilized rate modifier. Moreover, it also provides a higher reaction efficiency because the convection introduced by the rotation of the disk facilitates contact between the reagents in the solution and the immobilized rate modifier on the disk.

Figure 3.11 shows the signal profile obtained by using the immobilized CoPc on the disk and using the flow sample injection operational mode. Cobalt(II) phthalocyanine is only slightly soluble in water and can be easily immobilized on controlled-pore glass (CPG). The procedure used to attach the CPG-immobilized CoPc to the disk was similar to the one described earlier [16] using a double-sided tape. The effect of disk rotation is pronounced in this case. Without rotation, practically no chemiluminescence was observed for 8.8×10^{-6} M H_2O_2; but when the disk carrying the immobilized CoPc was rotated at 452 rpm, the chemiluminescence intensity resulted in a signal of 164 mV. The enhancement of chemiluminescence by the rotation is notable.

Signal-to-Noise Ratio

Table 3.1 compares the signal-to-noise ratios observed with the different configurations discussed in this chapter. As can be seen, the stopped-flow mode offers the highest signal-to-noise ratio. However, it is more complicated in operation than the other modes discussed here. The continuous sample feeding operation with two inlets (mode B) has good signal-to-noise ratio, but the consumption of reagents and sample with this mode is relatively large as it has to be operated at very high flow rates. Another continuous sample feeding operation, mode C, has the worst signal-to-noise ratio because the noise level is very high. The flow sample-injection seems to be the best operational mode because in addition to a good signal-to-noise ratio, it has relatively simple

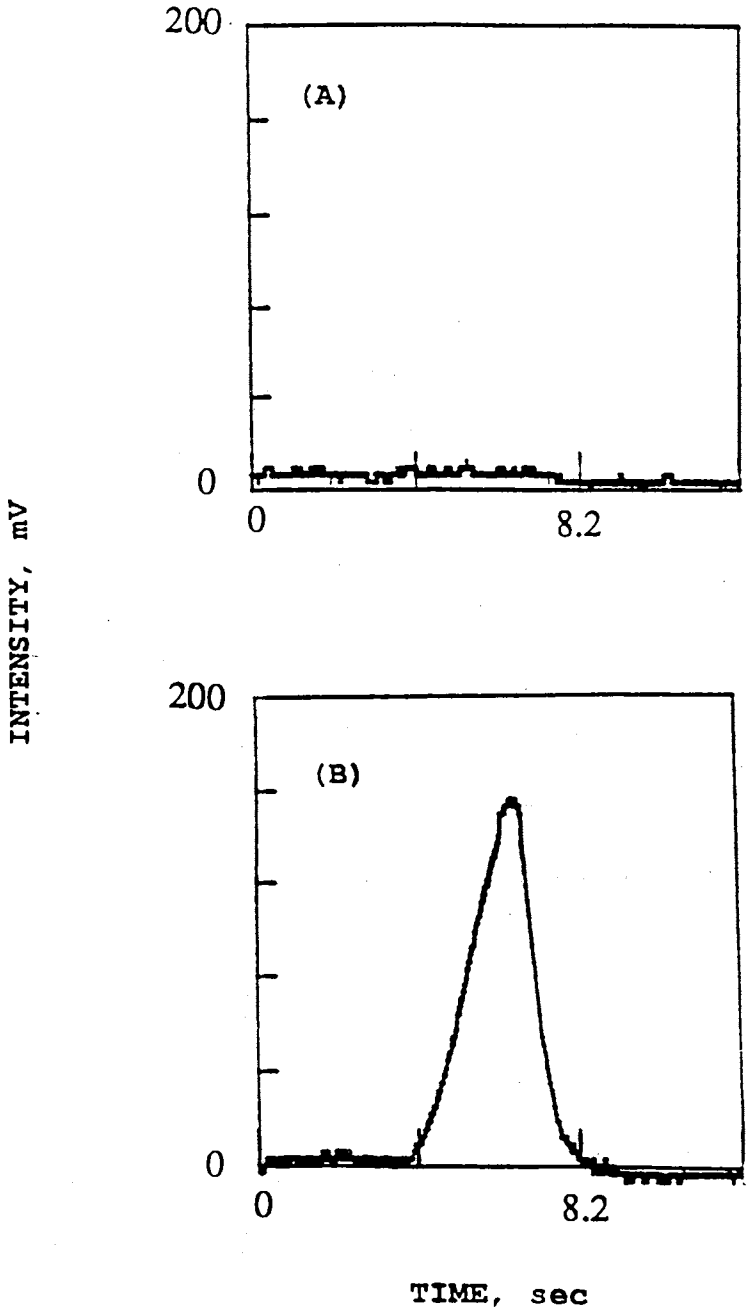

Figure 3.11. Signal illustrating the effect of disk rotation using CoPc as rate modifier immobilized on controlled-pore glass and using the flow sample-injection strategy of Figure 3.5A. A: no rotation. B: disk rotates at 452 rpm. Luminol: 5.0 x 10^{-3} M. H2O2: 8.8 x 10^{-6} M.

operation and consumes not so much reagents and sample when compared with the continuous sample feeding operations.

Table 3.1. Comparison of signal-to-noise ratios

op. mode[a]	(A)	(B)	(C)[b]	(D)
signal(mV)[c]	3094	14500	13280	12480
noise(mV)	4	12	45	4
signal/noise	976	1208	295	3120

(a) See Figure 3.5 for the configuration of each operational mode
(b) Without rotation.
(c) Concentration of H_2O_2 is 8.8×10^{-6} M.

Reduction of Pump Pulsation

The noise level is greatly increased by pump pulsation, especially in the continuous sample feeding operations. The level of noise can be as high as several hundred millivolts instead of several millivolts, if the pulsation is not suppressed. Pulsation is a periodical fluctuation in the flow rate because of the change in the pushing force created by the rotation of the head of the peristaltic pump. The extent of pulsation depends on the construction of the peristaltic pump head and the quality of the tubing. Pulsation cannot be completely eliminated, but it can be considerably reduced by adding a simple dampener consisting of a T-piece (one inlet for flow in, a second for flow out, and the third for the pulsation dampening as shown in Figure 3.12) and a piece of 30-cm Tygon tubing with an i.d. of 2.5 mm. One end of this tubing is connected to the third inlet of the T-piece. The other end is clamped before starting operation of the pump, trapping air inside the tubing. The air chamber, acting as a buffer for the change in pushing force, reduces pulsation considerably. In the flow system described in this chapter, each delivery tube was equipped with this simple device.

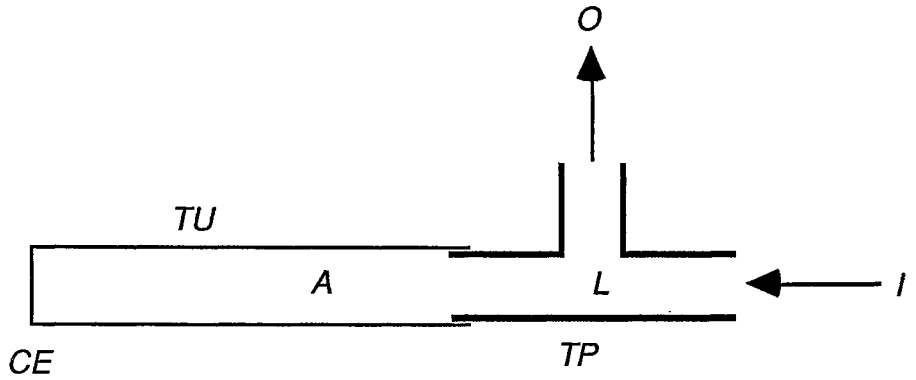

Figure 3.12. A dampening device for pump pulsation. TU: a piece of Tygon tubing. CE: the clamped end of the tubing. A: air trapped inside the tubing. TP: T-piece. I: the inlet of the T-piece for solution in. O: the inlet of the T-piece for solution out. L: liquid inside the T-piece.

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CHAPTER IV

CHARACTERISTICS OF CARBON DIOXIDE-ENHANCED CHEMILUMINESCENCENT REACTION OF LUMINOL IN THE ABSENCE OF ADDED OXIDANT

Introduction

Since it was first reported by Albrecht [1] in 1928, the chemiluminescent reaction of luminol with an oxidant (hydrogen peroxide in particular) has been studied extensively [2-4] and applied for the determination of various inorganic and organic species [5]. In actuality, this is the most frequently applied indicator reaction for metal ion determination based on the promotion of chemiluminescence (see Chapter II for details). At least 20 metal ions increase the rate of chemiluminescence for this reaction with limits of detection ranging from 10^{-8} to 10^{-11} M [6]. These low detection limits make the system interesting for pollution studies, analysis of biological materials, and metal ion detection in liquid chromatography.

Certain metal ions and metal-containing species, however, have been reported to enhance luminol chemiluminescence in aqueous solution in the absence of hydrogen peroxide or any other added oxidant. These include Fe(II) [7-9], Co(II) [8], Cu(II) [8], $Fe(CN)_6^{3-}$ [10-13], ferrioxalate [14], some metal-porphyrin complexes [15-18], V(II) [7], V(IV) [19], $AuCl_4^-$ [20], $SbCl_6^-$ [21], MnO_4^- [22], and Mn(II) (in the presence of high concentration of sodium chloride) [23]. Non-metal anionic species, such as OCl^-, also act as promoter in the presence of hydrogen peroxide. In the absence of hydrogen peroxide, however, OCl^- also reacts with luminol [24], and a detailed study of interferences and

optimization of the reaction has shown that it is suitable for the routine determination of OCl^- in drinking water [24].

As with most chemiluminescence systems, the mechanistic knowledge of the luminol chemiluminescent reaction is incomplete either in the presence of or in the absence of added oxidant. Over the years, a number of unusual and puzzling observations on the chemiluminescence of luminol have been reported. For example, Behrens *et al.* [25] reported that merely acidifying luminol in an alkali metal hydroxide solution yields light and White and Brundrett [26] noticed that the chemiluminescence emission is much stronger if hydrogen peroxide is present. Metallic impurities in the acid and dissolved molecular oxygen acting as oxidant could explain this observation. Althwarthan and Townshend [27], however, observed that after total exclusion of oxygen by bubbling nitrogen gas through the reaction mixture and enclosing the apparatus in a nitrogen-filled box (to prevent the diffusion of air *via* the plastic tubing used in their continuous-flow setup), light emission was reduced by only 28%. This led them to conclude that "molecular oxygen is unlikely to be the oxidant responsible for the chemiluminescent reaction". They also reported that addition of titanium(III) or iron(II) to a carbonate buffer of pH 10.4 (no luminol present) gave appreciable chemiluminescence (even in solution deaerated by bubbling nitrogen gas). They suspected the metallic species, acting as reducing agents, reduce molecular oxygen or traces of peroxide ions in the carbonate buffer. Possible glassware or plasticware contamination with luminol is not mentioned. The overall picture gets more complex with the finding that luminol is a mixture of five components and not a pure compound, and that different commercial sources provide different types of the "reagent" [28].

The effect of carbon dioxide on the chemiluminescence of luminol is another example of these intriguing observations. If a rapid stream of carbon dioxide gas is passed through a basic solution of luminol containing sodium chloride, hydrogen peroxide, and manganous chloride, the intensity of chemiluminescence emitted passes through four

maxima before the reaction ceases as the pH of the reaction mixture drops to 8 ~ 9 [28]. A similar enhancement effect of carbon dioxide on the luminol chemiluminescence was mentioned by Smith and co-workers when using hydrogen peroxide as oxidant and cobalt(II) as rate modifier [29], but no further work has been done in this field.

During the study on the chemiluminescence of luminol-hydrogen peroxide system stimulated by cobalt(II) phthalocyanine (CoPc), it was noticed that a hydrogen peroxide solution prepared with carbon dioxide-containing water (by bubbling carbon dioxide) gave much stronger chemiluminescence than the hydrogen peroxide solution of the same concentration prepared with plain water. Even in the absence of hydrogen peroxide, very intense light emission was detected when water with carbon dioxide was injected into the mixture of CoPc and luminol. A similar phenomenon was observed when Co(II) was used as rate modifier instead of CoPc. Bubbling carbon dioxide into the mixture of Co(II) (10^{-7} M) and luminol solutions in a strong alkaline medium resulted in intense light emission; the light was even visible in a partially darken room.

Because the effect of carbon dioxide on the chemiluminescence of luminol in the absence of any added oxidant had not been previously reported, and in consideration of its potential analytical applications, this phenomenon has been further investigated. The findings of this study are reported in this chapter.

Experimental Section

Reagents and Solutions

The water used for preparation of solutions was treated as described in Chapter III. The solutions of CoPc and $Fe_3(CN)_6^{3-}$ were as described in Chapter III.

The luminol used in this study was obtained from Aldrich (Milwaukee, WI) instead of Lancaster Synthesis (Windham, NH) because the batch-to-batch reproducibility of the luminol from Lancaster was not acceptable. It was prepared as a 5.0×10^{-3} M solution in

0.10 M potassium hydroxide (EM SCIENCE, Cherry Hill, NJ) and its pH was adjusted to the desired values with the same potassium hydroxide solution. This unbuffered solution of luminol was used in this study unless otherwise stated. Although sodium hydroxide was available with fewer metallic impurities than potassium hydroxide, the latter was used because of the much smaller alkaline error of the glass electrode towards K^+ rather than Na^+. The highest pH used in this work was 13.50, and by using as reference the Na^+ error for Sensorex electrode used in this work and the average relationship between the sodium and potassium errors [52], the expected corrections for our measurements ranged from 0.00 to -0.05 at pH 12.00 and pH 13.50, respectively. Considering an average uncertainty in pH measurements of ±0.02, the correction can be practically ignored except at pH 13.50.

For the study of the effect of buffer, two other solutions of luminol with the same concentration were prepared in 0.10 M phosphate buffer and 0.10 M carbonate buffer, respectively. All luminol solutions were allowed to stand for at least three days to stabilize before use.

Stock solutions of cobalt(II) and iron(II) (1.0×10^{-3} M) were prepared in 0.10 M hydrochloric acid (Fisher Scientific, Fair Lawn, NJ) from $Co(NO)_2.6H_2O$ (EM SCIENCE, Cherry Hill, NJ) and $Fe(NH_4)_2(SO_4)_2.6H_2O$ (Fisher Scientific, Fair Lawn, NJ), respectively. The stock solution of iron(III) (1.0×10^{-3} M) was prepared in 0.050 M sulfuric acid (Mallinckrodt, Paris, KY) from $Fe(NO_3)_3.9H_2O$ (Fisher Scientific, Fair Lawn, NJ). These stock solutions were stable for two weeks. The working solutions of these metal ions were prepared by diluting the stock solutions immediately before use. The acidities of cobalt(II), iron(II),and iron(III) solutions were provided by 1.0×10^{-4} M HCl, 1.0×10^{-3} M HCl, and 5.0×10^{-3} M H_2SO_4, respectively. The stock solution (1.0×10^{-3} M) and working solution of cobalt(II) *meso*-tetrakis(4-sulfonatophenyl)porphine ($CoTPPS_4$) (Midcentury, Posen, IL) were prepared in purified water.

Carbon dioxide was either generated by chemical reaction of reagent grade $CaCO_3$ with reagent grade HCl or obtained from Sooner Airgas (Shawnee, OK). The same behavior was observed regardless of the source of carbon dioxide gas.

Apparatus

The instrument used for the detection of chemiluminescence emission was described in Chapter III [30]. Figure 4.1 schematically shows the reagent transportation systems employed in this research. In Figure 4.1A, the luminol enters the flow cell through *C2* and *C3*, while the rate modifier solution (0.18 ml) is injected into the stream of luminol via *C1*. In this way, the flow rate of luminol is twice that of the rate modifier solution; therefore less time is needed to fill the flow cell with luminol solution. Consequently, the consumption of the rate modifier solution is decreased. Figure 4.1B illustrate the stopped-flow operational mode used for the determination of kinetic parameters. In the stopped-flow operation, the cell was filled with luminol solution and the flow halted. Then, a small amount of rate modifier solution (5 ~ 10 μl) was rapidly injected into the flow cell with a syringe. The intensity vs. time profile was then recorded.

Cyclic voltammetric measurements were performed with a BAS-100 electrochemical analyzer (Bioanalytical Systems, West Lafayette, IN), and absorption spectra data were obtained with a Perkin-Elmer Lambda 3840 UV/Vis linear-diode-array spectrophotometer operated by a Perkin-Elmer 7300 computer.

The CoPc content of saturated solutions was calculated on the basis of the Co(II) determined by inductively coupled plasma emission spectrometry (ICP) with a Jarrel Ash ICAP 61 instrument after evaporation of the solution and mineralization (with HNO_3) of the CoPc.

All pH measurements were made with an Orion Research (Cambridge, MA) model 701A digital pH meter equipped with an epoxy-body combination electrode (Sensorex, Stanton, CA).

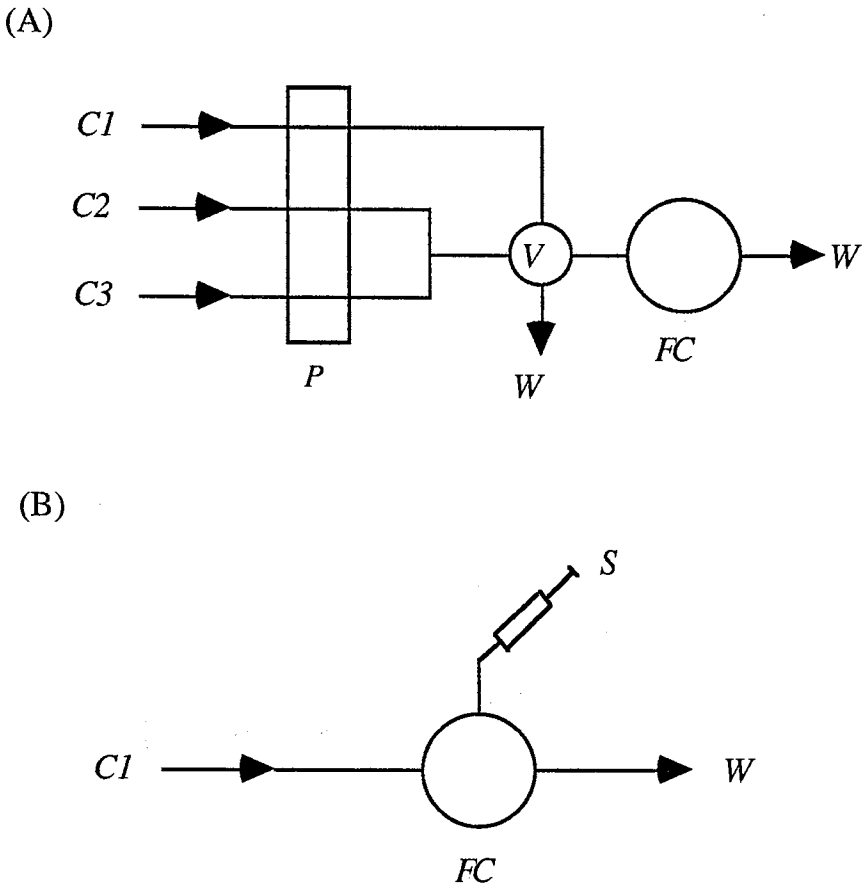


Figure 4.1. Schematic diagram of the reagent transport system. (A): Manifold for study of CO_2(g) effect. (B): Single-line manifold used to extract rate coefficients and for stopped-flow operation. C1 and C2: carrier lines for luminol solution. C3: carrier line for rate modifier. P: peristaltic pump, Gilson Minipuls 2 (Gilson Medical Electronics, Middleton, WI). SV: sample injection valve (Rheodyne type 50 four-way Teflon rotary valve). FC: flow cell (for details see Chapter III). S: syringe for injection of metal ion solution. W: to waste.

In the study of the effect of carbon dioxide, the solution containing the rate modifier was bubbled with $CO_2(g)$ for two minutes at 100 ml/min before injected into the flow cell.

Results And Discussion

Chemiluminescence Enhanced by Different Rate Modifiers

The effect of some metal ions and some metal complexes on luminol chemiluminescence were first examined in the absence of carbon dioxide as well as without any added oxidant. The species studied were Co(II), Fe(II), Fe(III), Cu(II), Mn(II), Cr(III), $Cr_2O_7^{2-}$, $Fe(CN)_6^{3-}$, CoPc, and $CoTPPS_4$, among which Co(II), Fe(II), Cu(II), Mn(II), Cr(III), and $Fe(CN)_6^{3-}$ have been known to be rate modifiers of the luminol chemiluminescent reaction in the absence of added oxidant [7-10]. Three species, Fe(III), CoPc, and $CoTPPS_4$, have not been reported to stimulate the chemiluminescence emission from luminol in the absence of added oxidant. Experimental results are summarized in Table 4.1, and are given as the relative chemiluminescence intensities (I_r) normalized to the weakest chemiluminescence emission observed with Cr(III). Cobalt(II) phthalocyanine is not very soluble in water and most common solvents, hence its effect is the result of a suspension of finely divided particles of CoPc as well as the dissolved CoPc (the soluble fraction was estimated as 0.35 by ICP determination). Therefore the relative intensity for CoPc is not listed in Table 4.1 because there is no basis for comparison. As can be seen from Table 4.1, hexacyanoferrate(III) produces the strongest chemiluminescence, while Cu(II), Mn(II), and Cr(III) cause relatively weak light emissions and $Cr_2O_7^{3-}$ stimulates no chemiluminescence. Intensity decreases in the order Fe(II) > Co(II) > Cu(II) > Mn(II) and parallels the observation given by Klopf and Nieman [8]. Cobalt(II) and cobalt(II)-containing species including CoPc and $CoTPPS_4$ all stimulate very strong light emission. Although the enhancement effect of Fe(III) is only 15% that of Fe(II), it is of interest to note that this species has been reported as inactive when hydrogen peroxide was used as oxidant unless diethylenetriamine was used as activator [37], and it also has been found as

Table 4.1. Optimum pH and relative intensity for luminol chemiluminescence caused by different rate modifiers in the absence and in the presence of carbon dioxide and in the absence of added oxidant.

rate modifier[a]	no CO_2 present		CO_2 present	
	I_r[b]	opt. pH	I_r'[b]	opt. pH
$Cr_2O_7^{2-}$	0	--	--	--
$Fe(CN)_6^{3-}$	205	13.0	1.0	13.0
Fe(II)	95	11.5	1.4	12.5
Fe(III)	14	12.5	2.5	12.8
Cu(II)	2	12.5	13.5	13.5
Mn(II)	2	12.5	44.6	13.2
Co(II)	90	11.8	86.8[c]	12.5
Cr(III)	1	12.0	112.0	12.5
$CoTPPS_4$	64	12.0	488.4[c]	13.0
CoPc	--	12.0	>500	>13.0

(a) The concentration of luminol solution (unbuffered): 5.0×10^{-3} M. Concentration of rate modifier is 1.0×10^{-5} M, except CoPc which was used as a 200-times dilution of a saturated aqueous solution with 0.10% ethanol and overall content of soluble and suspended CoPc equal to *ca.* 1.2×10^{-7} mole per liter.

(b) See text for the definition of I_r and I_r'.

(c) The comparison was made at a 1.0×10^{-7} M rate modifier concentration.

inactive in the absence of added oxidant [9]. The difference between these previous observations and the result obtained in this study seems to reside in a difference in pH conditions. In this work, no significant chemiluminescence emission was observed until the pH of luminol solution was greater than 11.50, while the pH in the previous reports were 11.4 [9] and in the range of 9.2 to 10.2 [37].

The effect of carbon dioxide on the luminol chemiluminescence was then examined in the presence of the same metal ions and complexes as rate modifiers. Under optimum pH conditions, the chemiluminescence intensity for each luminol/rate modifier/$CO_{2(g)}$ system was measured and compared with the intensity obtained in the absence of carbon dioxide. The magnitude of enhancement caused by carbon dioxide is expressed as I_r', which is equal to the ratio between the intensity obtained in the presence of carbon dioxide and the intensity obtained in its absence. The values of I_r' are also summarized in Table 4.1. As can be seen from Table 4.1, carbon dioxide causes an increase in chemiluminescence intensity with every rate modifier used in this study except $Cr_2O_7^{3-}$. The most dramatical enhancement is observed with CoPc, while the increase with $Fe(CN)_6^{3-}$ is so slight that it is negligible. The magnitude of enhancement (i.e., I_r') decreases in the order CoPc > $CoTPPS_4$ > Cr(III) > Co(II) > Mn(II) > Cu(II) > Fe(III) > Fe(II) > $Fe(CN)_6^{3-}$. When Co(II), Cr(III), CoPc, or $CoTPPS_4$ was used as rate modifier, the luminol chemiluminescence was very sensitive to the concentration of carbon dioxide in the solution of rate modifier; at the optimum pH, even a small amount of carbon dioxide produced a significant increase in chemiluminescence emission.

Effect of Carbon Dioxide on the Spectrum of Chemiluminescence Emission

Although the influence of carbon dioxide on the luminol chemiluminescence is pronounced, the spectrum of emitted light is not changed by its presence, and the maximum emission was observed at 425 nm which is the same as in the absence of carbon dioxide. This suggests that the light-emitting species is still the aminophthalate ion as has been

reported for other chemiluminescence systems using luminol [4]. The enhancement effect of carbon dioxide relies on its influence on particular steps of the overall chemiluminescence process. The presence of carbon dioxide changes the reaction conditions (e.g., optimum reaction pH and the concentrations of reactants), but does not change the species involved in the light emission process.

Effect of Carbon Dioxide on the Optimum pH for Chemiluminescence

The experiments to evaluate the effect of pH were carried out by adjusting unbuffered solutions of luminol to a given pH (by adding aqueous KOH or HCl). For each rate modifier, the intensity vs. pH profile was obtained both in the presence and in the absence of carbon dioxide gas and these are shown in Figure 4.2. It was noted that carbon dioxide enhanced the luminol chemiluminescence in the absence of added oxidant only when the pH of luminol solution was higher than a certain value; below such a critical value carbon dioxide greatly decreased the chemiluminescence intensity. This is illustrated in Figure 4.3 which shows the effect of carbon dioxide on the iron(II)-promoted chemiluminescence of luminol at two different pH values, one higher and the other lower than the critical pH of 11.90. At pH 12.98 the chemiluminescence intensity is increased by bubbling the solution containing Fe(II) with carbon dioxide gas, while at pH 11.60 the chemiluminescence intensity is decreased, and the longer the bubbling, the lesser intense is the chemiluminescence. The critical pH value is different for different rate modifiers. With an uncertainty of ± 0.10, the critical values are 11.30 for $CoTPPS_4$, 11.40 for Cr(III), 11.60 for Mn(II), 11.70 for CoPc, 11.80 for Co(II), 11.90 for Fe(II), 12.10 for Fe(III), 12.70 for $Fe(CN)_6^{3-}$, and 13.10 for Cu(II).

An associated effect of carbon dioxide on the optimum pH is to shift it to a more basic value compared to that observed in the absence of carbon dioxide. Hexacyanoferrate(III) is the only exception to such a trend. The extent of the change in optimum pH

Figure 4.2. Effect of pH on the chemiluminescence intensity in the presence of carbon dioxide (— • —) and in the absence of carbon dioxide (— o —) when different rate modifiers are used. [Luminol]: 5.0 x10^{-3} M.

(a) [Cr^{3+}]: 1.0 x 10^{-5} M; PMT voltage: -1100 V

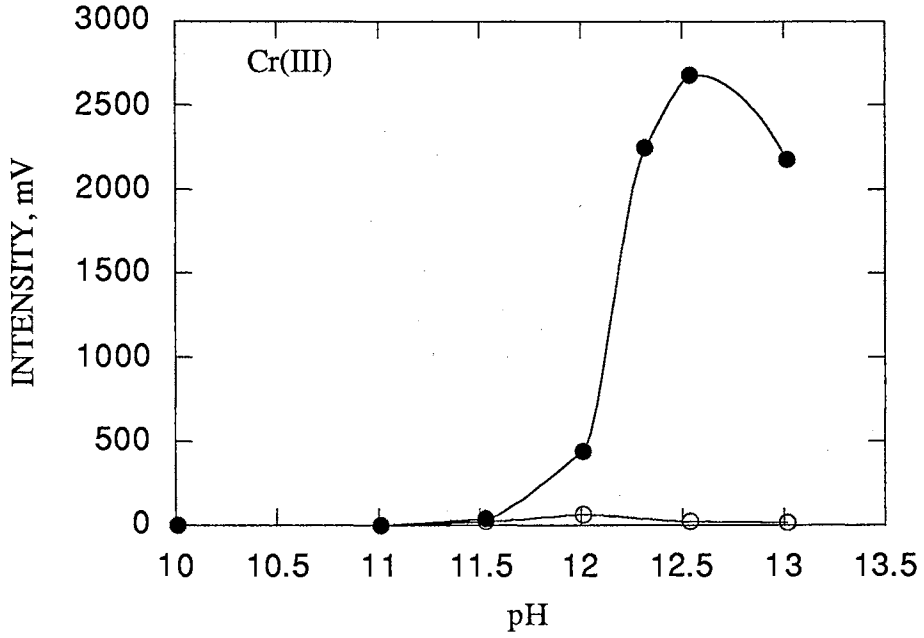
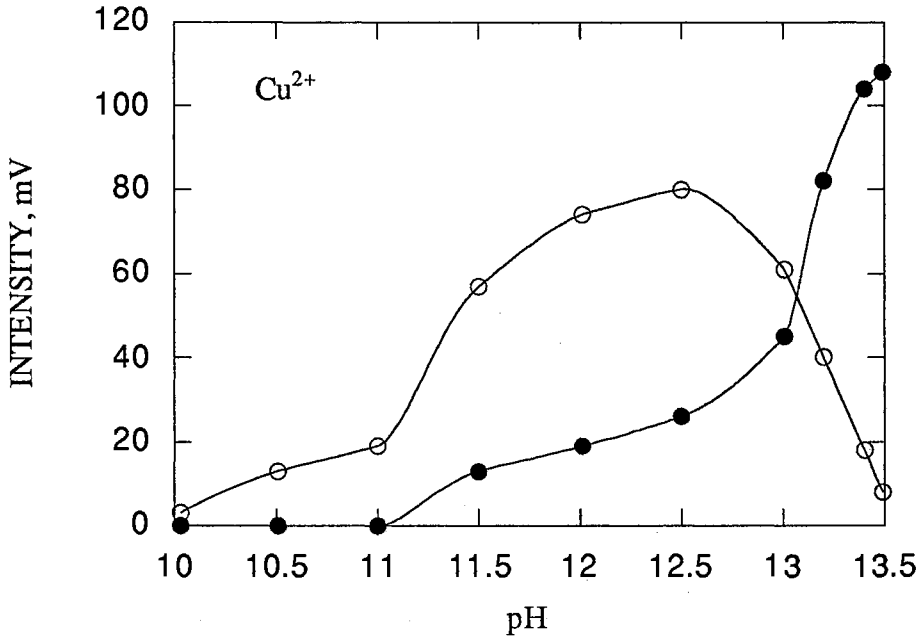


Figure 4.2 continued:

(b) [Cu^{2+}]: 1.0 x 10^{-5} M; PMT voltage: -1100 V



(c) [Mn^{2+}]: 1.0 x 10^{-5} M; PMT voltage: -1100 V

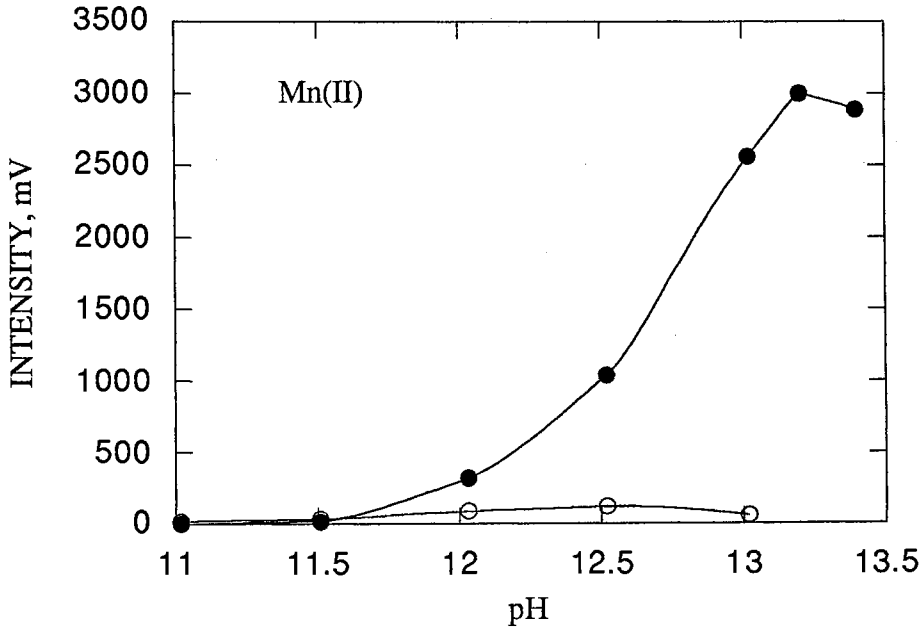
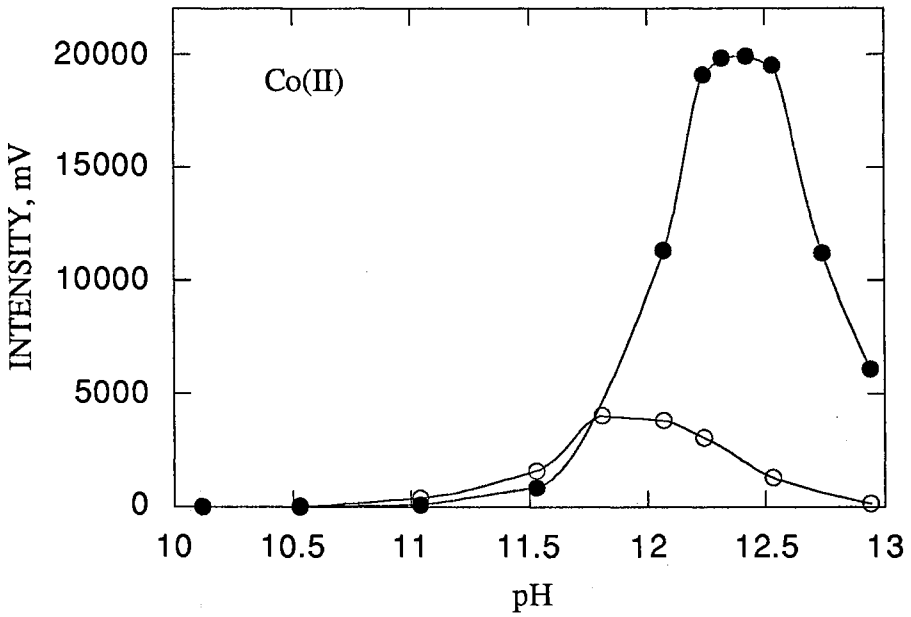


Figure 4.2 continued:

(d) $[Co^{2+}]$: 1.0 x 10^{-7} M; PMT voltage: -1000 V



(e) CoPc suspended in 0.1% ethanol; PMT voltage: -1000 V

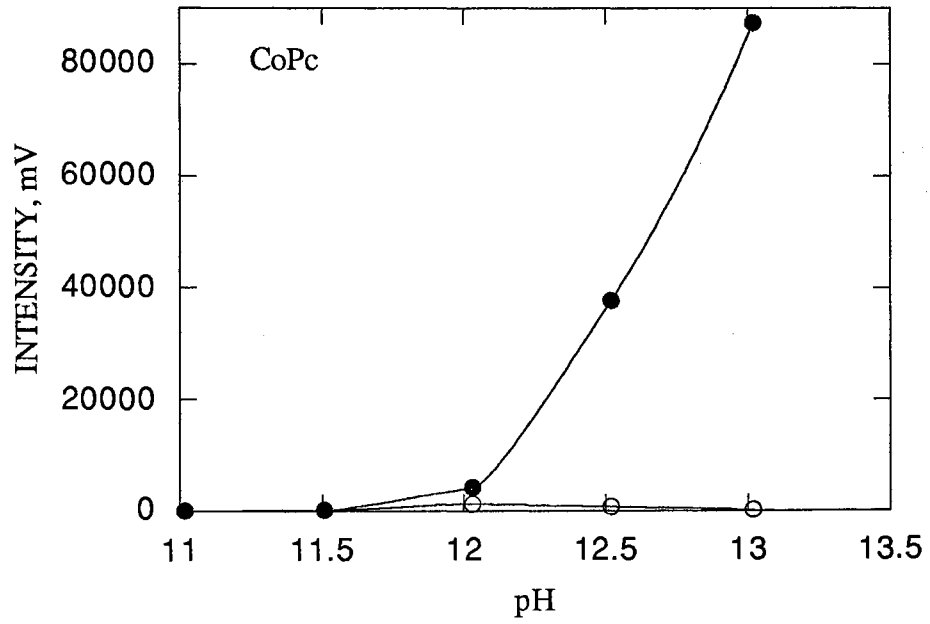
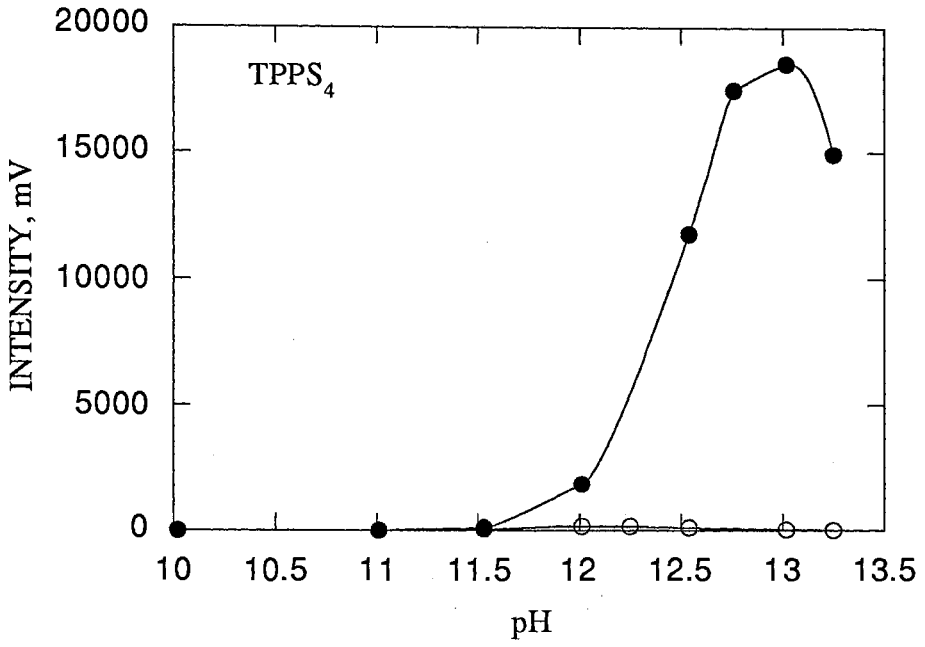


Figure 4.2 continued:

(f) [$CoTPPS_4$]: 1.0 x 10^{-5} M; PMT voltage: -1000 V



(g) [Fe^{3+}]: 1.0 x 10^{-5} M; PMT voltage: -1100 V.

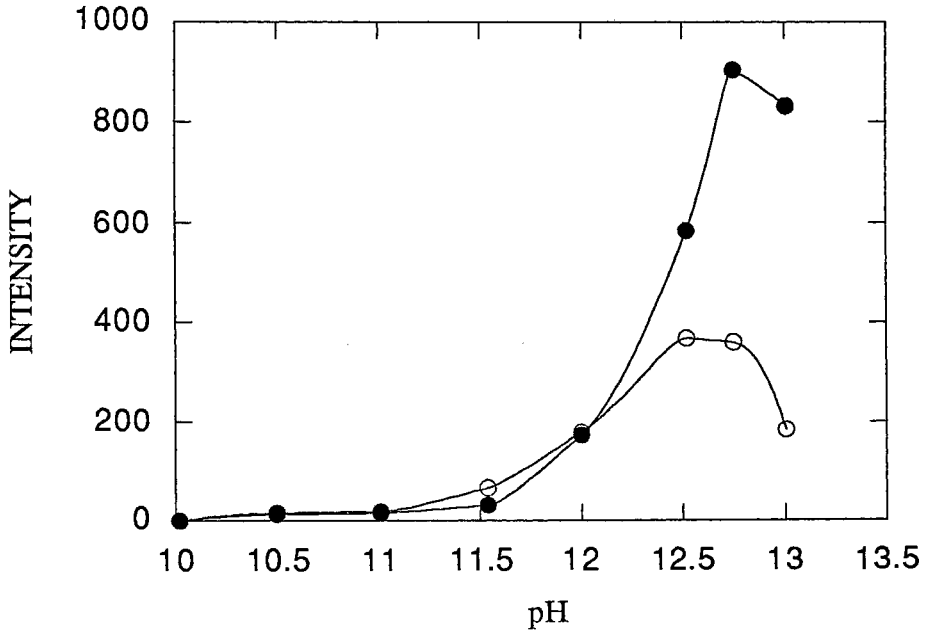
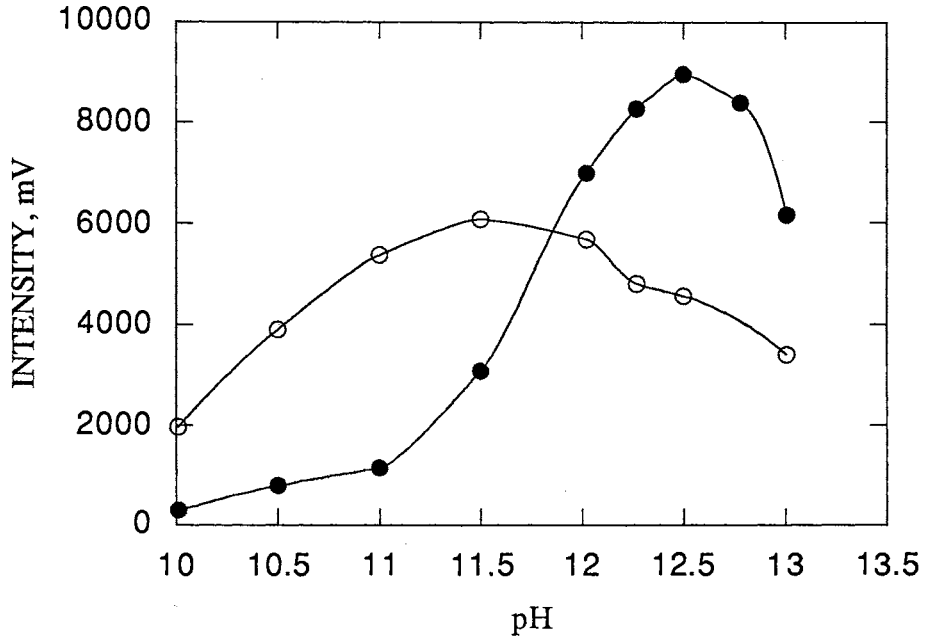
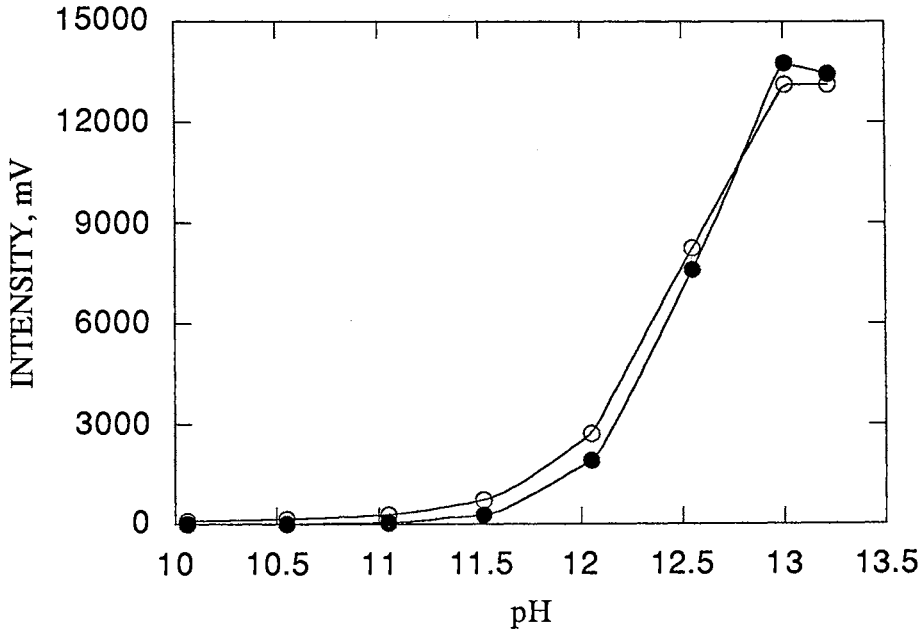


Figure 4.2 continued:

(h) $[Fe^{2+}]$: 1.0 x 10^{-5} M; PMT voltage: -1100 V.



(i) $[Fe(CN)_6^{3-}]$: 1.0 x 10^{-5} M; PMT voltage: -1000 V.



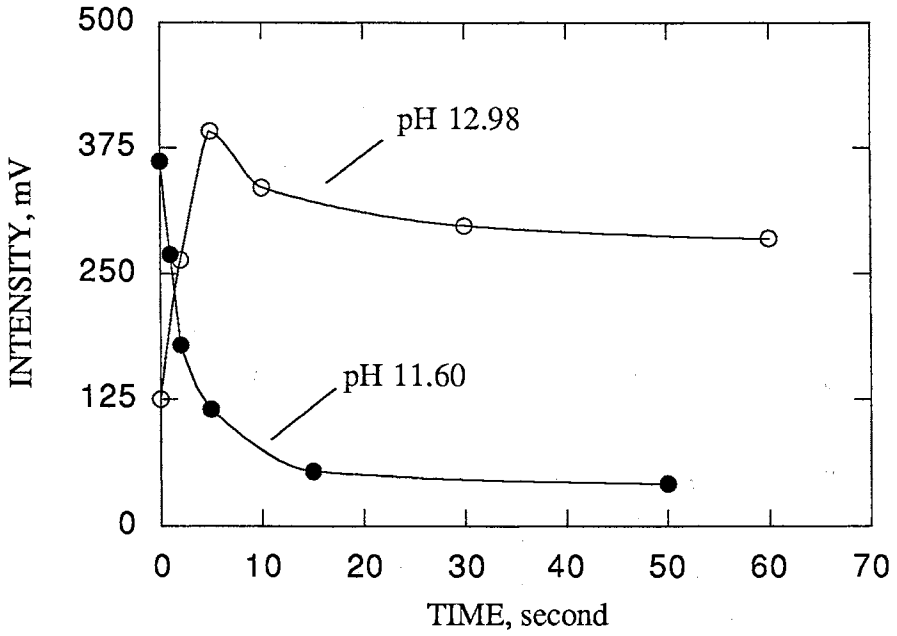


Figure 4.3. Effect of bubbling carbon dioxide on the chemiluminescence in the presence of Fe(II) as rate modifier at a pH smaller (pH 11.60) and a pH greater (pH 12.98) than the critical pH (11.90). [Luminol]: 5.0 x 10^{-3} M; [Fe^{2+}]: 1.0 x 10^{-5} M. Flow rate of CO_2 gas: 100 ml/min.

also depends on the rate modifier used for the chemiluminescent reaction. The largest shift was observed with CoPc, $CoTPPS_4$, Fe(II), and Cu(II) (about 1 pH unit), while the smallest, of only 0.3 pH unit, was observed with Fe(III) (see Table 4.1). These shifts cannot be considered to be due to the acidity of dissolved carbon dioxide because they were also observed with the luminol dissolved in phosphate buffers. For example, the change in optimum pH for Fe(II) was 1.0 pH unit when the luminol prepared in phosphate buffer solution was used; it is the same as that obtained with unbuffered luminol solution. A phosphate buffer solution has very good buffer capacity in the pH range used (~pH 12.5), so carbon dioxide as a weak acid and at very low concentration should not be expected to cause any noticeable change in pH.

For all rate modifiers tested, the optimum pH in the presence of carbon dioxide appears in the 12.5 to 13.5 range. In such strong basic solutions, luminol exists predominantly as a doubly negative ion. The first and second ionization constants for luminol have been estimated to be in the order of 10^{-6} and 10^{-13}, respectively [31]. The fraction of divalent anion can be roughly estimated to be only 0.031 at pH 11.5, but 0.24 and 0.76 at 12.5 and 13.5, respectively. This suggests that the doubly negative ion of luminol is the active species in the presence of carbon dioxide, as suggested for the luminol chemiluminescence observed with dissolved oxygen in an aprotic solvent [3].

Effect of Carbon Dioxide on the Optimum Luminol Concentration.

Carbon dioxide significantly changes the concentration of luminol producing the maximum chemiluminescence intensity. In the absence of carbon dioxide, the chemiluminescence intensity increases with the increase in the concentration of luminol, and a maximum is reached around 5×10^{-3} M. A further increase in luminol concentration results in a decrease in chemiluminescence. In the presence of carbon dioxide, however, the chemiluminescence intensity increases with the increase in the concentration of luminol

beyond 5 x 10^{-3} M. The effect was observed even at a concentration greater than 1.0 x 10^{-2} M. All rate modifiers included in this study exhibited the same behaviors.

Influence of Buffer Composition in the Presence of Carbon Dioxide

The enhancement of carbon dioxide on the luminol chemiluminescence is different with different composition of the buffer used to prepare the solution of luminol. Klopf and Nieman [8] studied the effect of buffer composition on the luminol chemiluminescence in the absence of added oxidant. They compared the intensities of chemiluminescence obtained with the luminol prepared in borate, carbonate, and ammonia buffers, as well as in unbuffered alkaline solutions. They showed that in the presence of Fe(II), Co(II), or Mn(II) as rate modifiers the luminol prepared in carbonate buffer gave rise to the strongest light emission, while the unbuffered luminol produced the weakest chemiluminescence. In the presence of carbon dioxide, however, the most intense chemiluminescence was observed in the unbuffered solutions in comparison with that observed in carbonate or phosphate buffers.

The carbonate anions have been found beneficial in enhancing luminol chemiluminescence when persulfate/perborate or persulfate/hydrogen peroxide were used as added oxidants [32]. Superoxide radical ($O_2^{-}\cdot$) is formed by the reaction between persulfate and perborate, and hydroxyl radical (HO·) by the reaction between persulfate and hydrogen peroxide. These radicals then produce carbonate radical ($CO_3^{-}\cdot$) and formate radical ($CO_2^{-}\cdot$), in the presence of carbonate, with a concomitant amplified oxidative effect and an increase in the chemiluminescence. In the absence of added oxidant, carbonate buffer is the best for luminol chemiluminescence because superoxide radicals can be formed through the oxidation reaction between luminol and oxygen (see discussion in sections about autooxidation of luminol and the mechanism of enhancement). The results of this study show that in the presence of carbon dioxide, however, the role of carbonate is relegated to insignificant.

Rate Coefficients for the Chemiluminescence Process in the Presence of Carbon Dioxide

Chemiluminescence emission is a kinetic-controlled process [48], and the intensity of chemiluminescence is proportional to the rate of the chemiluminescent reaction [5]. Therefore, the change in chemiluminescence intensity is the reflection of the change in the kinetic parameters of the reaction.

The kinetics of luminol chemiluminescence has been studied here, both in the absence and in the presence of carbon dioxide. The chemiluminescence intensity vs. time profile was recorded and the corresponding intensity vs. time data were read out by a Hewlett-Packard desktop computer to extract the kinetic parameters. It was found that for all rate modifiers, either with or without carbon dioxide, the chemiluminescence intensity rose rapidly to a maximum value and then slowly decayed to zero in less than 20 minutes. Carbon dioxide, however, increases the rate of the chemiluminescence process (both buildup and decay of light emission), as shown in Figure 4.4 when Co(II) was used as rate modifier.

Efforts have been made to find the rate coefficients for both the buildup and the decay processes. Unfortunately, this goal was not fully accomplished. For some rate modifiers, such as Cu(II) and Fe(III), the kinetic curves could not be accurately recorded because of the weak chemiluminescence emission even in the presence of carbon dioxide. On the other hand, kinetic profiles for Mn(II), Cr(III), CoPc, and $CoTPPS_4$ could be recorded only in the presence of carbon dioxide. The kinetic data collected from the systems for which intensity vs. time profiles were accurately recorded are listed in APPENDIX B.

The decay of chemiluminescence intensity was found to follow a first-order rate equation, while the buildup of light emission was not. This deviation from first-order behavior, however, may result from the instrumental limitations (slowdown of data acquisition by electronic filtering). Since the smallest response time the detection system

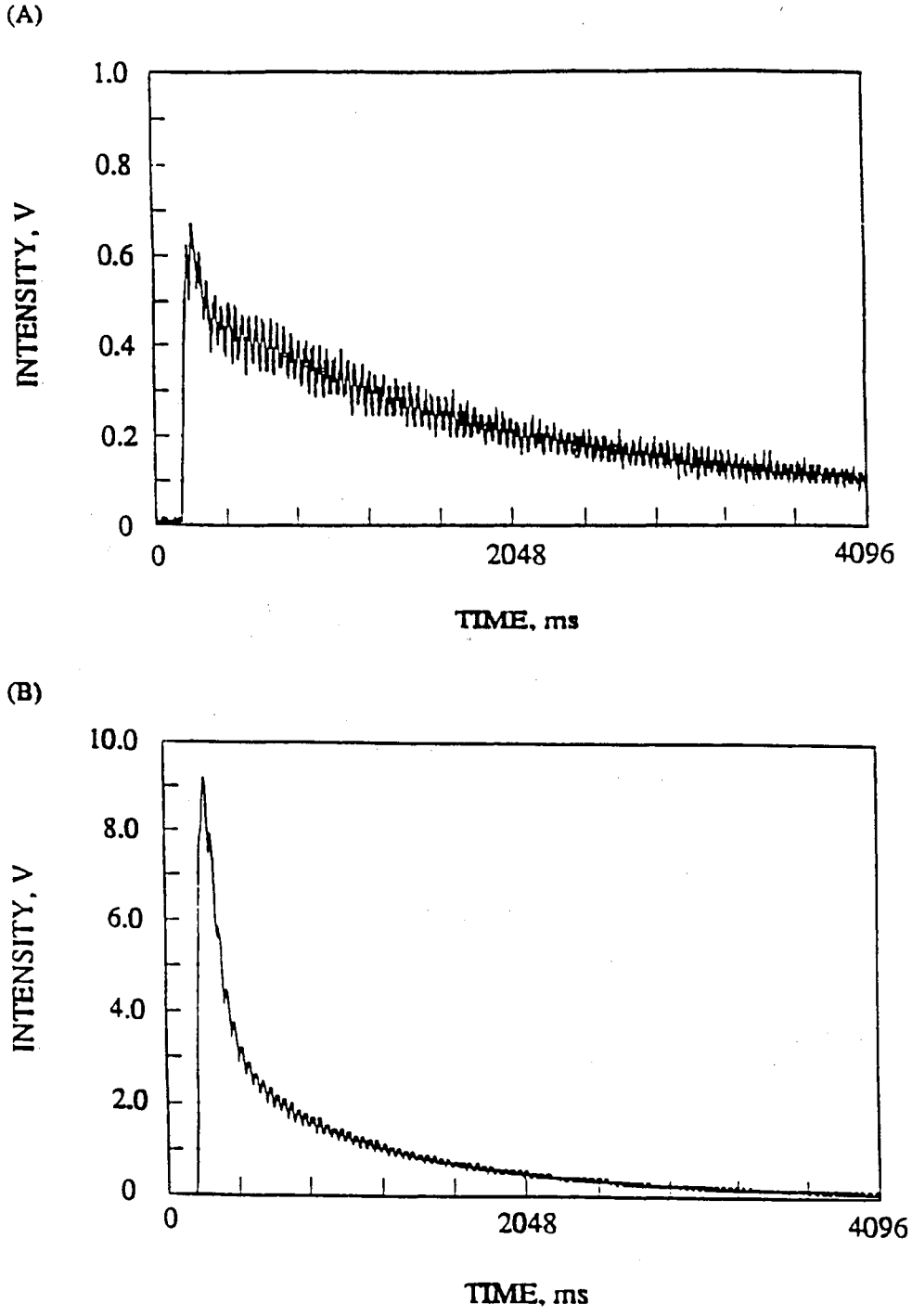


Figure 4.4. Typical profile of chemiluminescence intensity illustrating the buildup and decay of chemiluminescence. (A) in the absence of CO_2; (B) in the presence of CO_2. Rate Modifier: Co(II), 1.0 x 10^{-6} M; [Luminol]: 5.0 x 10^{-3} M; pH: 12.50; temperature: 25 ± 1°C; data acquired at a rate of 1 point per ms.

can provide without significantly increasing noise level is only 18 ms, the fastest process that can be accurately recorded by this instrument must have a rate coefficient smaller than 38 s^{-1} (for a first-order process). Otherwise, the recorded curve will correspond to the instrument response rather than the rate of the chemiluminescent reaction. Apparently, the rate of light emission is greater than the rate of data acquisition; therefore, the rate coefficient for the leading portion of the chemiluminescence peak was not amenable to determination.

The rate coefficients for chemiluminescence decay (k_d) in the presence of some rate modifiers have been calculated by using the data listed in APPENDIX B. A plot of log of chemiluminescence intensity vs. time is a straight line. The negative slope of this line equals k_d. The results for k_d are tabulated in Table 4.2 which also includes the enhancement factor, I_r', for comparison. In the absence of carbon dioxide, k_d is a constant no matter which rate modifier is used. In the presence of carbon dioxide, however, the k_d values follow the trend in I_r' values: the larger I_r', the larger k_d. Hence, the faster the decay of chemiluminescence, the larger the enhancement of light intensity by carbon dioxide.

What is the physical meaning of k_d? As early as 1930's, a key intermediate, the free radical of luminol, was identified in the chemiluminescent reaction of luminol when hydrogen peroxide was used as oxidant [31, 45]. Later, another key intermediate was also found in the chemiluminescent reaction of lucigenin in the absence of any added oxidant (dissolved oxygen was the oxidant) [46, 47]. Therefore, the process of the luminol reaction studied in this project can be expressed as:

$$\mathrm{L} \xrightarrow{k_1} \mathrm{M} \xrightarrow{k_2} \mathrm{P} + h\upsilon$$

where L, M, and P represent luminol, key intermediate, and final product of the reaction, respectively. This is only a simplified representation of the complicated process of chemiluminescent reaction. In reality, several steps may be involved in the formation of both M from L and P from M. Therefore, the rate coefficients, k_1 and k_2, are the overall

Table 4.2. Rate coefficients for the decay of luminol chemiluminescence in the absence and in the presence of carbon dioxide for some rate modifiers. Temperature: 25 ± 1°C.

	k_d, s^{-1}		
rate modifier	no CO_2	CO_2 present	I_r'
$Fe(CN)_6^{3-}$	0.15 ± 0.01	0.17 ± 0.01	1.0
Fe(II)	0.16 ± 0.02	0.21 ± 0.02	1.4
Mn(II)	--	0.36 ± 0.02	44.6
Co(II)	0.16 ± 0.02	0.45 ± 0.01	86.8
Cr(III)	--	0.48 ± 0.01	112.0
$CoTPPS_4$	--	0.51 ± 0.01	488.4
CoPc	--	0.55 ± 0.02	>500

values from the corresponding elementary steps. The overall rate of the reaction, according to this expression, can be written as:

$$\frac{d[P]}{dt} = k_2[M] \quad (1)$$

Because

$$\frac{d[M]}{dt} = k_1[L] - k_2[M] \quad (2)$$

and $k_1 >> k_2$ (as indicated by the tailing of the chemiluminescence intensity vs. time profile), the concentration of M increases with time at the beginning of the reaction. As most of L has been converted into M, k_1[L] << k_2[M]; then, equation (2) can be simplified as:

$$\frac{d[M]}{dt} = -k_2[M]$$

$$\frac{d[M]}{[M]} = -k_2 dt \quad (3)$$

By integrating equation (3), the following equation is obtained:

$$[M] = [M]_{t'} e^{-k_2(t-t')} \quad (4)$$

where $[M]_{t'}$ is the concentration of intermediate at time t' when all of luminol has been converted into intermediate and thus [L] is equal to zero. If [M] in equation (1) is replaced by equation (4),

$$\frac{d[P]}{dt} = k_2[M]_{t'} e^{-k_2(t-t')}$$

Because the chemiluminescence intensity is proportional to the rate of reaction,

$$I = K\frac{d[P]}{dt}$$

$$I = Kk_2[M]_{t'} e^{-k_2(t-t')} \quad (5)$$

$$\log I = -(k_2/2.303)(t - t') + \log (Kk_2[M]_{t'}) \quad (6)$$

where I is the chemiluminescence intensity and K is a proportional constant. Equations (5) and (6) are the mathematical expressions for the decay part of the kinetic curve far away from the peak of the curve. Equation (5) shows that the chemiluminescence intensity exponentially decreases with time, as experimentally observed. Equation (6) indicates that the log of chemiluminescence intensity vs. time should be a straight line with a slope equal to $-k_2/2.303$. From the definition of k_d, it follows that

$$k_d = \frac{k_2}{2.303}$$

This equation shows that the rate coefficient of chemiluminescence decay calculated from the kinetic curve is proportional to the rate coefficient for the decomposition of the key intermediate to produce light emission. The increase in k_d by carbon dioxide indicates an increase in k_2 and thus a possible change in the pathway of decomposition of the key intermediate or even a change in the nature of the key intermediate of the chemiluminescent reaction.

Considering another part of the kinetic process - the very beginning of the reaction, the concentration of the intermediate at this stage is so small that it is negligible compared to the concentration of luminol. Thus, equation (2) can be simplified to:

$$\frac{d[M]}{dt} = k_1[L]$$

Since

$$\frac{d[L]}{dt} = -k_1[L] \qquad (7)$$

therefore

$$\frac{d[M]}{dt} = -\frac{d[L]}{dt}$$

$$d[M] = -d[L]$$

$$[M] - [M]_0 = [L]_0 - [L] \qquad (8)$$

where $[M]_0$ and $[L]_0$ are the concentrations of intermediate and luminol at the beginning of the reaction (t = 0), respectively; [M] and [L] are the concentrations of intermediate and luminol at any time greater than zero, respectively. Because $[M]_0 = 0$, equation (8) can be simplified as follows:

$$[M] = [L]_0 - [L]$$

From equation (7):

$$[L] = [L]_0 e^{-k_1 t}$$

Therefore,

$$[M] = [L]_0 (1 - e^{-k_1 t}) \tag{9}$$

hence, replacing [M] in equation (1), the following equation is obtained:

$$\frac{d[P]}{dt} = k_2 [L]_0 (1 - e^{-k_1 t}) \tag{10}$$

An equation similar to equation (5) can be derived from equation (10):

$$I = K k_2 [L]_0 (1 - e^{-k_1 t})$$

where I is the chemiluminescence intensity and K is a constant. Therefore,

$$\frac{dI}{dt} = K [L]_0 k_1 k_2 e^{-k_1 t} \tag{11}$$

Equation (11) shows that an increase in k_2 also results in an increase in the rate of chemiluminescence buildup, dI/dt, as it was observed in this study.

Luminol "Autooxidation"

Hydrogen peroxide has been postulated to form via "autooxidation" of luminol under strongly basic conditions, and this hydrogen peroxide could be responsible for the sequential oxidation of luminol and the light emission in the absence of other oxidant [26]. The results collected in this study support some mechanistic postulations resulting from this so-called "autooxidation". It was found, for instance, that the age of the luminol solution

greatly affects the intensity of chemiluminescence, whether carbon dioxide is present or not. A freshly prepared luminol solution hardly produced detectable chemiluminescence in the absence of carbon dioxide, but use of the same solution even a day later results in easily detectable chemiluminescence. The presence of carbon dioxide permits detection of chemiluminescence with a freshly prepared solution of luminol, but the intensity was much smaller than that produced with a solution of luminol several days old. The trend of increase in the chemiluminescence intensity with increasing the age of the luminol solution was observed for as long as a month, but depended on how the luminol solution was stored. Storing luminol in a tightly closed container prolonged the trend of increasing luminescence with time. Cyclic voltammetry failed to detect peroxide and revealed only a shift of the oxidation peak of luminol; the magnitude of the shift increased with the age of the solution. Similar behavior was observed by direct addition of hydrogen peroxide to a freshly prepared solution of luminol. In consideration of the relative insensitivity of cyclic voltammetry to very low concentrations and the similar electrochemical behavior of luminol-hydrogen peroxide solution, the presence of peroxide cannot be ruled out as responsible for the increase in chemiluminescence intensity with increasing the age of the luminol solutions. As expected, and owing to rapid oxidation of luminol, solutions of luminol kept in open containers showed the opposite trend and after a few days such a luminol solution failed to produce chemiluminescence.

Although the so-called "autooxidation" of luminol is not well understood, experimental observations from this work point to dissolved oxygen as a very critical species for hydrogen peroxide formation, and this casts doubts about the legitimacy of inter- or intramolecular autooxidation of luminol species.

Figure 4.5 shows the change of chemiluminescence intensity of luminol/Co(II) system with the age of luminol solutions which were bubbled with oxygen, nitrogen, and air for 10 minutes, respectively, and stored in containers filled with the respective gases. Up to four or five days, on a relative basis, oxygen provided the environment for the most

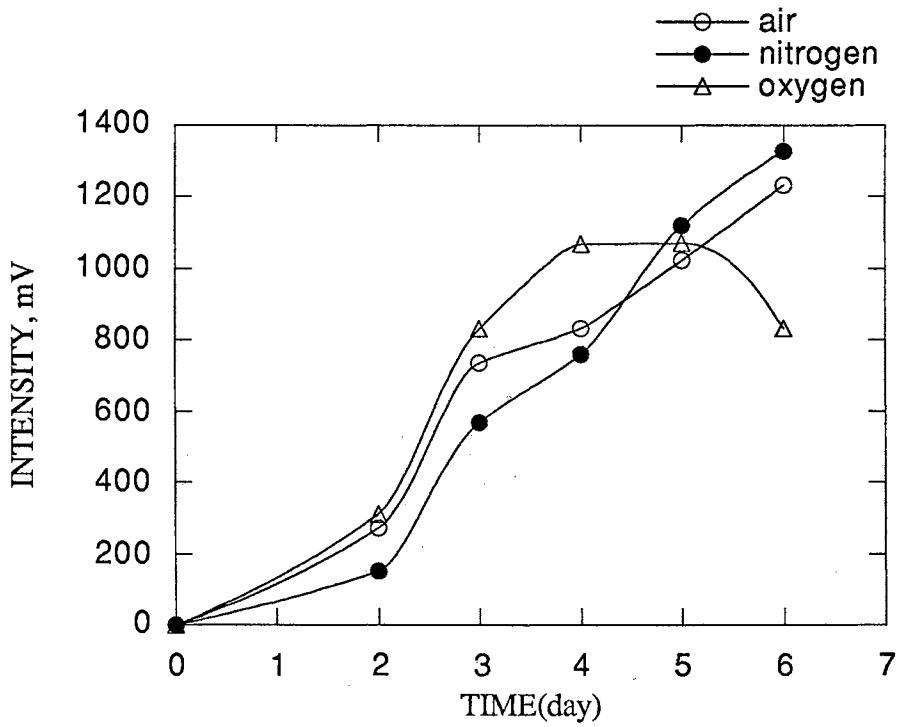


Figure 4.5. Variation of chemiluminescence intensity with the age of luminol solution saturated with air, nitrogen, and oxygen. No carbon dioxide present. [luminol]: 5.0 x 10^{-3} M; rate modifier: Co(II), 1.0 x 10^{-7} M; pH: 12.50.

intense chemiluminescence, while nitrogen the weakest light emission. After 5 days, however, the intensity in the presence of oxygen decreased but that in the presence of nitrogen continues the increasing trend and it became the one with the highest value. Since a 10-min bubbling of the corresponding gas was performed before each measurement, at a relatively high concentration level, oxygen can destroy the active species by a scavenging action. When luminol is stored under nitrogen atmosphere, this scavenging effect of oxygen is considerably diminished because of its low concentration in the solution, but residual oxygen still produces active species that accumulate with time, sustaining the increase in chemiluminescence under nitrogen beyond the fifth day. Storage under air, because of the predominant nitrogen content, parallels the trend observed with storage in a nitrogen atmosphere.

Excessive long standing changes the structure of luminol in solution. Evidence of this was observed spectrophotometrically by recording the absorption spectrum of luminol solutions at different storage time. Four absorption peaks were detected with fresh solutions, namely 195, 234, 302, and 350 nm. The absorbance of the peak at 195 nm slowly decreases with time, but the absorbance at other three peaks remains basically constant.

Species Responsible for the Enhancement of Luminol Chemiluminescence in the Presence of Carbon Dioxide

Carbonic acid and carbonate and bicarbonate ions are formed immediately after carbon dioxide gas dissolves in an aqueous solution so that the following equilibria can be established [51]:

$$CO_{2(g)} \leftrightarrow CO_{2(aq)}$$

$$CO_{2(aq)} + H_2O \leftrightarrow H_2CO_3$$

$$H_2CO_3 \leftrightarrow HCO_3^- + H^+$$

$$HCO_3^- \leftrightarrow CO_3^{2-} + H^+$$

Figure 4.6 illustrates the distribution of hydrated carbon dioxide (including $CO_{2(aq)}$ and H_2CO_3, expressed as H_2CO_3* in Figure 4.6, $[CO_{2(aq)}]/[H_2CO_3] = 650$ at 25°C [51]), HCO_3^-, and CO_3^{2-} with pH. As carbon dioxide gas was bubbled through the neutral or slightly acidic solution containing the rate modifier, the hydrated carbon dioxide and HCO_3^- are the most abundant species in the solution. These species will be converted into CO_3^{2-} after the rate modifier solution is mixed with the strongly basic solution of luminol, if they are not involved in other fast chemical reaction. Therefore, this question should be answered: which species, molecular CO_2 or carbonate ion (either HCO_3^- or CO_3^{2-}), is responsible for the enhancement of luminol chemiluminescence?

The following experiments were conducted to identify the species responsible for the chemiluminescence enhancement. Rate modifiers were prepared in the solutions containing carbonate (either HCO_3^- or CO_3^{2-}) rather than bubbled with carbon dioxide gas. The concentration range of carbonate was from 10^{-8} to 10^{-2} M. It was found that the chemiluminescence intensity did not increase as the total carbonate concentration was increased, with the exception of Co(II) as rate modifier. For Co(II) the chemiluminescence intensity started to increase at a total carbonate concentration higher than 1.0×10^{-5} M, reached to a maximum at 1.0×10^{-4} M, but sharply dropped to practically zero at a total carbonate concentration of 1.0×10^{-3} M. Even though carbonate increases the chemiluminescence intensity in the presence of Co(II) as rate modifier, the increase is much smaller than that caused by carbon dioxide gas. For the other rate modifiers the chemiluminescence remained constant upon increasing the total carbonate concentration but decreased to zero at a total carbonate equal to 1.0×10^{-2} M. The same results were observed no matter if $KHCO_3$ or K_2CO_3 was used as the source of carbonate. However, no matter what was the total carbonate concentration in the rate modifier solution, acidifying the solution to release carbon dioxide gas resulted in strong chemiluminescence emission. The change of chemiluminescence intensity with the concentration of carbonate in the presence of Co(II) and Fe(II) as rate modifiers is illustrated in Figure 4.7.

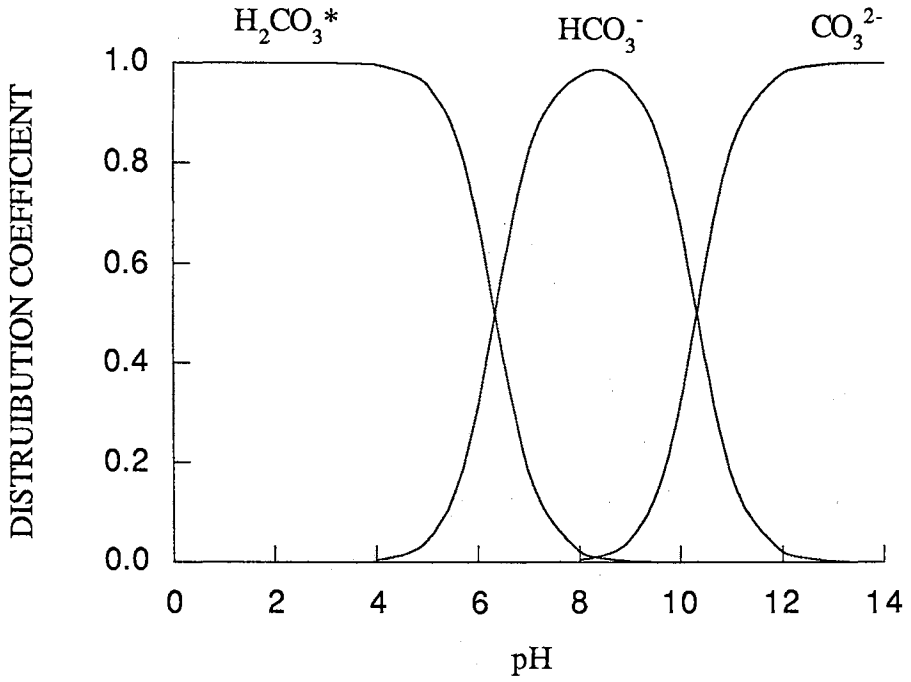


Figure 4.6. Distribution of hydrated CO_2 (H_2CO_3*), HCO_3^-, and CO_3^{2-} from pH 0 to 14. $H_2CO_3^* \rightarrow H^+ + HCO_3^-$, $pK_1 = 6.35$ [51]; $HCO_3^- \rightarrow H^+ + CO_3^{2-}$, pK2 = 10.33 [51].

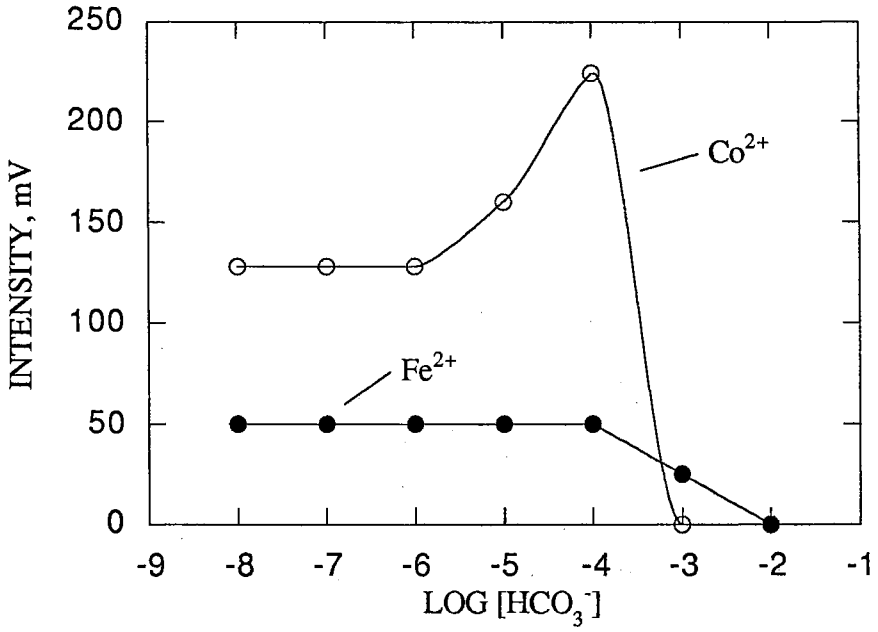


Figure 4.7. Effect of bicarbonate concentration in the rate-modifier solution on the chemiluminescence intensity. [Luminol]: 5.0 x 10^{-3} M; [Co(II)]: 1.0 x 10^{-7} M (PMT voltage: -1000 V); [Fe(II)]: 1.0 x 10^{-5} M (PMT voltage: -1100 V).

Experiments also have been performed by passing carbon dioxide gas through the luminol solution instead of the rate-modifier solution. Since the luminol solution is strongly basic (pH > 12), $CO_2(g)$ is completely converted into CO_3^{2-} immediately after it is mixed with the luminol solution. Bubbling for short time resulted in no change in the chemiluminescence intensity. However, bubbling for long time decreased the chemiluminescence intensity because the pH of luminol solution is decreased by the acid-base equilibria of dissolved carbon dioxide.

Evidently, molecular carbon dioxide is the reactive species and is responsible for the enhancement of luminol chemiluminescence.

Effect of Oxygen in Luminol and Rate Modifier Solutions

Dissolved oxygen has been identified by White *et al.* [3] as the oxidant in the luminol chemiluminescence in a dimethyl sulfoxide solution and in the absence of any other added oxidant. This conclusion was made on the basis of tracer experiments which showed that the product of the chemiluminescent reaction, aminophthalate, contained the oxygen atom present originally in the oxygen gas. In aqueous solutions, oxygen is also necessary for luminol chemiluminescence in the absence of an added oxidant. Baxendale [33, 34], for instance, found that aqueous basic solutions of luminol containing dissolved oxygen emitted light when subjected to pulses of 10 MeV electrons; no light was observed, however, if argon or nitrous oxide was first bubbled through to remove the oxygen [33]. In the presence of a metal ion [8] or a metal complex [35] as rate modifier, the displacement of dissolved oxygen by nitrogen in the luminol solution also decreased or totally suppressed the chemiluminescence.

In the absence of carbon dioxide, a similar phenomenon about the effect of replacing oxygen by nitrogen in the luminol solution was observed in this study. Moreover, the necessity of oxygen for luminol chemiluminescence is confirmed by the observation that the chemiluminescence intensity increases with the increase in the

concentration of oxygen in the luminol solution. The effect of nitrogen and oxygen normalized to a value of 100 for an air-saturated luminol solution is illustrated in Figure 4.8A. Bubbling the luminol solution with nitrogen (2 min at *ca* 100 ml/min) decreased the chemiluminescence intensity in the presence of all rate modifiers except Co(II); the reverse was observed by bubbling the luminol solution with oxygen at the same rate and for the same length of time. In the presence of Co(II) as rate modifier, no noticeable difference was observed. In the presence of hexacyanoferrate(III), bubbling nitrogen decreased the chemiluminescence intensity, but upon bubbling oxygen the chemiluminescence intensity was the same as that obtained with air-saturated solutions. From Figure 4.8A it can be seen that the rate modifier in whose presence a pronounced effect was observed, when bubbling nitrogen or oxygen, is Fe(III). It should be pointed out, however, that bubbling oxygen for more than 15 minutes reverses the trend, and the chemiluminescence intensity starts decreasing with increasing oxygen content.

Most literature references considering the effect of oxygen on the luminol chemiluminescence parallel the experimental design used to obtain the data depicted in Figure 4.8A. It is very interesting to note, however, that the bubbling of nitrogen or oxygen throughout the solution containing the rate modifier, instead of the one containing luminol, provides different results. Figure 4.8B summarizes the observations collected in such a case. Bubbling nitrogen into the solution containing the rate modifier resulted in decreasing the chemiluminescence intensity with respect to air-saturated solutions. Exception to this trend was observed with $Fe(CN)_6^{3-}$, where the intensity was slightly higher. Bubbling oxygen, however, also decreased the chemiluminescence intensity with respect to that observed with air-saturated solutions. Copper (II), in this case, is the notable exception. The decrease caused by oxygen is even greater than that caused by nitrogen. The results obtained with nitrogen and oxygen show that dissolved oxygen is necessary for chemiluminescence, but a high concentration of oxygen has an adverse effect. This adverse effect may be ascribed to the interaction between oxygen and the rate

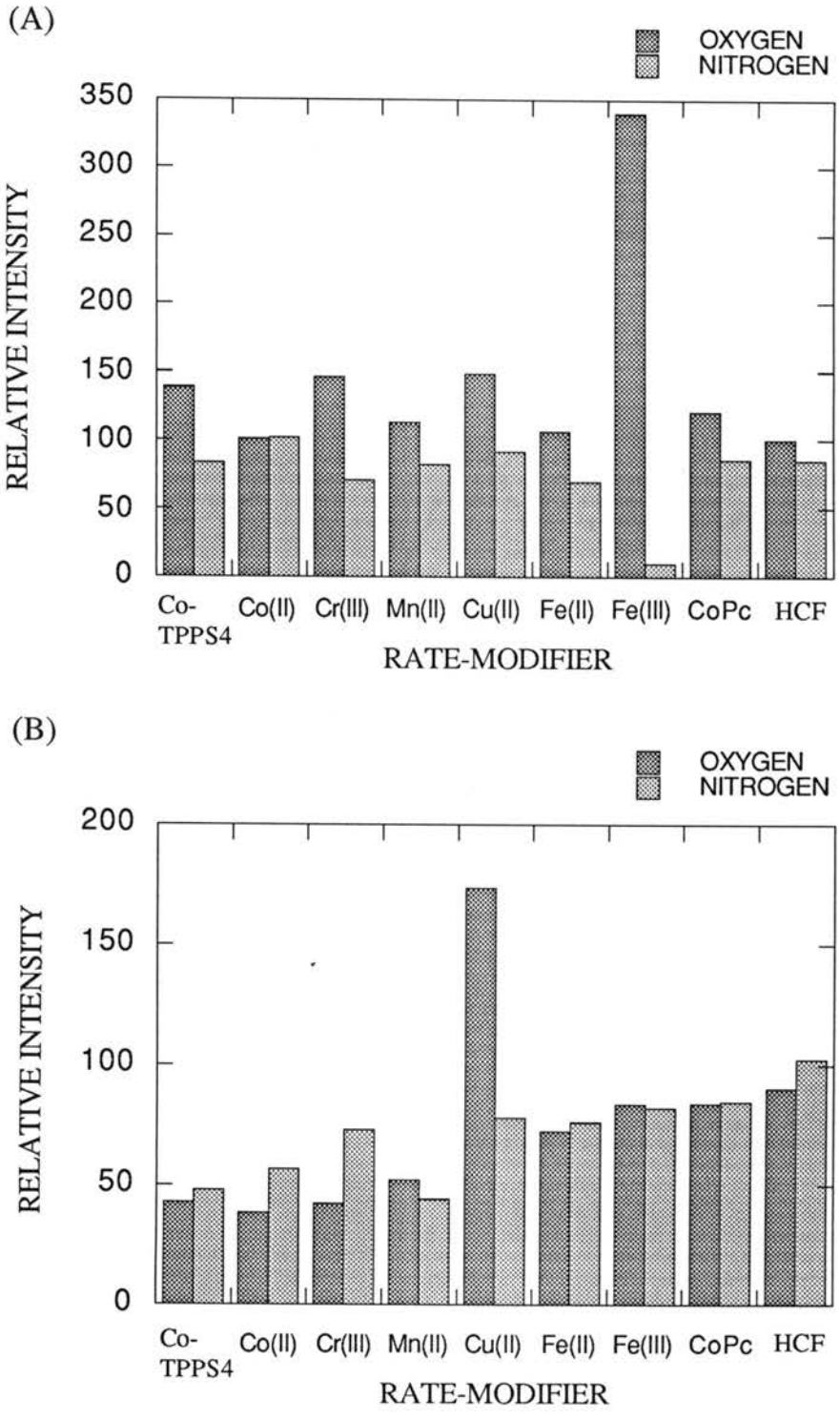


Figure 4.8. Normalized effect of bubbling oxygen at a rate of 100 ml/min for 2 min through the luminol solution (A) or rate modifier solution (B). HCF: $Fe(CN)_6^{3-}$.

modifier similar to the one observed between oxygen and Fe(II)-porphyrin [36]. The interaction results in the formation of a complex which has less "catalytic" power than the unoxygenated metal ion or chelate because oxygen blocks the active center (metal ion) in the rate modifier.

When carbon dioxide is present, however, the bubbling of nitrogen or oxygen into either the rate modifier or the luminol solution has little if any effect on the chemiluminescence intensity. Although oxygen is needed for the chemiluminescent reaction, in the presence of carbon dioxide the need becomes secondary and relegated to substantially lower concentration levels.

Dependence of Enhancement of Luminol Chemiluminescence on the Form of Rate Modifier

The oxidation state and chemical form of the metal ion acting as rate modifier (e.g. free or complexed) results in different effects on the luminol chemiluminescence. In some cases only one of two or more possible oxidation states is capable of promoting chemiluminescence. This type of behavior is also observed in the presence of carbon dioxide. Already mentioned are the different enhancements by Co(II) or the same metal ion complexed as CoPc and $CoTPPS_4$.

If the metallic species was in the form of a metal hydroxide suspension, a roughly 70% reduction in chemiluminescence intensity was observed in comparison with that observed with free metal ion. Complexation with EDTA resulted in practically total quenching of chemiluminescence. Copper(II) as $Cu(NH_3)_4^{2+}$ and Fe(III) as FeF_6^{3-} also exhibited the same quenching effect on the chemiluminescence, and carbon dioxide had practically no effect in such cases. Even for the Fe(II)-EDTA complex, the only metal-EDTA complex stimulating chemiluminescence from luminol, carbon dioxide failed to increase the chemiluminescence intensity.

The overall set of observations collected in these studies point to the relevance of the coordination geometry of the metal-ligand rate modifier. As shown in Figure 4.9,

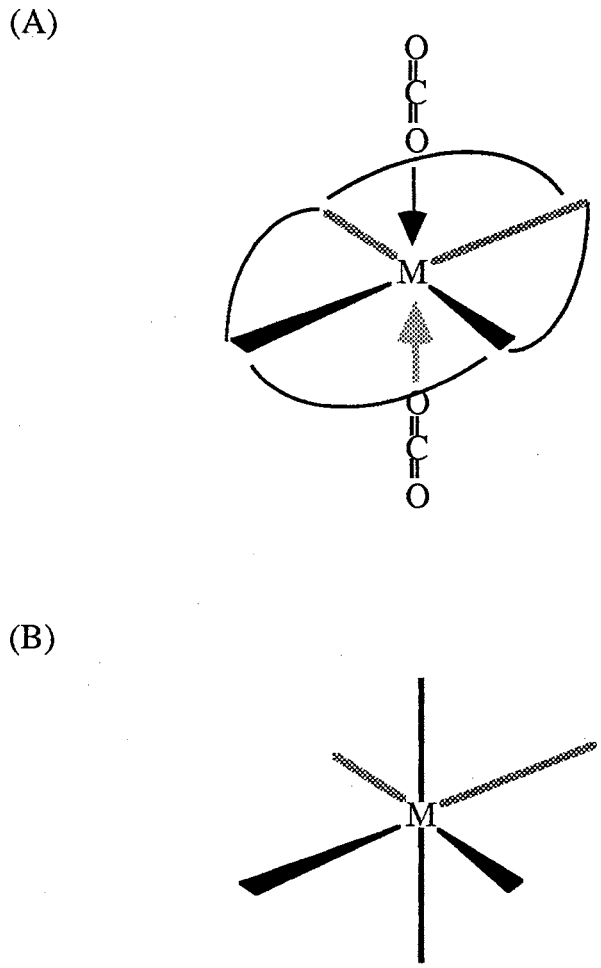


Figure 4.9. Coordination geometry of some metal complexes. M: positively charged metal ion. (A) Planar structure of CoPc or $CoTPPS_4$ providing two sites for interaction with carbon dioxide molecules. (B) octahedral structure of $Fe(CN)_6^{3-}$ or metal-EDTA complexes, and distorted octahedral structure of $Cu(NH_3)_4(H_2O)_2$ (two H_2O molecules occupying axial positions are more distant from M than are the four NH_3 molecules at equatorial positions); no interaction sites are available.

planar complexes such as CoPc and $CoTPPS_4$, and considering a coordination number of 6 for Co(II), provide two potential points for attachment of carbon dioxide molecules. The octahedral structures of $Fe(CN)_6^{3-}$ and the metal-EDTA complexes are coordinately saturated and preclude a similar interaction; therefore the enhancement of chemiluminescence is absent. The copper tetraamine complex has a structure similar to the one shown in Figure 4.9B because Cu(II) also combines with two H_2O molecules in addition to four NH_3 molecules [50]. These two H_2O molecules occupy the axial positions, but are more distant from the Cu(II) than are the four NH_3 molecules in the square planar arrangement. Therefore, the copper-amine complex has a distorted octahedral structure. Evidently the carbon dioxide enhancement is only possible if the $CO_2(g)$-metal interaction is feasible. This proposed interaction is in agreement with the knowledge that reactions leading to $CO_2(g)$-metal complex formation via addition or substitution nearly always can take place at room temperature and at normal pressure [49].

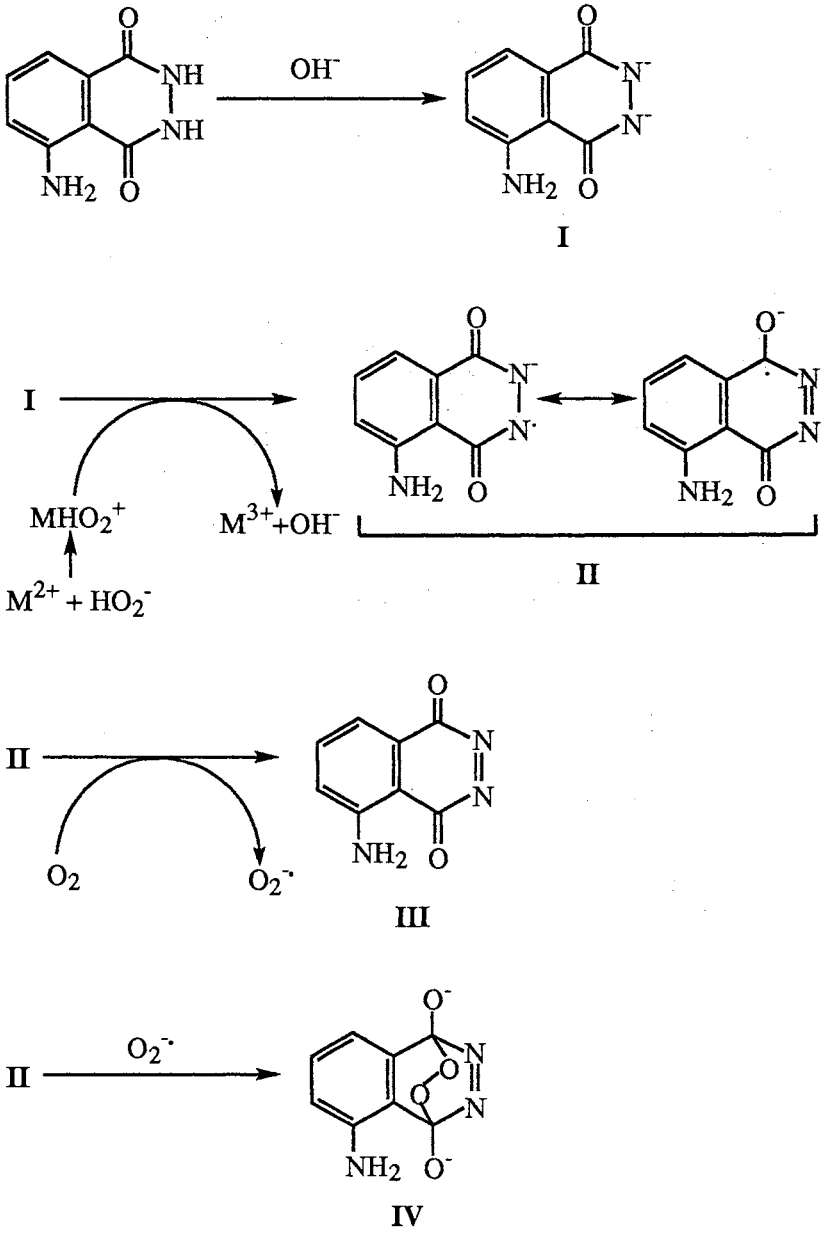
Possible Mechanism for the Carbon Dioxide Enhancement of Luminol Chemiluminescence

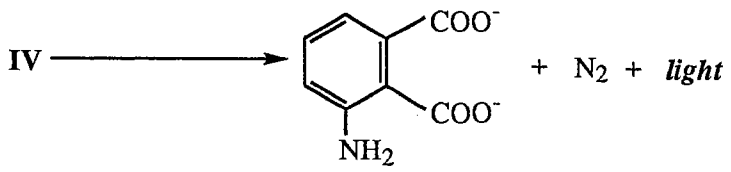
Although extensively studied, the mechanism for light production in the luminol chemiluminescent reaction is not fully understood [2]. It is commonly postulated that the chemiluminescent process is initiated by an one-electron oxidation of luminol to produce a free radical [33, 35, 39-42]. The research conducted by Fridovich *et al.* [39, 40] demonstrated that the superoxide radical anion $O_2^{-\cdot}$ is also a crucial intermediate in the chemiluminescent reaction of luminol. The luminol free radical can reduce $O_2(g)$ to $O_2^{-\cdot}$ and also reacts with $O_2^{-\cdot}$, yielding an unstable endoperoxide. The decomposition of the endoperoxide leads to an electronically excited aminophthalate [41].

Little have been researched, however, about the role of metal ions or complexes in the luminol chemiluminescence. In the study of the mechanism of cobalt(II)-promoted luminol chemiluminescent reaction with hydrogen peroxide, Burdo and Seitz [43]

postulated that cobalt(II) forms a complex with peroxide; this complex then reacts with luminol in basic media to produce a luminol free radical.

The observations collected in this study show that the dinegative ion of luminol, peroxide ion, oxygen, and carbon dioxide participate in the luminol chemiluminescent reaction in the absence of other added oxidant. By taking into consideration of previous studies and the observations collected in this study, the following mechanism can be postulated for the luminol chemiluminescence in the absence of added oxidant:





This mechanism explains, for instance, the effect of oxygen in the absence of carbon dioxide. Oxygen is necessary for chemiluminescence because it is the source of superoxide radical $O_2^{-}\cdot$, an important intermediate. Relatively high concentrations of oxygen, however, lead to a decrease in chemiluminescence because it: (1) competes with the peroxide ion for complexation with the rate modifier, and (2) favors the reaction between II and $O_2(g)$ (to form an inert compound III) over the reaction between II and $O_2^{-}\cdot$, deviating from the path that leads to chemiluminescence, i.e., the formation and then the decomposition of IV.

The enhancement of luminol chemiluminescence by carbon dioxide can be ascribed to facilitation of the formation of reaction intermediates critical to the chemiluminescence (i.e., luminol-based and superoxide free radicals). In a strong basic medium, carbon dioxide may react with peroxide to form peroxycarbonates [2]:

$$CO_2(g) + HOO^- \rightarrow {}^-OOC\text{-}O\text{-}OH \rightarrow {}^-OOC\text{-}O\text{-}O\text{-}COO^-$$
$$\mathbf{V} \qquad\qquad \mathbf{VI}$$

This is a nucleophilic addition reaction. It is facilitated by the interaction between the rate modifier and carbon dioxide as shown in Figure 4.9A. The decomposition of peroxycarbonate results in the formation of several free radical species:

$$\mathbf{V} \rightarrow CO_2^{-}\cdot + HO_2\cdot$$

$$\mathbf{VI} \rightarrow 2\ CO_3^{-}\cdot$$

The superoxide radical can be formed from the dissociation of $HO_2\cdot$ at the high pH used in this study ($HO_2\cdot \rightarrow H^+ + O_2^{-}\cdot$, pK_a of $HO_2\cdot$ = 4.8 [44]). Among the other radicals formed, $CO_3^{-}\cdot$ is a strong oxidant which can oxidize luminol into a free radical, and $CO_2^{-}\cdot$

can undergo further reaction to form $CO_3^{-\cdot}$ with the carbonate or bicarbonate ions produced when carbon dioxide is dissolved in alkaline solution [32]:

$$CO_2^{-\cdot} + HCO_3^- \rightarrow CO_3^{-\cdot} + HCOO^-$$

The production of luminol-based radical and superoxide free radical, as the result of reactions involving carbon dioxide, favors the formation of IV and thus results in the enhancement of luminol chemiluminescence.

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CHAPTER V

APPLICATION OF CARBON DIOXIDE-ENHANCED CHEMILUMINESCENCENCE OF LUMINOL IN THE ABSENCE OF ADDED OXIDANT I: DETERMINATION OF COBALT(II)

Introduction

The enhancement effect of carbon dioxide on the luminol chemiluminescence in the absence of added oxidant has several possible analytical applications. The Co(II)/luminol/$CO_{2(g)}$ system has been employed for the determination of cobalt(II). This system shows better analytical characteristics than most other chemiluminescence systems for cobalt(II) determination.

The methods of chemiluminometric determination of cobalt(II) have been mainly based on the Co(II)-promoted oxidation of luminol by hydrogen peroxide [1]. The enhancement effect of Co(II) on the chemiluminescence produced from the oxidation of lucigenin (bis-*N*-methylacridinium nitrate) [2, 3] or lophine (2,4,5-triphenylimidazole) [4, 5] by hydrogen peroxide in alkaline solution has also been applied to the determination of cobalt(II). The lucigenin system [2, 3] is quite selective for Co(II) and exhibits very wide linear dynamic range. Other chemiluminescence systems for cobalt determination include the Co(II)-promoted oxidation of certain organic compounds by hydrogen peroxide such as gallic acid [6], tartaric acid [7], quercetin [8], 4-diethylamino-phthalohydrazide [9], and pyrogallol [10]. All these systems have very high sensitivities for the Co(II) determination. The detection limit for Co(II) can be as low as 0.6 pg/ml, i.e., 1.0×10^{-11} M [11]. However, most of these systems suffer from severe interferences from some transition metal ions, such as Fe(II), Fe(III), Cu(II), Zn(II), Cr(II), and Mn(II), etc. Thus, in most

cases, masking reagents [7, 9] or pre-separation methods such as ion chromatography [1, 11] have to be used to reduce interferences.

Experimental Section

All reagents and solutions employed in this study were prepared in the same way as those in Chapter IV. Carbon dioxide gas was bubbled into cobalt(II) solutions with for 2 minutes before the new solution was injected into the flow cell. The flow rate of carbon dioxide gas was 100 ml/min.

The flow scheme represented by Figure 4.1A (in Chapter IV) was used in this study.

Results and Discussion

Optimum Conditions for Cobalt(II) Determination.

As discussed in Chapter IV and shown in Figure 5.1, luminol chemiluminescence in the presence of carbon dioxide increases with an increase in luminol concentration even beyond 10^{-2} M. However, 1.0 x 10^{-2} M luminol was selected to use in the experiment in order to reduce the consumption of luminol. Although this is not the optimum concentration, the sensitivity and reproducibility obtained at this concentration were satisfactory. A weak chemiluminescence emission was observed even when the concentration of luminol was zero (i.e., the cobalt(II) solution with carbon dioxide was directly injected into the water with a pH of 12.5). This phenomenon is similar to that observed by Townshend, *et al.* [12, 13]. They reported that chemiluminescence was produced when Fe(II) or Ti(III) solution was injected into a carbonate buffer (pH 10.4) and postulated that the reduction of oxygen molecules or traces of peroxide ions in solution might be responsible for the chemiluminescence emission. The nature of the emission is still not well understood.

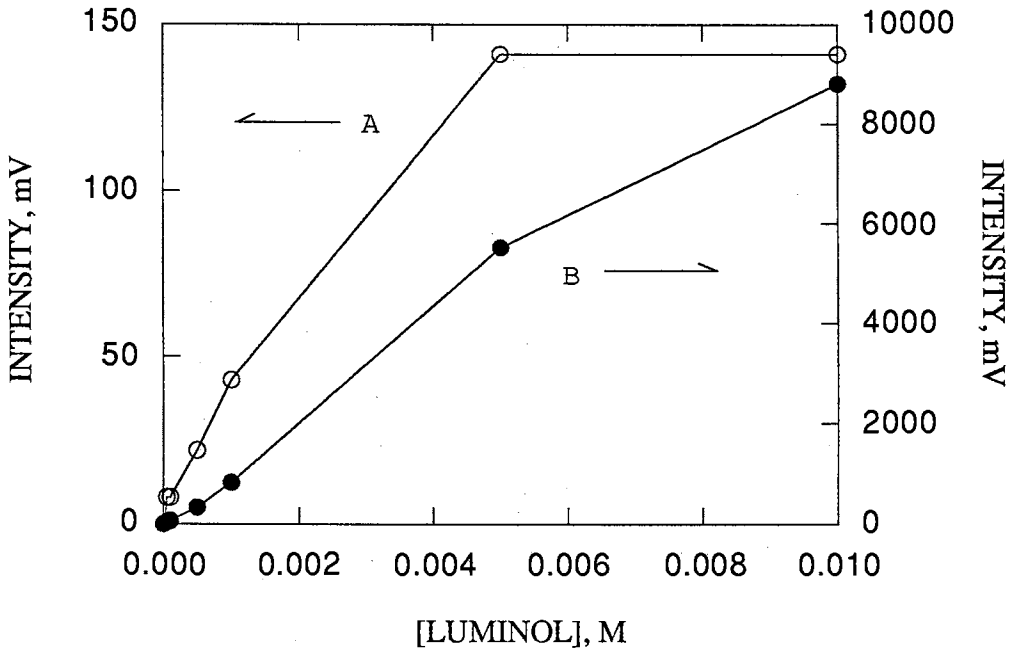

Figure 5.1. Effect of luminol concentration on the chemiluminescence intensity (A) in the absence and (B) in the presence of carbon dioxide. [Co^{2+}]: 1.0 x 10^{-7} M. Flow rate: luminol, 2.8 ml/min; Co^{2+}, 1.5 ml/min. Disk rotation velocity: 450 rpm. Volume of Co^{2+} injected: 0.29 ml.

It is known from Chapter IV that the optimum pH of a luminol solution is 12.5 in the presence of Co(II) as rate modifier. The acidity of the cobalt solution also influences the chemiluminescence. An acidity higher than that produced by 1.0×10^{-4} M HCl reduced the chemiluminescence intensity, and the higher the acidity, the lower the intensity. For example, the intensity obtained in 0.10 M HCl is only 80% of the intensity obtained in 10^{-4} M HCl. The reason for this may be that the solubility of carbon dioxide in aqueous solution decreases with an increase in the acidity of the solution. From 10^{-5} M to 10^{-4} M HCl, the chemiluminescence intensity was constant. An acidity less than 10^{-5} M HCl produced chemiluminescence of reduced intensity due to the formation of cobalt hydroxide. The intensity decreases with a decrease in the acidity of cobalt solution. Hence, all cobalt solutions were prepared at 10^{-4} M HCl for further experiments.

The time of bubbling carbon dioxide gas into the cobalt solution affected the chemiluminescence intensity. Although weak chemiluminescence was observed in the absence of carbon dioxide, a short-time bubbling with carbon dioxide resulted in a dramatic increase in chemiluminescence intensity. For example, at 5.0×10^{-3} M luminol, 1.0×10^{-8} M Co(II), and other optimum conditions, the intensity increased from 6.4 mV to 4960 mV when the bubbling time was increased from zero to 30 seconds. Bubbling more than 30 seconds caused a decrease in chemiluminescence intensity. However, the intensity, which is only 67% of the intensity with a 30-second bubbling, remained unchanged from 1 to 20-minute bubbling. In this study, all cobalt(II) solutions were bubbled with carbon dioxide for at least 2 minutes. Therefore, the fluctuation in the flow rate of carbon dioxide should not affect the reproducibility of chemiluminescence measurements.

It has been demonstrated that the flow cell with a rotating disk is an ideal reactor in continuous-flow chemiluminescence detection [15]. The rotation of the disk introduced convection and thus facilitated the mixing of luminol with cobalt(II) rapidly and thoroughly, and resulted in the enhancement of the chemiluminescence signal. Figure 5.2 shows the change of chemiluminescence intensity with the voltage applied to magnetic

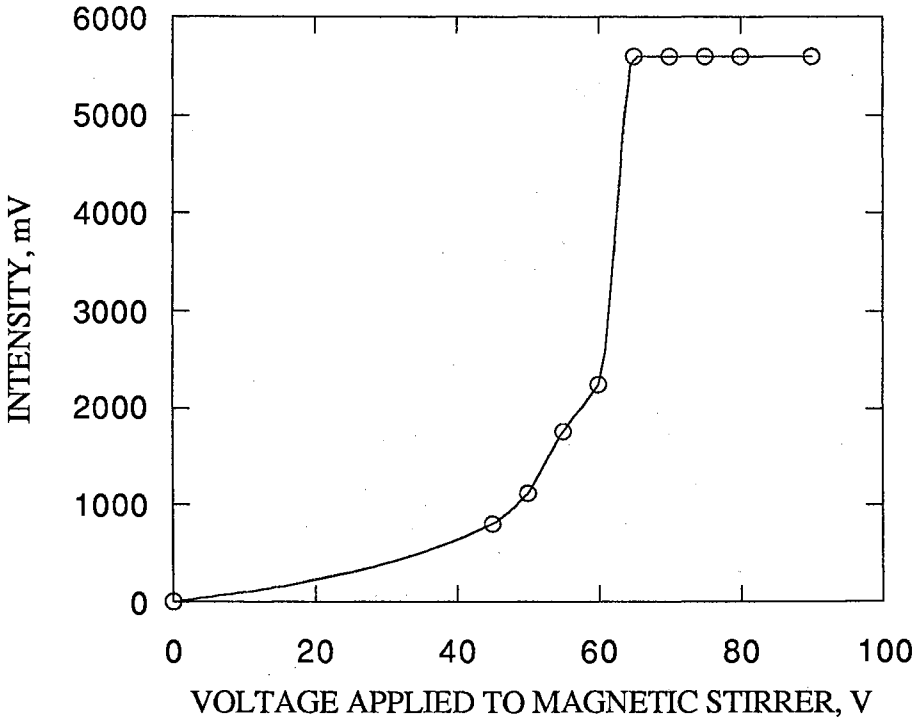

Figure 5.2. Effect of disk rotation on the chemiluminescence intensity. [Co^{2+}]: 1.0 x 10^{-7} M (bubbled with CO_2 at 100 ml/min for 2 min); [Luminol]: 1.0 x 10^{-3} M.

stirrer (i.e., the rotation velocity of the disk inside the flow cell). Higher voltage results in faster rotation. The relationship between the rotation velocity of the disk and the voltage applied to the magnetic stirrer has been given by Matsumoto, *et al.* [14]. As expected, the chemiluminescence intensity increased with an increase in the rotation velocity of the disk. The maximum intensity was reached at 65 V, which corresponds to a rotation velocity of 350 rpm. No further increase in intensity was observed beyond 350 rpm. It was noted, however, that no chemiluminescence emission could be detected if the disk was motionless.

The flow rate of the luminol solution influenced the chemiluminescence intensity. With an increase in the flow rate of the luminol solution, the intensity of chemiluminescence increased until 3.7 ml/min was achieved. Further increase in the flow rate did not change intensity. The flow rate of cobalt(II) solution did not affect the chemiluminescence intensity. In the experiment, the flow rate of cobalt(II) was maintained at only half of the optimum flow rate of luminol (3.7 ml/min) so as to save the consumption of the sample.

The optimum volume of a cobalt(II) solution injected into the luminol stream was found to be in the range of 0.14 to 0.18 ml. The injection of a smaller or greater volume of a cobalt(II) solution resulted in a less intense chemiluminescence. Moreover, injecting more than 0.18 ml cobalt(II) solution produced double-peak signals which were less reproducible than single-peak signals as the injection volume was equal to or less than 0.18 ml. The 0.18 ml volume was selected as the injection volume of cobalt(II) in further experiments.

Interferences from Other Metal Ions.

Many metal ions such as Cr(III), Mn(II), Fe(II), Fe(III), Ni(II) and Cu(II) interfere severely with cobalt(II)-promoted chemiluminescence of luminol when hydrogen peroxide is used as the oxidant [15]. The interferences from zinc(II) and cadmium(II) are so serious that a method for the determination of these two metal ions has been developed based on

their inhibition of the chemiluminescence of the Co(II)/luminol/H_2O_2 system [16]. In the absence of an added oxidant, iron(II), even at the same concentration as cobalt(II), can increase the cobalt(II)-promoted chemiluminescence of luminol 1000 times [17]. For the studies of the tolerance level of interferences in the cobalt(II)/luminol/ carbon dioxide system, the effects of the metal ions mentioned above, and other common metal ions, on the chemiluminescence intensity of 5.0×10^{-8} M cobalt(II) were examined under optimum conditions. The results are listed in Table 5.1.

As can be seen from Table 5.1, 200-fold excesses of Al(III), UO_2^{2+}, Zn(II), Fe(II) and Fe(III) and 100-fold excesses of Cd(II) and Cr(III) do not interfere; Mn(II), Ni(II) and Cu(II) interfere severely. Further studies showed that 50-fold excesses of Mn(II) and Ni(II) and 10-fold excess of Cu(II) caused an intensity change of less than ±5%. At least 1000-fold excesses of Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+} and Ba^{2+} had no effect on the chemiluminescence intensity.

Analytical Characteristics.

The calibration graph, obtained under the optimum conditions and prepared by plotting the log of chemiluminescence intensity vs. the log of cobalt(II) concentration, gave linear ranges with different slopes from 5.0×10^{-13} M to 1.0×10^{-9} M and from 1.0×10^{-9} M to 1.0×10^{-7} M. The linear regression equations for these two ranges are (blank readings being subtracted):

5.0×10^{-13} M to 1.0×10^{-9} M:

$$\log (\text{signal, mV}) = 4.098 + 0.240 \log [\text{Co(II)}]$$

1.0×10^{-9} M to 1.0×10^{-7} M:

$$\log (\text{signal, mV}) = 11.015 + 1.008 \log [\text{Co(II)}]$$

both with regression coefficient of *ca.* 0.999. It was noted that in order to get good linearity, the chemiluminescence intensity of the sample solution was subtracted from the intensity of blank solution before conversion logarithms, especially when the concentration

Table 5.1. Effect of metal ions on the chemiluminescence intensity of 5.0×10^{-8} M Co(II)

Foreign metal ion	Intensity change, %	
	5.0×10^{-6} M*	1.0×10^{-5} M*
Cu^{2+}	-81.8	-91.8
Ni^{2+}	-55.8	-74.6
Mn^{2+}	-28.6	-32.7
Cr^{3+}	-5.4	-32.1
UO_2^{2+}	+3.4	+5.2
Zn^{2+}	+1.8	+1.8
Cd^{2+}	+1.7	-8.5
Fe^{2+}	0	+2.1
Fe^{3+}	-0.5	-1.5
Al^{3+}	0	-1.8

* Concentration of foreign ion.

of cobalt(II) in the sample was below 10^{-9} M. As shown in Figure 5.3, up to 10^{-6} M cobalt(II) can be determined, but the curve begins to bend to the concentration axis beyond 10^{-7} M cobalt(II). The reason for the sudden change in linearity at 10^{-9} M Co(II) is not clear.

The relative standard deviation (RSD) for five measurements of a 1.0×10^{-9} M cobalt(II) solution was 3.4%. As faster injection of cobalt(II) results in stronger chemiluminescence, the reproducibility of chemiluminescence measurements largely depends on the reproducibility of injection when manual injection is employed. The use of an automatic injector should give lower RSD, and thus better reproducibility.

By using the calibration graph, cobalt(II) can be readily determined. Results for the analysis of several synthetic samples are listed in Table 5.2.

In conclusion, the carbon dioxide-enhanced, cobalt(II)-promoted luminol chemiluminescence has very high sensitivity for the determination of cobalt(II) and can tolerate interference from some transition metal ions. It may be applied to the direct determination of cobalt(II) in certain samples without the need for pre-separation or the use of masking agents. For comparison, Table 5.3 summarizes the characteristics of typical chemiluminescence reactions used to determine cobalt(II). As can be seen, the cobalt(II)-luminol-carbon dioxide system has the lowest detection limit, the widest dynamic range, and suffers less interferences from some transition metal ions.

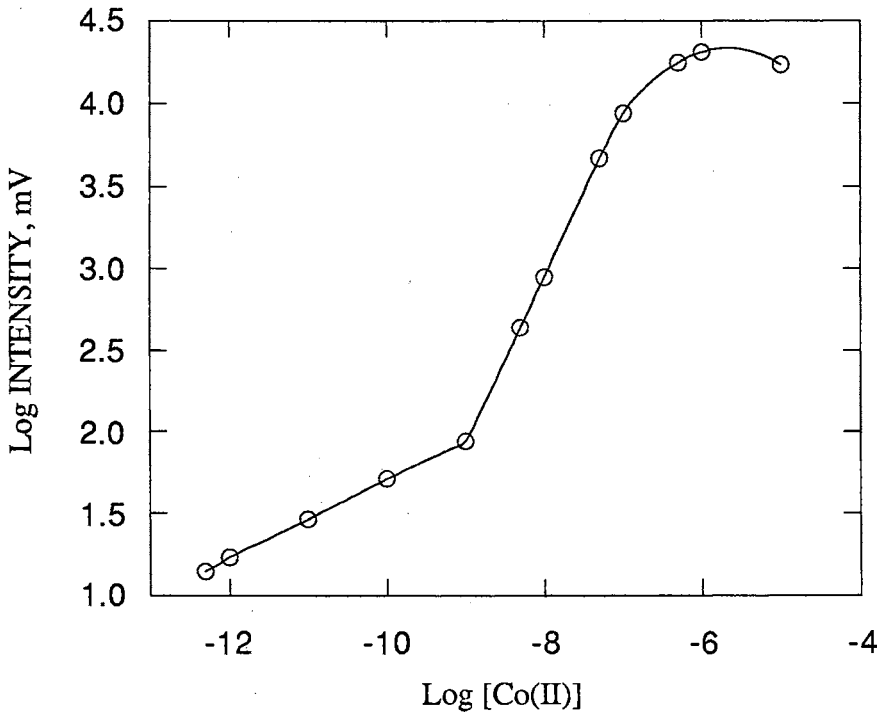

Figure 5.3. Calibration curve for the determination of cobalt(II). Acidity of Co^{2+}: 1.0 x 10^{-4} M HCl (bubbled with CO_2 for 2 min). [Luminol]: 5.0 x 10^{-3} M. Flow rate: luminol, 3.7 ml/min; Co^{2+}, 2.0 ml/min. Rotation velocity of the disk: 450 rpm. Volume of Co^{2+} injected: 0.18 ml.

Table 5.2. Determination of Co(II) in synthetic samples prepared by adding foreign ion species.

matrix	foreign ions added, M	Co(II) added, M	Co(II) found, M
deionized-distilled water	Fe(II) (1.0×10^{-10}) + Ni(II) (5.0×10^{-11})	5.0×10^{-11}	4.7×10^{-11}
	Fe(II) (5.0×10^{-8}) + Ni(II) (5.0×10^{-9}) + Cu(II) (1.0×10^{-9}) + Zn(II) (1.0×10^{-8}) + Mn(II) (5.0×10^{-7})	1.0×10^{-9}	8.9×10^{-10}
	Mn(II) (5.0×10^{-7}) + Cr(III) (5.0×10^{-7}) + UO_2^{2+} (5.0×10^{-6})	1.0×10^{-7}	9.6×10^{-8}
tap water*		1.0×10^{-8}	1.0×10^{-8}
		1.0×10^{-7}	9.8×10^{-8}
		1.0×10^{-6}	9.9×10^{-7}

* Tap water contained Co(II) was diluted ten times before measurement.

Table 5.3. Comparative data for typical chemiluminescence systems employed to determine Co(II).

System	Comments[a]	Reference
CO_2-luminol	Limit of detection: $5x10^{-13}$ M. Dynamic range: up to 10^{-7} M. Tolerance level to metal ions: Mn(II) and Ni(II), 50-fold excess, Cu(II): 10-fold excess.	this work
luminol-H_2O_2	Limit of detection: 1×10^{-11} M. Dynamic range: not available. Ion chromatography used for separation of Co(II), Cu(II), Fe(II), and Cr(III).	18
luminol-KIO_4	Limit of detection: 2×10^{-10} M. Dynamic range up to 2×10^{-7} M. Tolerance level to other metal ions: Cd(II) and Ni(II), 50-fold excess. Citrate used as masking agent.	19
O_2-DEAPH[b]	Limit of detection: 2×10^{-11} M. Dynamic range: up to 1×10^{-7} M. Determination in presence of pentadecyltriethylammonium bromide.	9
lucigenin-H_2O_2	Limit of detection: 3×10^{-10} M. Dynamic range: up to 3×10^{-3} M. Most metal ions do not interfere.	2 3
lophine-H_2O_2	Limit of detection: 2×10^{-9} M. Dynamic range: up to 2×10^{-7} M. Ion-exchange separation of Co(II) from Ca(II), Mg(II), Fe(II), and Mn(II).	5 20

Table 5.3 continued:

tartaric acid-H_2O_2	Limit of detection: 7 x 10^{-10} M (estimated from the mean background signal plus twice its standard deviation). Dynamic range: 6 x 10^{-8} M to 3 x 10^{-5} M. Citrate used as masking agent. Interference from Fe(II), Cr(III), and Mn(II) above a 10-fold excess.	7
gallic acid-H_2O_2	Limit of detection: 7 x 10^{-9} M. Dynamic range: 10^{-8} to 10^{-3} M. Mn(II), Cd(II), Ag(I), and Pb(II) interfere.	6
pyrogallol-H_2O_2	Limit of detection: 8 x 10^{-9} M. Dynamic range: up to 2 x 10^{-4} M. Mn(II) interferes above an 8-fold excess, and Cd(II) above an 80-fold excess.	10

(a) only those metal ions whose tolerance level is below a 100-fold excesses are included.
(b) DEAPH = 4-diethylaminophthalohydrazide.

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CHAPTER VI

APPLICATION OF CARBON DIOXIDE-ENHANCED CHEMILUMINESCENCENCE OF LUMINOL IN THE ABSENCE OF ADDED OXIDANT II: DETERMINATION OF CARBON DIOXIDE IN THE GAS PHASE

Introduction

The quantitative measurement of gaseous carbon dioxide is important in clinical health care and in environmental studies. Breath-by-breath monitoring of carbon dioxide, in addition to oxygen, is needed in intensive care units and operation rooms [1, 2]. For example, during an operation one serious problem can occur, namely the "disconnect" problem which arises when the gas line carrying forced ventilation to the patient's lungs becomes dislodged [3]. The continuous monitoring of the rhythm of exhaled carbon dioxide is a check that the forced ventilation is satisfactory, and any collapse of the rhythm provides early warning of a disconnection. As the primary anthropogenically-produced greenhouse gas, carbon dioxide is also a key chemical parameter in environmental research [4]. Widespread mapping of the partial pressure of carbon dioxide in the atmosphere is important for global atmospheric pollution control [5].

Traditionally, carbon dioxide in the gas phase is determined by absorption in a basic solution (followed by titration with acid), infrared absorption spectroscopy, or thermal conductivity measurement [6, 7]. Although these methods provide accurate analytical data, there is increasing interest in the development of inexpensive, portable sensors for carbon dioxide [5-17]. One of the active research areas is the solid state sensors which mainly include (1) the capacitor-type sensors using polycrystalline ceramics [18-20] and (2) solid

electrolyte sensors using the NASICON (sodium ionic conductor)-based electrochemical cell [21-23] or based on the lithium ionic conductor [24, 25]. Usually, these solid carbon dioxide sensors have a wide dynamic range (e.g., from 4 to 4 x 10^5 ppm [22]) and their response times are in the range of 8 s to 40 s. However, such sensors have to be operated at high temperature (600 to 1100 K) and are sensitive to temperature changes.

At present, the most commonly used carbon dioxide sensor is the Severinghaus-type sensor [26], which is based on the determination of the pH of a small volume of HCO_3^- solution by a glass electrode. A gas permeable membrane is used to hold the HCO_3^- solution around the pH electrode. When carbon dioxide reaches equilibrium on both sides of the membrane, the pH in the internal solution depends on the concentration of carbonic acid produced from the hydration of the permeated carbon dioxide, which in turn is proportional to the partial pressure of carbon dioxide in the sample:

$$CO_2(g)\ (\text{in sample}) \leftrightarrow CO_2(aq)\ (\text{in internal solution})$$

$$CO_2(aq) + 2H_2O \leftrightarrow HCO_3^- + H_3O^+$$

$$HCO_3^- + H_2O \leftrightarrow CO_3^{2-} + H_3O^+$$

This type of potentiometric carbon dioxide sensor is subject to the various drawbacks of the pH electrode [27], such as slow response, temperature effect, electrical resistance problems, reference contamination, etc.

To avoid the drawbacks associated with the potentiometric method of detection, optical carbon dioxide sensors have been developed in recent years [28-36]. At present, almost all optical sensors have the same sensing principle as the Severinghaus-type potentiometric sensor. The construction of such sensors is also the same as the Severinghaus-type sensor except that a piece of optical fiber is used to replace the pH electrode and a pH-sensitive dye is added to the internal HCO_3^- solution. The protonated form, HD, and the deprotonated form, D^-, of the dye have different absorption or fluorescence characteristics. The change of absorbance, fluorescence intensity, or fluorescence lifetime at a certain wavelength is proportional to the partial pressure of carbon

dioxide in the sample. Typically, these optical sensors show response up to ca. 15-20% (v/v) carbon dioxide, and their sensitivities are governed by the pK_a of the dye, the ionic strength, and the HCO_3^- concentration in the internal solution [28].

While avoiding problems inherent to the pH electrode, the current optical sensors retain the drawbacks of using an internal HCO_3^- solution (e.g., slow response time and short shelf life because of the loss of reagent in the internal solution). Recently, a novel range of colorimetric and fluorometric sensors for carbon dioxide were developed without any internal solution [29-32]. A phase transfer agent, tetraoctylammonium hydroxide, was used to replace the previously required HCO_3^- solution. This agent allowed a pH-sensitive dye to be incorporated into a plastic thin film, along with the water required for production of carbonic acid and protonation of the dye. This type of sensor exhibits fast response and recovery times (typically less than 3 s) and has a very long shelf life. However, the sensitivities are not high enough for the determination of carbon dioxide in the atmosphere (about 0.033% v/v).

Another strategy for solving the problem of reagent loss and changes in the chemical composition of the internal solution is to deliver continuously the reagent solution to the tip of the sensor, essentially forming a microoptical flow cell. As demonstrated by DeGrandpre [5], this so-called "renewable-reagent" fiber optic sensor has unprecedented stability while retaining high sensitivity for carbon dioxide. However, as most other optical sensors which use a membrane, it has a slow response time (more than 10 min). This makes it unsuitable for monitoring carbon dioxide in breath gas, which requires almost instantaneous response.

Because chemiluminescence determinations have unique advantages over colorimetric and fluorometric methods (e.g., high sensitivity and simple instrumentation), the development of carbon dioxide sensors based on chemiluminescence determinations represents an attractive area of research. However, such sensors have not been reported in the literature. The results obtained in the previous studies of this research project [37]

made it possible to develop a chemiluminescent carbon dioxide sensor. This chapter will discuss a novel sensing principle for carbon dioxide, the construction of a prototype carbon dioxide sensor, and the application of this sensor for monitoring carbon dioxide in respiratory gas and in the atmosphere on the basis of the enhancement of luminol chemiluminescence by carbon dioxide in the presence of cobalt(II) phthalocyanine as a rate modifier.

Experimental Section

Reagents and Solutions

The water used for preparation of solutions was treated as described in Chapter III. The luminol solutions were prepared in the same way as in Chapter IV except that the pH of the solution was adjusted to 12.6 with KOH.

The saturated cobalt(II) phthalocyanine (CoPc) solution was prepared by adding a small amount of solid CoPc (Pfaltz & Bauer, Flushing, NY) to a 4% (v/v) ethanol aqueous solution, stirring well, and then filtering through a No. 4 Whatman filter paper (W. & R. Balston, England). The filtrate was used as the working solution of CoPc. Compared to the unfiltered CoPc solution used in previous studies, this solution gave a chemiluminescence of 35% less intensity.

The air and pure carbon dioxide gases were purchased from Sooner Airgas (Shawnee, OK). The standard carbon dioxide gas [792 ppm (v/v) in air] was obtained from Jimmie Jones (Tulsa, OK). Other carbon dioxide gases of different concentrations were prepared by diluting either pure or standard carbon dioxide with air. The air was passed twice through 1.0 M sodium hydroxide solution before mixing with carbon dioxide gas. Two flow meters were used to control the relative amounts of carbon dioxide and air in the mixture. After the gases passed through the flow meters, they were combined at a T-tube which was connected to the inlet port of the reaction cell. The total flow rate of the gas entering the reaction cell was 57 ml/min.

Reaction Cell

The reaction cell used in this study was obtained by modifying the flow cell developed in Chapter III [38] so that it could be used for the chemiluminescent reaction between gas and aqueous solution. Figure 6.1 illustrates the details of the reaction cell. The cell body was constructed of PTFE and the external surface was painted black to make it light-tight. It was designed in such a way that half of the cell contained the chemiluminescent reagent and the other half was filled with sample gas (the depth of the reaction cell is 10 mm). To increase the contact between the chemiluminescent reagent and the sample gas, a miniature stirring bar (approximately 10 x 3 x 3 mm, PTFE-coated Micro Stir bar from Markson Science, Phoenix, AR) was placed inside the cell so that the solution could be stirred well. The volume of the cell was about 800 ml, but it was reduced to about 200 ml when the stirring bar is rotating.

To determine carbon dioxide in a sample, the gas is sent into the reaction cell through *IN1*, and the mixture of luminol and CoPc was pumped into the cell through *IN2*. A chemiluminescent reaction occurs at the interface between the solution and the gas. The light emitted from the cell is detected by a photo-detector (a RCA 1P28A side-on 9-stage photomultiplier tube) which is located just above the glass window (*GW*). The photomultiplier tube housing and the cell are interconnected with Canon-type optical connectors (*OC*), and therefore they can be easily separated from each other.

Instrumentation

The chemiluminescence detection system developed in Chapter III was used in this study except that the flow cell with a rotating disk was replaced with the gas-liquid reaction cell described in the above section. A magnetic stirrer was still used to drive the stirring bar inside the reaction cell.

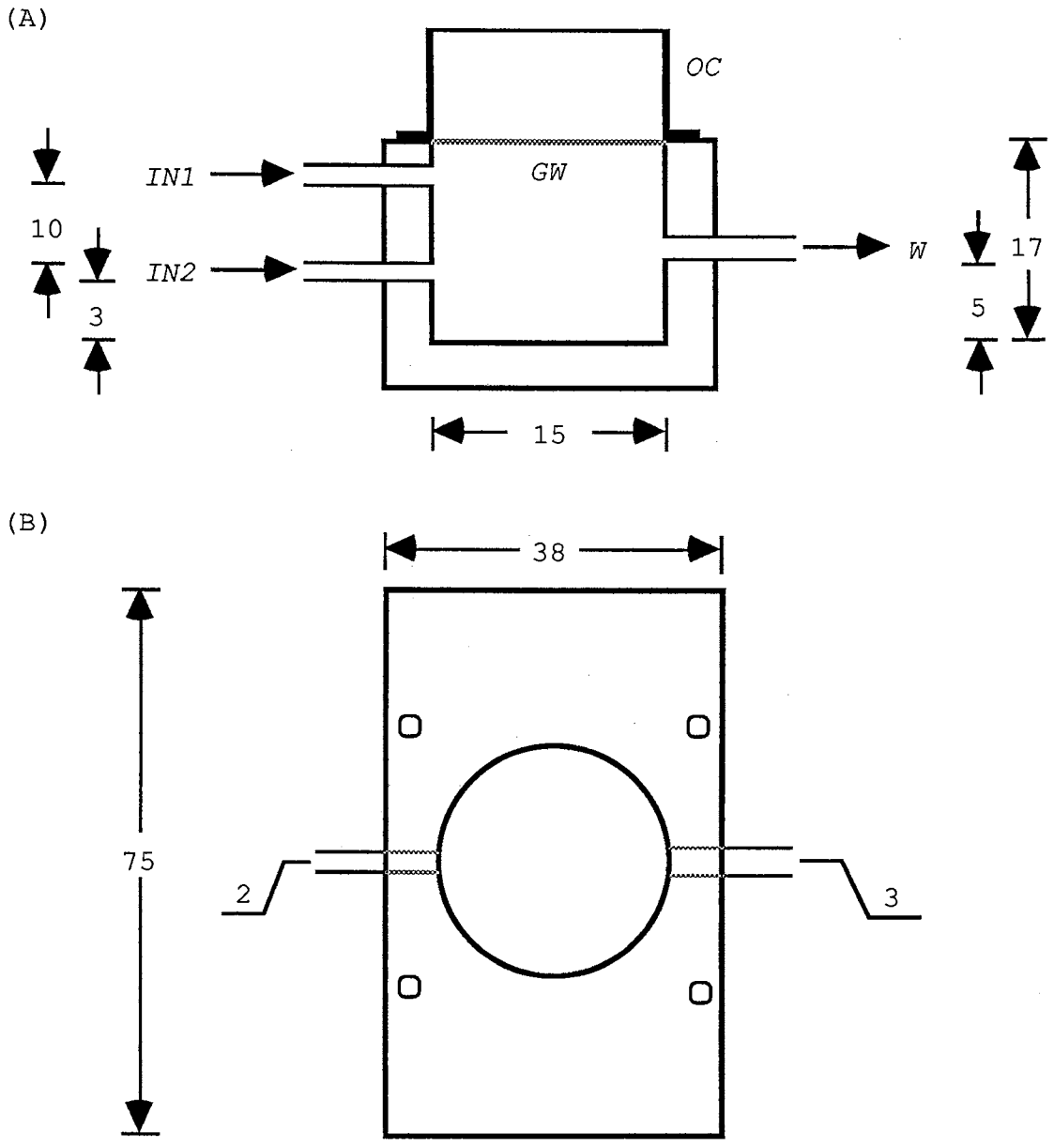

Figure 6.1. Schematic representation of (A) the cross-section view and (B) the top view of the reaction cell used in this study. IN1 and IN2: inlet ports for gas and the mixture of luminol and CoPc solutions, respectively; W: outlet port for waste solution and gas; GW: glass window; OC: optical connector. All measurements are in mm.

Figure 6.2A and 6.2B schematically illustrate the instruments used for monitoring carbon dioxide in atmospheric air and expired human breath, respectively. In Figure 6.2A, the gas is directly introduced into the reaction cell and the reagent mixture (luminol + CoPc) is continuously pumped into the cell. A steady-state chemiluminescence is reached in a short time. This scheme is suitable for the determination of carbon dioxide in low concentrations (less than 800 ppm). In Figure 6.2B, a four-way Teflon rotary injection valve (Rheodyne Type 50) is used to inject a small volume of sample gas (100 ml) into the reaction cell; the reagent mixture is the carrier solution. It is suitable for monitoring the gas in which the concentration of carbon dioxide is greater than 800 ppm.

Results and Discussion

As shown in previous studies [37], carbon dioxide can greatly increase the luminol chemiluminescence either in the presence or in the absence of hydrogen peroxide as an oxidant, and a range of metal ions and complexes can act as rate modifiers for this chemiluminescent reaction, which may include Fe(II), Fe(III), Cu(II), Mn(II), Cr(III), Co(II), CoPc, $CoTPPS_4$, and hexacyanoferrate(III). The enhancement of carbon dioxide is dependent upon which rate modifier is used in the chemiluminescent reaction. The largest enhancement was observed when CoPc was used as the rate modifier for the luminol chemiluminescence, either in the presence or in the absence of hydrogen peroxide. Therefore, the CoPc/luminol system was selected to determine carbon dioxide in the gas phase, and, in order to keep the background low, no hydrogen peroxide was used. The chemical reaction involved in this study can be written as:

$$\text{Luminol} + \text{CoPc} + CO_2 \rightarrow \text{Products} + h\nu$$

Determination of Carbon Dioxide in Atmosphere

Factors Affecting the Chemiluminescence. First of all, the effect of pH on the chemiluminescence of luminol/CoPc/carbon dioxide system was examined by adjusting

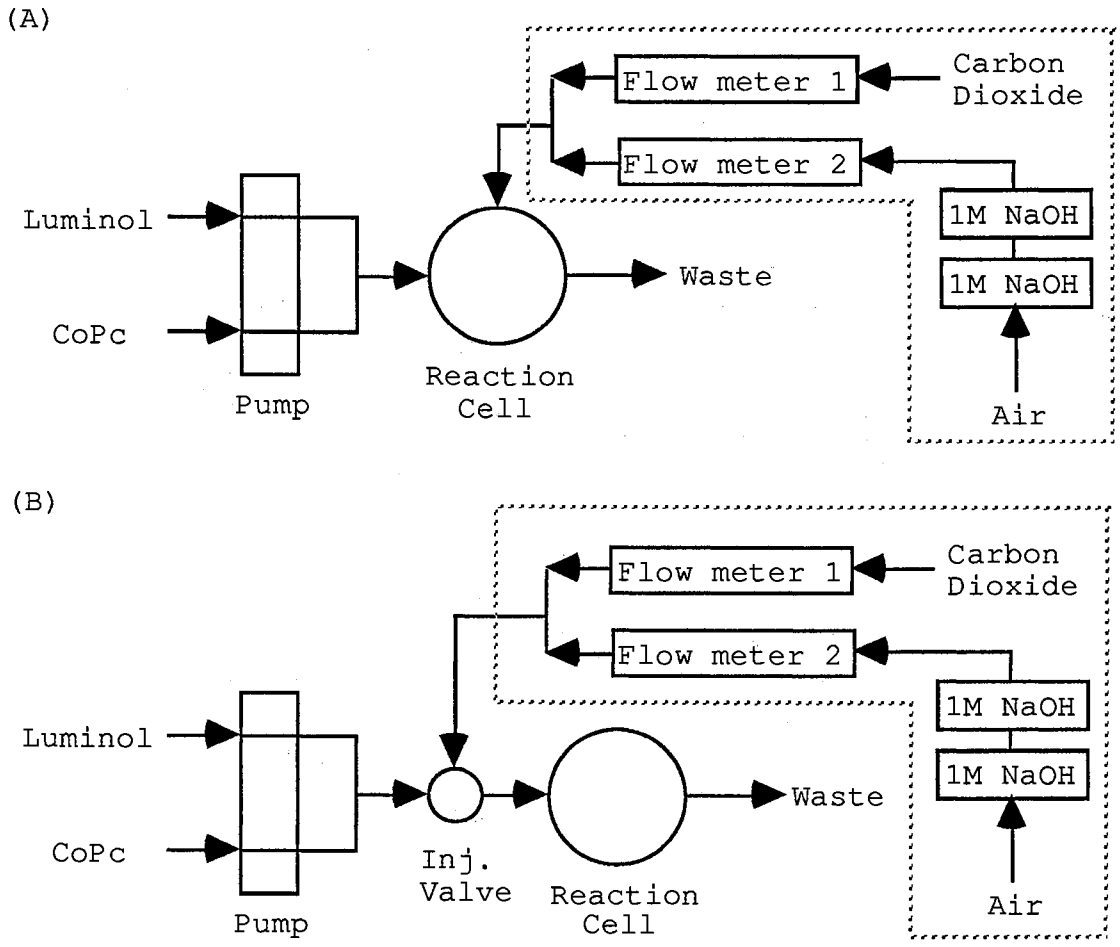

Figure 6.2. Schematic representation of the instrumentation used for determination of carbon dioxide in: (A) atmospheric air and (B) in expired human breath. Apparatus included in dashed line are only used to obtain calibration curve; otherwise, the gas of interest is directly introduced into the reaction cell or the injection valve.

the unbuffered luminol solution to the desired pH values with potassium hydroxide solution (Figure 6.3). Chemiluminescence was detected only when the pH of luminol solution was greater than 9.0. Two maximum intensities were observed in the pH range of 9.0 to 13.0: one was at pH 10.0 ~ 10.7, another was at pH 12.0 ~ 12.7, and the latter had higher intensity. Due to the difference in the reagent transport system, this pH profile is different to that obtained in the previous study which has only one maximum at pH ~13 [37]. The luminol solution of pH 12.6 (prepared in potassium hydroxide solution) was used in this study. In such a strongly basic medium, no buffer is needed; therefore the background chemiluminescence from the reagent mixture in the absence of carbon dioxide is greatly reduced. The concentration of luminol also affects the chemiluminescence intensity. In order to minimize the consumption of luminol, a 5.0×10^{-3} M luminol solution was used, although the chemiluminescence intensity increased with an increase in the concentration of luminol even beyond 1.0×10^{-2} M.

The stirring bar inside the reaction cell is designed to facilitate the contact between the sample gas and the reagent solution. As a result, the chemiluminescence was observed to increase with an increase in stirring velocity, while the background emission gradually decreased (Figure 6.4). The rotation velocity of the stirring bar is proportional to the voltage applied to the magnetic stirrer. The maximum intensity was reached when the voltage applied to the magnetic stirrer was 65 V, and beyond this value no change was observed for both signal and background.

Since the reagent mixture and the sample gas are continuously introduced into the reaction cell, the flow rates of reagent solution and sample gas affect the chemiluminescence. The chemiluminescence intensities of both sample and background increase with an increase in the flow rate of the reagent mixture. As shown in Figure 6.5, a maximum net signal is reached at 1.20 ml/min. Beyond 1.20 ml/min, the net signal begins to decrease. Therefore, 1.20 ml/min was selected as the total flow rate of the reagent mixture (luminol and CoPc in the mixture have the same flow rate). An increase in the

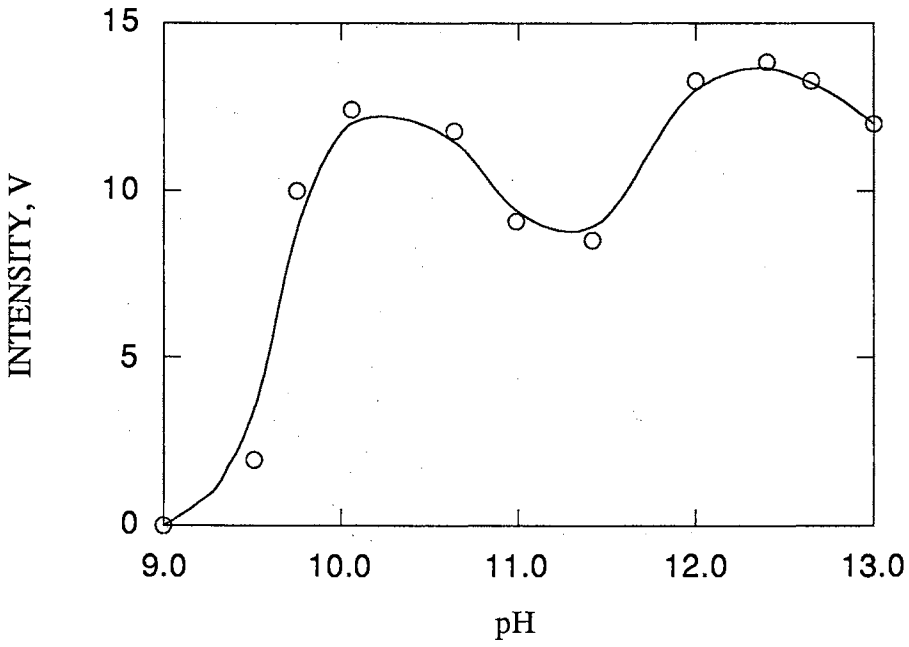

Figure 6.3. Effect of pH on the chemiluminescence of the luminol/CoPc/CO_2 system. [Luminol]: 5.0 x 10^{-3} M; CO_2: 320 ppm.

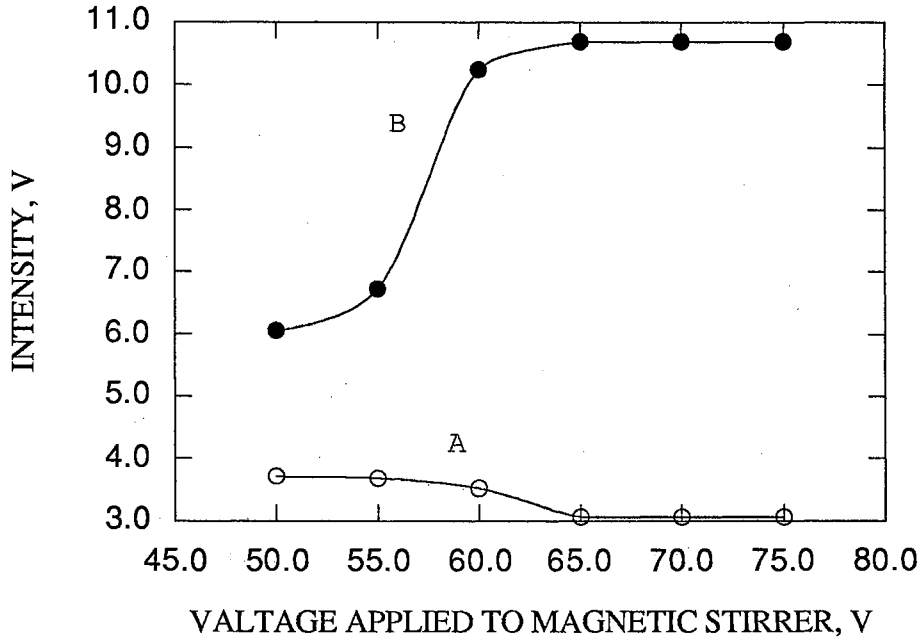

Figure 6.4. Change of (A) background emission and (B) net signal with stirring velocity. [Luminol]: 5.0 x 10^{-3} M; CO_2: 320 ppm.

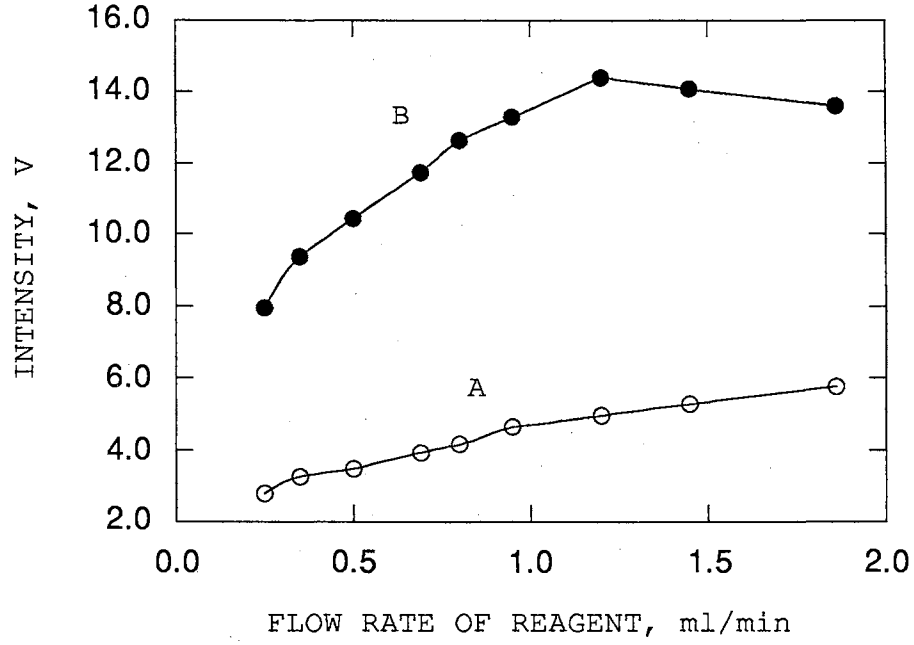

Figure 6.5. Influence of the flow rate of the reagent mixture on (A) the background emission and (B) the net signal. [Luminol]: 5.0 x 10^{-3} M; CO_2: 320 ppm.

flow rate of the sample gas also increases the chemiluminescence intensity. The optimum flow rate was found to be greater than 35 ml/min, above which the intensity of chemiluminescence did not change.

Calibration Curve. The log of chemiluminescence intensity vs. the log of carbon dioxide concentration in air is linear up to 800 ppm CO_2 (regression coefficient = 0.997) (Figure 6.6A). The limit of detection, defined as the concentration that corresponds to a signal of 3s (s is the standard deviation for the measurements of background emission), was found to be 2.04 ppm CO_2.

The standard deviation for 11 measurements of 320 ppm carbon dioxide was 1.2%. The carbon dioxide in several synthetic air samples were determined; the concentration of carbon dioxide in these samples ranged from 200 to 600 ppm. The recovery was found to be within 99 - 101%

Interferences. The concentration of carbon dioxide in the atmosphere is in the range of 300 to 1000 ppm; other harmful gases include SO_2 (10^{-3} to 10 ppm), NO_x (NO and NO_2, 10^{-3} to 10 ppm), and O_3 (10^{-3} to 1 ppm) [7]. These gases, with such low concentrations compared to carbon dioxide, are expected to introduce little, if any, interference in the determination of carbon dioxide.

Breath-by-Breath Determination of Carbon Dioxide

In the breathing process, the flow of expired gas is not constant. Therefore, the instrumentation used for the determination of carbon dioxide in the atmosphere (Figure 6.2A) is not suitable for monitoring carbon dioxide in breath. A new scheme, as shown in Figure 6.2B, has been developed for such a purpose. An injection valve was used to introduce the sample gas into the reaction cell by intercalating a small volume of sample gas in the flow stream of carrier solution, the pre-mixed luminol and the CoPc solution. In this way, the flow rate of the expired gas does not affect the chemiluminescence intensity.

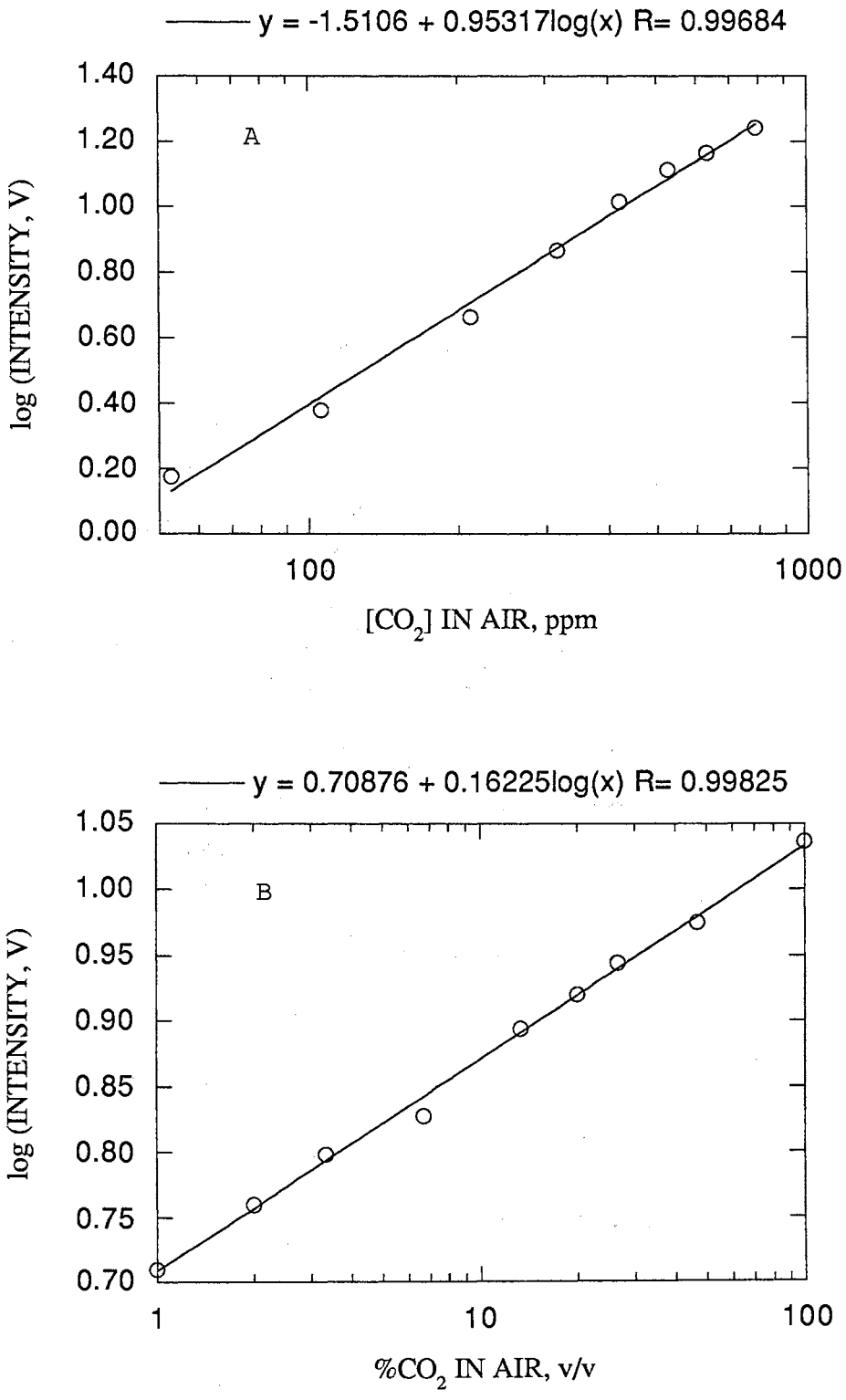

Figure 6.6. Calibration curves for carbon dioxide at (A) low concentration and (B) high concentration. [Luminol]: 5.0×10^{-3} M.

Two important parameters must be considered in the breath-by-breath determination, namely response time and recovery time. Here, response time is defined as the time for 100% signal to be reached, and the recovery time as the time for the signal to decay by 90%. These times determine if the respiratory rhythm can be detected. For example, the sum of response and recovery times must be smaller than 4 sec in order to get the respiratory curve of a normal human being with base line resolution because the human respiratory cycle takes about 4 sec. With the instrumentation shown in Figure 6.2B, both response and recovery times depend on, in addition to the characteristics of the chemiluminescent reaction, the flow rate of reagent mixture and the volume of sample gas to be introduced into the reaction cell. The faster the flow and the smaller the volume, the faster the response and recovery times. For example, the response and recovery times were 1.0 and 2.6 sec, respectively, when the injection volume of expired gas was 100 ml and the flow rate of reagent mixture was 5.2 ml/min.

Figure 6.7 shows a typical breath-by-breath response from a healthy human subject. It can be seen that satisfactory monitoring of the respiratory rhythm can be achieved.

The quantitative determination of carbon dioxide in breath is also feasible because the CO_2/luminol/CoPc system shows responses up to 100% carbon dioxide. A straight line was obtained by plotting the log of chemiluminescence intensity vs. the log of % (v/v) carbon dioxide (Figure 6.6B). From this calibration curve, the average concentration of carbon dioxide recorded in Figure 6.7 was found to be 4.78% (v/v).

Conclusions

The CO_2/luminol/CoPc system described herein is capable of determining carbon dioxide in atmospheric air and monitoring the breath-by-breath changes with unprecedented speed, sufficient sensitivity, and high accuracy. Based on this novel sensing principle for carbon dioxide, a more compact sensor can be constructed by adopting optical fibers and a

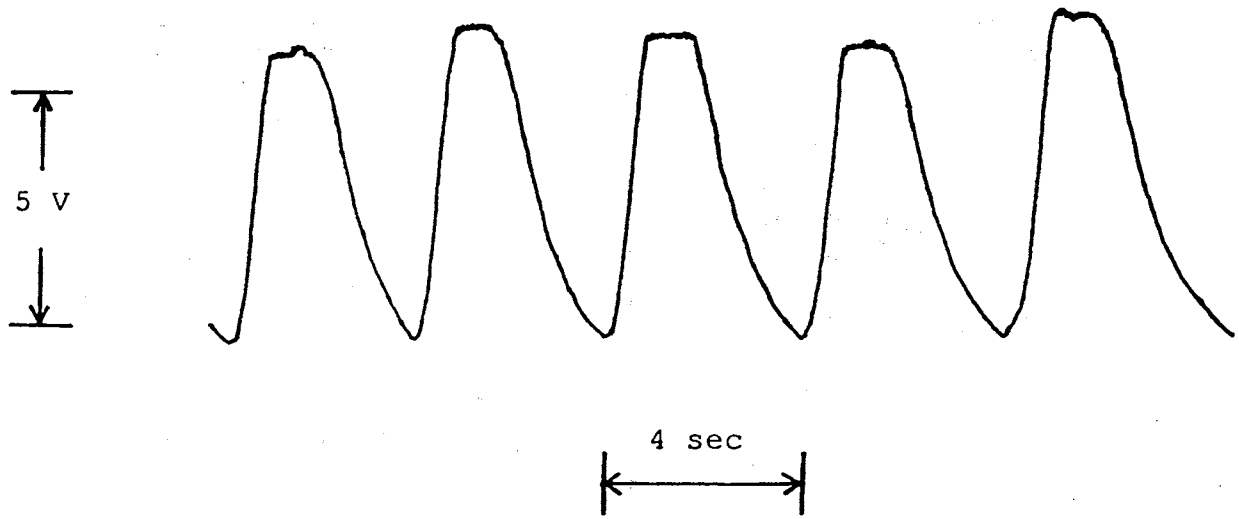

Figure 6.7. The respiratory rhythm recorded from a healthy human subject.

selective carbon dioxide permeable membrane. Such an optical sensor will greatly reduce the consumption of chemiluminescent reagent and permit remote sensing. Although the use of a membrane will slow the response time, it would allow the sensor to be used for the determination of carbon dioxide in solutions and exhaust where many interferences exist.

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APPENDIX

A. COMPUTER PROGRAMMS

(1) Program Used to Draw the Complete Graph in the Oscilloscope Window

```
0: ent "FULL SCALE mV:", V
1: wrt 705, "IN; SP1; IP1000, 1000, 4300, 6000;"
2: wrt 705, "PA1000, 1000; PD; TL4; XT;"
3: for K=1 to 10
4: wrt 705, "PR330, 0; XT;"
5: next K
6: wrt 705, "PA1000, 1000;"
7: for J=1 to 9
8: wrt 705, "PR0, 180; TL2; YT;"
9: next J
10: wrt 705, "PA1000, 2800, 4300, 2800, 4300, 1000;                PU;"
11: wrt 705, "SC1, 4096, 0", V, ";"
12: dim Y$[8208]
13: buf "data", Y$, 2
14: wtc 2, 3; wtc 2, 0; wait 75
15: tfr 2, "data", 4095
16: wtc 2, 3
17: for I=0 to 4095
18: itf(Y$[2I+1, 2I+2])->Y
```

```
19: Y*V*0.0005->Y
20: wrt 705, "PA", I, Y, ";PD"
21: next I
22: wtc 2, 3
23: wrt 705, "PU; SP0;"
24: end
```

(2) Program Used to Draw 1/2 Window of Oscilloscope

```
0: ent "FULL SCALE mV:", V
1: wrt 705, "IN; SP1; IP3000, 1200, 7560, 5800;"
2: wrt 705, "PA3000, 1200; PD; TL4; XT;"
3: for K=1 to 6
4: wrt 705, "PR760, 0; XT;"
5: next K
6: wrt 705, "PA3000, 1200;"
7: for J=1 to 9
8: wrt 705, "PR0, 460; TL2; YT;"
9: next J
10: wrt 705, "PA3000, 5800, 7560, 5800, 7560, 1200;                PU;"
11: wrt 705, "SC1, 2458, 0", V, ";"
12: dim Y$[6925]
13: buf "data", Y$, 2
14: wtc 2, 3; wtc 2, 0; wait 75
15: tfr 2, "data", 2458
16: wtc 2, 3
17: for I=0 to 2457
18: itf(Y$[2I+1, 2I+2])->Y
```

```
19: Y*V*0.0005->Y
20: wrt 705, "PA", I, Y, ";PD"
21: next I
23: wrt 705, "PU; SP0;"
24: end
```

(3) Program Used to Read I vs. t Data from a Curve

```
0: ent "FULL SCALE mV:", V
1: ent "START TIME ms:", S
2: ent "END TIME ms:", E
3: ent "TIME PER POINT FROM OSCILLOSCOPE ms:", P
4: ent "TIME INTERVAL DESIRED ms:", D
5: S/P->I;  E/P->F
6: dim Y$[8208]
7: buf "data", Y$, 2
8: wtc 2, 3; wtc 2, 0; wait 75
9: tfr 2, "data", 4095
10: wtc 2, 3
11: for J=0 to 4095
12: itf(Y$[2J+1, 2J+2])->Y
13: Y*V*0.0005->Y
14: if J<I; gto 18
15: if J>F; gto 19
16: if J*P=S;  prt "t", S, "I", Y
17: if J*P-S=D; gsb "SUBROUTINE"
18: next J
19: end
```

20: "SUBROUTINE": J*P->S

21: prt "t", S, "I", Y

22: ret

B. KINETIC DATA FOR SOME RATE MODIFIERS

(1) $Fe(CN)_6^{3-}$ in the Absence of CO_2

TIME, ms	INTENSITY, mV	TIME, ms	INTENSITY, mV
1700	528	4900	520
1800	552	5000	400
1900	456	5100	440
2000	504	5200	400
2100	448	5300	432
2200	472	5400	368
2300	400	5500	424
2400	456	5600	352
2500	400	5700	400
2600	424	5800	344
2700	360	5900	368
2800	392	6000	312
2900	368	6100	352
3000	352	6200	288
3100	312	6300	304
3200	328	6400	280
3300	296	6500	312
3400	312	6600	272
3500	256	6700	312

3600	312	6800	264
3700	248	6900	288
3800	304	7000	256
3900	248	7100	272
4000	232	7200	264
another set:		7300	240
4000	552	7400	224
4100	600	7500	232
4200	504	7600	208
4300	544	7700	232
4400	480	7800	200
4500	488	7900	240
4600	472	8000	176
4700	472	8100	208
4800	416		

(2) $Fe(CN)_6^{3-}$ in the Presence of CO_2

TIME, ms	INTENSITY, mV	TIME, ms	INTENSITY, mV
2000	1008	4100	344
2100	800	4200	368
2200	984	4300	344
2300	768	4400	320
2400	864	4500	280
2500	688	4600	320
2600	760	4700	280
2700	648	4800	288
2800	664	4900	248

2900	576	5000	280
3000	640	5100	248
3100	520	5200	256
3200	544	5300	240
3300	472	5400	240
3400	472	5500	208
3500	416	5600	224
3600	480	5700	192
3700	392	5800	224
3800	432	5900	208
3900	360	6000	216
4000	352		

(3) Co^{2+} in the Absence of CO_2

TIME, ms	INTENSITY, mV	TIME, ms	INTENSITY, mV
171	11.2	188	76.8
172	17.6	189	72.0
173	19.2	190	68.8
174	25.6	191	64.0
175	28.8	192	75.2
176	30.4	193	70.4
177	35.2	194	73.6
178	43.2	195	68.8
179	52.8	196	86.4
180	51.2	197	84.8
181	60.8	198	72.0
182	62.4	199	80.0

183	62.4	200	70.4
184	52.8	201	84.8
185	64.0	202	75.2
186	65.6	203	89.6
187	62.4	204	81.6

(4) Co^{2+} in the Presence of CO_2

TIME, ms	INTENSITY, mV	TIME, ms	INTENSITY, mV
127	80.0	221	4240
128	160	226	3920
129	240	231	3600
130	320	236	3440
131	400	241	3600
132	480	246	3600
133	560	251	3600
134	720	256	3440
135	880	261	3280
136	1040	266	3040
137	1200	269	2800
138	1360	319	2400
139	1440	369	2240
140	1680	419	2080
141	1760	469	1680
142	1920	519	1600
143	2080	569	1600
144	2160	619	1600
145	2400	669	1360

146	2480	719	1280
147	2560	769	1280
148	2720	819	1200
149	2880	869	1120
150	3040	919	1040
151	3040	969	1040
152	3200	1019	960
153	3280	1069	880
154	3360	1119	880
155	3440	1169	880
156	3600	1219	800
157	3600	1269	720
158	3760	1319	720
159	3920	1369	640
160	4000	1419	640
161	4160	1469	560
162	4320	1519	560
163	4320	1569	560
164	4480	1619	560
165	4480	1669	480
166	4640	1719	400
171	4880	1769	480
176	4880	1819	400
181	4800	1869	320
186	4560	1919	400
191	4240	1969	400
196	4080	2019	320

201	4240	2069	320
206	4480	2119	320
211	4560	2169	320
216	4400		

(5) Mn^{2+} in the Presence of CO_2

TIME, ms	INTENSITY, mV	TIME, ms	INTENSITY, mV
257	48.0	470	976
259	64.0	490	816
261	128	510	864
263	208	530	784
265	224	550	800
267	272	570	704
269	320	590	816
271	368	610	704
273	464	630	736
275	496	650	656
277	624	670	752
279	640	690	640
281	704	710	720
283	736	730	624
285	784	750	672
287	896	770	560
289	944	790	704
291	960	810	560
293	1056	830	576
295	1104	850	576

297	1184	870	640
299	1248	890	528
301	1312	910	624
303	1424	930	512
305	1456	950	560
307	1472	970	480
309	1472	990	560
310	1488	1010	496
315	1472	1030	528
320	1440	1050	448
325	1344	1070	496
330	1264	1090	464
335	1296	1110	528
340	1440	1130	448
345	1392	1150	480
350	1408	1170	416
355	1408	1190	464
360	1328	1210	416
365	1232	1230	496
370	1168	1250	416
375	1152	1270	416
380	1184	1290	432
385	1248	1310	416
390	1232	1330	400
395	1152	1350	416
400	1072	1370	368
405	992	1390	400

410	944	1410	368
430	1008	1430	416
450	880	1450	336

(6) CoPc in the Presence of CO_2

TIME, ms	INTENSITY, mV	TIME, ms	INTENSITY, mV
500	6880	1300	1920
550	5920	1350	1840
600	4880	1400	1600
650	4400	1450	1440
700	4560	1500	1520
750	4240	1550	1440
800	3520	1600	1280
850	3280	1650	1200
900	3360	1700	1200
950	3200	1750	1200
1000	2640	1800	960
1050	2480	1850	880
1100	2560	1900	960
1150	2400	1950	880
1200	2000	2000	720
1250	1920		

(7) Co^{2+} in the Presence of CO_2

TIME, ms	INTENSITY, mV	TIME, ms	INTENSITY, mV
400	5440	1100	1120
450	4240	1150	960

500	3120	1200	800
550	2640	1250	880
600	2000	1300	880
650	1920	1350	800
700	1840	1400	640
750	1680	1450	720
800	1360	1500	720
850	1360	1550	640
900	1360	1600	560
950	1200	1650	560
1000	1120	1700	560
1050	1120		

VITA

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