AN ABSTRACT OF THE THESIS OF

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Abstract approved: James D. Ingle, Jr.

This thesis is concerned with the development of analysis techniques based on chemiluminescence (CL). In general, CL is produced during the oxidation of ethanol and phenols by acidic permanganate. With a discrete sampling-system, detection limits of 0.07% (v/v) of ethanol and 0.03 μM of pyrogallol (Pg) were obtained.

Mn(IV) and Mn(III) were used as oxidants instead of MnO_4^-. Each species is discussed in terms of optimization of experimental conditions, calibration curves, and detection limits. The results are consistence with Mn(IV) being the actual oxidant for ethanol responsible for the CL signal. The identity of the actual oxidant of Pg is not clear.

Additional oxidants (AO) (Ce^{4+}, Ag^{2+}, $Cr_2O_7^{2-}$, IO_4^-, and $S_2O_8^{2-}$) were added to Pg/ethanol reaction mixture before injecting permanganate solution. In all cases, the CL signal of both analytes decreased since the AO oxidized them before permanganate was injected. Solid phase extraction (SPE) using ENVI-Chrom P tubes (styrene divinylbenzene) was tested for

retaining phenols in beverage samples (wine, whiskey, and rum) so that ethanol can be detected alone. The CL signal for phenol is reduced by the treatment but the recovery of ethanol is still not satisfactory.

Oxidation of Ethanol and Phenols with Permanganate for Chemiluminescence Analysis

by

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

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Jameela Isa Al-Mutawah, Author

To my mother...with love

إلى أمي ... مع حبي وتقديري وامتناني

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I would like to dedicate this achievement to my mother for her continual encouragement, support, and her faith in me.

I would like to thank all my family and friends whose support and encouragement made this work possible.

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF TABLES

OXIDATION OF ETHANOL AND PHENOLS WITH PERMANGANATE FOR CHEMILUMINESCENCE ANALYSIS

INTRODUCTION

Chemiluminescence (CL) is the emission of radiation, usually in the visible or near infrared region, as a result of a chemical reaction.[1] One of the reaction products (or intermediates) is formed in an excited electronic state and emits the radiation. Oxidation reactions are the most frequent source of CL, as they involve large free energy changes.

CL techniques are based on the fact that in a few reactions, a significant fraction of intermediates or products are produced in excited electronic states and the emission of photons from the excited molecules is a favorable deactivation process. Under appropriate conditions, the emission intensity related to the concentration of a reactant or catalyst in the CL reaction.[2]

Chemiluminescent reactions can occur very rapidly (< 1 s) or can be long lasting (> 1 d).[1] Such a wide range presents a challenge to the development of instrumentation for CL monitoring.[1] CL determinations often lack selectivity because other compounds or species present in the matrix also chemiluminesce or affect the primary CL reaction.

Recently it was noted that oxidation of ethanol and phenols by permanganate under very acidic conditions produces

CL.[1,3] The research in this thesis is concerned with a more detailed examination of these CL reactions and their potential analytical use. Goals included improving the detection limit for the determination of phenols and ethanol and making the CL technique useful for the determination of ethanol in complex real samples. The analysis was accomplished by a discrete sampling method in which permanganate was injected into a reaction mixture to initiate the reaction. Some of the important factors studied include:

- additional oxidants in conjunction with MnO_4^- to pre-oxidize phenols but not ethanol
- sensitizers (fluorophores that accept the energy from the excited state produced and luminesce with more efficiency) to enhance the CL signal
- a PMT with higher quantum efficiency in the red than that used in previous studies
- Mn(III) and Mn(IV) as oxidants instead of MnO_4^- to learn more about the species actually responsible for the actual oxidation of phenols and ethanol as proposed in a previous study.[3]

Several wine and liquor samples were tested. The use of CL technique for determining ethanol in these beverages samples is limited by the lack of selectivity due to oxidation and CL from phenolic compounds in these samples. Therefore, the separation of ethanol from the interference or masking of the interference prior to the analysis of the samples was

investigated. In particularly, solid phase extraction (SPE) techniques were employed in an attempt to remove phenols from the sample matrices.

HISTORICAL

The Discovery of CL

The early writings of Human civilization contain references about luminous animals such as fireflies and glow-worms. However, it was the Greeks and Romans who first reported their characteristics. Euripides (480-406 B.C.) described how the Bacchantes carried fire on their hair without being hurt.[4] Aristotle (384-322 B.C.) is one of the first writers to recognize 'cold light'. He mentioned the light of the dead fish and flesh and realized that they were different from other bodies which had colors and could be seen by day. Pliny the elder (A.D. 23-79) provides a description of several luminous organisms including a luminous jelly fish, the luminous bivalve, and glow-worm. During the middle Ages (A.D. 200-1400), few scientific observations were reported. St. Augustine (354-430) and Gregory of Tours (544-595) both mentioned the glow worms and the latter reported the northern lights. Sawed Isidore (560-636) also talked about the luminous stones, as did Pliny, and about the lighting beetles, as did Rabanus Maurus (776-856) later. Another Middle Ages reference to the glow worms was by Holy Hildegard (1099-1179) from Germany. Arab writers also mentioned some lighting insects. References to fireflies occur in the works of Ibn-al-Baithar (1197-1248) and of Isa Kamal-al-Din al-Damiri (1344-1405). Ibn-al-Baithar described "Hobaheb" as a beetle

with wings that lights during the night. Columbus (1446-1506) and John Davis (1550-1603) referred to a light, which may have been the luminous worm, in the sea water during their voyage to the new world. Gonzalo Fernandez de Ovido (1478-1557) mentioned in his book Historia four kinds of luminous things: centipedes, worms, the light of the tree, and an elaterid beetle.[4]

In spite of these early descriptions, serious scientific investigations of 'cold' light only began at the end of the 17th century. Robert Boyle, in 1668, described the shining wood and he compared it to burning coal. Newton, in 1704, said that the burning coal is red hot wood, emits light and shines when heated beyond a certain degree. By 1794 J. Hutton used the term 'incandescence' (Latin, *incandare* = to become white) to describe the emission of light by a body heated to high temperature.[5] In 1860, Kirchhoff established the dependence of the intensity and color of light emission on the temperature of the emitting body. Planck, in 1900, explained this property by considering the radiation to be emitted by oscillators, in discrete packets called 'quanta'. Then, Einstein, in 1904, defined the equation used to calculate the energy of these 'quanta'. After the discovery of the 'Bolognian Stone' in 1603, the term chemiluminescence (Latin, *lumen* = light) was first used by Eilhard Wiedemann, in 1888, to describe chemical reactions which emit light. He suggested that any light not due to temperature radiation be called a luminescence. In fact, he distinguished six types of lumines-

cence: photoluminescence, electroluminescence, thermoluminescence, triboluminescence, crystalloluminescence, and chemiluminescence.[5]

The luminescence of phosphorus has been observed since its discovery by Henning Brand in 1669. He produced a material, by reduction of the solid from distilled urine, which has a property of glowing in the dark. He called it phosphorus. By that time, it was not realized that living organisms were responsible for 'shining wood' and 'shining flesh'.[5] John Canton's, in 1768, observed light when heating oyster shells and sulfur.[4] Johann Florian Heller, in 1843, first reported that luminous fungus and bacteria were responsible for 'shining wood' and 'shining flesh'.[5] In 1667, Boyle made many experiments to show that light from these sources is dependent upon a plentiful supply of air. In 1672, he reported some observations on luminous meat. This was the first experimental demonstration that oxygen, or one of its derivatives, is required in bioluminescent and chemiluminescent reactions even though he was not aware of that since oxygen was discovered over 100 years later by Scheele and Priestley.[5]

Even though Baker in 1742 had suggested that fish luminescence might be due to animalcules, and later, Hulme in 1800 concluded that the light is a constituent principle of marine fishes, it was Michaelis (1830) and Ehrenberg (1834) who decided that this light must be the result of some living

things.[4] In 1854, Heller saw minute living strands as the source of the light from the damp wood. Experiments conducted by McCartney in 1810 and repeated by Harvey in 1926 showed that extracts of luminous jelly fish could glow without air.[5] This puzzle was resolved in 1962 by Shimomura by his discovery of proteins which could be extracted from luminous jelly fish containing an organic prosthetic group with oxygen in the form of a hydroperoxide covalently attached to it.[5] Organic chemiluminescent reactions usually involve oxygen, but there are many examples of inorganic chemiluminescent reactions which do not.[5]

Other critical requirements have been found for chemiluminescence and bioluminescence. Spallanzani in the 1790s showed that water was necessary for luminous wood or jelly fish to glow.[5] In 1821, Macaire concluded that the luminous material in glow-worms was composed mainly of 'albumine', and required oxygen.[5] Between 1885 and 1887, Raphael Dubois showed that extraction of luminous organ in cold water produced a suspension which glowed initially and then gradually faded away. The extraction in hot water, however, resulted in no emission. He concluded that luminescence was the result of a chemical reaction and must require a heat-stable factor.[4]

The man-made substance luminol was discovered in the mid-19th century, but only was reported to be chemiluminescent in 1928 by Albrecht.[5] The first synthetic chemiluminescent

organic compound, lophine (2,4,5-triphenylimidazole), was prepared in 1877 by Bronislaus Radziszewski. He observed that luminescence was produced when lophine was shaken with alkaline alcoholic solution in air and that hydrogen extinguished it. By 1880 he had formulated a long list of synthetic chemiluminescent organic compounds and characterized the first chemiluminescence spectrum of the organic compound, lophine.[5]

The discovery of hydrogen peroxide by Thernard in 1819 and that of ozone by van Marum in 1785 led to the discovery of many other synthetic reactions capable of producing light. In 1877, Radziszewski reported that lophine does not emit a light when heated by itself. This enabled Wiedemann in 1888 to distinguish chemiluminescence from incandescence. The difference between incandescence and luminescence was a source of confusion for several centuries. Now, the luminescence is defined as the emission of electromagnetic radiation in UV, visible, and IR light from atoms or molecules as a result of the transition of an electronically excited state to a lower energy state, usually the ground state. Chemiluminescence is luminescence as the result of a chemical reaction.[5]

Sources of Light

Cold light[6] can be classified, according to the method producing it, as follows:

Candoluminescence. When heating some bodies, their

temperature becomes higher and they give off light of shorter wavelength than would be expected. This is called candoluminescence, the luminescence of the incandescent solids.

Pyroluminescence or *Flame Luminescence*. This emission is produced when salts are held in a bunsen burner giving various colors because of the excitation of atoms or molecules.

Thermoluminescence. This luminescence is produced when minerals are heated slightly. It depends on some previous illumination or radiation of the crystals.

Fluorescence and *Phosphorescence*. These processes are described as the emission of radiation by substance after absorption of light. If the exciting radiant energy is light, this is called photoluminescence, if cathode rays, cathodoluminescence, if anode rays, anodoluminescence, and if x-rays, radioluminescence. Fluorescence and phosphorescence are specific types of photoluminescence and the lifetime of fluorescence is much shorter than that of phosphorescence.

Electroluminescence. It occurs when two surfaces are separated from each other, the capacity diminished and the voltage rises until a discharge takes place, exciting the surrounding gas to luminesce.

Sonoluminescence. Light appears when intense sound waves pass through fluids. It accompanies electroluminescence.

Galvanoluminescence. Occurs when solutions are electrolyzed. It appears at anode or cathode as a result of chemical reaction.

Triboluminescence. This process involves light produced by shaking, rubbing, or crushing crystals.

Crystalloluminescence. Light is observed when solutions crystallize.

Lyoluminescence (solution luminescence). It is the light accompanying the solution of colored (from exposure to cathode rays) crystals of lithium, sodium, or potassium chlorides.

Chemiluminescence (CL). It is the production of light during a chemical reaction at low temperature.

Bioluminescence. It is a special type of chemiluminescence in which compounds manufactured by luminous animals are oxidized producing excited reaction products and light production.

Nature of CL

There are different mechanisms[5] by which the excited molecule loses its energy:

1. luminescence

   $AB^* \rightarrow AB + h\nu$

2. molecular dissociation

   $AB^* \rightarrow A + B$

3. chemical reaction with another molecule

   $AB^* + Z \rightarrow AZ + B$

4. intramolecular energy transfer

   $AB^* \rightarrow AB$

5. intermolecular energy transfer

$$AB^{*} + CD \rightarrow AB + CD^{*}$$

6. isomerization

$$AB^{*} \rightarrow BA$$

7. physical quenching

$$AB^{*} + M \rightarrow AB + M^{*}$$

CL is the emission of radiation, usually in the visible or near IR region, as a result of a chemical reaction. One of the reaction products (or intermediates) is formed in an electronic excited state and emits the radiation on returning to the ground state.[1]

$$A + B \rightarrow C^{*} + D$$

$$C^{*} \rightarrow C + h\nu$$

where C^{*} represents the excited state of the species C.

From the above mechanisms, chemiluminescence can be divided into two main types, direct and indirect. In the direct type, the reaction product (or intermediate) is in an excited state and is responsible for light emission as it falls to the ground state. The reaction can be represented by the following reaction sequence[3]

$$L + R \rightarrow I^{*} \rightarrow I + h\nu \rightarrow P$$

where L is the initial form of the luminescing species, R represents necessary reagents, I^{*} is the intermediate in its excited state, I is the intermediate in its ground state, and P is the final product. In some CL reactions, the final product acts as the luminescing species (P^{*}).

$$L + R \rightarrow P^* \rightarrow P + h\nu$$

For indirect CL, the excited product (or intermediate) is not the actual light emitter, but it transfers its energy to an acceptor which then emits light.[1] By transferring the excitation energy to an efficient fluorophore (F) added to the system, a considerable increase in luminescence may be achieved.

$$P^* + F \rightarrow P + F^*$$

$$F^* \rightarrow F + h\nu$$

This process is called sensitization.[1]

The number of photons emitted per unit time is proportional to the number of excited molecules which in its turn is proportional to the rate of reaction.[7] In most CL reactions, peak-shaped CL signal is observed which decays due to the decrease in rate of formation of the excited molecules as the reactants are consumed.[3]

Oxygen, or any one of its derivatives, is often a necessary reactant for the chemiluminescence in both organic and inorganic reactions. For a reaction to be chemiluminescent, there must first be sufficient energy available for the formation of the electronically excited state (i.e., the reaction must be exothermic). In general, the free energy must be large enough that $-\Delta G > hc/\lambda$, where λ is the wavelength of emission. For visible photons, ΔG must be at least between 40 and 70 kcal/mol.[3,8] Secondly, the formed excited state needs a pathway by which this energy can

be channelled. If all the energy is lost as heat, there will not be any chemiluminescence. Finally, the excited product must be capable of losing its energy as a photon, or be capable of energy transfer to an acceptor.[5]

The CL quantum efficiency is often low because a small fraction of reactants are converted to excited species or because of the availability of other pathways by which the excited molecule can dissipate its excess energy and return to the ground electronic state.[3]

For most CL reactions, there is only one emitting species; therefore, no wavelength discrimination is needed. In some cases the excited product (or intermediate) is an inefficient emitter. In these cases, photon counting, which is more complex and expensive, has been used to improve the detection limit.

CL is an attractive spectroscopic detection technique for some situations. The excitation energy is generated by a chemical reaction, as opposed to an external light source with other spectroscopic methods. There are no problems with sample irradiation and light scattering or source instability. Therefore, if the CL efficiency is high, CL can result in better detection limits than other emission or luminescence techniques, as there is rarely any background emission or scattering to increase the noise level.[9]

Oxidation reactions are the most frequent source of CL as they often involve large free energy changes.[10] Because few

compounds chemiluminesce, there should be minimal interference from endogenous compound in complex matrices.[9] Hundreds of inorganic and organic chemical reactions are known to produce visible light. There are four principal factors[5] which characterize a particular chemiluminescent reaction:

1. The brightness of light emission,
2. The state in which the chemiluminescent reaction occurs (gas, liquid, solid, or any interface),
3. Whether the primary substance in the chemiluminescent reaction is organic or inorganic,
4. The existence of an acceptor substance (sensitizer).

One of the most attractive features of luminescence methods is the potential of good detection limits many orders of magnitude better than those obtained with absorption spectroscopy. Another advantage is often a larger dynamic range than that obtained with absorption methods. Also, the selectivity of the CL technique can be better than those of the absorption methods and so it is useful for chemical speciation studies.[11] CL analysis is also attractive because of the simplicity of the instrumentation.

CL Instrumentation

There are four different CL instrument designs[12] or systems for mixing the sample with reagents: discrete sampling, stopped flow, continuous flow, and centrifugal analyzer. In the discrete sampling system, all solutions

(reagents and analyte) except one are placed in the reaction cell and the remaining solution then is injected with a syringe. In stopped-flow system, the sample and reagents are mixed simultaneously and rapidly. For continuous flow systems, the reagents and a blank solution are pumped through tubing and combined and the analyte solution is injected into the blank stream. A flow system provides rapid and reproducible sample injection and mixing with reagents while a discrete sampling system can provide better detection limits and is simpler and lower in cost. In the centrifugal analyzer, mixing is produced by centrifugal action.

Determination of Trace Organics

Determination of trace organic species plays an important role in the life sciences and ecology. About 5% of analytical publications deal with the determination of trace organics in food samples, agricultural specimens, and air or water sources. The trace analysis of organic compounds directly relevant to mankind obviously contributes to the development of fields that are currently of public interest, such as protection of the environment or purity of the food, and also to biochemistry, clinical chemistry and medicine.[13]

Organic analyses are needed to protect our health and our environment and to ensure the nutritional value of our food.[14] The concentrations of organic species produced by man and those naturally occurring need to be determined. Quality

control of many organic products of chemical industry is now usually necessary. Solvent and many reagents must meet certain purity standards.[13]

There are some difficulties associated with organic trace analysis.[13] First, the number of organic compounds present in a real sample is large which often makes complex separation schemes necessary. Second, selective methods with sufficiently low detection limits are needed. Methods and equipment that meet these criteria are often available, but they are often very expensive and require special trained technicians. A third problem is the similarity (structure or reactivity) of many organic compounds which makes them difficult to separate and determine. Fourth, many organic compounds are unstable towards hydrolysis, oxidation and microbiological attack which is difficult to control. A fifth problem is that the chemical identity of the analyte and potential interferents is not known. Hence, it is difficult to predict the chemical reactions or separation conditions that might be useful. The sixth problem is contamination control. With relatively insensitive methods, contamination is not considered as a big problem. As a method became more sensitive, background contamination should be taken into consideration.

Determination of Alcohols and Phenols

In drink and liquor formulations, aromatic substances plays an important role because they introduce individual flavours that may affect consumer preferences. These aromatic substances originate mostly from natural sources (essential oils, extracts of roots, leaves, flowers, etc.), although these natural products are tending to be replaced with synthetic substances.[15] Phenols are generated by a number of processes[16] including the petrolum industry,[17] the pulp and paper industry,[18] and in the synthesis of plastics and pharmaceuticals.[19] Chlorinated phenols have been used as insecticides, antiseptics and disinfectants, and have been found in drinking water following chlorination.[20] Alcohols and phenols have been determined using many different methods:

1. *Amperometry*. Recent literature contains a large number of reports dealing with the determination of phenolic compounds using HPLC[21-24] followed by fluorescence detection,[21,24] UV absorbance detection[22,24] or electrochemical detection.[24-26] Electrochemical detectors for liquid chromatography (LC) are often based upon a thin-layer cell with working electrodes.[27] Both twin electrode steady-state amperometry (four-electrode configuration) and simple thin-layer hydrodynamic amperometry (three-electrode configuration) have been used to detect catecholamines in blood plasma.[27] Kissinger[28] has recently reviewed the general area of amperometric and coulometric detectors for LC. The determination of phenols using HPLC and

post-column reaction detection has been studied by Bigley and Grob.[29] Trace phenolic compounds were determined in water by reversed phase LC with electrochemical detection using a carbon-polyethylene tabular anode.[30] LC columns have been used to separate phenol mixtures in water samples.[31-33] Electrochemical detection of phenols after LC separation was demonstrated in 1973 by Takata and Muto.[33]

Carbon paste has been used as oxidative working electrode but it is unsuitable for LC eluents containing methanol or acetonitrile.[30] Glassy carbon is preferred because it performs well in an electrochemical detector once the surface has been rigorously polished.[34]

2. *Gas Chromatography* (GC). To identify and quantitatively measure total phenols at ppb levels, procedures generally involve solvent extraction followed by GC-ECD or selective derivatization and GC-MS. Lamparski and Nestrick[35] have reported the GC analysis of phenol and substituted phenols following the production of the heptafluorobutyryl derivatives in a benzene extract. Hoshika and Muto[36] have reported the separation and quantification of eight phenols by GC after conversion to their corresponding bromophenols. Coutts *et al*[37] formed the acetate esters of six phenols before extraction with methylene chloride from water and analysis by GC. Alcohols were detected at the femtogram level as pentafluorophenyl-dimethylsilyl ethers.[38] Fluorocarbon containing dimethylsilyl ethers were investigated using GC of sterols

with electron capture detection (ECD). ECD gives nearly 100% ionization of certain halogen-containing compounds in GC.[13] GC-ECD is also used in the determination of phenols as 2,4-dinitrophenyl ethers[39] and in the determination of phenolic compounds in water samples after converting phenols by means of pentafluorobenzoyl chloride to the corresponding acyl derivatives.[40] Pentafluorophenyldialkylchlorosialanes were used as versatile derivatizing reagents for GC-ECD.[41]

GC-MS was used to detect the fragments of pentafluorobenzoyl derivatives of phenol and 4-chloro-2-methyl-cresol[40] and flophemesyl derivatives.[41-43] GC can also be used with a flame ionization detector (FID)[44,45] or a flame photometric detector (FPD).[46] Disadvantages of GC methods are sample preparation time, cost of MS equipment, incomplete recoveries for most phenols, and the lack of detector selectivity when only phenols are desired.[30]

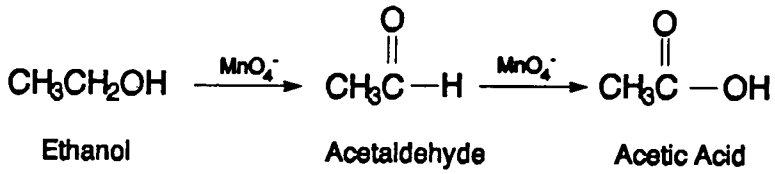
3. *Fluorescence*. Phenols were detected by both TLC[47] and HPLC[48] after reacting with dansyl chloride to produce fluorescent derivatives. Fluororganic labelling for pesticide residue analysis has been used.[49] The use of fluorescence detection in LC is now recognized as a powerful method which provides detection limit and specifity advantages.[48,50] Several investigators have already reported the use of fluorescence detection in conjunction with HPLC.[48,51-53]

4. *Spectrophotometry*. Methods of the determination of alcohol and phenols also include LC with spectrophotometric

(UV) detection.[15,54] Spectrophotometry is unsatisfactory when used alone due to the possibility of interferences, which are difficult to eliminate even with complex sample purification procedures; furthermore, it does not allow detection of very low concentrations. A combination of the high separation efficiency of HPLC with the sensitivity and selectivity of the photometric detector can be used for better results.

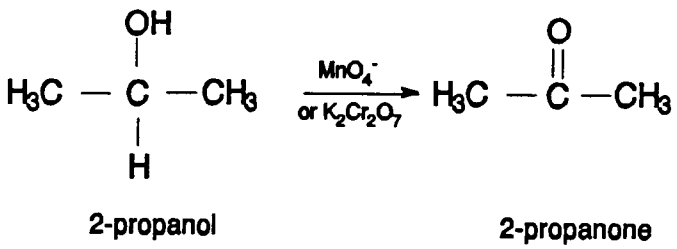
Oxidation of Alcohols

Primary alcohols upon oxidation with permanganate undergo the following reactions.[55]



The carbon bearing the OH becomes oxidized, first to an aldehyde and then to an acid. Aldehydes are generally more rapidly oxidized than the alcohols so that they are not readily obtained by this process.

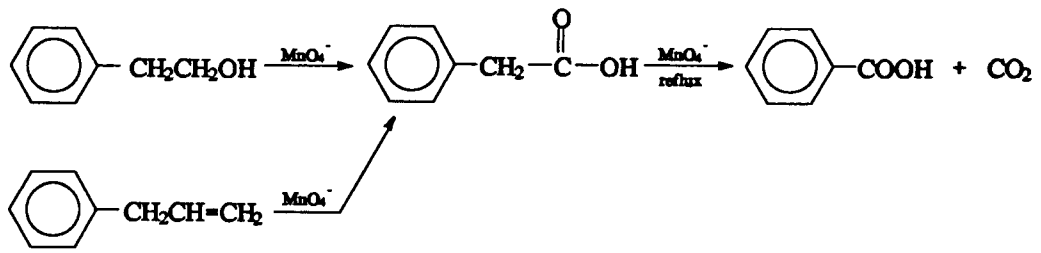
Secondary alcohols react to give ketones.[55]



Since ketones have no hydrogen on the carbon atom bearing the oxygen, they are resistant to further oxidation and good yields of ketones may be obtained.

Tertiary alcohols do not react under conditions that oxidize primary and secondary alcohols. Under vigorous conditions tertiary (as well as secondary) alcohols are degraded into small fragments.[55]

All of these oxidation reactions can be carried out when the groups concerned are on the side chain of an aromatic nucleus. The conditions required for the oxidative removal of such a chain are more drastic than those needed in the above reactions.[55]



The research in this thesis is based on the chemiluminescence produced in the oxidation of alcohols and phenols by potassium permanganate in nitric acid. The oxidation of ethanol to acetaldehyde by acid permanganate is a two-electron process, and to acetic acid is a four-electron process.[56] The stoichiometric reactions of ethanol oxidation are shown below.[57] The reaction products depend on the molar ratio of ethanol to permanganate.[4] When ethanol is in molar

excess relative to permanganate, it takes 0.4 mol of MnO_4^- to oxidize 1 mol of ethanol and the product is acetaldehyde.

$$5CH_3CH_2OH + 2MnO_4^- + 6H^+ \rightarrow 5CH_3CHO + 2Mn^{2+} + 8H_2O$$

If the oxidation proceeds to acetic acid, it takes 0.8 mol of MnO_4^- to oxidize 1 mol of ethanol.

$$5CH_3CH_2OH + 4MnO_4^- + 12H^+ \rightarrow 5CH_3COOH + 4Mn^{2+} + 11H_2O$$

The oxidation of ethanol has been discussed in many studies. Different mechanisms have been postulated based on the following:

1. Oxidation by Mn^{3+} and Mn^{4+} in acidic solution.[56-60]
2. Hydride transfer to the oxidant.[61,62]

The first mechanism has been extensively studied.[56-60] According to Tompkins[63] the oxidation of alcohols by permanganate takes place first by a reduction of MnO_4^- by Mn^{2+} ions to form Mn^{3+} or Mn^{4+} which then oxidizes the alcohol. It has been confirmed that some Mn^{2+} is always present in freshly prepared permanganate solution.[3]

The oxidation of alcohols does not take place by direct attack of MnO_4^-.[58] Transient manganese ions of valency states higher than 2+ and lower than 7+ are involved instead.[64,65] These intermediate oxidation states are produced when permanganate is attacked by Mn^{2+} in acidic solution as in the following reactions:

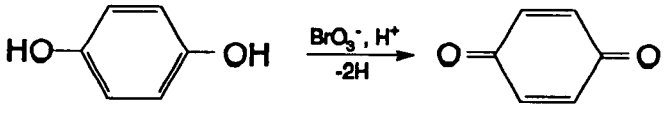
$$2MnO_4^- + 3Mn^{2+} + 16H^+ \rightarrow 5Mn^{4+} + 8H_2O$$

$$MnO_4^- + 4Mn^{2+} + 8H^+ \rightarrow 5Mn^{3+} + 4H_2O$$

Oxidation of Phenols and Quinones

Phenol, and particularly the phenolate ion exhibit marked nucleophilic activity, undergoes substitution reactions readily. As a consequence of this ready availability of electrons in the phenolic ring, this substance is easily attacked by oxidizing agents (electrophilic agents). The products of the reaction are complex, and extensive degradation of the molecule results. Most phenols, particularly in alkaline solution, are subject to air oxidation, and they develop color on standing. This susceptibility is increased in polyhydroxy aromatic compounds, although in many of these cases, well-defined products can be obtained.[55]

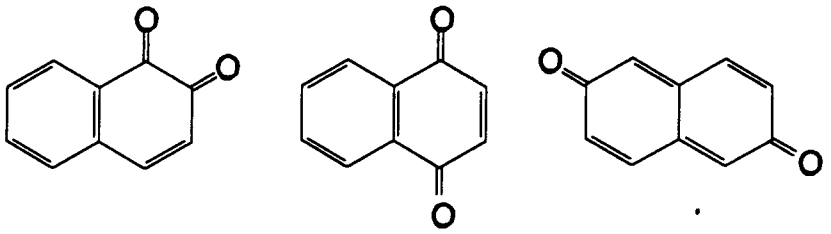
For example,[55] colorless hydroquinone (1,4-dihydroxy-benzene) is oxidized under mild conditions to a yellow substance called quinone.



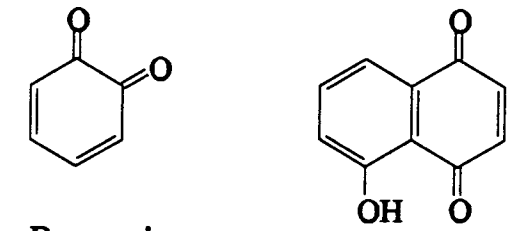
Quinone

Quinone may also be obtained as one of the products of the oxidation of phenol and certain other electron-rich benzene derivatives. Quinone is the parent of a class of compounds called 'quinones'. Quinones may be formed from appropriately substituted dihydroxy aromatic compounds under much milder conditions than those required for the oxidation of simple

phenols, and the reaction is usually reversible under comparably mild conditions. The two hydroxyl groups need not be located in the same ring, but they must be located in such positions that all sp^2-hybridized carbon atoms can participate in π bonds.[55] Some representative quinones are:



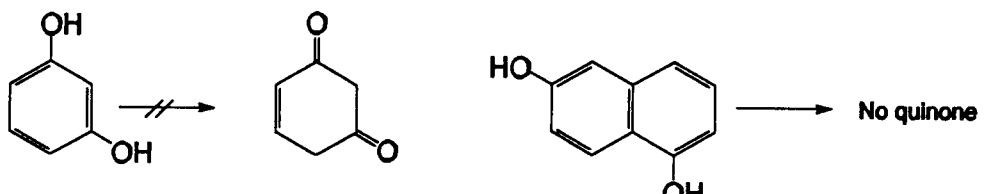
Naphthaquinones



o-Benzoquinone

Juglone

Dihydroxy aromatic compounds which cannot give rise to fully bonded structures do not yield quinones, for example, m-dihydroxybenzene.



m-Dihydroxybenzene

(Resorcinol)

Not known

No quinone

Quinones are important as coloring agent (pigments and dyes), and are often found in natural products. They are also

important in oxidation-reduction systems since the quinone-hydroquinone reaction is one of the few readily reversible redox reactions in organic chemistry.[55]

$KMnO_4$ as an Oxidizing Agent

Potassium permanganate is not a common oxidant such as oxygen or hydrogen peroxide in the CL literature. However, its use as a CL oxidant has increased in the last ten years. Usually very acidic reactions are employed and often sensitizers are added as summarized in Table 1. Except for sulfite and loprazolam, all the analytes in the table have phenolic groups. Al-Tamrah and Townshend[66] reported the determination of sulfite using the flow injection CL. Riboflavin or 3-cyclohexylaminopropanesulphonic acid (CAPS) were added to the reaction mixture to enhance the CL signal. Yamada and Nakada[67] investigated the same CL reaction using different sensitizers. Brilliant sulfaflavine and riboflavine phosphate gave a signal enhancement by a factor of 330. Abbott *et al.*[9,10,68] have determined morphine in body fluids by HPLC with CL detection. They found that polyphosphoric acid

provided a larger signal than orthophosphoric, hydrochloric or sulfuric acid. SO_2 in air was detected by Stauff and Jaeschke[69] after being absorbed in tetrachlo- romercurate. Nakagama and Yamada[70] have determined polyphenols with and without metal catalysts. They achieved a detection limit of picomol for adrenaline.

Table 1. CL Analytical Methods Based on $KMnO_4$ as the Oxidant

Analyte	Detection Limit	[Acid][a]	$[MnO_4^-]$[a] (mM)	Notes	Ref
sulfite	5 ng 1.2 ng	10^{-2} M H_2SO_4	5 X 10^{-3}	RF CAPS	66
sulfite	0.9 ng 1.8 ng	10^{-2} M H_2SO_4	2 X 10^{-3}	RFP BSF	67
morphine	0.7 pg	0.01 M poly-phosphoric	6 X 10^{-1}		9
morphine	0.7 pg	0.1 M poly-phosphoric	6 X 10^{-1}		10
morphine	1 fmol	0.2 M poly-phosphoric	6 X 10^{-1}		68
SO_2 in air[b]	0.5 ng	10^{-3} M H_2SO_4	2 X 10^{-2}		69
poly-phenol	1 pmol	10^{-3} M H_2SO_4	1	catalysts	70
loprazolam	163 ng	0.94 M formic	2 X 10^{-1}		71
Naphthol	5 X 10^{-7} M	0.2 M H_2SO_4	2 X 10^{-2}	R-B	72
Burpenor-phine hydro-chloride	1 X 10^{-8} M	0.05 M poly-phosphoric	1 X 10^{-2}		73

[a] Initial concentration
[b] absorbed in tetrachloromercurate
RF = riboflavin
CAPS = 3-cyclohexylaminopropanesulphonic acid
RFP = riboflavin phosphate
BSF = brilliant sulfaflavin
R-B = rhodamine B

Andrews and Townshend[71] have studied seven different benzodiazepine compounds. Only loprazolam gave CL with acidic permanganate. They studied the effect of different acids with adjusted pH and formic acid gave the highest signal. Of twelve metal ions studied, only Fe^{2+} and Mn^{2+} significantly affected the emission and decreased the CL signal. Also, Rhodamine B and fluorescein at 1×10^{-4} M attenuated the signal.

Naphthol has been determined by Al-Tamrah and Townshend.[72] 2×10^{-5} M Rhodamine B was observed to enhance the CL signal; however, it decreased the signal when higher concentrations were used. Burpenorphine hydrochloride has been determined by Alwarthan and Townshend[73] using polyphosphoric acid in the carrier stream.

In the same laboratory at Oregon State University as this researcher, Montalvo[3] was concerned about the application of the CL techniques based on permanganate oxidation. She demonstrated that chemiluminescence is produced during the oxidation of hydroxy-containing compounds by permanganate at low pH and discussed the possibility of having Mn(VII), Mn(IV), or Mn(III) as the attacking species. The Mn(VI) and Mn(V) species are ruled out because there is no indication of the green manganate anion in acid media and Mn(V) is unknown. She mentioned three possible schemes involving Mn(VII), Mn(IV), or Mn(III) as the attacking species for ethanol:

Scheme I (Mn(VII)):

$$Mn(VII) + CH_3CH_2OH \rightarrow Mn^{V}O_4H^{2-} + CH_3HC{=}OH$$

$$CH_3HC{=}OH \rightarrow CH_3CHO + H^+$$

$$Mn^{V}O_4H^{2-} \rightarrow Mn(II) + Mn(VII)$$

Scheme II (Mn(IV)):

$$CH_3CH_2OH + Mn(IV) \rightarrow CH_3CH_2O\text{-}Mn(III) \rightarrow CH_3CHO + Mn(II) + 2H^+$$

Scheme III (Mn(III)):

$$Mn(III) + CH_3CH_2OH \rightarrow CH_3CH_2O\text{-}Mn(III)$$

$$CH_3CH_2O\text{-}Mn(III) \rightarrow CH_3CH_2O + Mn(II) + H^+$$

$$CH_3CH_2O + Mn(III) \rightarrow CH_3CHO + Mn(II) + H^+$$

Scheme I was ruled out since Mn(VII) cannot be the attacking species if an induction period occurs before the maximum CL intensity as was observed. According to Waters,[74] the reduction of Mn(VII) to Mn(III) in acidic medium is more probable than reduction of Mn(VII) to Mn(IV). The conversion of Mn(VII) to Mn(III) should be slow because of the change from tetrahedral to octahedral symmetry.[75]

Montalvo found that the addition of Mn(II) to the reaction mixture decreased the CL intensity. It was suggested that Mn(IV) is the oxidant (Scheme II) because of the following equilibrium equation:

$$Mn(IV) + Mn(II) \rightarrow 2Mn(III)$$

As Mn(II) is added, the equilibrium between Mn(III) and Mn(IV)

is shifted towards Mn(III). This should decrease the concentration of Mn(IV) and therefore the intensity if Mn(IV) is the oxidant. The CL signal should increase if Mn(III) is the oxidant.

When Fe(II) is added to the reaction mixture, it causes a rapid reduction of Mn(VII) to Mn(III) or Mn(IV) such that the induction period is eliminated and the CL intensity is increased. Both a sharp CL peak with no induction period and the normal slow peak (reduced in intensity) were seen with the mole ratio of Fe(II) to permanganate less than three. The slow peak is eliminated when this mole ratio is 3.0.

$$MnO_4^- + 3Fe^{2+} + 8H^+ \rightarrow Mn^{4+} + 3Fe^{3+} + 4H_2O$$

This fact also suggests that Mn(IV) is the attacking species rather than Mn(III). If Mn(III) was responsible, the mole ratio at which the slow peak is eliminated would be 4.0.

$$MnO_4^- + 4Fe^{2+} + 8H^+ \rightarrow Mn^{3+} + 4Fe^{3+} + 4H_2O$$

Methods were developed for the determination of ethanol in gin, the antioxidants propyl gallate and BHA in lard, pyrogallol (Pg) in hair dye and vanillin in vanilla extract. Some of her results are summarized in Table 2.

Table 2. Some Applications of CL in Acidic Permanganate[3]

Analyte	[HNO_3] % (w/v)	[$KMnO_4$] (M)	Expected Conc.	Measured Conc.
Pg in hair dye	55	1×10^{-2}	-	0.055% (w/v)
PG in lard	50	1×10^{-3}	0.0012% (w/w)	0.00117% (w/w)
Vanillin in vanilla extract	95	1×10^{-3}	0.2% (w/v)	0.31% (w/v)
EtOH in Gordon's Gin	95	1×10^{-2}	40.0% (v/v)	39.58% (v/v)
EtOH in Beefeater Gin	95	1×10^{-2}	47.0% (v/v)	47.07% (v/v)
EtOH in Tanqueray Gin	95	1×10^{-2}	47.3% (v/v)	47.40% (v/v)

EXPERIMENTAL

Instrumentation

A discrete sampling system based on a design reported earlier[12,76] was used for all the CL measurements. It consists of a sample cell, an injector, a mixing device, a photodetector, a signal processor, and a data recorder.

Sample Cell. A 1-cm pathlength plastic sample cell was used. It was housed in the sample chamber which is made of aluminium and painted black providing a dark environment to protect the detector from external light.

Injector. A Hamilton Precision Liquid Dispenser was used as a device to provide a repeated, automatic, and accurate dispensing of preset amount of liquid. It was loaded with a 3-mL plastic syringe and adjusted to deliver 1.00 mL. The final reagent (usually the oxidant) was injected into the sample cell, through a septum in the compartment lid, to initiate the reaction. The external plastic tubing, connecting the syringe to the needle that pierces the septum, is encased in black, opaque heat shrink preventing light piping to protect the photodetector from excessive illumination and to ensure light-tight seals.

Mixing Device. Since the poor mixing leads to poor precision and makes the measurement of fast emission unreliable, a magnetic stirrer under the sample cell with a small stirring bar in the cell were used to provide rapid and efficient mixing of the reaction mixture.

Detector. Initially an RCA 1P28 photomultiplier tube (PMT) and later a Hamamatsu R928 PMT was used to detect the light produced in the reactions studied with no wavelength discrimination. The latter PMT has a better red response.

Signal Processor. The current output from the photodetector is converted into voltage signal and amplified so it can be plotted on a chart recorder. A Keithley Current Amplifier (model 427) was used with a rise time of 300 ms and usually a gain of 10^7 V/A. A Keithley High Voltage Supply (model 244) was used at 500 V as the PMT power supply. A Spectrum Filter and Amplifier (model 1021) was connected between the current-to-voltage converter and recorder and used with a 1-Hz cut-off frequency and a gain of 10.

Data Recorder. The signal in the analog form was recorded on a Heath Schlumberger chart recorder model SR-205. Typically, the chart speed was set to 0.5 in/min and the range was adjusted to 500 mV.

Solution Preparation

All solutions were made in deionized water (dw) obtained from a Millipore (Milli-Q) reagent-grade water system fed by house deionized water. All weighing was accomplished with Mettler balance type H15 to 0.1 mg. Volumetric glassware was used for the preparation or dilution of the solutions.

A 1 x 10^{-2} M $KMnO_4$ stock solution was prepared by dissolving 0.158 g of reagent grade $KMnO_4$ (MW=158.04, Baker) in 100 mL of dw. Daily, 50 mL of working solution were prepared from the stock solution. It was stored at room temperature.

A 4 x 10^{-3} M pyrogallol (Pg) stock solution was prepared by dissolving 0.126 g of reagent grade Pg (MW=126.11, Mallinckrodt) in 250 mL of dw. The flask containing the solution was covered with aluminum foil to prevent decomposition of Pg when exposed to light. The solution was refrigerated until needed.

The stock ethanol solution was the absolute solution (200 proof, Midwest Grain). The diluted solutions were prepared as % (v/v) using volumetric flasks and diluting the appropriate amount of 100% ethanol to volume with dw. The solutions were refrigerated until needed. All the refrigerated solutions were allowed to warm up to room temperature before being used.

All the major solutions used in the experiments are listed in Table 3 along with their source and preparation.

Different sensitizers were used as listed in Table 4.

Table 3. Major Stock Solutions

Solution	Source	Preparation Procedure
1 X 10^{-2} M $KMnO_4$ MW=158.04	JT Baker RGT (3228)	0.158 g diluted to 100 mL
4 X 10^{-3} M Pg MW=126.11	Mallinckrodt RGT (1732)	0.126 g diluted to 250 mL
Ethyl alcohol 200 proof, 100%	Midwest Grain	Refrigerated
58 mM Ce^{4+} $(NH_4)_2Ce(NO_3)_6$ MW=548.26	Matheson & Bell (8565)	ceric ammonium nitrate (CAN) in 3 M H_2SO_4
50 mM Ce^{3+} $Ce(NO_3)_3 \cdot 6H_2O$ MW=44.23	Matheson & Bell (7423)	2.171 g diluted to 100 mL
20 mM $K_2S_2O_8$ MW=270.32	Mallinckrodt AR (7076)	0.270 g dissolved in 25 mL dw, 0.5 mL $AgNO_3$ (0.01) added, diluted to 50 mL with dw.
20 mM $K_2Cr_2O_7$ MW=294.19	Mallinckrodt AR	0.588 g diluted to 100 mL
10 mM AgO MW=123.87	Apache (6993) 99+%	0.124 g dissolved in 50 mL 1% (v/v) HNO_3 and diluted to 100 mL with dw
20 mM KIO_4 MW=230.00	Mallinckrodt AR	0.460 g dissolved in 50 mL dw, gently heated and diluted to 100 mL
1 M Mn^{2+} $MnSO_4 \cdot H_2O$ MW=169.01	Mallinckrodt AR (6192)	4.225 g diluted to 25 mL
5 mM Fe^{2+} $FeSO_4 \cdot 7H_2O$ MW=278.02	Mallinckrodt AR (5056)	0.139 g dissolved in 50 mL dw, acidified with 1 mL HNO_3, 1% (v/v), to prevent oxidation of Fe^{2+}, and diluted to 100 mL with dw
200 μM Mn^{3+} $Mn(OOCCH_3)_3 \cdot 2H_2O$ MW=268.10	Alfa Product (10724)	0.0268 g dissolved in 20 mL H_2SO_4 (50%) and diluted to 100 mL with dw

Table 3 (continued)

100 mM Mn^{4+}	Mnadal's method[77]	0.790 g $KMnO_4$ dissolved in 30 mL H_2SO_4 (9 M) with stirring for 8 hrs, left overnight and diluted to 50 mL with 9 M H_2SO_4
1 M NaF MW = 41.99	Baker & Adamson, RGT (2250)	1.050 g diluted to 25 mL
HNO_3 conc. = 70.4%	Mallinckrodt AR (2704-07)	conc. acid diluted (v/v) with dw
H_2SO_4 conc. = 96.1%	JT Baker AR (9681-3)	conc. acid diluted (v/v) with dw
HCL conc. = 37.0%	Mallinckrodt AR (2612-07)	conc. acid diluted (v/v) with dw
H_3PO_4 conc. = 85.0%	Mallinckrodt AR (279)	conc. acid diluted (v/v) with dw
$H_4P_2O_7$ conc. = 97.0%	Aldrich d = 2.060	conc. acid diluted (v/v) with dw

Table 4. Sensitizers[a]

Sensitizer	λ_{max} (nm)[b]	Source	Mass used (g)
Pyronin B MW = 1042.20	553	Sigma	0.2606
Rhodamine B MW = 479.00	543	Sigma	0.1198
Eyosin Y MW = 691.90	514	Sigma	0.1730
Acridine Orange MW = 370.00	489	Sigma	0.0925
Brilliant Salfaflavine MW = 418.40	422	Sigma	0.1046
BBQ mW = 675.00	306	Exciton	0.1688
p-Terphenyl mW = 230.31	276	Exciton	0.0575
Coumarin 450 MW = 217.00	366	Exciton	0.0543
Stilbene 420 MW = 563.00	349	Exciton	0.1408
LD 688 MW = 355.00	516	Exciton	0.0888
Rhodamine 575 MW = 414.49	518	Exciton	0.1036
Fluorescein 548 MW = 401.20	512	Exciton	0.1003
Rhodamine 590 MW = 530.38	530	Exciton	0.1326
Coumarin 521 MW = 283.33	452	Exciton	0.0708
Fluorol 555 MW = 324.41	442	Exciton	0.0811

[a] Sensitizer solutions of 0.25 mM were made by diluting the weighed amount to 100 mL with absolute ethanol and diluting 1 mL of the prepared solution to 10 mL with water.
[b] Wavelength of maximum absorption.

Methodology for Chemiluminescence Analysis

The general procedures used during this study are outlined below following the injection procedure previously described[12,28]. With the shutter closed and sample lid open, 1.0 mL of the blank (dw) or the analyte solution, 0.5 mL of an acid solution (usually HNO_3), and 1.0 mL of dw were added to the reaction cell with Eppendorf pipets. The lid of the chamber was lowered and the instrument was covered with a dark cloth to prevent light leaks. The shutter was opened and 1.0 mL of the permanganate solution was injected through the rubber septum, by the precision liquid dispenser, into the cell. This standard procedure was used for optimization studies, calibration curves, and sample analysis, and as the control for additive studies.

To study the effect of other species denoted additives, x mL of an additive solution and y mL of dw were added in place of 1 mL dw. The additive was typically an additional oxidant, sensitizer, metal ion, or anion. In most experiments, the total volume of the reaction mixture was kept constant, 3.5 mL, by the addition of dw so $x + y = 1$ mL. This procedure was followed to keep the fraction of light reaching the detector constant.

After injection, the CL signal was recorded by the chart recorder and the shutter then was closed. The reaction solution was removed from the cell by suction with disposable pipet tip attached to a vacuum aspirator flask. The sample

cell was rinsed five times with dw. When ethanol was used, the brown precipitate (MnO_2) produced in the reaction was removed by filling the cell with 50% (v/v) HCl for 3 min, then it was removed by aspiration and the cell was rinsed 10 times with dw. This cleaning procedure is useful so the same cuvette at the same position can be used for all the reactions for more reproducible results.

Studies of Alcoholic beverages

Beverage Samples. Absolute ethanol and five alcoholic beverages samples were tested for their CL signal. The alcoholic beverages are listed below:

Sample # 1: Ernest and Julio Gallo (1991) Sauvignon blanc
11% alcohol by volume

Sample # 2: Ron Bacardi Superior Rum
40% alcohol by volume

Sample # 3: Sutter Home (1990) White Zinfandel
9% alcohol by volume

Sample # 4: Sutter Home (1989) Cabernet Sauvignon
12% alcohol by volume

Sample # 5: Seagram's (Seven Crowns) Whiskey
40% alcohol by volume

Solvent Extraction. Solvent extraction was tested as a technique to separate alcohol from polyphenols in wine samples. A mixture of ethanol and Pg was used as a model for

this study. Also a phenol and a wine sample were tested with the same purpose. Different solvents were tested to extract Pg from a mixture of Pg and ethanol so ethanol could be detected alone. In a separatory funnel, 10 mL of the test solution was mixed with 10 mL of solvent (benzene, toluene, and trichloromethane) and shaken well. The aqueous layer was separated from the solvent layer and tested for any change in the CL signal.

Solid Phase Extraction (SPE). As an attempt to separate ethanol from Pg, a special SPE tube (ENVI-Chrom P) recommended by Supelco was used. The highly crosslinked, specially cleaned styrene-divinylbenzene resin was developed specifically for extraction of polar aromatic compounds from aqueous samples. The general SPE procedure was carried out following the standard procedure recommended by the manufacturer.[78]

The tubes were inserted into a Baker spe-12G column processor which was attached to the house vacuum line through a side-arm flask. The column was conditioned by passing 6 mL of ethyl acetate through the column at about 2.5 mL/min. Next, 6 mL of methanol was passed through the column at the same flow rate followed by 6 mL of deionized water. Dryness of column was avoided between steps. Finally, the sample was applied to the column and the solution was drawn through the column and collected in a 10 mL test tube for analysis. The vacuum was adjusted during the application of the sample (17-

20 in-Hg) so that the flow rate was approximately 2.5 mL/min. Air was drawn through the column for about 2 min to remove any remaining liquid in the column. Later, the column was cleaned by adding 2 mL of ethyl acetate with the vacuum off to allow the solvent to soak into the packing for 1 min. Next, 5 mL ethyl acetate was added and allowed to drip at a dropwise rate. The tube conditioning procedure was repeated to get the column ready for the next sample.

RESULTS AND DISCUSSION

This thesis work, in general, is concerned with the CL produced during the oxidation of alcohols (ethanol) and phenols (Pg) with permanganate or related oxidants. Alcohols and phenols were studied under different conditions to see if there is any difference in their behaviour which can be used to distinguish them and to better understand the nature of the CL reactions. Separation methods to isolate ethanol from phenols were also considered.

Effect of Different Oxidants

The use of oxidants (in addition to permanganate) was first considered. Some oxidants were previously studied by Drew Reynolds[79] (undergraduate student) in the same laboratory as this researcher. He tried to oxidize Mn(II) to Mn(III) and Mn(IV) which can then oxidize Pg (as proposed by Montalvo[3]). He added $MnSO_4$ into the reaction cell with Pg and injected various oxidants. None of the oxidants gave a signal except $NaBiO_3$. Then he added the oxidants to the reaction cell initially and then injected $MnSO_4$. He did not see any signal for any of the oxidants using this method. He suggested that the oxidants were oxidizing Pg directly. To prove that, he mixed the oxidants in the reaction cell with Pg and HNO_3 and injected MnO_4^- to see if there was any reaction between Pg and

the oxidants. All of the oxidants suppressed the CL signal of Pg.

In this section, some of Reynolds experiments were repeated and then extended. The effect of different additional oxidants (AO) on the CL signal was tested during the oxidation of pyrogallol with permanganate in acidic solution. These oxidants were added to the reaction cell before the injection of permanganate solution. One purpose was to confirm if the oxidants reacted only with Pg or also with lower oxidation states of Mn or in some way altered the CL reaction.

Ceric Ion. The peak for standard run (no additives) for Pg is shown in Figure 1a. The CL peak is sharp with a shoulder. The first tested AO was Ce^{4+}. The reaction mixture was:

1 mL Pg (1 or 4 mM)

0.5 mL nitric acid (50% or 95% (v/v))

0.25 mL Ce^{4+} (2, 8, 10, or 32 mM)

0.75 mL water

1 mL MnO_4^- (2 or 10 mM), injected

When a 2 mM Ce^{4+} solution was added to 1 mM Pg, the signal decreased with a more distinct shoulder. As the concentration of Ce^{4+} was increased to 8 mM, the CL signal decreased and it disappeared on using 32 mM Ce^{4+}. The purple color of permanganate in the reaction mixture became darker as the concentration of Ce^{4+} solution increased. The results suggest that Ce^{4+} oxidizes the Pg.

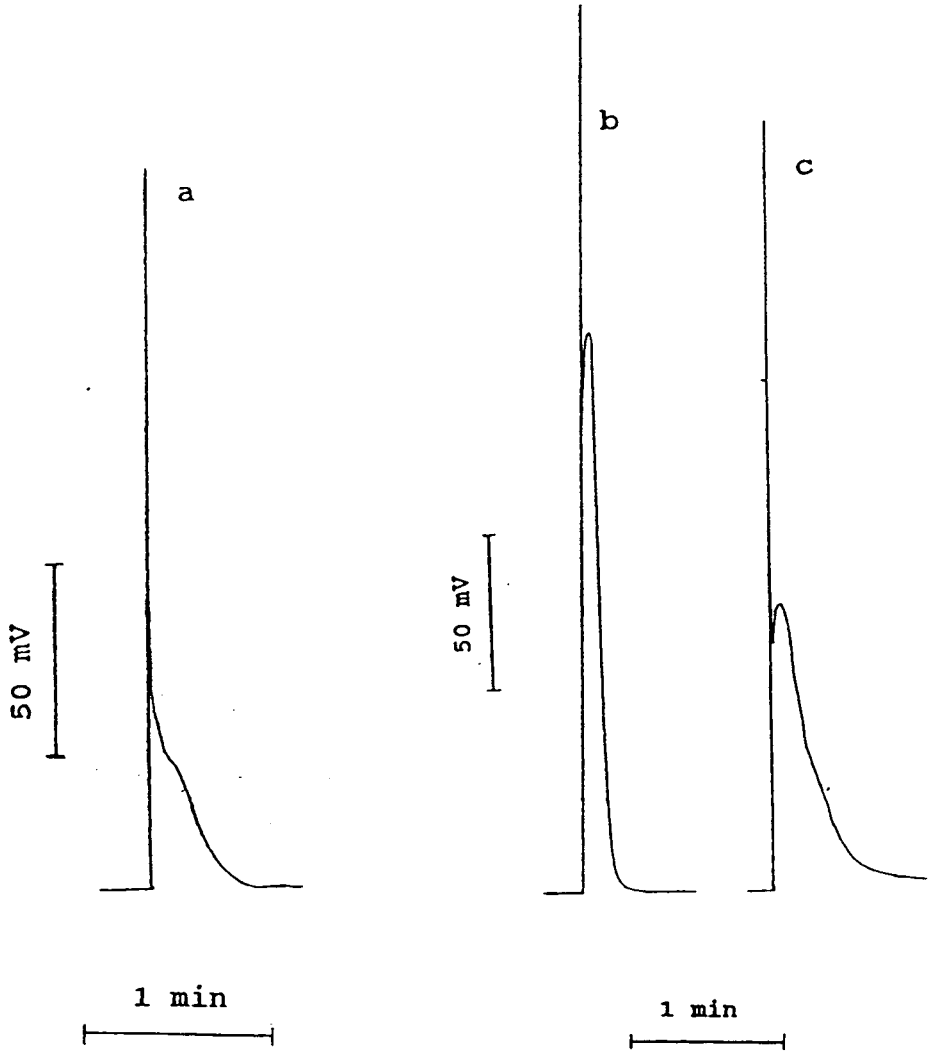


Figure 1. Typical shape of CL signals for Pg. Reaction mixtures (a): 1 mL of 1 mM Pg, 0.5 mL of 50% (v/v) HNO_3, 1 mL of water, and 1 mL of 2 mM $KMnO_4$ (injected); (b): 1 mL of 4 mM Pg, 0.5 mL of 95% (v/v) HNO_3, 1 mL of water, and 1 mL of 10 mM $KMnO_4$ (injected); (c): same as b except 1 mL of 10 mM Ce^{4+} instead of water.

With a higher concentration of Pg (4 mM), nitric acid (95%) and permanganate (10 mM), a larger initial CL signal was observed and a second distinct peak appeared (Figure 1b). With a 10 mM Ce^{4+} solution (as shown in Figure 1c), the height of the first peak decreased and the second peak became more distinct but lower in height. Doubling the volume of Ce^{4+} solution decreased the initial signal by more than 14 times and the second peak disappeared. Delaying the injection of permanganate solution for 30 s after adding Ce^{4+} caused no difference in the signal height and indicates that the Ce^{4+} oxidation is rapid.

The results of further studies using different concentrations of all the reagents is summarized in Table 5. The CL intensity without Ce^{4+} is 184 mV. The highest CL signal was observed when the molar ratio of permanganate to Pg was 10:4 as in experiments no. 2, 12 and 14. Here and elsewhere, the molar ratio refers to ratio of the number of moles of oxidant to the number of moles of Pg in the mixed reaction mixture (calculated as though no reaction occurred). The signal decreased as the concentration of Ce^{4+} increased. In the cases where Ce^{4+} was present in excess by a factor of 4, as in experiment no. 3, 10 and 11, no signal or a very small signal was observed. With a concentration of Ce^{4+} to obtain 1:1 molar ratio with Pg, as in experiment no. 5, the signal was small with no second peak. Because the oxidation of Pg to orthoquinone involves 2 electrons, a molar ratio of 2 is theoretically required for complete oxidation by Ce^{4+}. In

experiment no. 6 where the concentration of permanganate solution was decreased from 10 to 2 mM, the signal increased and the second peak was observed as tailing. By replacing the 95% nitric acid with 50%, little difference was observed.

Table 5. The Effect of Ce^{4+} on the CL Signal of Pg[a]

	Pg		HNO_3	Ce^{4+}		MnO_4^-	CL Signal (mV)	
#	mM	mL	%	mM	mL	mM	1st	2nd
1	3.97	1.00	95	4.00	1.00	10.0	140	43
2	3.97	1.00	50	4.00	1.00	10.0	145	41
3	1.00	1.00	50	4.00	1.00	10.0	0	0
4	1.00	1.00	50	2.50	1.00	10.0	24	0
5	1.00	1.00	50	1.00	1.00	10.0	44	0
6	1.00	1.00	50	1.00	1.00	2.00	85	8
7	1.00	0.75	50	1.00	0.75	2.00	54	0
8	1.00	1.00	50	1.00	0.75	2.00	83	9
9	1.00	1.25	50	1.00	0.75	2.00	114	15
10	1.00	1.00	50	4.00	1.25	10.0	0	0
11	1.00	1.00	50	4.00	0.75	10.0	0	0
12	3.97	1.00	50	4.00	0.75	10.0	152	45
13	3.97	1.00	50	4.00	1.25	10.0	144	26
14	3.97	1.00	50	4.00	0.50	10.0	149	52

[a] Volume of HNO_3 was 0.50 mL and of $KMnO_4$ was 1.00 mL in all experiments.

The experiment was repeated using a lower Pg concentration to see if the same behavior occurs. Only one peak without a shoulder is observed. As shown in Figure 2, the Pg

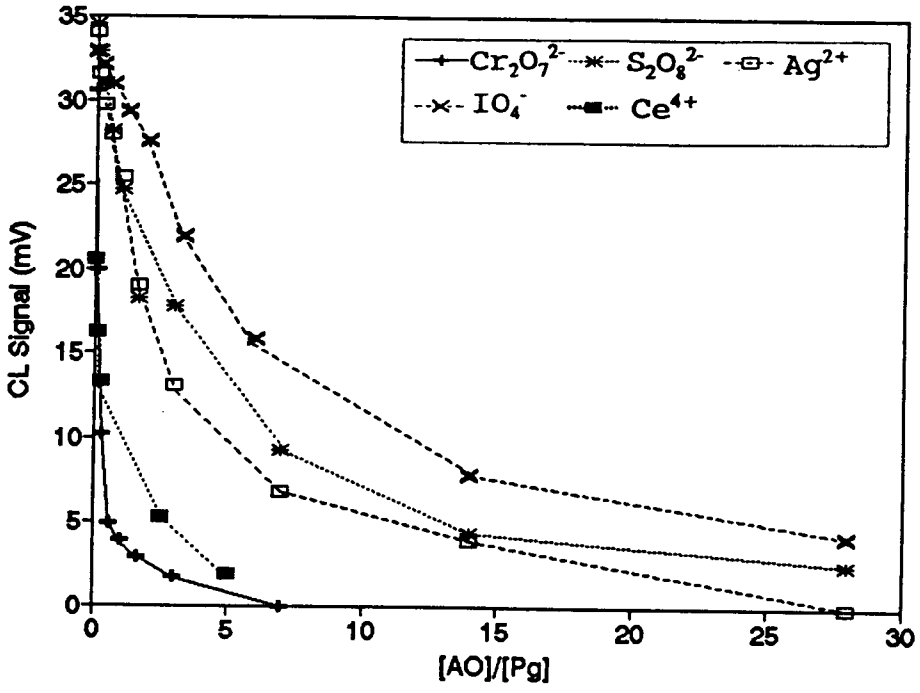


Figure 2. The effect of the concentration of an additional oxidant (AO) on the CL intensity of Pg.
Reaction mixture: 100 μM Pg (variable volume), 0.5 mL of 50% (v/v) HNO_3, AO (variable volume), dw (variable volume to keep total volume at 3.5 mL), and 1 mL of 200 μM $KMnO_4$ (injected). $[Ce^{4+}]$ = 1 mM, $[Cr_2O_7^{2-}]$ = 100 μM, $[S_2O_8^{2-}]$ = 100 μM, $[Ag^{2+}]$ = 100 μM, and $[IO_4^-]$ = 200 μM.

CL signal decreases as the concentration of Ce^{4+} increases. The signal is not zero when the molar ratio is 2. Apparently an excess of Ce^{4+} is required for complete oxidation of the Pg.

Two further possibilities were considered. First, Ce^{4+} may react with manganese in lower oxidation states before reacting with Pg. Second, the permanganate may react with a reduced form of Ce before reacting with Pg. The latter possibility was tested by making the following mixtures of permanganate and Ce^{3+} solution in test tubes:

1 mL MnO_4^- (2 mM) + 1 mL Ce^{3+} (2 mM)

1 mL MnO_4^- (2 mM) + 1 mL Ce^{3+} (50 mM)

1 mL MnO_4^- (200 μM) + 1 mL Ce^{3+} (100 μM)

No change was observed in the color of permanganate solution which suggests that permanganate does not react with Ce in its lower oxidation state. There is no evidence that Ce^{4+} reacts with Mn in its lower oxidation states.

Dichromate. The second tested AO was dichromate ($Cr_2O_7^{2-}$). The injection of permanganate was delayed for 15 and 60 s. The typical reaction mixture was:

1 mL Pg (4 mM)

0.5 mL HNO_3 (50% (v/v))

1 mL $Cr_2O_7^{2-}$ (1 to 20 mM)

1 mL MnO_4^- (10 mM), injected

The molar ratio of $Cr_2O_7^{2-}$ to Pg was changed until the signal disappeared. When a high concentration (20 mM) of $Cr_2O_7^{2-}$ was used, the signal was small with tailing. As the concentration of $Cr_2O_7^{2-}$ decreased, the signal increased and the second peak became more distinct. With 1 mM $Cr_2O_7^{2-}$, the signal was 5 times greater than with 5 mM and tailing was observed instead of second peak. The CL signal and the tailing decreased as the delay time increased which suggests that $Cr_2O_7^{2-}$ oxidizes Pg more slowly than Ce^{4+}. A brown precipitate formed slowly when the reaction was conducted in a test tube.

The dependence of the CL signal on the molar ratio of $Cr_2O_7^{2-}$ to Pg with a lower Pg concentration than above is shown in Figure 2. The CL signal disappears above about a molar ratio of 5.

Persulfate. The third AO was persulfate ($S_2O_8^{2-}$). The solution was prepared in 0.01 M $AgNO_3$ which provides Ag^{2+} as a catalyst. At higher concentrations of Pg (4 mM) and $S_2O_8^{2-}$ (20 mM), there was a sharp peak with a shoulder. The signal decreased as the concentration of $S_2O_8^{2-}$ solution increased. The dependence of the CL signal on the molar ratio of $S_2O_8^{2-}$ to Pg with a lower concentration of Pg is shown in Figure 2. A large excess of persulfate is required to oxidize the majority of the Pg.

Silver(II). The fourth AO was Ag(II). The solution was prepared by dissolving AgO salt in 1% (v/v) nitric acid.

Table 6 summarizes the solution conditions and results in different experiments.

There was no effect on the signal height by delaying the injection of permanganate solution. The peak shape did not change from the typical one (Figure 1a) by the addition of Ag^{2+}. The signal decreased as the molar ratio of Ag^{2+} to Pg increased as shown in Figure 2 and a large excess was required for complete oxidation.

Table 6. The Effect of Ag^{2+} on the CL Signal of Pg[a]

	Pg		Ag^{2+}		CL Signal
#	μM	mL	μM	mL	mV
1	100	1.00	water	1.00	110
2	100	1.00	100	1.00	130
3	100	2.00	-	-	174
4	100	1.75	100	0.25	161
5	100	1.50	100	0.50	152
6	100	1.25	100	0.75	143
7	100	1.00	100	1.00	130
8	100	0.75	100	1.25	97
9	100	0.50	100	1.50	67
10	100	0.25	100	1.75	35
11	50	0.25	100	1.75	20
12	25	0.25	100	1.75	0

[a] Reaction mixture contains 1 mL of 200 μM $KMnO_4$ and 0.5 mL of 50% (v/v) HNO_3.

Periodate. The fifth AO was periodate (IO_4^-). The typical reaction mixture was:

1 mL Pg (4 mM)

0.5 mL HNO_3 (50% (v/v))

1 mL IO_4^- (20 mM)

1 mL MnO_4^- (10 mM), injected

At high concentrations of Pg, the signal height decreased with more tailing as IO_4^- was added. The delay time between adding IO_4^- and injecting MnO_4^- has no effect on the peak height or shape. Two resolved peaks were observed by increasing the volume of Pg solution to 1.25 mL and only one peak was observed by reducing the concentration of Pg, IO_4^-, and MnO_4^- by a factor of 50. The shape of the second peak became sharper as the molar ratio of IO_4^- to Pg decreased. With a lower Pg concentration, the signal height decreases with the molar ratio of IO_4^- to Pg as shown in Figure 2. The CL signal is 0 mV for molar ratio of 58.

All of the tested oxidants oxidize Pg although the efficiency varies. Many of the oxidants resulted in a second peak either as a shoulder of the first peak or as a separate peak. With higher concentrations of Pg, the shape and intensity of the shoulder or second peak appear somewhat dependent on the AO. The additional oxidants appear to increase the time period over which the oxidation of Pg persists and hence the duration of the CL. They may stabilize or change the formation kinetics of Mn(III) or Mn(IV).

Effect of Different Acids

Different acids, HNO_3, HCl, H_2SO_4, H_3PO_4, and a mixture of HNO_3 and H_3PO_4, were used to observe their effect on the CL signal. Typical peaks and the reaction conditions are shown in Figure 3. As previously noted, the peak has a shoulder using HNO_3 as the acid. With HCl there is a second peak, but it is not well resolved; while with H_2SO_4 the two peaks are resolved better. Use of H_3PO_4 results in two peaks and the second one is broad and considerably delayed. With the acid mixture, there are two peaks also. The first one was shorter than that with H_3PO_4 and the second one appeared as tailing.

In general, the second peak became distinct, resolved, and larger when 1.25 mL Pg was used instead of 1.0 mL. Overall signal height of the first peak was not affected much by the type of acid. The height and shape of the second peak; however, changed with different acids. Further studies were conducted with lower concentrations of Pg and permanganate (100 μM and 200 μM, respectively). The results obtained with different concentrations of the acids are summarized in Table 7 and Figure 4. The CL signal appeared as one peak.

For all acids, there is a dramatic increase in signal from 0.01 to 10% acid. From 10% acid to 100% acid, the signal height varies slightly for most acids but decreases significantly with acid concentration only with HNO_3. Possibly HNO_3 oxidizes the Pg.

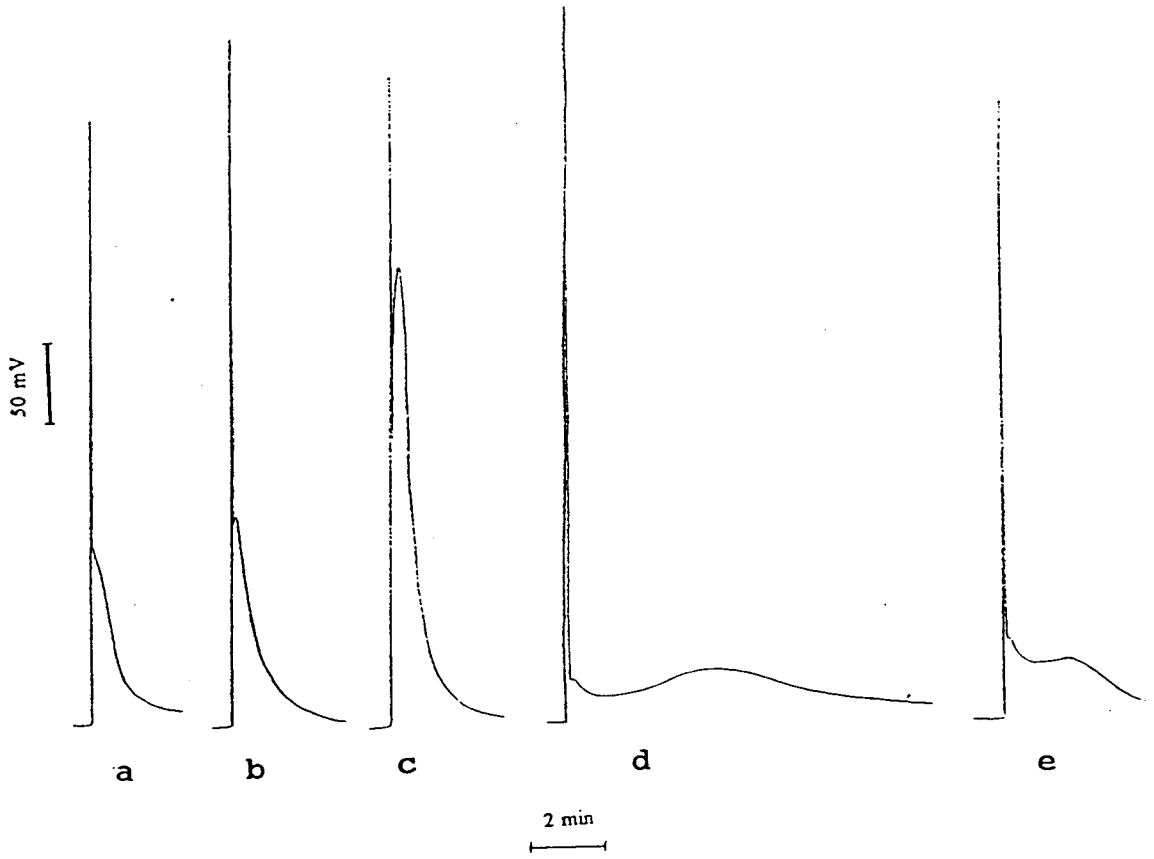


Figure 3. The effect of type of acid on the shape and intensity of the CL signal of Pg.
Reaction mixture: 1 mL of 4 mM Pg, 0.5 mL Of 50% (v/v) acid, 1 mL of water, and 1 mL of 10 mM $KMnO_4$ (injected).
a. HNO_3; b. HCl; c. H_2SO_4; d. H_3PO_4; e. H_3PO_4/HNO_3.

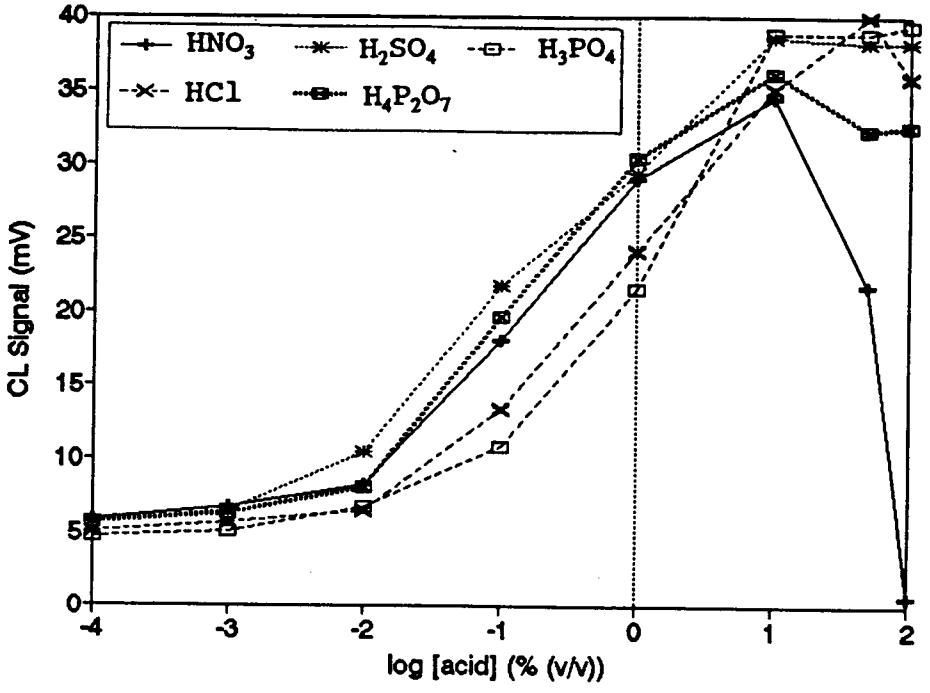


Figure 4. Dependence of CL intensity of Pg on the type and concentration of acid.
Reaction mixture: 1 mL of 100 μM Pg, 0.5 mL of acid, 1 mL of water, and 1 mL of 200 μM $KMnO_4$ (injected).

Table 7. Dependence of CL Signal of Pg on the Type and Concentration of Acid

	Peak Height (first peak) (mV)				
Conc. (%)	HNO_3	H_2SO_4	H_3PO_4	HCl	$H_4P_2O_7$
100	0.6	38	39	36	33
50	22	38	39	40	32
10	35	39	39	35	36
1	29	29	22	24	30
0.1	18	22	11	13	20
0.01	8.2	10	6.7	6.7	8.0
0.001	6.7	6.5	5.1	5.7	6.3
0.0001	5.9	5.7	4.7	5.1	5.7
RSD[a] (%)	1.1	1.0	6.7	19	4.4

[a] RSD for 50% (v/v) acid.

All the acids provide approximately the same maximum signal (35-40 mV) at the optimum acid concentrations. Sulfuric acid provides good precision and the reaction resulted in a second peak. The reactions with phosphoric acid and polyphosphoric acid are slow with broad peaks and the precision is poor. Likewise, the precision with hydrochloric acid is not good. Nitric acid was chosen as the best acid because it produced a high CL signal with relatively good precision. It was also chosen by Montalvo[3] for CL analysis of ethanol based on CL intensity, precision, duration of CL, and detection limits.

Effect of Metal Ions

The effect of Fe(II) and different oxidation states of Mn on the CL signal of Pg was studied. First, two ions were tested, Mn(II) and Fe(II). The reaction mixture was:

1 mL Pg (4 mM)

0.5 mL HNO_3 (50% (v/v))

1 mL water or metal ion (1 to 5 mM)

1 mL MnO_4^- (10 mM), injected

Addition of Mn(II) to the reaction mixture of Pg before injecting permanganate caused little observable difference in the CL signal as shown in Figure 5. The peak has the same shape as that of the control solution except it is slightly lower. When Fe(II) was used, a second peak became more resolved as the concentration of Fe(II) increased. Again the first peak height did not change much. When lower concentrations of all the reagents were used (100 μM Pg, 200 μM MnO_4^-), the CL signal appeared as one peak and it decreased very slightly as the concentration of the additive ion increased from 1 to 5 mM.

Intermediate oxidation states of Mn were tested because Montalvo proposed that they are involved in the CL reaction. When 200 μM Mn(III) was injected (instead of permanganate) into a reaction mixture of 100 μM Pg and 50% HNO_3, no signal was observed. With the injection of a 50 mM Mn(III) solution, as shown in Figure 6, a signal was produced which decreased

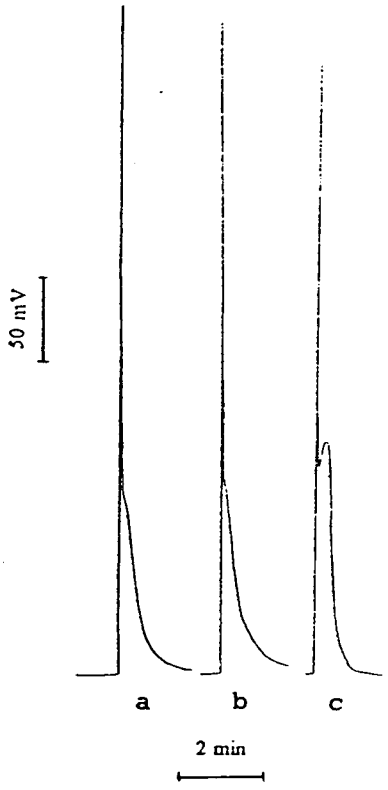


Figure 5. The effect of metal ions on the CL signal of Pg. Reaction mixture: 1 mL of 4 mM Pg, 0.5 mL of 50% (v/v) HNO_3, 1 mL of water or additive, and 1 mL of 10 mM $KMnO_4$ (injected). a. water; b. 5 mM Mn^{2+}; c. 5 mM Fe^{2+}.

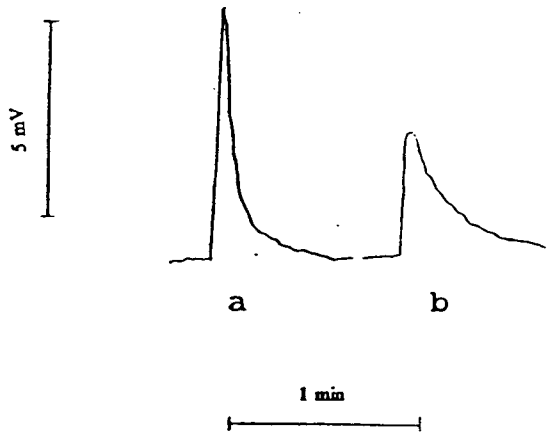


Figure 6. The effect of injecting Mn(III) on the CL signal of Pg.
Reaction mixture: 1 mL of 100 μM Pg, 0.5 mL of 50% (v/v) HNO_3, 1 mL of water, and 1 mL of Mn(III), (injected).
a. 50 mM Mn(III); b. 20 mM Mn(III)

by 60% as the concentration of Mn(III) decreased from 50 to 20 mM.

As shown in Figure 7, the addition of 1 mM Mn(III) to reaction mixture containing 4 mM Pg before the injection of 10 mM permanganate caused a distinct and large second peak. The height of the second peak decreased as the concentration of Mn(III) solution decreased from 1 mM to 200 μM.

From the above observations, Mn(III) can be seen to play an important rule in the oxidation of Pg. At higher concentrations of Mn(III), but not lower, a CL signal was observed with injection of Mn(III) which indicates the oxidation of Pg.

When Mn(III) was added to the cell before injecting permanganate solution, the height and the shape of the signal changed. At higher concentrations a second peak was observed, and at lower concentrations the signal height decreased. This fact indicates that Mn(III) is involved in the equilibria that affect the CL signal and may be the oxidant that produces CL.

The effect of Mn(IV) on the CL signal was also evaluated. After preparation of the 10 mM Mn(IV) solution as outlined in Table 3, the color of the solution turned from purple to yellowish brown, which is the color of Mn(IV) solution. The color was stable but that of the diluted solution (200 μM) turned pink after few days (possibly Mn(III)).

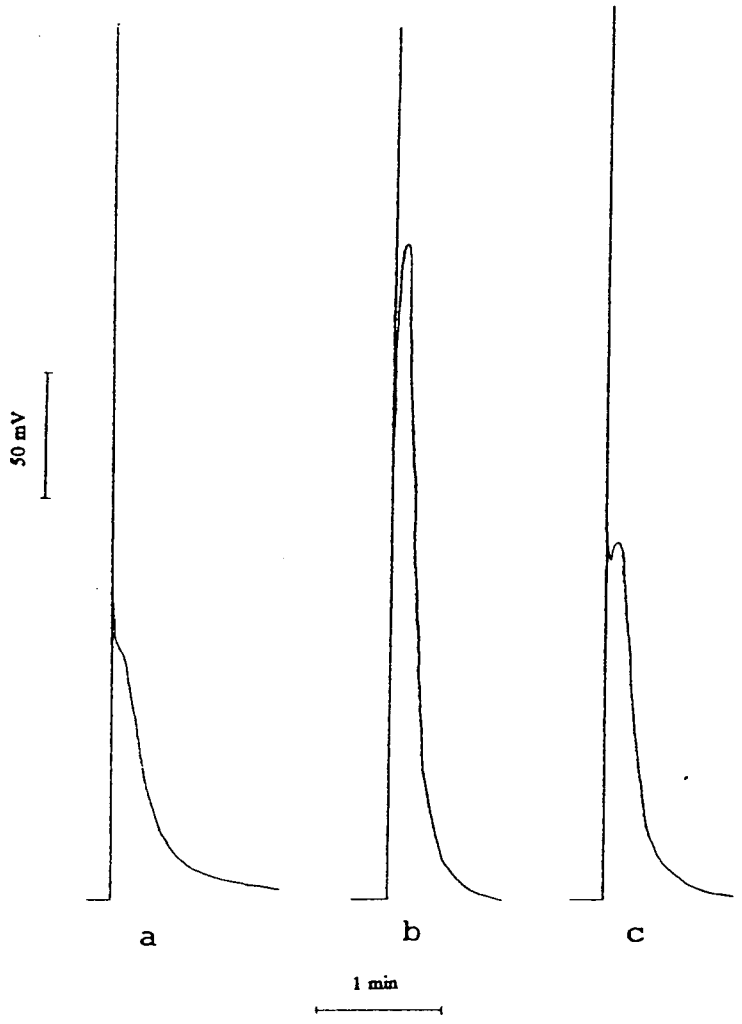


Figure 7. The effect of adding Mn(III) on the CL signal of Pg. Reaction mixture: 1 mL of 4 mM Pg, 0.5 mL of 50% (v/v) HNO_3, 0.25 mM of water or Mn(III), 0.75 mL of water, and 1 mL of 10 mM MnO_4^-, injected.
a. water; b. 1 mM Mn(III); c. 200 μM Mn(III).

When Mn(IV) solution was injected into the reaction cell, there was no signal observed even with various concentrations of all the reactants (100 μM to 4 mM Pg and 200 μM to 10 mM Mn(IV)). It appears that Mn(IV) is not the species responsible for the CL signal. Addition of a Mn(IV) solution to the cell before injecting permanganate resulted in a signal lower than that of the control as shown in Figure 8. The signal decreased as the in-cell concentration of the Mn(IV) solution increased (see Table 8). Therefore, Mn(IV) may oxidize Pg but is not directly responsible for the CL signal.

Table 8. The Effect of Mn(IV) on the CL Signal of Pg[a]

	Mn(IV)		Peak height
#	μM	mL	mV
1	water	1.00	30
2	200	0.25	23
3	200	0.50	11
4	200	0.75	4.1
5	200	1.00	1.8

[a] Reaction mixture: 1 mL Pg (100 μM), 0.5 mL HNO_3 (50% (v/v)), and 1 mL $KMnO_4$ (200 μM).

A mixture of permanganate (10 mM) and Mn(II) (10 mM) was prepared in 9 M sulfuric acid. The product of the mixture is believed to be Mn(IV). When this solution was injected into the reaction cell, a single peak was observed which is lower by 65% than that produced by injecting permanganate solution

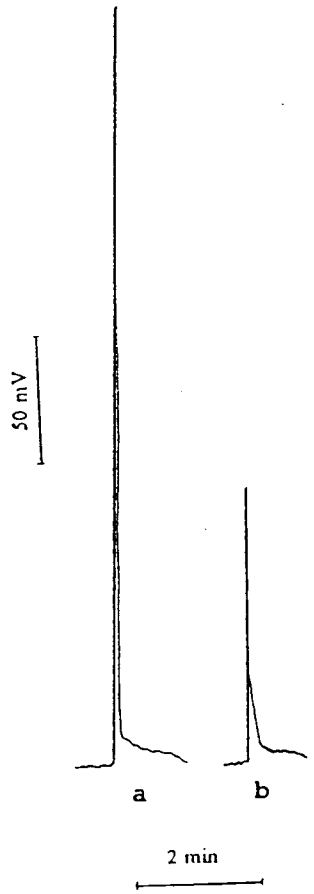


Figure 8. The effect of adding Mn(IV) on the CL signal of Pg. Reaction mixture: 1 mL of 100 μM Pg, 0.5 mL of 50% (v/v) HNO_3, 1 mL of water or Mn(IV), and 1 mL of 200 μM $KMnO_4$ (injected). a. water; b. 100 μM Mn(IV).

alone. A dilution of the Mn(II)/Mn(VII) mixture to 200 μM produced an instable solution which changed from yellow to pink within few minutes.

Effect of Additives on Pg/Ethanol Mixtures

The next set of studies was concerned with determining if additives affected the CL of ethanol and Pg differently. The reaction mixture was:

0.5 mL Pg (0.5 mM)

1 mL ethanol (10% (v/v))

0.5 mL HNO_3 (95% (v/v))

0.5 mL water or additive

1 mL MnO_4^- (10 mM), injected

The effect of different additional oxidants was first tested on Pg/ethanol mixtures. Ce^{4+} solutions of different concentrations (1 to 32 mM) were added to the reaction cell before injecting the permanganate solution. The ethanol signal was affected very slightly by the concentration of the Ce^{4+} solution. The Pg signal, on the other hand, decreased as the concentration of the Ce^{4+} solution increased and decreased by a factor of 7 with 32 mM Ce^{4+}. The same general effect was observed by adding $Cr_2O_7^{4-}$. IO_4^- had a less effect on both signals.

Apparently, the oxidation of Pg by Ce^{4+} and $Cr_2O_7^{4-}$ is very fast, and ethanol is mainly oxidized by permanganate. The oxidation of ethanol is a slower reaction and a brownish

solution with a precipitate (MnO_2) is formed. The reaction cell was cleaned with 50% HCl solution between runs.

When 20 μL of 10 mM permanganate solution was added to the reaction cell before the injection of permanganate, the Pg signal decreased but that of ethanol did not since the oxidation of ethanol is a slow reaction.

The effect of some other additives (F^-, $P_2O_7^{4-}$, and Mn^{2+}) on the oxidation of Pg and ethanol was also studied. For 100 μM Pg, F^- has no effect while 1 M $P_2O_7^{4-}$ and 1 M Mn^{2+} decreased the signal slightly. For ethanol, 1 M F^- and 1 M $P_2O_7^{4-}$ decreased the peak height to about half while 1 M Mn^{2+} suppressed it almost completely as shown in Figure 9. These additives probably affect the CL reaction by changing the concentrations and rates of production and disappearance of intermediate oxidation states of Mn. Pyrophosphate and fluoride are known to complex Mn(III)[3] and should stabilize it and reduce formation of Mn(IV).

The results of Montalvo[3] show that Mn^{2+} decreased the signal of 7.5 mg/L (~60 μM) Pg by 20%, while it enhanced that of 150 mg/L (~1.2 mM) Pg by 40%. By adding Mn^{+2} before injection of dichromate, Montalvo did not get any signal. Based on the following equilibria, she suggested that the CL pathway does not include oxidation of the Pg by Mn(III).

$$Cr(VII) + \text{substrate} \rightarrow Cr(IV) + \text{product}$$

$$Cr(IV) + Mn^{2+} \rightarrow Mn(III) + Cr(III)$$

$$2\ Mn(III) \rightarrow Mn(II) + MnO_2$$

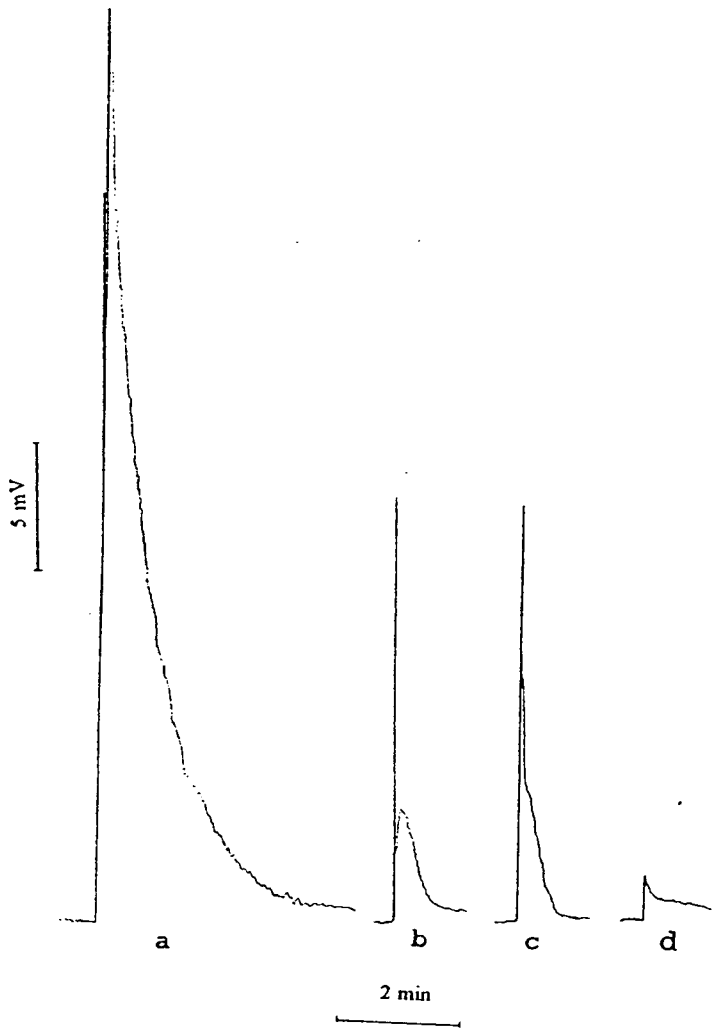


Figure 9. The effect of additives on the CL signal of ethanol. Reaction mixture: 1 mL of 50% (v/v) ethanol, 0.5 mL of 95% (v/v) HNO_3, 1 mL of water or additive, and 1 mL of 10 mM $KMnO_4$ (injected).
a. water; b. 1 M F^-; c. 1 M $P_2O_7^{4-}$; d. 1 M Mn^{2+}.

Montalvo mentioned that Mn^{2+} decreased the ethanol signal significantly. Mn^{2+} would be expected to increase the rate of the reduction of MnO_4^- to Mn(III) or Mn(IV) as in the following equilibria:

$$MnO_4^- + 4\ Mn^{2+} + 8\ H^+ \rightarrow 5\ Mn(III) + 4\ H_2O$$

$$2Mn(III) \rightleftarrows Mn(II) + Mn(IV)$$

Mn(III) and Mn(IV) solutions were injected separately into the reaction cell mixture which contains ethanol. With a 50 mM Mn(III) solution as the injected oxidant, the signal was broad in the case of the control and it was eliminated by the addition of 1 M Mn(II) and suppressed by the addition of F^- and $P_2O_7^{4-}$ by 95 and 90%, respectively. When a 10 mM Mn(IV) solution was injected, the behavior was different as shown in Figure 10. Both F^- and $P_2O_7^{4-}$ enhanced the signal. On the other hand, 1 M Mn(II) eliminated the signal. The presence of Mn(II) should reduce the relative concentration of Mn(IV) by shifting the equilibrium reaction between Mn(II) and Mn(IV) to Mn(III). This shifting would reduce the CL signal if Mn(IV), rather than Mn(III), was the oxidant resulting in CL. Because Mn(II) also completely depressed the ethanol signal when Mn(III) was injected, this experiment does not suggest which Mn species is responsible for the CL oxidation of ethanol. Complexation of Mn(III) by F^- and $P_2O_7^{4-}$ would be expected to suppress the signal with Mn(III) but not Mn(IV) injected as was observed.

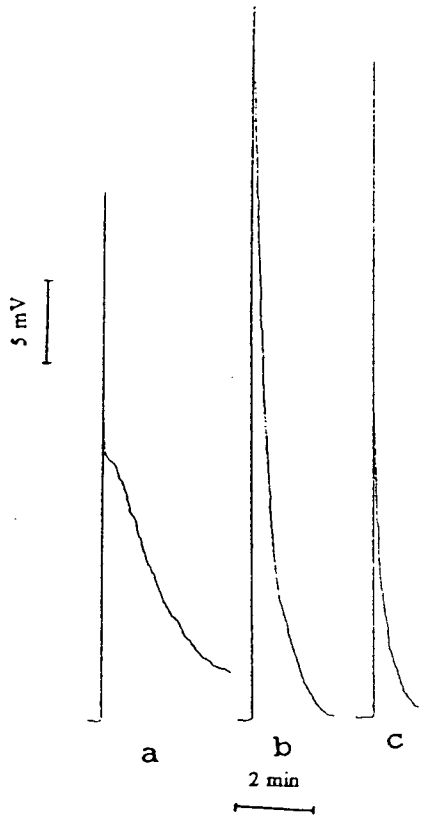


Figure 10. The effect of additives on the CL signal of ethanol while injecting Mn(IV) solution.
Reaction mixture: 1 mL of 50% (v/v) ethanol, 0.5 mL of 95% (v/v) HNO_3, 1 mL of water or additive, 1 mL of 10 mM Mn(IV) (injected).
a. water; b. 1 M F^-; c. 1 M $P_2O_7^{4-}$.

Effect of Permanganate Concentration

Permanganate solutions of different concentrations were injected into the reaction cell containing Pg. At a high concentration (1 mM) of permanganate solution, all the Pg was oxidized and the signal was high. When permanganate solution was injected again into the same reaction mixture, no additional signal was observed. Since the oxidation of Pg is a two-electron process, it requires 200 μM of MnO_4^- to oxidize 100 μM Pg (assuming a $1e^-$ reduction of Mn(IV) to Mn(III) or Mn(III) to Mn(II)). Figure 11 shows that 200 μM MnO_4^- was the lowest concentration that oxidized the entire amount of Pg.

With a lower permanganate concentration, not all the Pg was oxidized by the first injection and another injection oxidized the remaining Pg as shown in Figure 11. In some cases where the concentration of permanganate solution was very low, even a second injection did not oxidize the entire amount of Pg. Figure 11d shows two, almost equal peaks heights and the first one has no tailing because not all the Pg has been oxidized.

Effect of Sensitizers

Different fluorescence dyes were tested for their effect on the CL signal. It was hoped that they would enhance the CL signal by capturing the excitation energy and becoming exciting species themselves. The signal would be enhanced if the fluorescence quantum efficiency of the dye is greater than

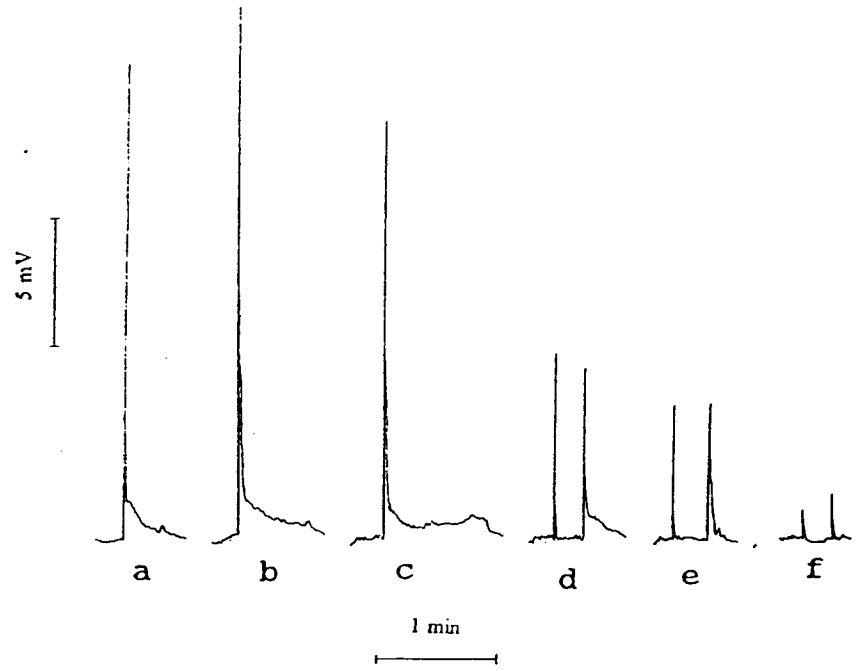


Figure 11. CL signal of Pg with double injection of permanganate solution.
Reaction mixture: 1 mL of 100 μM Pg, 0.5 mL of 50% (v/v) HNO_3, 1 mL of $KMnO_4$ (double injected).
[$KMnO_4$]: a. 1 mM; b. 400 μM; c. 200 μM; d. 100 μM; e. 50 μM; f. 20 μM.

that of the original excited species. The reaction mixture was:

1 mL Pg (100 μM) or water

0.5 mL HNO_3 (50%)

100 μL dye (0.25 mM) or water

0.9 mM water

1 mL MnO_4^- (1 mM), injected

The CL signal of Pg without added sensitizers was 21 mV. All of the dyes tested increased the signal by 10 mV or more as shown in Table 9. The experiment was repeated with the Pg solution replaced by water. Many of the dyes still gave high signals by themselves so they are oxidized and produce CL. Others did not chemiluminesce and appear to slightly enhance the CL signal by energy transfer from the excited species and subsequent CL. Dyes which are oxidized by MnO_4^- cannot be used as sensitizers for the CL reaction of Pg since they interfere with the Pg oxidation. The enhancement from the dyes that are not oxidized is not sufficient to use them as sensitizers.

After completion of the reaction, the reaction mixture was colorless when the dye was used with Pg and pink when either Pg or the dye was used alone. When a 0.5 mM permanganate solution was injected, the reaction mixture was colorless and a double injection produced a second peak. Therefore, a 1 mM permanganate solution was used to obtain the data in Table 9 to ensure there was enough permanganate to oxidize both the Pg and the dye.

Table 9. The Effect of Sensitizers on the CL Signal of Pg

Sensitizer	CL Signal (mV) (w/ Pg)	CL Signal (mV) (w/o Pg)
Pyronin B	37	32
Rhodamine B	35	30
Eyosin Y*	34	9.8
Acridine Orange	37	30
Brilliant Salfaflavine*	32	6.1
BBQ*	32	1.2
p-Terphenyl*	34	1.0
Coumarin 450	35	31
Stilbene 420*	32	7.5
LD 688	32	18
Rhodamine 575	36	31
Fluorescein 548*	34	0.6
Rhodamine 590	37	29
Coumarin 521	34	33
Fluorol 555	34	32

* Dyes gave small CL signals by themselves.

Additional Studies of Additives

For further experiments, the R928 PMT was substituted for the 1P28 and the effect of a few additives were tested. Under the same solution and other instrumental conditions, the CL signal for 100 μM Pg using the new PMT is slightly higher than that observed with the old PMT as shown in Figure 12. The tail of the peak is also broader yielding a broader peak with the new PMT. The baseline peak-to-peak noise (dark current

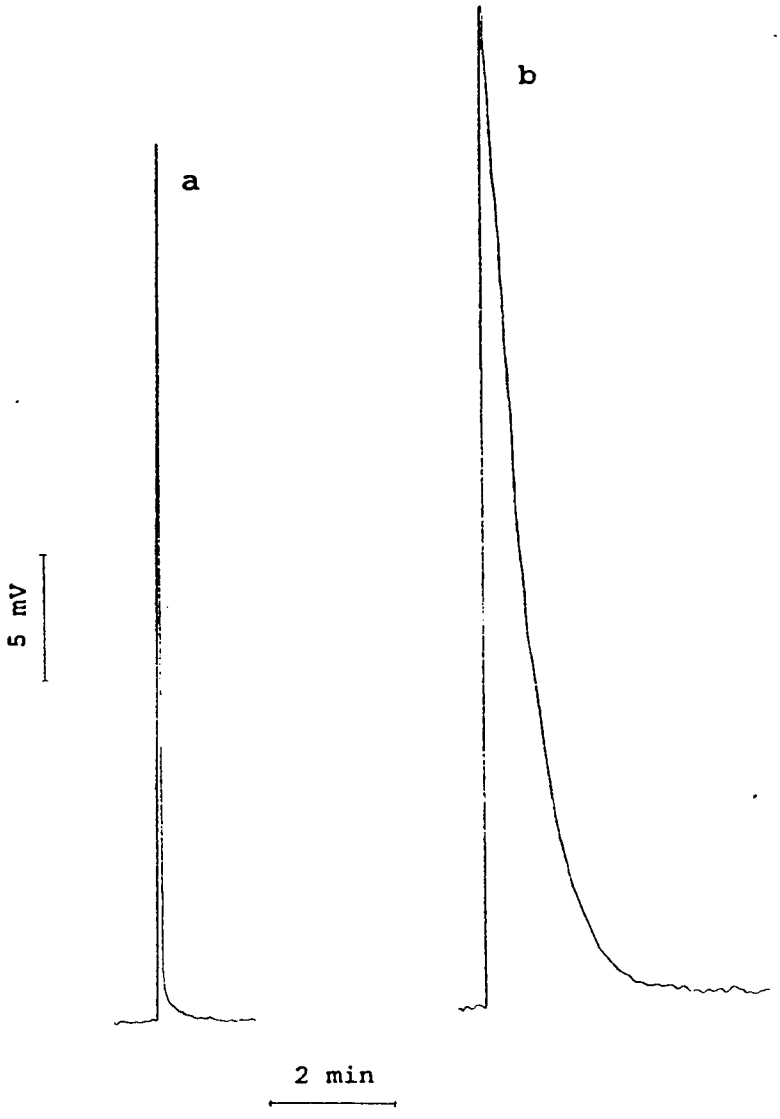


Figure 12. CL signal of Pg with different PMT's.
Reaction mixture: 1 mL of 100 μM Pg, 0.5 mM of 50% (v/v) HNO_3, 1 mL of water, and 1 mL of 200 μM $KMnO_4$, injected.
a. 1P28; b. R928

noise) is about twice as great with the new PMT.

A mixture of Pg and ethanol was first tested. The basic reaction mixture was:

1 mL Pg (5 mM)

1 mL ethanol (10%)

0.5 mL HNO_3 (50%)

1 mL MnO_4^- (10 mM)

With the above mixture of Pg and ethanol, only a Pg CL peak was observed because the Pg reaction is very fast compared to the ethanol reaction, and $[MnO_4^-]/[Pg] = 2$ such that all the permanganate is consumed. When ethanol was tested alone, a broad peak was observed. With 0.25 mL of 5 mM Pg in the reaction mixture, both peaks were observed. When 95% acid was used, the Pg peak height did not change, but the ethanol peak became higher and more distinct.

Ce^{4+} (32 mM) was added to a mixture of 100 μM Pg and 10% ethanol using a 10 mM permanganate solution as the oxidant. Both the Pg and ethanol signals decreased indicating the oxidation of both analytes by Ce^{4+} and that the concentration of Ce^{4+} needed to reduce Pg completely (Figure 2) results in some oxidation of ethanol (about 28%).

Before injecting the 10 mM solution, 100 μL of a permanganate solution (200 μM) was added manually to the reaction cell. Lower signals for both ethanol and ethanol/Pg mixture were obtained due to oxidation of some of both species. It appears difficult to preferentially reduce Pg in the presence of ethanol.

The result of delaying the injection of permanganate solution is summarized in Table 10. The majority of the oxidation occurs rapidly.

Table 10. Dependence of the CL Signal on the Injection Time of Permanganate

Ce^{4+}		Time Delay	Peak Height
Conc. (mM)	Vol. (mL)	(min)	(mV)
-	-	0	43
4	1	0	14
4	1	10	10

Reaction mixture: 1 mL Pg (100 μM), 0.5 mL HNO_3 (50% (v/v)) and 1 mL $KMnO_4$ (200 μM).

Other oxidants were tested for their effect on ethanol and ethanol/Pg mixture. The oxidant concentrations used were in great molar excess relative to Pg. The reaction mixture was:

1 mL ethanol (10%)

0.25 mL Pg (400 μM) or water

0.5 mL HNO_3 (95%)

0.25 mL AO or water

1 mL MnO_4^- (10 mM)

The results are summarized in Table 11.

Table 11. The Effect of Additional Oxidants on the CL Signal of Ethanol and Pg

Analyte	AO (Conc.)	EtOH Signal (mV)	Pg signal (mV)
EtOH	-	284	-
EtOH	IO_4^- (20 mM)	118	-
EtOH	Ag^{2+} (10 mM)	284	-
EtOH	$Cr_2O_7^{2-}$ (3 mM)	288	-
EtOH	Ce^{4+} (4 mM)	269	-
EtOH+Pg	-	294	339
EtOH+Pg	IO_4^- (20 mM)	100	122
EtOH+Pg	Ag^{2+} (10 mM)	224	335
EtOH+Pg	$Cr_2O_7^{2-}$ (3 mM)	216	180
EtOH+Pg	Ce^{4+} (4 mM)	190	98

The CL signals of Pg and ethanol are overlapping in the first few seconds of the reaction but the ethanol peak maximum appears much later as shown in Figure 13. The difficulty is that both species react with permanganate and the concentration of permanganate affects the CL. The additional oxidants oxidize both Pg and ethanol so both signals are affected. Ideally an additional oxidant would oxidize only Pg so that the Pg signal would be eliminated and the ethanol signal could be measured without interference.

N-Bromosuccinimide (NBS) is used in the oxidation of alcohols to aldehydes and ketones.[80] To see its effect on the CL signal, it was added to the reaction cell containing the

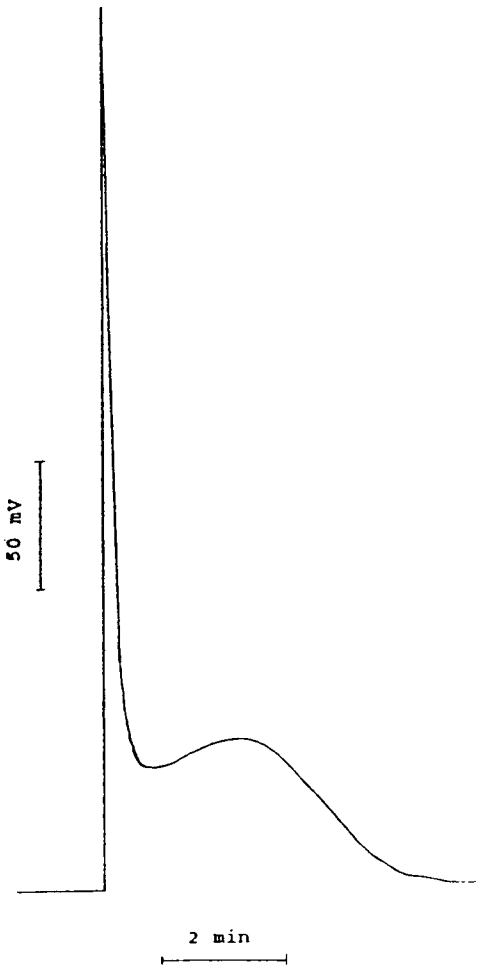


Figure 13. Typical shape of CL signal for ethanol/Pg mixture. Reaction mixture: 0.5 mL of 4 mM Pg, 1 mL of 10% (v/v) ethanol, 0.5 mL of 50% HNO_3, 0.5 mL of water, and 1 mL of 10 mM MnO_4^-, injected.

following reaction mixture:

1 mL Pg (100 μM) or ethanol (10%) or both

0.5 mL HNO_3 (10 or 100%)

1 mL water or NBS (20 mM)

1 mL MnO_4^- (20 mM), injected

NBS has a great effect on the Pg. It reduced the Pg signal by a factor of 40 while it increased that of ethanol slightly. Permanganate was present in great molar excess relative to Pg. The small signal observed might be due to significant absorption by the dark purple colored permanganate. With 4 mM Pg instead of 100 μM, a very high signal was observed (4.5 V) which decreased by adding NBS by a factor of 13. For ethanol, 100% (v/v) solution gave a signal of 2.3 V which increased to 2.4 V by the addition of NBS. NBS was tested with blank solution (water) to see if it reacts with permanganate. No signal was observed.

By using NBS as an additional oxidant, there was more selectivity observed in oxidizing Pg relative to ethanol with NPS compared to other oxidants tested. Overall, by choosing the right volume and concentration of NBS, the Pg signal can be suppressed and only that of ethanol is detected. Addition of 1 mL of 30 mM NBS caused the signal of 100 μM Pg to decrease from 41 mV to about 1 mV, and that of 10% (v/v) ethanol to increase from 82 mV to 90 mV.

Optimization with Permanganate as the Oxidant

The effect of HNO_3 concentration on the CL signals of ethanol and Pg with a constant MnO_4^- concentration is shown in Figures 14 and 15. For ethanol, higher acid concentrations increased the CL signal with the largest signal at 95-100%. For Pg however, higher acid concentration decreased the signal and the optimum concentration was 10%. The optimum acid concentration found by Montalvo[3] was 95% for ethanol and 50% for Pg.

The effect of permanganate concentration on the CL signal of ethanol and Pg with the "best" HNO_3 concentrations determined above is shown in Figures 16 and 17. The CL signal for ethanol is greatest at a permanganate concentration of about 15 mM and decreases above this concentration because purple permanganate absorbs the CL radiation.[3] For Pg, the CL signal decreases when using permanganate concentration above about 300 μM. At and above this concentration, the color of the reaction mixture after the reaction was pink and the permanganate is present in molar excess relative to Pg.

For all concentrations tested, ethanol is in molar excess relative to permanaganate as shown in Table 12. Whether the oxidation of ethanol by permanganate produces acetic acid or acetaldehyde depends on the molar ratio of permanganate to ethanol.[3] The intensity of CL signal is proportional to the rate of oxidation which in turn is proportional to the ethanol and permanganate concentrations.

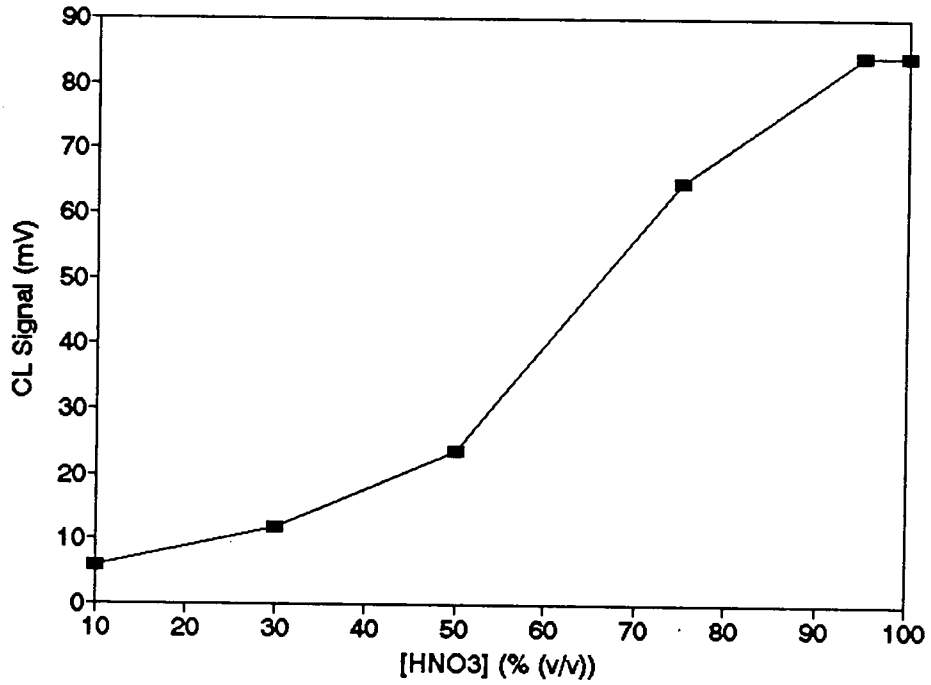


Figure 14. Optimization of nitric acid concentration for ethanol with permanganate as the oxidant.
Reaction mixture: 1 mL of 10% (v/v) ethanol, 0.5 mL HNO_3, 1 mL of water, and 1 mL of 10 mM $KMnO_4$ (injected).

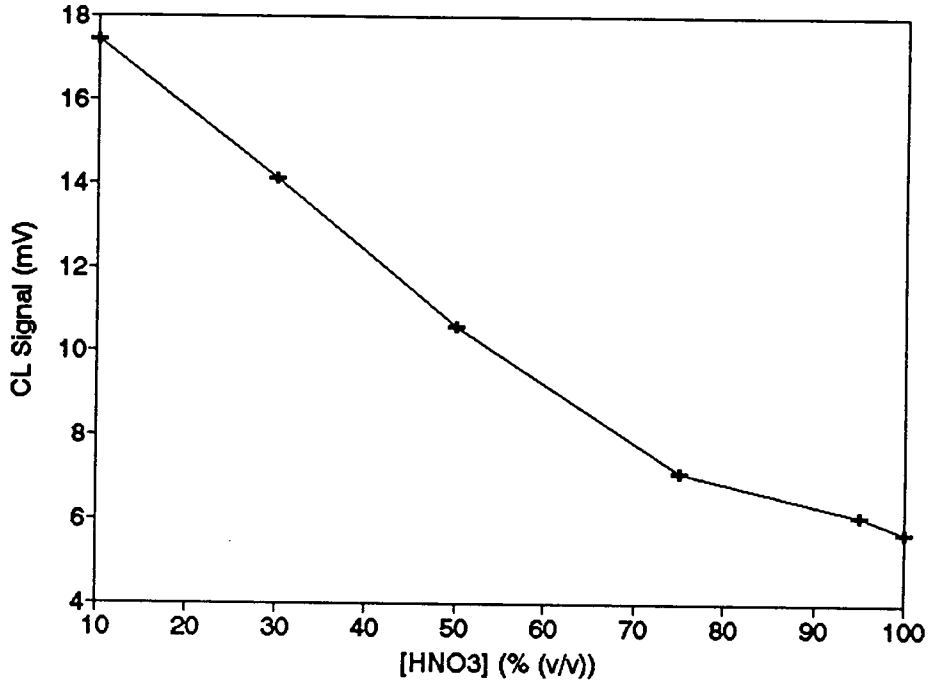


Figure 15. Optimization of nitric acid concentration for Pg with permanganate as the oxidant.
Reaction mixture: 1 mL of 100 μM Pg, 0.5 mL of HNO_3, 1 mL of water, and 1 mL of 200 μM $KMnO_4$ (injected).

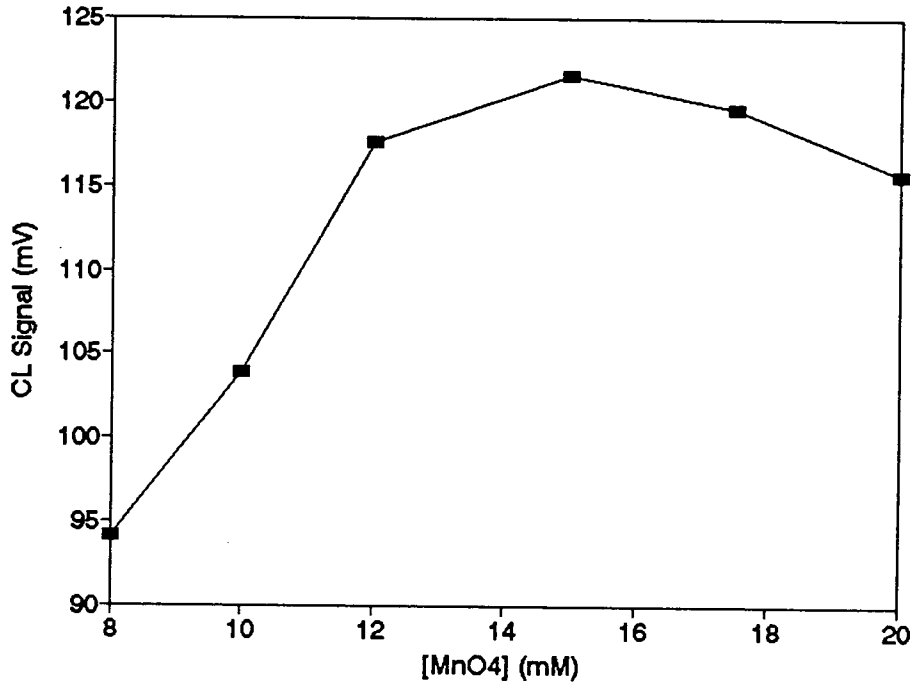


Figure 16. Optimization of permanganate concentration for ethanol.
Reaction mixture: 1 mL of 10% (v/v) ethanol, 0.5 mL of 95% (v/v) HNO_3, 1 mL water, and 1 mL of $KMnO_4$ (injected).

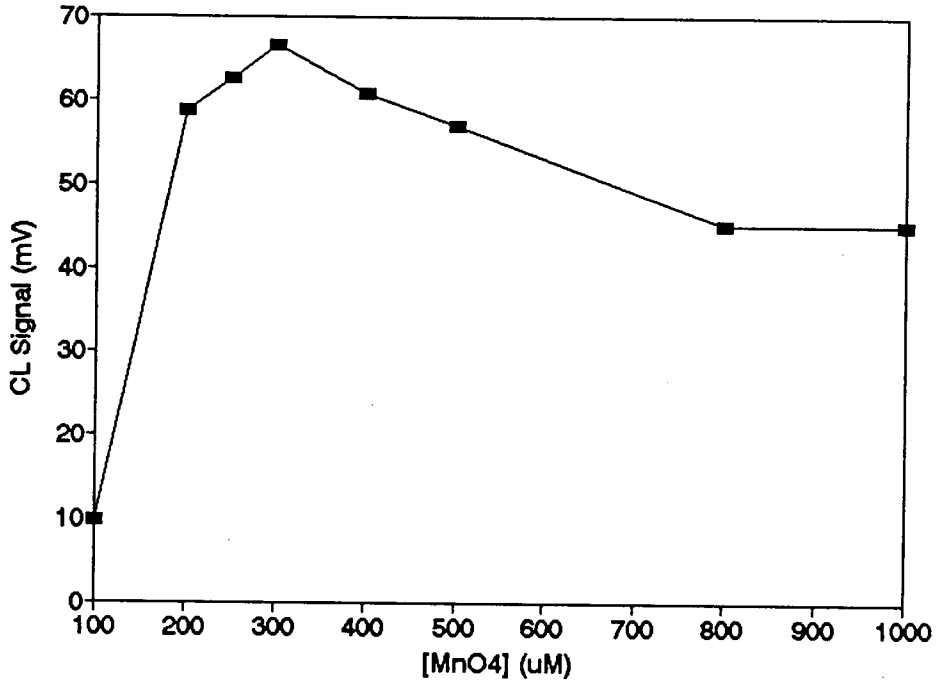


Figure 17. Optimization of permanganate concentration for Pg. Reaction mixture: 1 mL of 100 μM Pg, 0.5 mL of 10% (v/v) HNO_3, 1 mL of water, and 1 mL of $KMnO_4$ (injected).

Table 12. Dependence of the Molar Ratio of Ethanol on the Ethanol Concentration

EtOH Conc. (% (v/v))	EtOH Conc. (% (w/v))	EtOH Conc. (M)	Molar Ratio[a]
100	78.9	17.1	0.00088
80	63.1	13.7	0.0010
60	47.3	10.3	0.0015
40	31.6	6.87	0.0022
20	15.8	3.43	0.0044
10	7.89	1.72	0.0087
5	3.95	0.859	0.018
4	3.16	0.687	0.022
3	2.37	0.515	0.029
2	1.58	0.343	0.044
1	0.789	0.172	0.087
0.5	0.395	0.0859	0.18
0.1	0.0789	0.0171	0.88

[a] $[MnO_4^-]/[ethanol]$; concentration of MnO_4^- is 1.5×10^{-2} M.

Figure 18 shows a calibration curve for ethanol with the optimum concentrations of HNO_3 and permanganate. Above 60% (v/v) ethanol, the slope of the curve increases rapidly. At higher ethanol concentrations the reaction appears faster, the peak maximum occurs earlier, and the permanganate is consumed more rapidly reducing attenuation of CL radiation due to absorption. The calibration curve is reasonably linear below about 40% (v/v) ethanol as shown in Figure 18b. The detection limit for ethanol was calculated to be 0.07%(v/v) (1.2×10^{-2} M) from the ratio of the peak-to-peak baseline noise (0.6 mV)

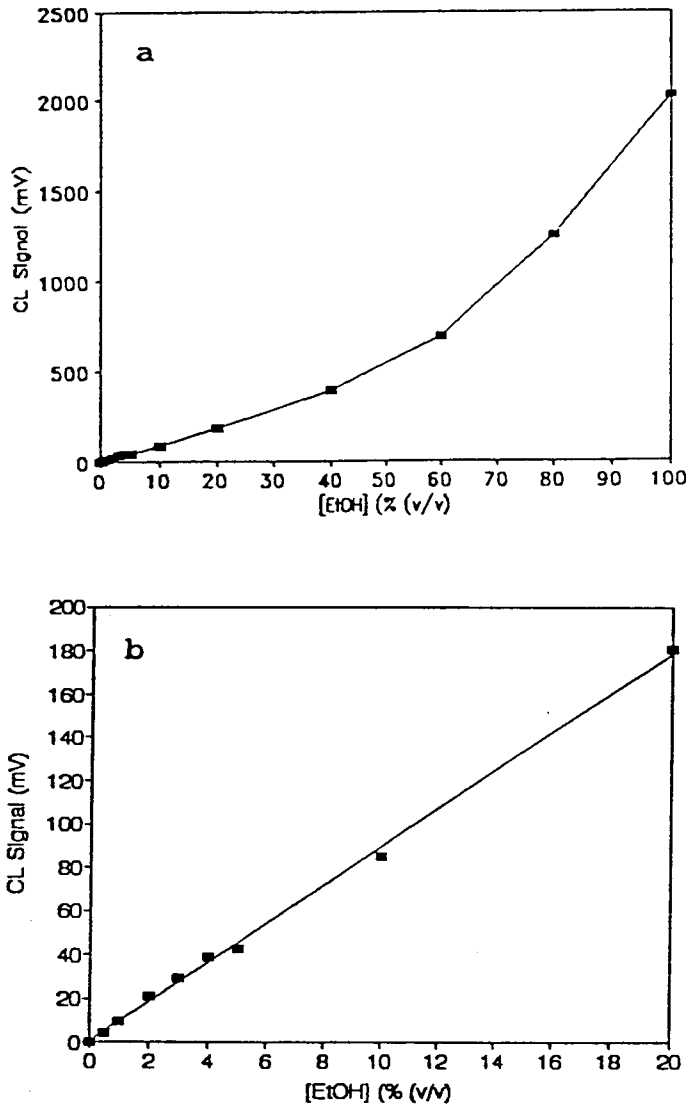


Figure 18. Calibration curve for ethanol with permanganate as the oxidant.
Reaction mixture: 1 mL of ethanol, 0.5 mL of 95% (v/v) HNO_3, 1 mL of water, and 1 mL of 15 mM $KMnO_4$ (injected).
a. full range; b. lower concentrations

to the calibration curve slope (9.0 mV/%(v/v)).

Figure 19 shows a calibration curve for Pg with the optimum concentrations of HNO_3 and permanganate. The curve is linear below about 2.5 μM (see Figure 19b). Above this concentration, the curve flattened; the reaction is very fast and the real CL maximum might not be measured because of the response of the recorder. For all Pg concentrations tested, permanganate was in excess (if the oxidation of Pg is a $2e^-$ reaction). The reaction mixture was pink after the completion of the reaction and some absorption of radiation by remaining MnO_4^- occurs. The detection limit for Pg was calculated to be 0.03 μM from the ratio of the peak-to-peak baseline noise (0.4 mV) to the calibration curve slope (11.6 mV/μM).

Optimization with Mn(IV) as the Oxidant

With Mn(IV) as the injected oxidant, the acid concentration was optimized for Pg and ethanol. The reaction mixture was:

1 mL Pg (100 μM) or ethanol (10%)

1 mL water

0.5 mL HNO_3

1 mL Mn(IV) (10 mM), injected

Next, the concentration of Mn(IV) was optimized for Pg and ethanol using the optimum acid concentration.

The optimum acid concentration for Pg was 10% and that for ethanol was 100%. The optimum concentration of Mn(IV) for

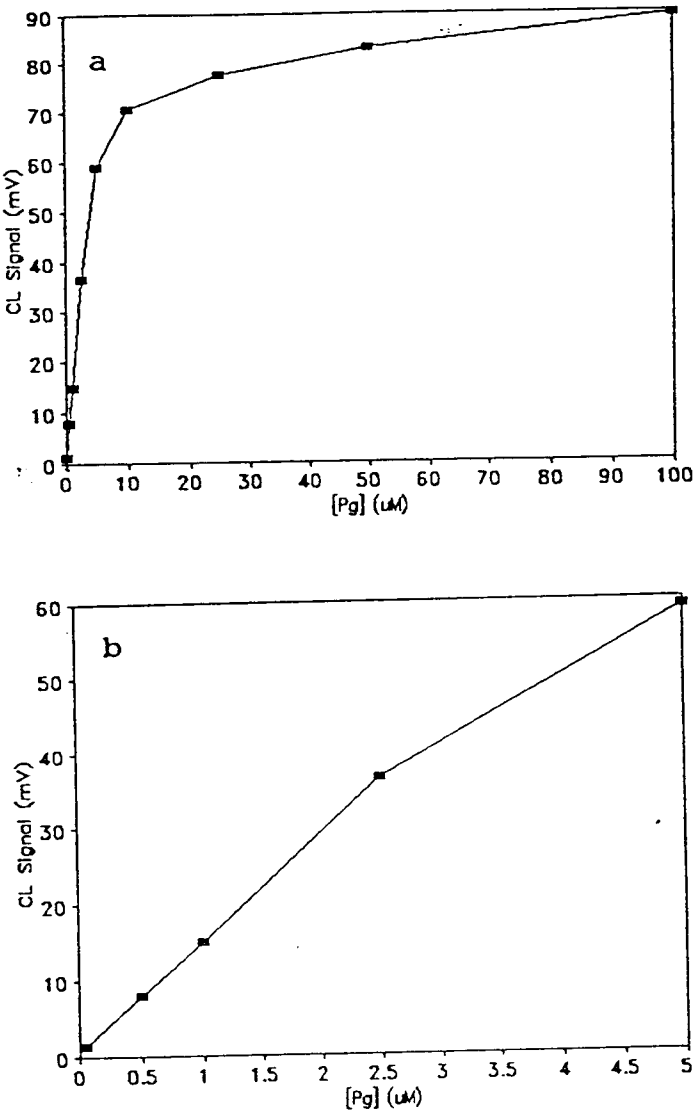


Figure 19. Calibration curve for Pg with permanganate as the oxidant.
Reaction mixture: 1 mL of Pg, 0.5 mL of 10% (v/v) HNO_3, 1 mL of water, and 1 mL of 300 μM $KMnO_4$ (injected).
a. full range; b. lower concentrations

Pg was 2 mM and for ethanol was 20 mM. Compared to MnO_4^- as the oxidant, the maximum Pg signal is obtained at a much higher oxidant concentration with Mn(IV). The Mn(IV) solutions with concentrations of 1 mM and lower were not stable and turned from yellow to pink within an hour. Figures 20 and 21 show calibration curves for ethanol and Pg with the optimum concentration of HNO_3 and Mn(IV). The turnover in the calibration curve may due to some form of quenching at high concentrations of Pg. The detection limits of ethanol and Pg with Mn(IV) were calculated to be 0.02% (v/v) (3 mM) and 4 μM, respectively.

The effect of addition of Mn(II) to the reaction mixture before injecting Mn(IV) was evaluated with the following reaction mixture:

1 mL Pg (100 μM) or ethanol (10%)

0.5 mL HNO_3 (10 or 100%)

1 mL water or Mn(II) (1 M) added

1 mL Mn(IV) (2 or 20 mM), injected

With 20 mM Mn(IV), the Pg signal increased by a factor of 16 and that of ethanol decreased by a factor of 30. These observations suggest that Mn(IV) is the oxidant for ethanol as Mn(II) would shift the equilibrium between Mn(IV) and Mn(III) toward Mn(III), while Mn(III) is the oxidant for Pg because Mn(II) would hinder Mn(III) from converting to Mn(IV).

Both a 2 and a 20 mM Mn(IV) solutions were prepared in 5 M sulfuric acid since with 9 M acid, the solution is very viscous and the injector does not always function well. The

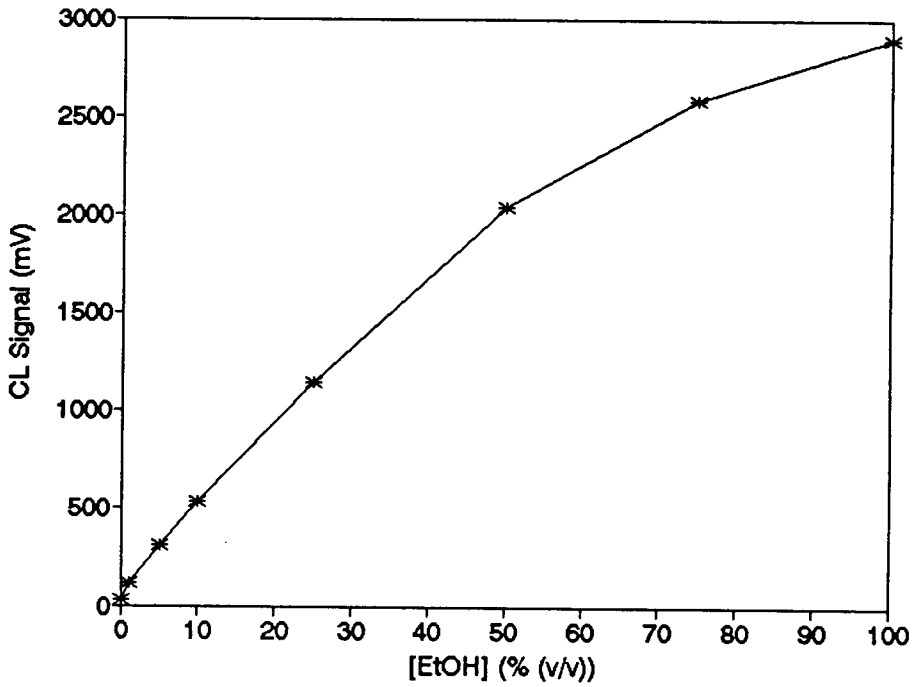


Figure 20. Calibration curve for ethanol with Mn(IV) as the oxidant.
Reaction mixture: 1 mL of ethanol, 0.5 mL of 100% (v/v) HNO_3, 1 mL of water, and 1 mL of 20 mM Mn(IV) (injected).

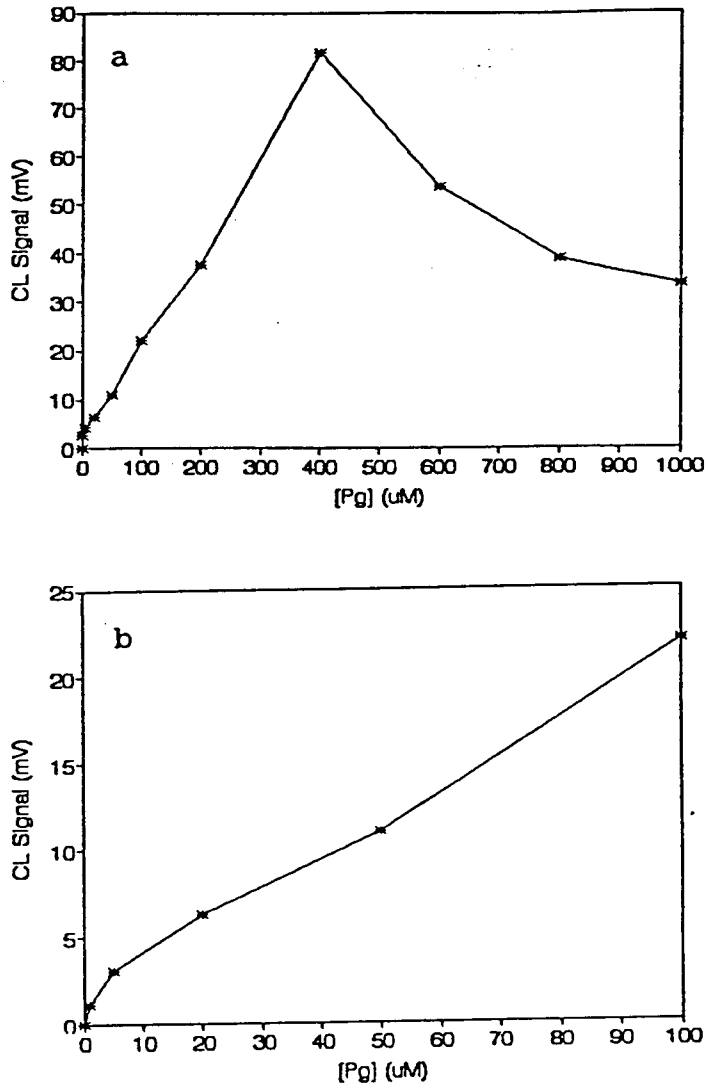


Figure 21. Calibration curve for Pg with Mn(IV) as the oxidant.
Reaction mixture: 1 mL of Pg, 0.5 mL of 10% (v/v) HNO_3, 1 mL of water, and 1 mL of 2 mM Mn(IV), (injected).
a. Full range; b. Lower concentrations.

color of the 20 mM solution did not change during storage overnight; however, the CL signals of ethanol and Pg with the aged solution were about 20% greater compared to that obtained with the fresh solution.

The color of the 2 mM Mn(IV) solution turned pink during overnight storage and no CL signal observed upon injecting the aged solution. With the fresh 2 mM Mn(IV) solution, both the Pg and ethanol signals decreased (by about 25 and 30%, respectively) with the addition of 1 M Mn(II). The fresh solution was pale yellow and it gave a higher signal with 100 μM Pg than the 20 mM solution. Possibly the more concentrated solution which is dark in color absorbs more of CL radiation.

Optimization with Mn(III) as the Oxidant

Mn(III) was injected into reaction mixture of Pg instead of permanganate. HNO_3 and Mn(III) concentrations were optimized for 100 μM Pg and 10% (v/v) ethanol. The Mn(III) stock solution was prepared in 50% (v/v) H_2SO_4 and all solutions were diluted with 50% H_2SO_4. Even with a lower acid concentration than used in previous studies (90% (v/v)), the solution is very viscous and it was difficult to keep the injected volume constant.

The optimum acid concentration for Pg was 1% (v/v) and that of Mn(III) was 25 mM. A calibration curve for Pg with Mn(III) is shown in Figure 22. For ethanol, the optimum acid concentration was 100% (v/v) and that of Mn(III) was 25 mM.

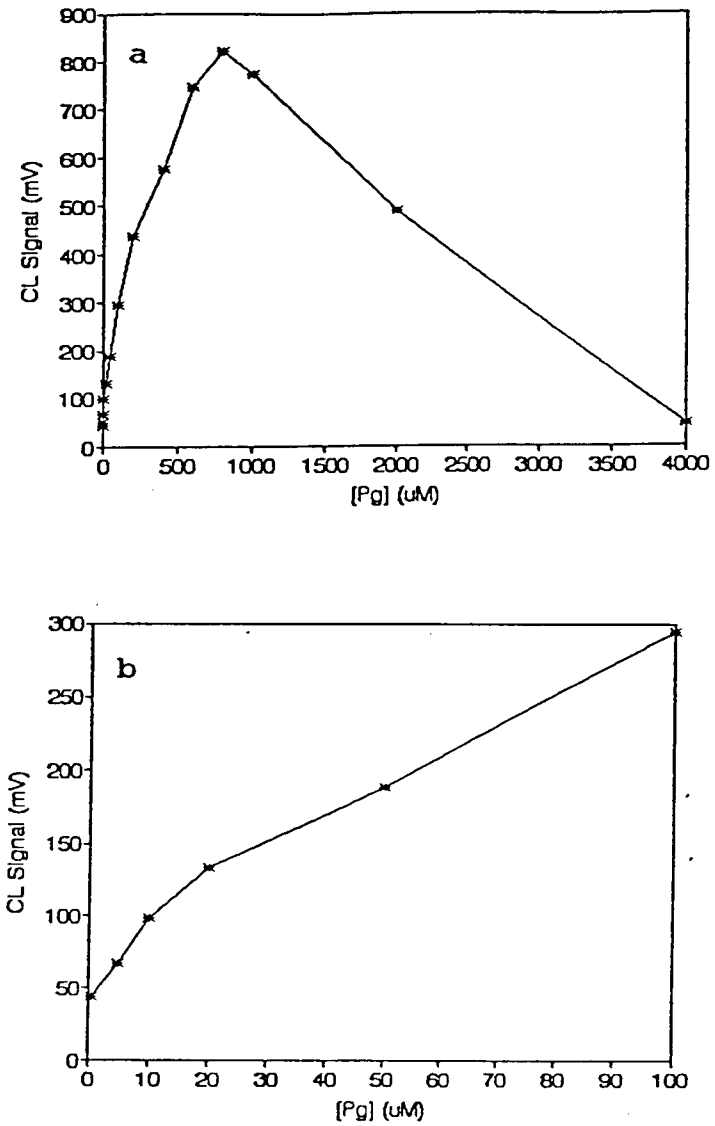


Figure 22. Calibration curve for Pg with Mn(III) as the oxidant.
Reaction mixture: 1 mL of Pg, 0.5 mL of 1% (v/v) HNO_3, 1 mL of water, and 1 mL of 25 mM Mn(III), injected.
a. Full range; b. Lower concentrations.

A calibration curve for ethanol with Mn(III) is shown in Figure 23. The detection limits for ethanol and Pg were calculated to be 0.2% (v/v) and 0.4 μM, respectively.

Comparison of MnO_4^-, Mn(IV) and Mn(III) as Oxidants

Table 13 shows a comparison of $KMnO_4$, Mn(IV) and Mn(III) as oxidants for Pg and ethanol. The concentration for all three forms of the Mn oxidant was adjusted to be the same to allow comparison. The 1 mM oxidant concentration is considerably less than the optimum for Mn(III) and may account for the absence of a CL signal.

Table 13. Comparison of MnO_4^-, Mn(IV) and Mn(III) as Oxidants for Pg and Ethanol

	Pg Signal (mV)[a]		EtOH Signal (mV)		
	MnO_4^-	Mn(IV)	MnO_4^-	Mn(IV)	Mn(III)
	87.2	3.4	148	180	52.8
	78.9	3.7	145	179	43.3
	82.7	3.9	151	174	48.7
	89.0	3.1	152	182	45.1
	88.6	2.9	155	180	50.9
	86.3	3.1	150	184	51.3
Avg	85.5	3.4	150	180	48.7
SD	3.6	0.4	3.3	2.9	3.4

[a] no signal for Pg with Mn(III).
Reaction mixture for Pg: 1 mL of Pg (100 μM), 0.5 mL of acid (10%) and 1 mL of oxidant (1 mM) injected. For ethanol: 1 mL of ethanol (10%), 0.5 mL of acid (100%) and 1 mL of oxidant (15 mM) injected.

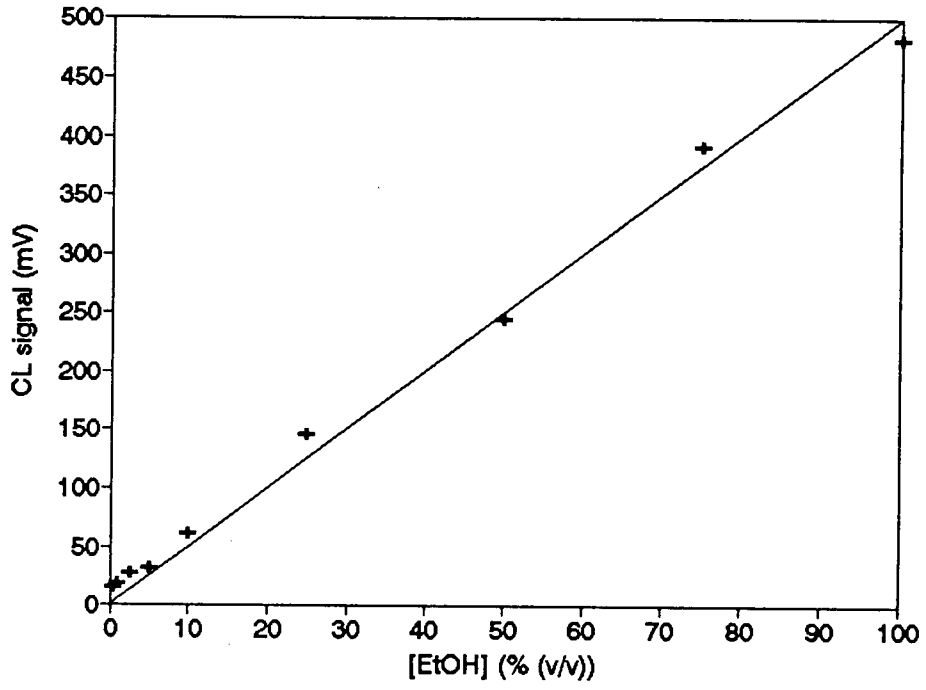


Figure 23. Calibration curve for ethanol with Mn(III) as the oxidant.
Reaction mixture: 1 mL of ethanol, 0.5 mL of 100% (v/v) HNO_3, 1 mL of water, and 1 mL of 25 mM Mn(III) (injected).

Table 14 shows a comparison of the calibration slopes for all three oxidants with Pg and ethanol with the optimum conditions. For ethanol, data in Table 13 are consistent with Mn(IV) as the oxidant which provides the highest signal with Mn(IV). The lower signal with Mn(III) could be due to rapid conversion of some Mn(III) to Mn(IV).

Table 14. Calibration Slopes for Pg and Ethanol with Different Oxidants

	Calibration Slope	
Oxidant	Pg (mV/μM)	Ethanol (mV/%(v/v))
MnO_4^-	12	9.0
Mn(IV)	0.22	46
Mn(III)	2.0	4.9

With optimized conditions, (Table 14), Mn(IV) is more clearly the best oxidant for ethanol. For Pg, MnO_4^- is a more efficient oxidant than either Mn(III) or Mn(IV) when all oxidants are adjusted to the same concentration. Mn(IV) is the poorest oxidant.

Beverages Samples

Initial Studies of a Wine Sample. An undiluted wine sample (#1) was tested for its ethanol signal. The reaction mixture was:

0.25 mL wine sample (undiluted)

0.5 mL HNO_3 (95% (v/v))

1 mL MnO_4^- (10 mM), injected

A very high signal (2.7 V) was obtained and the solution was colorless after the reaction. Here and in the remaining studies, the current gain was decreased if the CL signal exceeded 0.5 V. All reported signals are normalized to gain of 10^7 V/A. Multiple injection of permanganate solution produced multiple signals as shown in Figure 24 and the solution remained colorless after each injection. This behavior suggests species in addition to alcohol are being oxidized. A 100% (v/v) ethanol would typically give a signal of 2 V under these conditions.

In a test tube, 10 mM permanganate solution was added to a mixture of 1 mL wine and 0.5 mL HNO_3 (95%). A brown precipitate started to form after adding 25 mL of permanganate solution. With a 1:20 diluted wine sample (about 0.6% (v/v) or 0.1 M alcohol), the first brown color in the reaction cell appeared after the second injection of 10 mM permanganate solution. The very large ratio of permanganate to alcohol suggests the presence of species other than alcohol are being oxidized by permanganate and produce CL.

The concentrations of acid and permanganate were optimized for a 1:10 diluted wine sample (#1) which yields both a sharp initial peak and a broad second peak. For the second peak which is assumed to be due to ethanol, the optimum acid concentration is 95% and the optimum permanganate concentration is 30 mM. A calibration curve for ethanol with

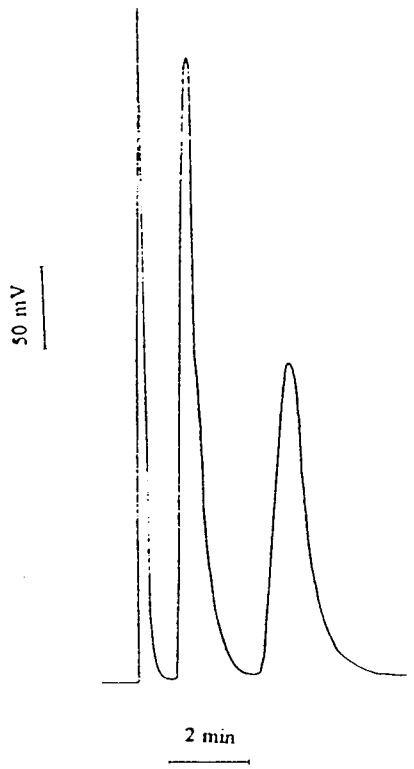


Figure 24. Multiple injections of permanganate solution into wine sample #1.
Reaction mixture: 0.25 mL of undiluted wine (sample # 1), 0.5 mL of 95% (v/v) HNO_3, 1 mL of 10 mM $KMnO_4$ (multiple injected).

these optimum concentrations is shown in Figure 25. It is quite non-linear because of the high concentration of colored permanganate. Figure 26 shows the signal height of different dilutions of wine sample #1. The curve is not linear due to the absorption of some radiation by the colored permanganate. The concentrations of ethanol in the diluted samples were calculated from the ethanol calibration curve (Figure 25) and the measured signals. The results are shown in Table 15.

Table 15. Calculation of Ethanol Concentration in Diluted Wine Sample.

Sample (% (v/v))	Ethanol Conc. in Diluted Sample (% (v/v))	Ethanol Conc. in Original Sample (% (v/v))
1	1.7	170
5	15	300
10	73	730

Ethanol concentrations in those samples of dilutions more than 10% (v/v) were not calculated because they gave signals higher than that of 100% ethanol. The calculated ethanol concentrations in the original samples depend on the dilution used and are much higher than theoretically possible. This could be due to the presence of other species which produce or enhance the CL signal by many fold.

Addition of 1.0 mL of 20 mM NBS to a 1:10 diluted wine sample caused polyphenol signal to decrease from 0.90 to 0.82

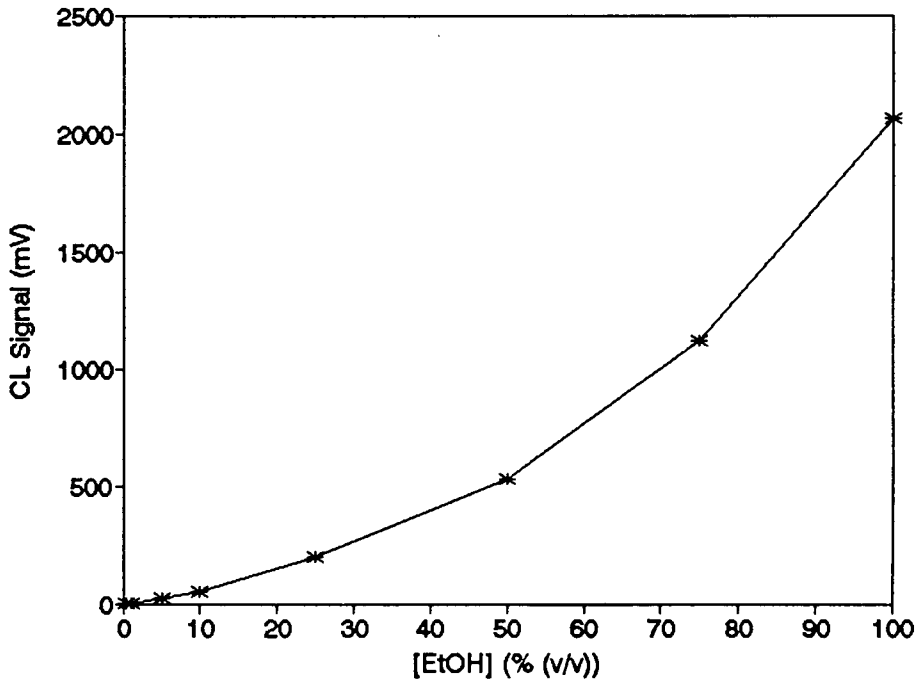


Figure 25. Calibration curve for ethanol with 30 mM permanganate as the oxidant.
Reaction mixture: 1 mL of ethanol, 0.5 mL of 95% (v/v) HNO_3, 1 mL of water, and 1 mL of 30 mM $KMnO_4$ (injected).

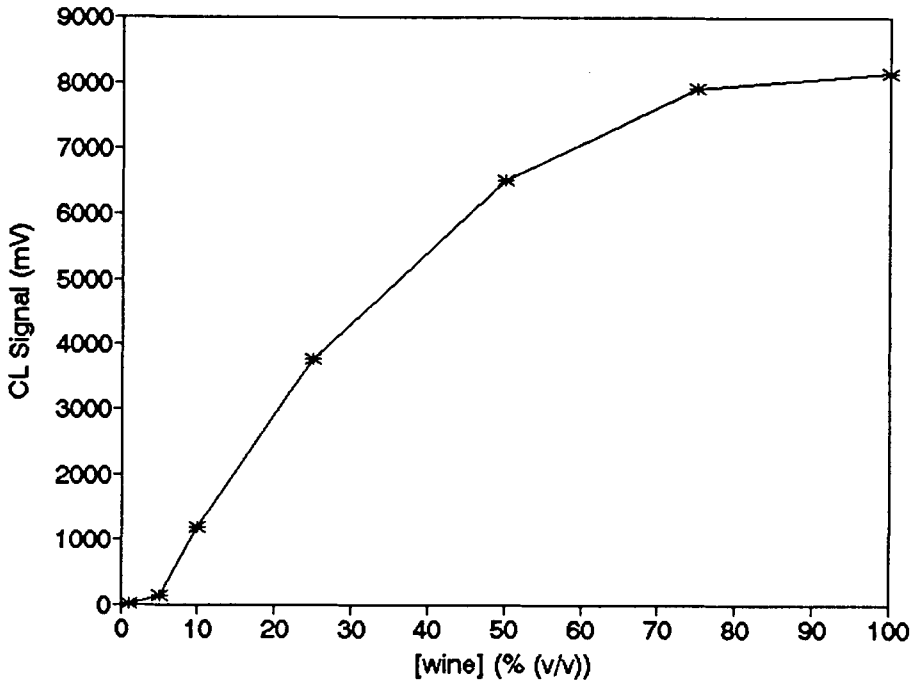


Figure 26. CL signal of different dilutions of wine sample with 30 mM permanganate as the oxidant.
Reaction mixture: 1 mL of wine (sample # 1), 0.5 mL of 95% (v/v) HNO_3, 1 mL of water, and 1 mL of 30 mM $KMnO_4$ (injected).

V and that of ethanol to decrease from 1.25 V to 1.12 V. Without NBS, the solution after the completion of the reaction was colorless which suggests that permanganate was a limiting reagent and completely reduced in the reaction. With NBS, the solution after the reaction completed was yellow.

Solvent Extraction. All attempts to remove Pg from aqueous mixture of Pg and ethanol by solvent extraction failed. Apparently Pg did not separate from ethanol because it is highly polar and soluble in water. The separated aqueous layer contains both Pg and ethanol. With benzene (dipole moment = 0), two separated layers were formed and the signal of both Pg and ethanol did not change from that before extraction. With toluene (dipole moment = 0.36) and trichloromethane (dipole moment = 1.01), the mixture was slightly soluble and the signals of both Pg and ethanol were slightly smaller than those before the extraction procedure.

Similar results were obtained with wine sample #1. A 3.5 mM Phenol solution, when tested by itself, did not show any difference in the signal height after the extraction. Solvent extraction, therefore, appears not selective and useful for the separation of polyphenols from alcohols.

Comparison of Beverages Samples. All five alcoholic beverages were compared and the results obtained are summarized in Table 16.

Table 16. CL Signal of Double Injecting Permanganate Solution into Beverages Samples

	Undiluted Samples		1:10 Dilution
sample	1st injection (V)	2nd injection (V)	CL signal (V)
EtOH (100%)	3.67	1.18	0.0618
#1 (11%)*	25.9	15.7	1.25
#2 (40%)	0.980	0.167	0.0226
#3 (9%)	26.0	18.6	1.667
#4 (12%)	39.4	25.5	0.902
#5 (40%)	3.55	0.470	0.043

* Percentages indicate the concentrations of ethanol (v/v) in samples.
Reaction mixture: 1 mL of sample, 0.5 mL of HNO_3 (95%) and 1 mL of $KMnO_4$ (30 mM), injected.

Very high signals were obtained from three samples and considerably exceeded that of the absolute ethanol. For the undiluted samples, the reaction mixture was colorless after the reaction completed. Permanganate was injected again to oxidize the remaining alcohol. Samples no. 2 and 5 gave a brown precipitate. The other three samples were still colorless even after the second injection.

With the diluted samples, lower signals were obtained and the reaction mixture of samples no. 1, 3, and 4 were yellow after the completion of the reaction. The other two samples gave brown precipitate.

Figure 27 shows the CL signal after injecting permanganate solution into 1 to 10 dilution of different

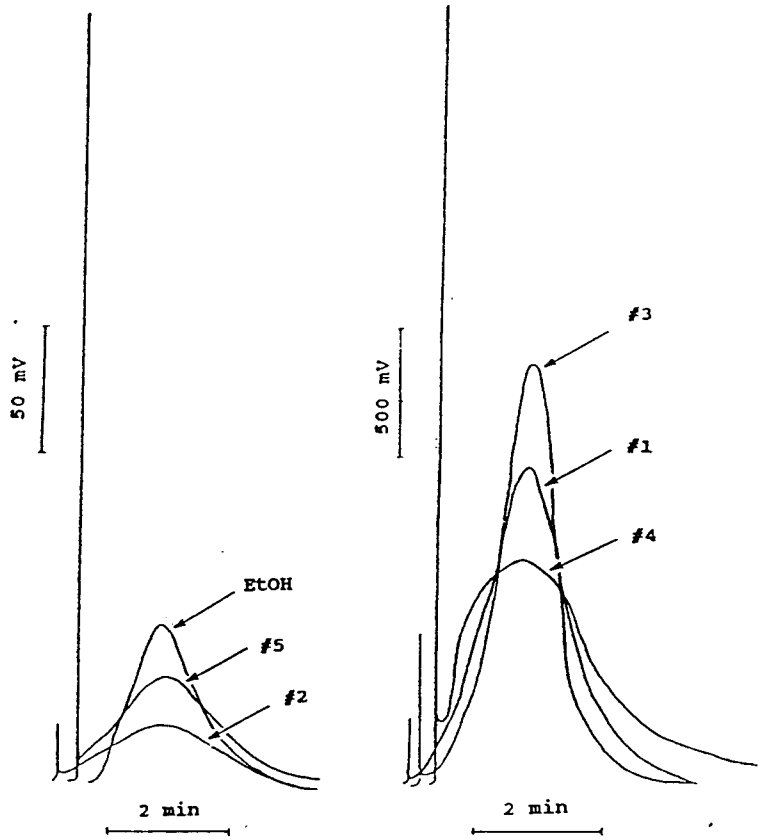


Figure 27. CL signals of different beverage samples and ethanol.
Reaction mixture: 1 mL of 10% (v/v) beverage sample or ethanol, 0.5 mL of 95% (v/v) HNO_3, 1 mL of 30 mM $KMnO_4$ (injected). Numbers refer to the samples as listed in experimental section. The ethanol concentrations in the diluted samples are: #1, 1.1%; #2, 4%; #3, 0.9%; #4, 1.2%; #5, 4% (v/v).

beverages samples. Ethanol concentrations in the samples were calculated from the CL signals of 1:10 dilutions of samples and pure ethanol and compared to the real concentrations. The results are summarized in Table 17.

Table 17. Calculation of Ethanol Concentration in the Beverages Samples

	Ethanol Conc. (% (v/v))	
Sample	Expected	Calculated
#1	11	2020
#2	40	37
#3	9	2700
#4	12	1460
#5	40	70

Samples no. 2 and 5 are closest to the real ethanol concentrations. Sample no. 2 has the best agreement with an error of 7.5%. The other samples resulted in signals of 100 fold or more higher than the theoretical ethanol contents. All data indicate that CL is not produced by ethanol alone, but there are other species which are either oxidized and produce CL or affect the CL of ethanol by sensitizing or some other way.

On the labels of samples no. 1, 3 and 4, it is shown that they contain sulfite which is known to be chemiluminescent species as reported in previous studies.[66,67] Sulfite was tested for its effect on the CL signal of ethanol.

The reaction mixture was:

1 mL SO_3^{2-} (10 mM)

1 mL water or ethanol (10% (v/v))

0.5 mL HNO_3 (95% (v/v))

1 mL MnO_4^- (15 mM), injected

Sulfite by itself gave a signal of 28 mV. When it was added to ethanol, two signals were obtained, a sharp one (530 mV) and a broad one (69 mV) similar in shape to the ethanol signal. Ethanol by itself gave a signal of 133 mV. Sulfite, therefore, is oxidized and consumes some of the permanganate. Sulfite reacts fast and ethanol gives a lower signal because some of the permanganate is consumed.

Solid Phase Extraction

Initial Studies. The sample volume was adjusted to prevent exceeding the recommended amount of the analyte in the column (100 mL of a 2.5 mg/L sample). Throughout this study, 5 mL of 100 μM Pg (or 10% ethanol) was used unless otherwise stated.

First, 10 mL Pg (100 μM) at pH 7.3 was passed through the column. The collected solution was treated again with a clean column. The procedure was repeated using a Pg sample adjusted to a lower pH (3.8) by adding concentrated nitric acid. Also 10 mL of ethanol (10%) and then 10 mL of a Pg/ethanol mixture (final concentration is 50 μM Pg and 5% ethanol) were treated with the same procedure.

The results in Table 18 show that some Pg has been retained in the column. Double treating the sample decreases the signal more and lowering the pH decreases it even more. The pH has a great effect on the separation process. A lower pH produces more protonated Pg which is a neutral species retained more by hydrophobic interactions with the column packing. The ethanol CL signal also decreases with treatment; this is probably due to column dispersion and dilution by the water present in the column before the addition of the sample. The first 3 mL of the eluted sample were discarded to reduce this effect.

Table 18. The Effect of SPE on the CL Signal of Pg and Ethanol

	pH	CL Signal (mV)		
Sample		untreated	1st treatment	2nd treatment
Pg	7.3	163	28.2	13.3
Pg	3.8	156	2.4	0.8
EtOH	7.8	43	35.3	-
Pg (50 μM)*	3.8	146	12.5	-
EtOH (5%)*	7.8	46	32.5	-

* Final concentrations of analytes in mixture.
Reaction mixture for Pg: 1 mL of 100 μM Pg, o.5 mL of 10% (v/v) HNO_3, 1 mL of water, and 1 mL of 300 μM MnO_4^-, injected. Reaction mixture for ethanol and Pg/ethanol mixture: 1 mL of mixture or 10% (v/v) ethanol, 0.5 mL of 100% (v/v) HNO_3, 1 mL of water, and 1 mL of 15 mM MnO_4^-, injected

Different sample volumes of 100 μM Pg were pipetted into the column and collected by vacuum elution. The results

are summarized as follows: a 5-mL sample gave the lowest signal (1.6 mV) indicating a better separation of Pg, a 10-mL sample gave a higher signal (12.5 mV), and a 20-mL sample gave a very high signal (105.9 mV) due to break through.

A 10-mL sample was placed on the column and 2 mL fractions were collected as they were vacuum eluted. The results are summarized in Table 19. At the third fraction, there is a sudden increase in the CL signal of both Pg and ethanol but the ratio of the ethanol to Pg signal is highest. At the fifth fraction, ethanol gave the highest signal, but at the same time Pg CL was very high. A 5-mL sample was used for further studies with discarding the first 3-mL fraction.

Table 19. The Effect of Sample Volume on the CL Signal of Pg and Ethanol

	CL Signal (mV)					
Fraction #	1	2	3	4	5	6
Pg	0.8	3.9	14.1	46.3	63.5	69.2
EtOH	3.3	8.2	59.4	71.5	79.3	81.0
Pg*	12.9	64.3	133	148	145	155
EtOH*	1.2	9.8	51.8	69.8	77.6	77.6

* Analytes in mixture.
Reaction mixture for Pg: 1 mL treated solution, 1 mL water, 0.5 mL HNO_3 (10%), and 1 mL $KMnO_4$ (300 μM), injected.
Reaction mixture for EtOH and Pg/ethanol mixture: 1 mL treated solution, 1 mL water, 0.5 mL HNO_3 (100%), and 1 mL $KMnO_4$ (15 mM), injected.

Application to Beverages Samples. SPE was applied to all five beverages samples to attempt to separate polyphenols from the samples so that ethanol can be detected alone. Exactly 5 mL of undiluted sample was pipetted through SPE column and the first 3 mL were discarded. The CL signals for the collected solutions were obtained and summarized in Table 20.

Table 20. The Effect of SPE on the CL Signal of Ethanol and Beverages Samples

	Polyphenol Signal (V)		EtOH Signal (V)	
Sample	untreated	treated	untreated	treated
#1 (11%)*	5.2	0.90	4.1	2.9
#2 (40%)	1.2	0.063	0.57	0.28
#3 (9%)	11.9	1.3	5.5	3.5
#4 (12%)	18.2	1.1	0	4.1
#5 (40%)	1.7	1.4	1.6	0.28
EtOH (40%)	0.53	0	0.56	0.33

* Ethanol concentration (v/v) in the original samples. Reaction mixture: 1 mL of sample, 1 mL of water, 0.5 mL of 100% HNO_3, and 1 mL of 15 mM $KMnO_4$, injected.

Both the Pg and ethanol signals decreased after treatment. The decrease in the CL signal is more noticeable for the polyphenol signal. The signals for sample no. 2 show the largest change. Polyphenol signals in samples no. 1, 3, and 4 also decreased indicating that some of the polyphenols or other species enhancing the CL signal are retained in the column. The ethanol signal also decreased by treatment except for sample no. 4 which gave a very high polyphenol signal

before treatment and seems to consume most of the permanganate. After treatment, the polyphenol signal was less and a CL signal for ethanol was observed as some permanganate remained after reacting with the polyphenols. Ethanol showed an initial sharp peak before the primary broad peak, probably due to contamination, and it disappeared after treatment. Samples no. 2 and 5 shown a better agreement of ethanol signal to that of pure ethanol.

Diluted ethanol and beverages samples were treated with the SPE column. The concentration of the standard ethanol was chosen to be the same as that in the diluted samples. The solution pH was lowered to about 0.8 by adding 1 mL concentrated HNO_3 to a 10 mL sample. The results are summarized in Table 21. The CL signal of ethanol in sample no. 2 shows a better agreement to that of the pure ethanol with an error of 12%.

Table 21. The Effect of SPE on the CL Signal of Diluted Ethanol and Beverages Samples

	Polyphenol Signal (mV)		EtOH Signal (mV)	
Sample	untreated	treated	untreated	treated
EtOH (4%)	-	-	45.5	25.1
#2 (4%)*	65.9	9.4	58.8	28.2
#5 (4%)*	166	67.1	78.4	36.1

* Ethanol concentration (v/v) in the diluted samples.

In general, both Pg and ethanol signals were reduced by the SPE treatment. Overall, the technique appears not to be useful for the separation of polyphenols from ethanol.

A C-18 column was tested for the separation of Pg from aqueous samples using the same solvents as ENVI-Chrom P. Identical signals for treated and untreated samples were obtained indicating no separation of the polyphenol.

CONCLUSIONS

As a general observation, CL is produced during the oxidation of ethanol and phenols by permanganate, Mn(IV), and Mn(III) in acidic media. This fact can be used as the basis for the determination of ethanol or phenols in aqueous solutions.

When additional oxidants were used to oxidize polyphenols in mixtures of polyphenol/ethanol to allow determination of ethanol without interference, both CL signals were reduced indicating the oxidation of both analytes. None of the additional oxidants was found to selectively oxidize polyphenols. N-Bromosuccinimide (NBS) provided the best selectivity in oxidizing polyphenols. The ethanol signal was slightly enhanced or not affected by the use of NBS, but only if the proper amount of NBS was added which requires prior knowledge of the polyphenol concentration. Also, NBS decreased both the ethanol and polyphenol signals in a wine sample suggesting that too much NBS was added.

Most of the sensitizers used in this study are oxidized by permanganate and produced CL. The signal enhancement from the other sensitizers that are not oxidized is not sufficient to warrant their use as sensitizers.

All the results of using different states of Mn as oxidants are consistent with Mn(IV) being the actual oxidant for ethanol responsible for the CL signal. Pg gave the highest signal and calibration slope with permanganate as the

oxidant; however, the identity of the actual oxidant of Pg is not clear. The addition of Mn(II) to a Pg or ethanol reaction mixture before injecting Mn(IV) provided some idea of the nature of the oxidizing species. Mn(II) decreases the ethanol signal while it increases the Pg signal. This fact supports the conclusion that Mn(IV) is the actual oxidant for ethanol because Mn(II) shifts the equilibrium between Mn(III) and Mn(IV) toward Mn(III) and hence the CL signal decreases. For Pg, Mn(III) is more probably the oxidant since Mn(II) keeps Mn(III) from converting to Mn(IV) and hence the CL signal increases.

The CL signal of ethanol is suppressed when Mn(II) is added before injecting permanganate. Apparently, Mn(II) changes the concentrations and rates of production and disappearance of intermediate oxidation states of Mn. Pyrophosphate and fluoride form complexes with Mn(III), so they stabilize it and reduce the formation of Mn(IV). The ethanol signal is suppressed by these two anions before the injection of permanganate or Mn(III). They enhance the ethanol signal when Mn(IV) is injected.

Mn(IV) can be used instead of permanganate for ethanol determination because of the bigger CL signal it gives, less absorption of radiation because of its color, and lower signal for Pg which lessens the interference effect. Mn(IV) also gives the largest calibration slope and the best detection limit for ethanol.

Among the five beverages samples tested, the ethanol

concentrations estimated were closer for the rum and whiskey samples. The other three samples produced very high CL signals (higher than that of absolute ethanol) which suggests the presence of other species that enhance the signals by sensitizing or being oxidized themselves. Sulfite is known to produce a CL signal on oxidation by permanganate. Since it presents in the last three samples, it could be one species that also affects the CL signal.

As an attempt to separate polyphenols from ethanol, solvent extraction was tested on Pg/ethanol mixture and some alcoholic beverages samples. None of the tested solvents (benzene, toluene, or trichloromethane) was found to be a good solvent for the separation.

Solid phase extraction has some effect on the CL signals. Some polyphenols are adsorbed by passing a sample through a special SPE column (ENVI-Chrom P) as evidenced by a decreased Pg CL signal. For a Pg/ethanol mixture and beverages samples, the ethanol signal also decreased. Therefore, SPE is not considered as a useful technique to remove polyphenols from the beverages samples.

High performance liquid chromatography (HPLC) can be used for the separation of phenols from real samples, followed by CL detection.

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