AN ABSTRACT OF THE THESIS OF

Socorro I. Vasquez de Montalvo for the degree of Doctor of Philosophy in Chemistry presented on September 30, 1986

Title: Analytical and Mechanistic Studies of the Chemiluminescence Oxidation of Hydroxy-Containing Compounds

Abstract approved: 

This thesis is concerned with the development and use of routine analysis techniques based on chemiluminescence (CL) detection for: 1) ethanol in gin down to the 0.3% (v/v) level; 2) the antioxidants propyl gallate and BHA in lard to levels as low as 0.3 and 0.2 mg/L, respectively; 3) pyrogallol in hair dye with a detection limit of 0.7 mg/L; 4) vanillin in vanilla extract to a concentration as low as 0.5 mg/L. All these determinations are based on the oxidation of these hydroxy-containing compounds by permanganate in highly acidic solutions.

Each technique is discussed in terms of optimization of experimental conditions, calibration curve and detection limit data, and application to real samples. In most cases, a solvent extraction separation step is required to reduce interference effects. The results of analyses of real samples are compared to those obtained with accepted analytical techniques.

It is shown that CL is produced during the oxidation of most aliphatic alcohols and phenols by permanganate. The duration of the CL signal is correlated to the rate of oxidation and is generally much less for phenols than for aliphatic alcohols.
The nature of the CL reaction for aliphatic compounds was investigated in some detail. Studies support the hypothesis that Mn(IV) is the species that attacks the alcohol to produce an excited intermediate. The reaction products acetaldehyde and acetic acid were detected and characterized as final products for the ethanol CL reaction by $^1$H NMR, $^{13}$C NMR, and GC-MS.
Analytical and Mechanistic Studies of the Chemiluminescence Oxidation of Hydroxy-Containing Compounds

by

Socorro I. Vasquez de Montalvo

A THESIS

submitted to

Oregon State University

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Completed September 30, 1986

Commencement June 1987
ACKNOWLEDGEMENTS

To Roberto and Jeanne for their patience and love.

To my parents, Fabio and Socorro for their confidence in me and for the best inheritance of all: a college education.

To my host family, the Robinsons, for their love and support.

To Dr. Ingle, for his patience and advice.

To Dr. Krueger, for listening to me in hard times and being so understanding.

To the rest of the famous four study group, Marcia, Claudia and Tim, for their friendship and moral support.

To Cecilia for being a good friend.

To Hector, Liu, Dean, Jeff and Joe for their respect and concern.
"NOTHING IS ETERNAL, MY SUNRISE HAS COME . . .
FEELS LIKE BEING BORN AGAIN"

Herman Hesse
# TABLE OF CONTENTS

## INTRODUCTION

| Introduction | 1 |

## HISTORICAL

| Production of Chemiluminescence | 4 |
| Sensitized Chemiluminescence | 5 |
| Nature of the Emitting Species | 8 |
| Chemiluminescence Instrumentation | 10 |
| Ethanol Determination | 15 |
| Oxidation of Alcohols | 18 |
| Oxidation of Aldehydes and Ketones | 19 |
| Oxidation of Phenols | 24 |
| Antioxidants in Consumer Products | 25 |
| Oxidation of Alcohols | 27 |
| Analysis Techniques | 31 |
| Analytical Determination of Antioxidants | 34 |
| Extraction Techniques | 34 |
| Analytical Techniques | 35 |
| Determination of Vanillin in Vanilla | 35 |
| Summary of Analytical Techniques | 36 |
INSTRUMENTATION

Chemiluminescence Instrumentation

Auxiliary Instrumentation

EXPERIMENTAL

Introduction

Solution Preparation

Methodology for Chemiluminescence Analysis

Preliminary Studies

Coffee

Model compounds

Other samples

Pyrogallol chemiluminescence

Spectral studies

Optimization

Other studies

Extraction and separation of pyrogallol from hair dye

Analysis of hair dye

Spectrophotometric determination

Chemiluminescence determination

BHA and Propyl Gallate Chemiluminescence

Optimization

Extraction and separation of the antioxidants from lard

Initial studies

HPLC extraction procedure

Chemiluminescence extraction procedure

Analysis of lard sample
HPLC analysis
Chemiluminescence determination
Interference study
Vanillin Chemiluminescence
Optimization
Extraction of vanillin from the vanilla extract
Analysis of vanilla extract
Spectrophotometric method
Chemiluminescence analysis
Interference study
Ethanol Chemiluminescence
Optimization
CL and absorption spectra
Reaction product studies
Effect of other species and deaeration on the chemiluminescence intensity
Cations and manganese dioxide
Anions
Methanol and hydrogen peroxide
Other oxidants
Other solvents
Activators
Deaeration
Cyclobutanol study
Ethanol determination in gin
Interference study
RESULTS AND DISCUSSION

Preliminary Studies

Coffee

Model compounds

Other samples

Pyrogallol Chemiluminescence

Initial studies

Absorption spectra

Fluorescence spectra

Optimization

Effect of cations

Other oxidants

Determination of pyrogallol in hair dye

BHA and Propyl Gallate Chemiluminescence

Optimization

Determination of PG and BHA in lard

HPLC analysis

Chemiluminescence analysis

Interference study

Vanillin Chemiluminescence

Optimization

Determination of vanillin in vanilla extract

Interference study

Recommendations

Ethanol Chemiluminescence

Optimization

Absorption spectra
Chemiluminescence spectra

Reaction product studies

Effect of other species and deaeration on the chemiluminescence intensity

  Effect of Mn(II)
  Effect of Fe(II)
  Effect of Mn(III)
  Effect of anions
  Effect of CH₃OH and H₂O₂
  Effect of other oxidants
  Effect of solvent
  Effect of activators

  Deaeration

Cyclobutanol studies

Determination of ethanol in gin

Interference study

CONCLUSIONS

BIBLIOGRAPHY

APPENDIX I

APPENDIX II
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Diagram of the low electronic levels for production of recombination of peroxy radicals and certain acceptors</td>
<td>11</td>
</tr>
<tr>
<td>2. Variation of the fluorescence spectrum of $1 \times 10^{-4}$ M 2-acetonaphthone with pH and Hammett acidity ($H_0$)</td>
<td>16</td>
</tr>
<tr>
<td>3. Mechanism proposed by Banerji and Sengupta for the oxidation of primary alcohols by K$_{2}$MnO$_4$</td>
<td>26</td>
</tr>
<tr>
<td>4. Hydride transfer mechanism</td>
<td>27</td>
</tr>
<tr>
<td>5. Proposed aldehyde oxidation mechanism</td>
<td>29</td>
</tr>
<tr>
<td>6. The major synthetic antioxidants in common food use</td>
<td>32</td>
</tr>
<tr>
<td>7. Some naturally occurring antioxidants</td>
<td>32</td>
</tr>
<tr>
<td>8. Schematic diagram of the discrete sampling CL photometer</td>
<td>38</td>
</tr>
<tr>
<td>9. Configuration of KIM microcomputer system</td>
<td>40</td>
</tr>
<tr>
<td>10. KIM system memory map</td>
<td>41</td>
</tr>
<tr>
<td>11. Diagram of the circuitry and hardware used to control the automatic injector</td>
<td>44</td>
</tr>
<tr>
<td>12. Procedure for extraction of PG in oils and fats</td>
<td>62</td>
</tr>
<tr>
<td>13. Modification of extraction procedure for BHA and PG given by Schwien and Conroy</td>
<td>65</td>
</tr>
<tr>
<td>14. Typical CL peak for coffee</td>
<td>88</td>
</tr>
<tr>
<td>15. Dependence of CL signal for coffee on KOH concentration</td>
<td>89</td>
</tr>
<tr>
<td>16. Dependence of CL intensity for coffee on oxidant concentration</td>
<td>90</td>
</tr>
<tr>
<td>17. Phenols, diphenols, and triphenols in roasted coffee</td>
<td>92</td>
</tr>
<tr>
<td>18. Dependence of CL intensity on pH for some model compounds</td>
<td>94</td>
</tr>
<tr>
<td>19. Dependence of CL intensity of some real samples on pH</td>
<td>104</td>
</tr>
<tr>
<td>20. Dependence of CL signal of coffee on pH</td>
<td>105</td>
</tr>
<tr>
<td>21. Dependence of CL intensity of humic acid on pH</td>
<td>106</td>
</tr>
<tr>
<td>22. Typical peak for pyrogallol (Py)</td>
<td>108</td>
</tr>
</tbody>
</table>
23. Dependence of the CL signal on cut-on wavelength for Py
24. Absorption spectrum for 25 mg/L Py
25. Absorption spectrum for 1% (w/v) nitric acid vs. dw
26. Absorption spectra of Py CL reaction mixture and blank
27. Dependence of CL intensity of Py on nitric acid concentration
28. Dependence of CL intensity of Py on concentration of KMnO₄
29. Unweighted calibration curve and confidence bands for the determination of Py by spectrophotometry
30. Weighted calibration curve and confidence bands for the determination of Py by spectrophotometry
31. Unweighted calibration curve and confidence bands for the determination of Py by CL
32. Weighted calibration curve and confidence bands for the determination of Py by CL
33. Dependence of antioxidant CL signals on acid concentration
34. Dependence of antioxidant CL signals on KMnO₄ concentration
35. Unweighted calibration curve and confidence bands for the determination of BHA by CL
36. Weighted calibration curve and confidence bands for the determination of BHA by CL
37. Unweighted calibration curve and confidence bands for the determination of propyl gallate by CL
38. Weighted calibration curve and confidence bands for the determination of propyl gallate by CL
39. Dependence of CL intensity of vanillin on HNO₃ concentration
40. Dependence of CL intensity of vanillin on KMnO₄ concentration
41. Unweighted calibration curve and confidence bands for the determination of vanillin by spectrophotometry
42. Unweighted calibration curve and confidence bands for the determination of vanillin by CL
43. Weighted calibration curve and confidence bands for the determination of vanillin by CL
44. Effect of type of acid on CL intensity of ethanol
45. Dependence of the CL intensity of 12.5% (v/v) ethanol on the concentration of HNO₃ for different KMnO₄ concentrations
46. Effect of the concentration of KMnO₄ on the shape of calibration curves for ethanol
47. Simplex optimization of reagent concentrations for the determination of ethanol
48. Typical ethanol calibration curve
49. Typical ethanol calibration curve at low concentrations
50. Typical peak shapes for the CL signals obtained from the oxidation reaction of ethanol by KMnO₄ in acidic medium
51. Absorption spectra of reagents
52. Absorption spectra taken during the oxidation reaction of ethanol by KMnO₄ in acidic medium
53. Absorption spectra taken during the oxidation reaction of ethanol by KMnO₄ with different acids
54. Absorption spectra taken during the oxidation reaction of ethanol by KMnO₄ in acidic medium with and without the presence of Mn(II)
55. Absorption spectra taken during the oxidation reaction of ethanol by KMnO₄ with and without Fe(II) added
56. Dependence of the transmitted CL signal for ethanol on the filter cut-on wavelength
57. CL spectra of the oxidation reaction of ethanol by KMnO₄
58. CL spectra of the oxidation reaction of ethanol by KMnO₄ in the presence of Fe(II)
59. ¹³C NMR spectrum of the solid obtained after reaction of stoichiometric amounts of mandelic acid and KMnO₄ in acidic medium
60. ¹H NMR spectrum of reaction products after reaction of an excess of mandelic acid with KMnO₄ in acidic medium
61. GC chromatogram of reaction products recovered from vacuum distillation in the oxidation of isopropanol by KMnO₄ in acidic medium
62. GC chromatogram of the reaction products recovered from vacuum distillation in the oxidation of ethanol by KMnO₄ in acidic medium

63. Effect of Mn(II) on the CL intensity of ethanol

64. CL peak shapes for the reaction of 6% (v/v) ethanol with KMnO₄ in acidic medium with two injections of KMnO₄ in the presence of Fe(II)

65. CL peak shapes for the reaction of 3% (v/v) ethanol with KMnO₄ in acidic medium

66. CL calibration curves for ethanol with and without Fe(II) added

67. Effect of anions on the CL intensity of ethanol

68. Effect of other anions on the CL intensity for ethanol

69. Effect of deaeration on the CL intensity of ethanol

70. Three point calibration curve for the determination of ethanol in gin (Gordon’s)

71. Three point calibration curve for the determination of ethanol in gin (Beefeater and Tanqueray)
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. The Fluorescence and Phosphorescence of Naphthaldehydes</td>
<td>15</td>
</tr>
<tr>
<td>and Acetonaphthones</td>
<td></td>
</tr>
<tr>
<td>II. Dependence of the Reaction Rate on Substrate Concentration</td>
<td>20</td>
</tr>
<tr>
<td>III. Oxidation of Alcohols, RCH₂OH, by Acid Permanganate in Acidic</td>
<td>21</td>
</tr>
<tr>
<td>Solution</td>
<td></td>
</tr>
<tr>
<td>IV. Summary of the Official Analytical Techniques for the Different</td>
<td>36</td>
</tr>
<tr>
<td>Analyses</td>
<td></td>
</tr>
<tr>
<td>V. Pin Connections for 6522 ADC Interface</td>
<td>42</td>
</tr>
<tr>
<td>VI. Major Stock Solutions</td>
<td>48</td>
</tr>
<tr>
<td>VII. Dependence of Unreacted Ethanol on the Reagent Concentration</td>
<td>78</td>
</tr>
<tr>
<td>VIII. Study of Effect of Fe(II) Concentration</td>
<td>81</td>
</tr>
<tr>
<td>IX. Dilution of Gin Samples</td>
<td>85</td>
</tr>
<tr>
<td>X. Effective Concentration of Ethanol Before and After Standard</td>
<td>85</td>
</tr>
<tr>
<td>Addition</td>
<td></td>
</tr>
<tr>
<td>XI. CL Signals for Model Compounds</td>
<td>95</td>
</tr>
<tr>
<td>XII. Relative Oxidation Potentials</td>
<td>102</td>
</tr>
<tr>
<td>XIII. Movement of Seven-Dimensional Simplex for Pyrogallol (Py)</td>
<td>115</td>
</tr>
<tr>
<td>XIV. Effect of Cations on the CL Signal for the Reaction of Py</td>
<td>119</td>
</tr>
<tr>
<td>XV. Spectrophotometric Determination of Py</td>
<td>122</td>
</tr>
<tr>
<td>XVI. CL Determination of Py</td>
<td>123</td>
</tr>
<tr>
<td>XVII. Determination of PG in Peanut Oil</td>
<td>131</td>
</tr>
<tr>
<td>XVIII. Determination of PG and BHA in Lard by HPLC</td>
<td>132</td>
</tr>
<tr>
<td>XIX. Determination of BHA in Lard by CL</td>
<td>134</td>
</tr>
<tr>
<td>XX. Determination of PG in Lard by CL</td>
<td>139</td>
</tr>
<tr>
<td>XXI. Interference Study for PG</td>
<td>140</td>
</tr>
</tbody>
</table>
XXII. Spectrophotometric Determination of Vanillin in Vanilla Extract  

XXIII. Chemiluminescence Determination of Vanillin in Vanilla Extract  

XXIV. Interference Study for Vanillic Acid  

XXV. Effect of Different Mineral Acids on CL Intensity  

XXVI. Dependence of Molar Ratio of Ethanol on the Ethanol Concentration  

XXVII. Effect of Fe(II) and Mn(II) on the CL Intensity of Ethanol  

XXVIII. Effect of Mn(II), Mn(III) and Glacial Acetic Acid on the CL Intensity of Ethanol  

XXIX. Effect of the Solvent on the CL Intensity for Ethanol  

XXX. Effect of DBA on the CL Intensity of Ethanol and Isopropanol  

XXXI. Effect of Diacetyl on the CL Intensity for Ethanol and PG Reactions  

XXXII. Results in the Determination of Ethanol in Gin  

XXXIII. Determination of Ethanol in Gin  

XXXIV. Effect of Glucose on the CL Determination of Alcohol
Chemiluminescence (CL) may be defined as the process occurring when an exothermic chemical reaction produces a species in an electronically excited state which emits light as it returns to the ground state. To observe significant CL, as for photoluminescence techniques, the pathway of radiative emission must be a favorable process compared to the usual thermal dissipation processes which increase the translational and vibrational energy of a system.

Chemiluminescence systems may be divided into two classes based on the origin of the emitting molecule. In the first class, the emitter is an intermediate or product produced in the chemical reaction (direct CL). In the second class, there is energy transfer between an electronically excited product or intermediate molecule and a second substance (the acceptor) which then becomes the emitter (sensitized CL). Much of the attention for CL is being directed toward the development of more efficient systems and to mechanistic studies concerning the luminescence process. CL offers considerable potential for evaluating the energy distribution among reactants and products in chemical reactions, for probing the kinetics of a chemical process, and for determining a variety of analytes.

Investigation of CL systems has been difficult due to the often complex, multi-step chemical reactions involved and the variety of parameters which influence light emission. CL determinations often
lack specificity because other compounds present in the matrix also chemiluminesce or affect the primary CL reaction. However, analytical methods based on CL offer many advantages. For example, the instrumentation required is simple so there is no need for highly qualified personnel. Also, spectral interferences are often minimal, since all the radiation arises from an intermediate or the product of the reaction. Moreover, calibration curves are sometimes linear over several orders of magnitude, and the time for analysis is short.

This research is concerned with three major topics:

1) Development and optimization of a new analytical technique based on the CL produced during the oxidation of organic compounds in solution with MnO$_4^-$.

2) Mechanistic studies of the CL reaction which mainly consisted of establishment of possible reaction pathways in terms of intermediates and reaction products.

3) Application of the developed CL technique in the determination of:

   a) EtOH in gin
   b) the antioxidants propyl gallate and BHA in lard
   c) pyrogallol (Py) in hair dye
   d) vanillin in vanilla extract

Most mechanistic studies were concerned with the CL oxidation of ethanol. To study the reaction kinetics, the rates of formation or decay of species involved in the CL reaction were monitored with CL and absorption techniques. The CL intensity-time profile indicates the portion of the overall reaction directly related to the light emission process. The light produced in these reactions was characterized in
terms of spectral distribution and total emission by means of a multiple signal spectrometer developed in our research group. The CL spectrum gives some insight into the nature of the emitting species. The products of the reaction were characterized by $^1$H NMR, $^{13}$C NMR and GC-MS. The effect of addition of such species as Mn(II) and Fe(II) to the CL reaction mixture provides evidence about the particular oxidation state of manganese responsible for the oxidation leading to CL.

For polyphenols, characterization of final compounds formed in the CL reaction was difficult because the products from oxidation of polyphenols are mostly complicated mixtures of dimeric, polymeric, and quinonoid compounds. Also, with analytes such as propyl gallate, BHA, pyrogallol (Py), and vanillin, most of the reaction end products are colored, causing spectral interferences with the CL system. For polyphenolic compounds the reaction time is on the order of milliseconds, whereas for the reaction time for the CL oxidation of alcohols is in the order of minutes.

Most of the CL determinations developed required the use of solvent extraction methods to separate the analyte from interferences in the sample matrix. A critical comparison of the analysis results obtained by CL to the results found by reference methods is also included.
Chemiluminescence (CL) has been a fascinating phenomenon to man since antiquity. The early scientific literature reflects this interest. In those times, chemiluminescence observations were concerned for the most part with luminescing living organisms found among plants and animals. Representative examples of these organisms include fireflies, marine fireworms, and a number of unicellular organisms (dinoflagellates) which are often the cause of the beautiful "phosphorescence" and "red tides" found in the seas.

By the end of the eighteenth century, light emission had been reported in the air oxidation of phosphorus, the neutralization of alkaline oxides with concentrated acids, and the reaction of peroxides with iodine and chlorine compounds. In the nineteenth century, CL was observed during thermal decomposition reactions and oxidation of a number of aldehydes, alcohols, sugars, amines, amides, and other nitrogen compounds with oxygen, ozone, or peroxides in alkaline media.

Two reports from 1887 later generated interest. First was Radziszewski's discovery that greenish light is emitted during the reaction of lophine (2,4,5-triphenylimidazole) with oxygen in the presence of strong base. Second was Eder's accidental observation of chemiluminescence from alkaline pyrogallol when it was being employed as a developer for photographic plates.

Research in chemiluminescence is now carried out in many laboratories throughout the world. The high level of interest in
Chemiluminescence is reflected by several recent conferences or symposia devoted to this subject.5

Production of Chemiluminescence

Direct chemiluminescence may be represented by the following reaction sequence:

\[ L + X + C \rightarrow I^* + \ldots \rightarrow I + h\nu \rightarrow P + P' \]  

(1)

where \( L \) is the initial form of the luminescing species, \( X \) represents necessary reagents, \( C \) denotes species which may accelerate or retard the rate of reaction, \( I^* \) is the luminescing species, \( I \) is the same species in its unexcited ground electronic state, \( P \) is the final product of \( I \), and \( P' \) denotes other products formed from \( L \) by non-luminescent reaction pathways. In some CL reactions, the luminescing species is a final product of the reaction (e.g., \( I^* = P^* \)).

The number of excited molecules per \( \text{cm}^3 \) produced per unit time (\( dn_{I^*}/dt \)) is proportional to the reaction rate (\( -dn_L/dt \)). The instantaneous CL intensity, the number of photons emitted per unit time (\( \Phi_{CL} \)), is proportional to (\( dn_{I^*}/dt \)). Thus the CL intensity is proportional to the reaction rate.6

In most chemiluminescence reactions, the reactants are rapidly mixed and a peak-shaped CL signal is observed. The decay in the CL intensity occurs because the rate of formation of \( I^* \) decreases as the reactants are consumed. The CL quantum efficiency (\( \phi_{CL} \)) equals the total number of photons emitted during the course of the reaction divided by the number of molecules, of the initial form of CL species (\( L \)), that react. It is the product of two different quantum
efficiencies:

\[ \phi_{CL} = \phi_{ES} \times \phi_{L} \]  

(2)

where \( \phi_{ES} \) is the excited state production quantum efficiency and \( \phi_{L} \) is the luminescence quantum efficiency.

For most reactions, \( \phi_{CL} \) is very low because either \( \phi_{ES} \) or \( \phi_{L} \) is small. The excitation efficiency is often low because the chemical reaction does not provide sufficient free energy (\( \Delta G \)) to produce excited species or the reaction pathway that produces an excited product or intermediate is not favorable. In general, the free energy must be large enough that

\[ -\Delta G(\text{kcal/mol}) \geq \frac{hc}{\lambda(\text{excit.})} = 28600(\text{kcal/mol})/\lambda(\text{excit.}) \]  

(3)

where \( \lambda \) has units of nanometers. For visible CL emission, \( -\Delta G \) must be at least between 40 and 70 kcal/mol.²

The luminescence quantum efficiency is often very low since there are other routes of deactivation that compete with photon emission (quenching, energy transfer, intramolecular radiationless deactivation). These physical processes can be described as follows:

direct CL;

\[ I^* \xrightarrow{k_L} I + h\nu_p \]  

(4)

internal conversion of \( I^* \);

\[ I^* \xrightarrow{k_{ic}} I + \text{heat} \]  

(5)

deactivation of \( I^* \) by quenchers \( Q \);

\[ I^* + Q \xrightarrow{k_q} I + Q^* \]  

(6)

transfer of excitation energy to an acceptor molecule \( A \);
\[
I^* + A \xrightarrow{k_A} I + A^*
\]

(7)

acceptor luminescence (sensitized CL);

\[
A^* \xrightarrow{k_{L'}} A + h\nu
\]

(8)

non-radiative deactivation of \( A^* \);

\[
A^* \xrightarrow{k_{ic'}} A + \text{heat}
\]

(9)

\[
A^* + Q \xrightarrow{k_{q'}} A + Q
\]

(10)

where:

\( k_L \) = rate constant for luminescence emission from radiative transition, \( s^{-1} \)

\( k_{ic} \) = rate constant for intramolecular radiationless transition (internal conversion), \( s^{-1} \)

\( k_q \) = rate constant of quenching of \( I^* \) by other compounds, \( s^{-1} \)

\( k_A \) = rate constant for transfer of excitation energy to an acceptor

\( k_{L'} \) = rate constant for luminescence emission by \( A^* \), \( s^{-1} \)

\( k_{ic'} \) = rate constant for internal conversion of \( A^* \), \( s^{-1} \)

\( k_{q'} \) = rate constant of quenching of \( A^* \) by other compounds, \( s^{-1} \).

Note that an acceptor is just a specific type of quencher which can be excited and emit a photon. Activators and quenchers may be reactants, intermediates, or products involved in the reaction or other species in the sample.

The overall instantaneous intensity of CL is given by\(^6\)

\[
\Phi_{CL} = (k_L n_{I^*} + k_{L'} n_{A^*}) \cdot V = \{\phi_L (1 - \phi_{LA}) + \phi_A \cdot \phi_{LA}\} \phi_{ES} \cdot (-dn_L/dt) \cdot V
\]

(11)

where
\[ \phi_L = \text{luminescence quantum yield} \]
\[ \phi_L = \text{luminescence quantum yield of the excited product or intermediate} \]
\[ \phi_{LA} = \text{energy transfer yield between the donor and the acceptor (dependent on acceptor concentration)} \]
\[ \phi_A = \text{luminescence quantum yield of the excited activator} \]
\[ \phi_{ES} = \text{excitation yield} \]
\[ -(dn_L/dt) = \text{reaction rate, molecules/cm}^3\text{-s} \]
\[ n_i = \text{number density (molecules/cm}^3\text{) of species} \]
\[ V = \text{volume of solution from which the CL is observed, cm}^3 \]

The CL intensity changes with time not only because the reaction rate decreases but also due to changes in \( \phi_L, \phi_{LA}, \phi_A, \) and \( \phi_{ES} \) during the course of the reaction. These yields can vary as the concentrations of reactants, intermediates and products change during the reaction.

When energy transfer to an acceptor is unfavorable \( (\phi_{LA} = 0) \), equation 11 reduces
\[ \Phi_{CL} = \phi_L \cdot \phi_{ES} \cdot (-dn_L/dt) \cdot V = \phi_{CL} \cdot (-dn_L/dt) \cdot V \]

(12)

In this case, the CL spectrum corresponds to that of species I* (or P*).

The luminescence quantum yield \( (\phi_L) \) and the lifetime \( (\tau_L) \) can be measured independently. Their ratio should be equal to the rate constant for radiative deactivation of the excited product or intermediate \( (k_L) \):
\[ k_L = \phi_L / \tau_L \]

(13)

Sensitized Chemiluminescence

The phenomenon of sensitized CL is useful when \( \phi_L \) is too low for direct CL measurements. Addition of compounds capable of accepting
energy (acceptor A) induces energy transfer processes according to equation 7. Emission of light by the activator may take place after energy transfer as indicated equation 8. When sensitized CL is much more favorable than direct CL, equation 11 reduces to

\[ \Phi_{CL} = \Phi_A^{\phi_{LA}} (-dn_L/dt)V \]  

(14)

Naturally, the spectrum, quantum yield and lifetime are characteristic of the acceptor molecule, rather than that of the primary excited species. If the CL spectrum and the fluorescence spectrum of the acceptor coincide, the emission transition arises from the first excited singlet state. The enhancement coefficient for CL with an acceptor (K) increases with the acceptor concentration according to 6

\[ K = I/I(n_A=0) \]  

(15)

\[ 1/(K-1) = \phi_L/\phi_A - \phi_L + [1/(\phi_A - \phi_L)](k_L/k_A)(1/n_A) \]  

(16)

CL reactions occur mostly by oxidation. This probably is due to the fact that oxidation reactions can be quite exothermic and the oxidation products (ketones, aldehydes, \( \text{O}_2 \)) have low energy levels that may become efficiently populated (i.e., the yield \( \Phi_{ES} \) is relatively high) 6 and emit in the visible region.

Since the probability of excitation is higher for a lower excited level, and the triplet levels of organic molecules, in particular ketones, always lie lower than singlets, it would be expected than mainly triplets become populated. 6 Energy transfer from a triplet donor to a triplet acceptor can occur readily. However, this would scarcely enhance CL, as the probability of triplet-singlet radiative deactivation in the acceptor molecule is usually low. 1 Therefore efficient sensitized CL would be expected to occur when energy transfer
from the excited triplet of the donor to an excited singlet of an acceptor is possible. However, the rate constant of the triplet-singlet energy transfer is often very low due to spin restrictions.

The energy transfer rate constant appears to be high only in the case of strong spin-orbit coupling in the acceptor molecule. For example, 9,10-dibromoanthracene (DBA) can be an efficient acceptor for sensitized CL. This compound is activated mainly by triplet-singlet energy transfer. These concepts are illustrated in Figure 1 which is a diagram of electronic levels of different species. Under certain conditions, oxidation of hydrocarbons and derivatives occurs by a radical chain mechanism and produces peroxy radicals. These can combine to produce oxygen and excited ketones such as acetophenone, cyclohexanone and biacetyl. These excited species become the donors in the presence of acceptors such as naphthalene, dibromoanthracene, and rubrene. Cyclohexanone and acetophenone levels are approximately the same. Anthracenes increase the CL of cyclohexanone and acetophenone, due to an efficient triplet-singlet energy transfer, and further radiative deactivation of the acceptor. However, anthracenes quench biacetyl due to triplet-triplet energy transfer and to the low probability of triplet-singlet deactivation. Rubrene enhances CL in all cases since its excited singlet state lies lower than all triplets.

Nature of the Emitting Species

In order for a compound to chemiluminesce, two basic requirements must be met. First, a reaction must produce sufficient energy to push a compound to an excited electronic state. Second, the excited
Figure 1. Diagram of the low electronic levels for products of recombination of peroxy radicals and certain acceptors (activators and quenchers).
compound must be fluorescent or phosphorescent, or must be able to transfer its energy to a compound that is. It is with the second of these requirements that we are concerned here.

According to Worthy, one of the termination products of organic reactions involving oxidation is an electronically excited ketone. Phosphorescence and/or fluorescence of carbonyl containing compounds have been extensively studied. Acetone phosphorescence can be observed quite readily in fluid solution of ordinary organic solvents, either by chemi-excitation or photo-excitation techniques.

The intensity of direct chemiluminescence (acetone fluorescence and phosphorescence) for the thermal decomposition of tetramethyl-1,2-dioxetane into two molecules of acetone as shown below:

\[
\begin{align*}
\text{CH}_3 - & \text{C} - \text{C} - \text{CH}_3 & \Delta & \rightarrow \text{CH}_3 - & \text{C} - \text{CH}_3 + \text{CH}_3 - & \text{C} - \text{CH}_3 \\
\text{CH}_3 & & & & \text{CH}_3 & \\
\end{align*}
\]

is given by

\[
\phi_{CL} = (\phi^S \phi_F + \phi^T \phi_p) k_1 [S]
\]  

where:

\(\phi^S\) = excitation efficiency for formation of excited singlet ketone

\(\phi^T\) = excitation efficiency for formation of excited triplet ketone

\(\phi_F\) = fluorescence yield of acetone
\[ \phi_p = \text{phosphorescence yield of acetone} \]
\[ k_1 = \text{rate constant for disappearance of substrate (s}^{-1}) \]
\[ [S] = \text{concentration of substrate in moles/L} \]
\[ \phi_{CL} = \text{CL intensity in einsteins/L s} \]

In the presence of air, the phosphorescence of reaction is not observed due to quenching of the triplet state by oxygen \( (\phi_{SF} > \phi_{T0p}) \).

In molecular systems, the first excited singlet state can be unimolecularly deactivated as shown below, by fluorescence emission (A), \( S \rightarrow T \) intersystem crossing (B), \( S_1 \rightarrow S_0 \) internal conversion (C), and chemical decomposition (D): \( ^{19} \)

\[
\begin{align*}
S_1^* & \rightarrow T_1^* \quad \text{(B)} \\
& \rightarrow S_0^* + \text{heat} \quad \text{(C)} \\
& \rightarrow \text{products} \quad \text{(D)} \\
& \rightarrow S_0 + \nu \quad \text{(A)}
\end{align*}
\]

As for simple ketones in the gas phase, the nonradiative processes predominate over the radiative process by at least two orders of magnitude (i.e., process (A) accounts for less than 1% of the overall deactivation processes for the excited singlet ketones). It is well accepted that the quantum yield of the \( S_1 \rightarrow T_1 \) intersystem crossing process is \( \sim 1.0 \) for acetone. \( ^{18} \) For acetaldehyde, the fluorescence spectrum of the singlet \( n \rightarrow \pi^* \) transition has been recorded. \( ^9 \) The wavelength of maximum emission is 420 nm. Excitation wavelengths used are 325 and 337 nm.

Aromatic aldehydes and ketones are better known for their phosphorescence than for their fluorescence. This is usually attributed to low lying \( n, \pi^* \) states associated with the carbonyl group which enhance
the rate constant for singlet-triplet intersystem crossing. Some aromatic aldehydes and ketones with medium sized aromatic systems and large polycyclic aromatic hydrocarbons have been found to fluoresce in hydroxylic, highly polar solvents (but not in hydrocarbon solvents) at room temperature.\textsuperscript{16,17}

Benzaldehyde and acetophenone do not fluoresce even in water. However, intense fluorescence from the conjugate acids is observed in concentrated acid media. Moreover, in acidic media, phosphorescence from the cations derived from these compounds is obtained. Phosphorescence of benzaldehyde and acetophenone have been shown to originate from $3\pi^*, \pi^*$ states, because protonation raises the energies of $n,\pi^*$ states.\textsuperscript{17}

Naphthaldehydes and acetonaphthones do not fluoresce in hexane, but they do in water. In concentrated acid solutions these compounds are protonated in the ground electronic state, at the carbonyl oxygen atoms. It has been proved\textsuperscript{20} that these compounds are considerably stronger bases in the lowest excited singlet states than in the ground state.\textsuperscript{16} This is of considerable analytical importance because if prototropic equilibrium is established within the lifetimes of the lowest excited singlet states of these compounds, the most desirable pH or Hammett acidity conditions in which to carry out chemiluminescence methods has to be determined prior to analyses.

Table I shows the relationship between fluorescence and phosphorescence spectra of naphthaldehydes and acetonaphthones and the explanation of the phenomenon. Variation of the fluorescence spectrum of acetonaphthone with pH is shown in Figure 2.
Table I. The Fluorescence and Phosphorescence of Naphthaldehydes and Acetonaphthones

<table>
<thead>
<tr>
<th>Phenomenon</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral species do not fluoresce in hexane but produce intense fluorescence in water.</td>
<td>Lowest excited singlet states of these compounds in hexane of the $n,\pi^<em>$ type which favors radiationless population of the triplet state. In water, the energy of the lowest $n,\pi^</em>$ state is raised by hydrogen bonding in the ground state and the energy of the lowest $\pi,\pi^<em>$ state is lowered by dipole-dipole interaction in the excited state so that fluorescing $\pi,\pi^</em>$ state is lowest in water.</td>
</tr>
<tr>
<td>Neutral species phosphoresce in aqueous solutions, cations do not exhibit phosphorescence.</td>
<td>Protonation of these compounds raises the energy of the low lying $n,\pi^<em>$ singlet states, which favors phosphorescence in the neutral molecules, making the lowest excited singlet states of the cations $\pi,\pi^</em>$ states.</td>
</tr>
</tbody>
</table>

The first fluorescence band (A,B,C,D) corresponds to the fluorescence of the neutral species. As the pH decreases, a red-shifted band is observed due to the formation of the protonated acetonaphthone.

Chemiluminescence Instrumentation

Analytical chemiluminescence techniques are based on detection of the light emitted during a CL reaction. Because of the low light levels involved, the photomultiplier tube (PMT) is the most used detector. Image intensifiers can also be used in conjunction with photographic film or plates to increase the detectability for low light levels. Normally the light emitted at all wavelengths is measured (i.e., no wavelength selection).
Figure 2. Variation of the fluorescence spectrum of $1 \times 10^{-4}$ M 2-acetonaphthone with pH and Hammett acidity ($H_0$).

(A) pH=4.9  (B) pH=3.0  (C) pH=2.0  (D) pH=0.9  (E) $H_0=0.7$

Solution CL spectra are difficult to obtain, first because the CL intensity is relatively low, and second because the typical duration of the CL signal is on the order of seconds which is not compatible with conventional scanning monochromators. Spectra are important to obtain
for the elucidation of the mechanisms involved and identification of the luminescing species.

Various designs of CL instruments have been proposed. They can be roughly classified into two types, discrete sampling systems and flow systems, based on the way the sample and reagents are mixed. In the discrete sampling instruments (used in this work), the sample and all reagents except one are mixed in the reaction cell. The cell is placed as close as possible to the detector (usually a PMT) to maximize the CL signal collection efficiency. A lid is placed over the reaction cell, and the final reagent is injected into the cell, the reaction mixture is mixed, and the CL signal versus time is recorded continuously.

In continuous flow CL methods, the sample and reagent solutions are continually pumped through tubing, combined and mixed, and sent to a flow cell. The main advantage of this system is that a steady signal is produced. A scanning monochromator can easily be used to obtain a CL spectrum. Reagent consumption with this technique is much greater compared with the discrete sampling approach. The CL signal observed is due to the emission of photons that occurs during the residence time of the reaction mixture in the sample cell. Thus only part of the CL versus time profile may be observed and information about reaction kinetics is not obtained unless flow rates are varied. For fast CL reactions, it is possible for the CL signal to decay significantly between the point of mixing and observation.

Recently, intensified diode arrays (IDA) have been used for the acquisition of fast CL spectra. A multiple signal spectrometer has been developed which is capable of measuring molecular chemiluminescence, fluorescence and absorption signals and spectra simultaneously.
over the 300-800 nm range. The injection system, data acquisition, and data handling are completely automated.

Ethanol Determination

Determination of ethanol in liquors is of importance. The regulations given by the Department of the Treasury (Bureau of Alcohol, Tobacco and Firearms) specify that in order to determine the concentration of alcohol, hydrometers have to be used. This procedure of analysis is also given by the Association of Official Analytical Chemists (AOAC). These hydrometers are furnished to Internal Revenue officers and are graduated to read the proof of aqueous alcoholic solutions.

Proof is defined as the ethyl alcohol content of a liquid at 60 degrees Fahrenheit (15.5 °C) stated as twice the percent of ethyl alcohol by volume. Because of temperature-density relationships and the selection of 60 °F for reporting proof, corrections are necessary for hydrometer readings at temperatures other than 60 °F.

Ethanol in other types of samples has been determined with many different methods. These include:

a) Amperometry. An oxygen electrode in a flow cell was used to monitor the oxygen concentration by the aerobic oxidation of EtOH, catalyzed by alcohol oxidase \( \text{C}_2\text{H}_5\text{OH} + \text{O}_2 \rightarrow \text{CH}_3\text{CHO} + \text{H}_2\text{O}_2 \). This enzyme was immobilized by covalent attachment via glutaraldehyde to the inside walls of nylon tubing. The enzyme columns are inserted into a flow system. The effluent is fed into a flow cell containing the oxygen electrode which amperometrically monitors the decrease in oxygen
concentration. This method was used in the determination of alcohol in blood.

b) Automated densitometer.\textsuperscript{32} The EtOH content of spiritous beverages was determined by a fully automated U tube densitometer. The microprocessor, an Intel 8080 CPU, controls the switch for the pump, probe operation, turntable rotation and transfers the density value to a printer after an equilibrium delay time.

c) Gas chromatography.\textsuperscript{33,34} A mixture containing methanol, ethanol, water, propanol, butanol, pentanol, hexanol and heptanol was separated on a 3 mm x 2 m GDX-303 column at 195 °C with a H\textsubscript{2} flow rate of 63.5 mL/min by a refractometer detector. Methanol, EtOH and isopropanol have also been determined in human blood by a flame-ionization detector using isopropanol as internal standard, Chromosorb 101 as the column packing, N\textsubscript{2} as the carrier gas and a column temperature of 130 °C. The determination range was 0.02-0.56% (v/v).\textsuperscript{35}

d) Titration.\textsuperscript{36} The titrimetric determination of EtOH is based on the oxidation of alcohol by potassium dichromate in sulfuric acid and subsequent determination of excess potassium dichromate by titration with I\textsubscript{2}. The liberated I\textsubscript{2} is then titrated with Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3}.

Oxidation of Alcohols

\textbf{Introduction}

The chemiluminescence technique developed in this thesis research is based on oxidation of ethanol by potassium permanganate in nitric acid. Although the CL from this reaction has not previously been
reported, the oxidation of ethanol by permanganate has been studied, and some relevant information is summarized below.

The oxidation of ethanol by acid permanganate is a two-electron process, which eventually gives acetic acid.\(^{37}\) According to Banerji\(^{38}\) the reaction stoichiometry is as follows:

\[
4\text{MnO}_4^- + 12\text{H}^+ + 5\text{CH}_3\text{CH}_2\text{OH} \rightarrow 5\text{CH}_3\text{COOH} + 4\text{Mn}^{2+} + 11\text{H}_2\text{O} \quad (19)
\]

With a large excess of the alcohol relative to permanganate, the reaction follows a pseudo-first order kinetics. The rate of disappearance of permanganate under these conditions is first order with respect to permanganate.\(^{38}\) The order with respect to the alcohol is also one as shown in Table II in which \(k\) is the rate constant determined from the initial rate.\(^{39}\) The rate increases linearly with acidity. This linear dependence might be attributed to the protonation of permanganate anion to give permanganic acid, a more powerful oxidant.

Table II. Dependence of the Reaction Rate on Substrate Concentration\(^a\)

<table>
<thead>
<tr>
<th>[EtOH] (\times 10^2) (M)</th>
<th>(k_1 \times 10^4) (s(^{-1}))</th>
<th>(k_1 \times 10^2/\text{[EtOH]})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>9.55</td>
<td>9.55</td>
</tr>
<tr>
<td>2.0</td>
<td>19.0</td>
<td>9.50</td>
</tr>
<tr>
<td>4.0</td>
<td>37.5</td>
<td>9.40</td>
</tr>
<tr>
<td>6.0</td>
<td>57.0</td>
<td>9.50</td>
</tr>
<tr>
<td>8.0</td>
<td>76.8</td>
<td>9.60</td>
</tr>
</tbody>
</table>

\(^a\) \([\text{KMnO}_4], 1 \times 10^{-4} \text{ M}; \text{[H}^+\], 1.0 \text{ M}; \text{[NaF]}, 0.01 \text{ M}; \text{Temp.}, 25 \, ^\circ\text{C}\)

The oxidation of ethanol by acid permanganate under nitrogen fails to induce polymerization of acrylonitrile. The formation of free
radicals is, therefore, unlikely.

Table III shows the third order rate constant for the acid permanganate oxidation of ten primary aliphatic alcohols at different temperatures. From this table, three trends can be observed. First, for a given alcohol, the reaction rate increases with temperature as expected. Second, the rate increases with chain length and branching. Third, electronegative substituents decrease considerably the ease of oxidation. No systematic study on the effect of structure in the permanganate oxidation of alcohols has been reported. However, it might be speculated that the inductive effect may be responsible for this effect of substituents on the reaction rate.

Table III. Oxidation of Alcohols, RCH₂OH, by Permanganate in Acidic Solution.

<table>
<thead>
<tr>
<th>R</th>
<th>25°C</th>
<th>30°C</th>
<th>35°C</th>
<th>40°C</th>
<th>45°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₃</td>
<td>955</td>
<td>1300</td>
<td>1740</td>
<td>2300</td>
<td>3100</td>
</tr>
<tr>
<td>C₂H₅</td>
<td>1410</td>
<td>1910</td>
<td>2500</td>
<td>3300</td>
<td>4360</td>
</tr>
<tr>
<td>n-C₃H₇</td>
<td>1730</td>
<td>2300</td>
<td>3000</td>
<td>4000</td>
<td>5100</td>
</tr>
<tr>
<td>i-C₃H₇</td>
<td>2340</td>
<td>3100</td>
<td>4000</td>
<td>5100</td>
<td>6500</td>
</tr>
<tr>
<td>t-C₄H₉</td>
<td>3850</td>
<td>4920</td>
<td>6200</td>
<td>7800</td>
<td>9800</td>
</tr>
<tr>
<td>C₆H₁₁</td>
<td>1820</td>
<td>2400</td>
<td>3150</td>
<td>4070</td>
<td>2550</td>
</tr>
<tr>
<td>(cyclohexyl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>87.0</td>
<td>128</td>
<td>182</td>
<td>257</td>
<td>370</td>
</tr>
<tr>
<td>CH₃OCH₂</td>
<td>72.5</td>
<td>110</td>
<td>157</td>
<td>240</td>
<td>330</td>
</tr>
<tr>
<td>ClCH₂</td>
<td>6.9</td>
<td>10.7</td>
<td>16.6</td>
<td>25.0</td>
<td>38.0</td>
</tr>
<tr>
<td>BrCH₂</td>
<td>8.5</td>
<td>13.2</td>
<td>20.0</td>
<td>28.8</td>
<td>43.0</td>
</tr>
</tbody>
</table>
The oxidation of alcohols by acid permanganate is autocatalytic and probably involves the Mn(III)/Mn(IV) couple. Addition of sodium fluoride suppresses the autocatalysis by decreasing the concentration of aquo complexes of Mn$^{3+}$ or Mn$^{2+}$, although it does not affect the initial rate.

For many years, oxidation of primary alcohols by oxidants has been studied. These studies have led to the postulation of the following mechanisms:

1) Oxidation by tri- and tetravalent states of manganese in mineral acid solutions.$^{37,38,40-49}$

2) A direct hydride transfer to the oxidant.$^{50,51}$

The first mechanism has been extensively studied by many researchers.$^{37,38,40-48}$ According to Tompkins$^{41}$ the oxidation of organic compounds involves a preliminary reduction of MnO$_4^-$ by Mn$^{2+}$ ions to form the Mn$^{4+}$ ion, which in the absence of reducing agents, hydrolyzes in weak acid solutions to manganese dioxide. It has been confirmed that some Mn$^{2+}$ is always present in freshly prepared permanganate solutions.

At room temperature, dilute aqueous solutions of alcohols (if aldehyde-free) are not immediately attacked by acid permanganate.$^{40}$ Hence the oxidation of primary and secondary alcohols must be brought about by transient manganese ions of higher valency state than Mn$^{2+}$ and of lower valency state than Mn$^{7+}$. Intermediate oxidation states of Mn can be produced by the following two slow irreversible reactions.

\[
2\text{MnO}_4^- + 3\text{Mn}^{2+} + 16\text{H}^+ \longrightarrow 5\text{Mn}^{4+} + 8\text{H}_2\text{O} \quad (20)
\]

\[
\text{MnO}_4^- + 4\text{Mn}^{2+} + 8\text{H}^+ \longrightarrow 5\text{Mn}^{3+} + 4\text{H}_2\text{O} \quad (21)
\]
The Mn(III) can be observed as it produces a cherry-red color in solution.

From potentiometric measurements, Grube and Huberich calculated for the redox equilibrium

\[ 2\text{Mn(III)} \rightleftharpoons \text{Mn(II)} + \text{Mn(IV)} \]  

an equilibrium constant \( K = \frac{[\text{Mn}^{2+}][\text{Mn}^{4+}]}{[\text{Mn}^{3+}]^2} \) of about \( 10^{-2.2} \) in 7.5 M H\(_2\)SO\(_4\).\(^{45}\)

The equilibrium constant of the following reaction

\[ 2\text{Mn}^{3+} + 2\text{H}_2\text{O} \rightleftharpoons \text{Mn}^{2+} + \text{MnO}_2(\text{s}) + 4\text{H}^+ \]  

has been estimated to be about \( 10^9 \) M\(^3\).\(^{45}\) The Mn\(^{3+}\) ion has therefore a strong tendency to disproportionate into the +2 and +4 states in neutral aqueous solutions. The rate of disproportionation is reduced by increasing the H\(^+\) concentration; this rate becomes unappreciable in solution when [H\(^+\)] is 3 M or greater.\(^{45}\) The stability of Mn(IV) should also increase in aqueous medium of high acidity according to equation below.

\[ \text{Mn}^{4+} + 2\text{H}_2\text{O} \rightleftharpoons \text{MnO}_2 + 4\text{H}^+ \]  

(24)

The Mn\(^{3+}\) ion forms strong complexes with some anions.\(^{49}\) When Mn(III) is stabilized by complex formation (indicated by a cherry red color), the rate of substrate oxidation by Mn(III) should be reduced and some delay in the precipitation of manganese dioxide during oxidation should be expected.

Experimentally it has been found that high concentrations of Mn\(^{2+}\) retard the oxidation of alcohols. From equation (22), increasing the concentration of Mn\(^{2+}\) reduces the equilibrium concentration of Mn(IV) and hence the rate of oxidation by this cation. However, if the concentration of Mn(II) is very small, this
should increase the initial rate of oxidation and reduce the induction period (i.e., the time from mixing to reach the maximum rate). According to Kemp and Waters,\textsuperscript{44} however, only a small concentration of Mn(II) such as 0.04 M should effectively eliminate any oxidation of organic substrates by Mn(IV) in solutions of Mn(III). The same authors also determined that Mn(II) retards oxidations involving Mn(III).

**Effect of other species**

If a good reducing agent such as hydrogen peroxide or ferrous ion is present during the oxidation, the induction period is greatly reduced and alcohols are oxidized immediately.\textsuperscript{40} This again suggests that the Mn(VII) does not directly attack the alcohols, but it has to be reduced first to Mn(IV) or Mn(III). Only in the presence of Fe(II) or other good reducing agents, the manganese end product of permanganate oxidation in acid solution is Mn(II). Otherwise most organic compounds reduce permanganate to manganese dioxide even in acidic solution. If both Mn(II) and Fe(II) are added together, the extent of oxidation due to Mn(IV) should diminish since addition of Mn(II) displaces the equilibrium in favor of Mn(III) and this would react rapidly with Fe(II).\textsuperscript{40}

Often, anions are added to the reaction mainly to complex and to reduce the activity of Mn(III). Fluoride is added to permanganate oxidation reaction mixtures to suppress the precipitation of manganese dioxide or the oxidation of transient Mn(III) or Mn(IV) species. The aqueous MnF\textsuperscript{2+} species should still be a powerful oxidant. However, the concentration of Mn(IV) in equilibrium with it must be greatly
reduced, unless Mn(IV) fluoride complexes are formed extensively.\textsuperscript{48}

Pyrophosphate ions retard oxidations mainly by complexing Mn(III) almost completely and preventing the formation of Mn(IV) in equilibrium with it.\textsuperscript{40} Perchlorate ions form unstable complexes with Mn(III). Hence HClO\textsubscript{4} is extensively used in different studies. Because acetate ions form Mn(III) complexes (in glacial acetic acid) which are polymeric and invariably contain Mn(II), they are unsuitable for kinetic studies.\textsuperscript{48} If the oxidation of the substrate is enhanced by replacing H\textsubscript{2}O with acetic acid, it means the reaction does not depend on a solvolysis step because the reduction of the nucleophilicity of the solvent would have retarded the reaction.

Mechanisms

According to Merz, Stafford and Waters,\textsuperscript{40} the mechanism of oxidation of primary and secondary alcohol includes the following steps:

1) Coordination of the Mn(IV) ion with the unshared electron pair of an oxygen atom on the alcohol (and one electron transfer):

\begin{equation}
R_2 - C - O + Mn^{4+} \xrightarrow{rds} R_2 - C - \overset{\text{o}}{\text{O}} - Mn^{3+}
\end{equation}

2) Loss of proton from the oxonium cation to give an unstable mono alkyl derivative of manganic hydroxide:

\begin{equation}
R_2 - C - \overset{\text{o}}{\text{O}} - Mn^{3+} \xrightarrow{\text{fast}} R_2 - C - \overset{\text{\text{o}}}{\text{\text{O}}} - Mn^{3+} + H^+
\end{equation}
3) Transfer of the two covalent electrons of the manganic complex to the manganese nucleus, and simultaneous release of proton from the C-H bond of the carbinol group:

$$R_2 - C \cdot Ho \cdot Mn^{3+} \rightarrow R_2C = \cdot Ho + H^+ + :Mn^{2+}$$

(27)

However, Banerji and Sengupta\textsuperscript{38} proposed Mn(III) as the attacking species. The mechanism is shown in Figure 3.

$$R - OH + Mn(III) \xleftrightarrow{\text{fast}} Mn - O (\text{complex})$$

$$Mn - O \xrightarrow{\text{rds}} RO^\cdot + Mn(II) + H^+$$

$R = CH_3$ or $C_2H_5$

$$CH_3O^\cdot + Mn(III) \xrightarrow{\text{fast}} CH_2O + Mn(II) + H^+$$

$$C_2H_5O^\cdot + Mn(III) \xrightarrow{\text{fast}} CH_3CHO + Mn(II) + H^+$$

The reacting species are formed as follows:

$$Mn(II) + MnO_4^- \leftrightarrow Mn(III) + MnO_4^{2-}$$

$$Mn(VI) + Mn(II) \xrightarrow{\text{fast}} 2 Mn(IV)$$

$$Mn(IV) + Mn(II) \xrightarrow{\text{fast}} 2 Mn(III)$$

Figure 3. Mechanism proposed by Banerji and Sengupta for the oxidation of primary alcohols by KMnO$_4$
The second type of mechanism involves a direct attack of the alcohol by Mn(VII), suggests that the C-H bond is ruptured in the rate-determining step, and includes a hydride transfer to the oxidant. Figure 4 shows such a mechanism, proposed by Littler and others.39,50,52

\[
\begin{align*}
\text{O} - \text{Mn}^{\text{VII}} & \rightarrow \text{O} + \text{H} \quad \text{CR}_2 \quad \text{O} - \text{H} \\
\text{O} - \text{Mn}^{\text{V}} & \rightarrow \text{OH} + \text{R}_2\text{C} = \text{O}^+ - \text{H} \\
\text{R}_2\text{C} & = \text{O}^+ - \text{H} \rightarrow \text{R}_2\text{C} = \text{O} + \text{H}^+ \\
\text{Mn}^{\text{V}} & \rightarrow \text{Mn}^{\text{II}} + \text{Mn}^{\text{VII}}
\end{align*}
\]

Figure 4. Hydride transfer mechanism.

Oxidations of alcohols by Mn(III) and H MnO\textsubscript{4} are considered one-electron processes in which a complex is formed containing one molecule of alcohol and one molecule of Mn(III) or -OMnO\textsubscript{3} species.50,51

**Strained alcohols**

When cyclobutanol is oxidized by MnO\textsubscript{4}\textsuperscript{-} in the presence of Mn(II), neither precipitate formation of MnO\textsubscript{2} nor the transient appearance of
the cherry red color of manganese (III) is clearly observed in contrast to oxidation of non-strained alcohols. Furthermore, the oxidation rate in the present of Mn(II) increases rather than decreases.\(^5\)\(^3\) This suggests that Mn(III) is very reactive toward cyclobutanol and is primarily responsible for the observed oxidation cleavage reaction. Strain-free alcohols react only slowly with Mn(III) in acidic medium.\(^5\)\(^3\)

Oxidation of Aldehydes and Ketones

A mechanism involving complex formation is suggested by Wiberg and Stewart for the oxidation of aldehydes and ketones in acid solution.\(^4\)\(^8\) The principal observations concerning the acid catalyzed reactions are:

1) The rate of reaction is proportional to the first powers of the permanganate and the substrate concentrations.

2) The rate-controlling step involves the cleavage of the aldehyde C-H bond.

3) The oxygen introduced into the aldehyde is derived mainly from the oxidizing agent, indicating that a bond is formed at some time between the aldehyde carbon and an oxygen of the oxidizing agent. A mechanism based on these observations is shown in Figure 5.\(^5\)\(^4\)

Oxidation of Phenols

Phenol in aqueous solution is completely and rapidly oxidized by potassium permanganate while 2,4,6-trinitrophenol is very
RCHO + H₃O⁺ ⇌ RCHOH + H₂O

RCHOH + MnO₄⁻ ⇌ R - C - O MnO₃

OH

R - C - H → B → R - CO₂H + HB + MnO₃⁻

O - MnO₃

3MnO₃⁻ + H₂O fast → 2MnO₂ + MnO₄⁻ + 2OH⁻

B = base

Figure 5. Proposed aldehyde oxidation mechanism.

stable.³⁷ This observation led to the conclusion that electron withdrawing groups in aromatic rings of phenol tend to stabilize the phenol to permanganate oxidation.

In 1919, Hinshelwood suggested that the oxidation of dinitrophenol and related compounds is brought about by bimolecular collisions between the organic molecule and the MnO₄⁻ ion.⁴⁹,⁵⁵ However, Alexander and Tompkins⁴⁹ proved that the oxidation proceeds through the action of the intermediate oxidation states (e.g., Mn (IV)) and that direct oxidation by the MnO₄⁻ is negligible.⁵⁶ Mononitrophenols and hydroxybenzoic acids are also instantaneously oxidized by KMnO₄ in acid.⁵⁵

The instability of phenol in the presence of MnO₄⁻ suggests
that the reactivity is due to the production of the tautomeric keto-form

\[
\text{O}^-
\]

Substitution might be expected to influence tautomerization and hence alter the reactivity. Phenol oxidation is very important as such and in the biosynthesis of natural products. The first step in the oxidation of phenol is the formation of a phenolate radical.\(^43\) It is considered that these radicals give stable molecular products by coupling in pairs.

Pyrocatechols (1,2-dihydroxybenzenes) are oxidized to o-quinones, but the products are much less stable than those of the para isomers. The mechanism is shown below:

In aqueous solution hydroxylated compounds or polymers are produced. Resorcinols do not form diradicals since they cannot be stabilized because meta quinones are impossible.

Hydroquinones are easily and rapidly oxidized to quinones, by the same path as pyrocatechols. Polymeric and "humic acid like" products
are expected to be formed afterwards. Pyrogallols are oxidized to ortho quinones which in time dimerize to purpurogallin (1) as shown below.43

![Diagram showing the oxidation of pyrogallols to purpurogallin](image)

Antioxidants in Consumer Products

Food antioxidants are additives and as such are part of the ingredient listing on the package. The combined effects of consumer activist groups and storms of controversy regarding tests of carcinogenic compounds have produced a very wary consumer and "the natural food syndrome". Because of education about ingredient listings, food additives are not welcome these days by naturalists. However, he or she may drive 70 miles an hour, puff at a cigarette, gobble up fat, sugar, alcohol and calories, but he or she will walk a mile to pay twice as much for a label that says "all natural".19

Antioxidants can be classified as natural (Figure 6) or synthetic (Figure 7). We will be interested in the second kind, synthetic antioxidants.
Figure 6. The major synthetic antioxidants in common food use.

Figure 7. Some naturally occurring antioxidants.

The regulations for food additives are specified in the Food, Drug and Cosmetics Act of 1938. The Act was amended in 1958 with the
Delaney Clause which is concerned with the induction of cancer in animal species and in man.

The limit of food additives is 0.02% by weight of the fat or oil content of the food. Combinations of additives, in general, may only add up to 0.02%. The main acid synergists, such as citric acid, ascorbic acid, phosphoric acid, are substances without restriction since they are natural human metabolites and vitamins. Tocopherols are unregulated for similar reasons.

Antioxidants are added to oils and fats to help prevent autooxidation which causes flavor deterioration and formation of carcinogens. Oxygen radicals are often responsible for this deterioration. Hence, compounds which are capable of trapping the oxygen can reduce considerably the rate at which the fat or oil becomes rancid.

One antioxidant, BHA, has been criticized as being potentially dangerous, even though this antioxidant has been extensively tested for cancer risk. In fact, the antioxidant can block the action of some carcinogens.

Another antioxidant, pyrogallol, has been used extensively as an antioxidant preservative in photography and in various dye products for furs and hair. However, this compounds is very poisonous. Ingestion may cause severe gastro-intestinal irritation, convulsions and even death. Poisoning and death have occurred from percutaneous absorption.

The determination of pyrogallol in consumer products is of extreme importance. The amount of pyrogallol added to hair dye is considered proprietary by many cosmetic companies.
Pyrogallol is considered by law to be a color additive. The general provision stating that a cosmetic is adulterated if it is, bears or contains an unsafe color additive does not apply to hair dyes because there is a separate provision for coal-tar hair dyes. This reflects an apparent inconsistency in the statute, since neither the general color additive provision nor the separate provision for coal-tar hair dyes is applicable to non-coal-tar hair dyes such as pyrogallol. It has been held that Congress did not intend to deprive the FDA of the authority to regulate non-coal-tar colors in hair dyes. However, pyrogallol is not subject to certification for the protection of the public health and is exempt from the certification requirements of section 706(c) of the Act. Because there are no regulations for non-coal-tar dyes such as pyrogallol, the amount of Py in commercial products is not specified. This means that there is not a value with which to compare the results obtained in this present work.

Analytical Determination of Antioxidants

**Extraction techniques**

Most analytical techniques include a cleanup procedure in which antioxidants are extracted from the complex sample. Satisfactory extraction of antioxidants from foods is not simple, particularly when antioxidants are present at low levels. Problems are usually associated with incomplete extraction of the antioxidants or with the co-extraction of potentially interfering substances.

The most useful solvents for extracting food antioxidants from fat
are acetonitrile and 70-95% water-alcohol mixtures. The fat is usually dissolved in hexane or petroleum ether and the antioxidant is extracted into the polar solvent.

**Analytical techniques**

A variety of analytical techniques have been developed over the years to determine antioxidants which include:

a) GC-MS. This combination has proved to be a most powerful tool for the structural analysis of compounds.

b) Reversed phase chromatography mostly utilizing UV detection at 280 nm. This technique has the advantage over GC-MS of being non-destructive and affording the isolation of relative labile oxidation products for further structural studies and research.

c) Size exclusion methods.

d) GC using flame ionization detection.

e) Colorimetric methods.

**Determination of Vanillin in Vanilla**

Vanillin occurs naturally in vanilla, potato parings, siam benzoin, etc. It is extensively used as a flavoring agent in confectionery, beverages, foods, galenicals (remedies prepared from herbs and other vegetable drugs, according to an official formula), and perfumery. Therefore, determination of vanillin is of some importance. Vanillin can be analyzed by a variety of analytical techniques including:
1) Reverse phase HPLC mostly using UV detection at 275 nm\textsuperscript{62-65}

2) GC-MS\textsuperscript{66,67}

Summary of Analytical Techniques

Table IV summarizes the methods recommended by the Association of Official Analytical Chemists for the species determined by CL in this work.

Table IV. Summary of the Official Analytical Techniques for the Different Analyses.

<table>
<thead>
<tr>
<th>ANALYSIS</th>
<th>OFFICIAL TECHNIQUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol in hard liquor</td>
<td>Precision hydrometers\textsuperscript{29}</td>
</tr>
<tr>
<td>Vanillin in vanilla extract</td>
<td>Ultraviolet Spectrophotometry\textsuperscript{28}</td>
</tr>
<tr>
<td>Antioxidants in food:</td>
<td></td>
</tr>
<tr>
<td>a) Propyl Gallate in lard</td>
<td>Gradient Liquid Chromatography (UV detection)\textsuperscript{28}</td>
</tr>
<tr>
<td>b) Butylated hydroxyanisole (BHA) in lard</td>
<td></td>
</tr>
<tr>
<td>Pyrogallol in hair dye</td>
<td>Spectrophotometry\textsuperscript{28}</td>
</tr>
</tbody>
</table>
Chemiluminescence Instrumentation

A schematic diagram of the CL photometer used for the investigation is shown in Figure 8. A detailed description can be found elsewhere.68-70 Briefly, the discrete sampling CL photometer consists of 1 cm pathlength spectrophotometer sample cell housed in an aluminum sample chamber. A brass temperature controlled jacket around the sample cell is connected to a thermostated water bath. The contents of the sample cell are mixed with a magnetic stirrer and a stirbar. The final reagent is delivered to the cell by means of a pneumatic syringe to initiate the reaction. A portion of the light emitted during the reaction is impinging on the PMT-shutter assembly. The resulting photoanodic current is converted to a voltage, electronically filtered, and displayed on a recorder.

A microprocessor control and data acquisition system based on a KIM single board microcomputer was incorporated into the system by Marino.70 The computer system is configured to trigger the injector and to digitize and store the CL signal versus time data. The duration of data collection and the number of data points collected are controlled by the user. Dark signal data are acquired before injection for an equivalent time period. The net peak height and peak area are calculated and reported. With repetitive runs, the mean and standard deviation are also reported. During the course of the research, the KIM was irreparably damaged by an unexplained water spill. The microcomputer system was replaced by another KIM microcomputer system.
Figure 8. Schematic diagram of the discrete sampling CL photometer. I/V - current to voltage converter.
built by Dewald and configured as shown in Figure 9.

Details about the microprocessor system, opto-isolated TTY interface, KIM peripherals, 8/12 bit ADC, and software can be found in other publications. The new memory map is shown in Figure 10. The I/O board contains two 6522 I/O chips giving 32 I/O lines, 8 control lines and four interrupt timers. Each 6522 is assigned 1 K of memory space. Only one I/O chip was used. It was addressed at starting location 8000 by configuring the first six switches (starting from the left, component side up) of the 8-pin dip switches on the board to 100000 (1 indicates closed and 0 indicates open).

For automatic injection of the last reagent, a low going pulse is sent to the triggering circuitry of the pneumatic syringe through port A in the 6530 I/O chip on the KIM. The 6522 I/O chip on expansion board is used to interface to the ADC. The I/O lines from the 6530 and 6522 were connected to 25-pin female amphenol D sockets mounted to the microcomputer box. Table V summarizes the connections between the amphenol connector and the ADC. For the 6530, pin 13 of the other amphenol connector is connected to PA0.

A copy of the machine and BASIC programs used is found in appendix I. The machine program used basically corresponds to the one developed by Marino, however since the location of the 6522 I/O ports was changed from memory location 1600 to memory location 8000, these had to be changed in the machine program.

For the BASIC program, major changes were introduced. The maximum acquisition time allowed in the old version is 30 s. For the CL reaction of aliphatic alcohols the maximum CL signal occurs over 30 s after injection. Therefore the maximum acquisition time was increased
Figure 9. Configuration of KIM microcomputer system.
### Figure 10. KIM system (KMS) memory map.

See Figure 10 in ref. 58 for details on locations 0 to 2000.
Table V. Pin Connections for 6522 ADC Interface.

<table>
<thead>
<tr>
<th>6522</th>
<th>ADC&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Color Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PA0</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>PA1</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>PA2</td>
<td>23</td>
</tr>
<tr>
<td>4</td>
<td>PA3</td>
<td>22</td>
</tr>
<tr>
<td>5</td>
<td>PA4</td>
<td>21</td>
</tr>
<tr>
<td>6</td>
<td>PA5</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>PA6</td>
<td>--</td>
</tr>
<tr>
<td>8</td>
<td>PA7</td>
<td>18</td>
</tr>
<tr>
<td>9,10</td>
<td>N.C.</td>
<td>--</td>
</tr>
<tr>
<td>11,12</td>
<td>N.C.</td>
<td>--</td>
</tr>
<tr>
<td>13</td>
<td>GND</td>
<td>GND</td>
</tr>
<tr>
<td>14</td>
<td>PB0</td>
<td>13</td>
</tr>
<tr>
<td>15</td>
<td>PB1</td>
<td>12</td>
</tr>
<tr>
<td>16</td>
<td>PB2</td>
<td>11</td>
</tr>
<tr>
<td>17</td>
<td>PB3</td>
<td>10</td>
</tr>
<tr>
<td>18</td>
<td>PB4</td>
<td>9</td>
</tr>
<tr>
<td>19</td>
<td>PB5</td>
<td>8</td>
</tr>
<tr>
<td>20</td>
<td>PB6</td>
<td>7</td>
</tr>
<tr>
<td>21</td>
<td>PB7</td>
<td>6</td>
</tr>
<tr>
<td>22,23</td>
<td>N.C.</td>
<td>--</td>
</tr>
<tr>
<td>24,25</td>
<td>N.C.</td>
<td>--</td>
</tr>
</tbody>
</table>

N.C. = no connection.

<sup>a</sup> Numbers correspond to amphenol connector pin no.

to 6 minutes. The time (t) between data points is determined by the numbers loaded into the register of timer 2 of the 6522 and in the x register of the 6502. From the user-selected number of data points (N) and the total measurement time (T), BASIC calculates the number (y) to
load into the timer 2 register. The timer outputs a pulse every time it counts down from y to 0. Every pulse decrements the number in the x register (x) by one. When the x register reaches 0, a start conversion pulse is sent to the ADC.

In the original program, x was 16 (HEX 10). The maximum value of y is 32,768 ms. Thus the maximum time between data points (t_m) was 16 x 0.0328 s = 0.524 s. Note that t_m x N must be greater than T. For t_m = 0.0328 s, and T = 30 s, the minimum value of N is 58.

The value in the x register (location 0102) was changed to 255 (HEX FF) to increase the maximum time between data points to 255 x 0.03285 = 8.36 s. The minimum number of data points that can be taken was changed to 75 since 75 x 8.36 s = 627 s > 600 s.

The circuitry for triggering the syringe was modified as shown in Figure 11. Basically, a 2-input NOR gate was added to the circuit to protect the system against stray signals that triggered the injector at unwanted times. One of the gate inputs is grounded while the other is connected to the trigger line which is normally in a high state (+5 V). Before injection the output of the NOR is logic 0. A low-going pulse from the computer causes the output of the NOR gate to go momentarily high. The high-going pulse triggers the 555 timer to activate the injector. With this modification, the problem of stray signals was solved.

Auxiliary Instrumentation

All pH measurements were made with a Chemtrix type 60A pH meter and a combination pH electrode. Spectrophotometric data were obtained
Figure 11. Diagram of the circuitry and hardware used to control the automatic injector. The dashed line shows the connections prior to modification with the NOR gate.
using either a Varian Cary model 118C double beam spectrophotometer or a HP 8451A diode array spectrophotometer. Fluorescence spectra were acquired on a Varian model SF-330 spectrofluorometer.

HPLC data were obtained with a chromatograph equipped with a SP 8700 solvent delivery system, a Linear strip chart recorder, a 20 μL Rheodyne sample loop injection valve, and a spectroflow monitor SF 770 with a GM 770 monochromator from Schoeffel Instruments Corp. to measure A at 280 nm. The column is a 10 cm length stainless steel and 4.6 mm i.d. column packed with octadecyl silica (C-18) bonded phase on 5 μm beads from Brownlee laboratories.

NMR spectra were measured with a Varian FT-80A spectrometer. The GC-MS was done on a Finnigan 9610 gas chromatograph interfaced with a data system. The column for GC was a Porapak Q 60-200/10. For the MS a Finnigan Model 4023 with a 45,000 ion source was used. The electro-energy was 70 eV and ion source temperature was maintained at 190 °C.

Melting point was obtained from a Buchi instrument.
Introduction

This section deals with the experimental procedures developed and used during the course of the present work. It includes, first, a description of the solution preparation procedures; second, a summary of the general procedure and instrumental conditions utilized for CL analysis; and third, several sub-sections which give a detailed accounting of the different steps involved in the study of particular chemical systems or samples. These latter sections include information about a) optimization and calibration methods for the techniques used, b) sample acquisition, c) sample preparation (extraction), d) determination of the respective analytes in samples, e) mechanistic studies, and f) interference studies.

Successful scientific research relies heavily on careful and detailed observations. Observations lead to scientific progress, development of more sophisticated instruments, a better understanding of scientific phenomena, and new analytical techniques. This research started from initial observations of key phenomena and from previous results obtained in our research group.

A previous study in our laboratory revealed that natural products or beverages containing natural products, such as coffee, tea, alcoholic beverages and pop, chemiluminesce when oxidized by potassium permanganate or hydrogen peroxide in a highly basic medium. These samples contain one or more of the following hydroxy-containing compounds: a) alcohols, b) polyphenols, and c) sugars. The idea that
the -OH group could be responsible for the observed chemiluminescence started the chain of experiments that gave form to this work.

Solution Preparation

All through this research, aqueous solutions were prepared with deionized water (dw) from a Millipore Milli-Q system connected to the house deionized water. All weighings were made with a Mettler type H15 balance to 0.1 mg.

The major stock solutions prepared for the experiments and their respective sources are listed in Table VI. Unless stated otherwise, dw was the dilution solvent. Volumetric glassware was always used for the preparation or dilution of stock solutions to make standards. For new solutions, glassware was soaked overnight with a 2% (v/v) solution of nitric acid in a Branson model 52 ultrasonic cleaner and then rinsed ten times with water. When flasks were to be refilled with the same type of solution, they were first rinsed with 50% (v/v) nitric acid and then ten times with water.

Potassium permanganate solutions were stored in black Teflon bottles in the dark. For their first use, these bottles were washed with 50% (v/v) HCl and then rinsed ten times with dw. When the cleaned Teflon bottles were not in use, they were filled with water until needed. Old permanganate solutions were always discarded. After rinsing with 50% (v/v) HCl, the previously used dark bottles were filled with 1% (v/v) HCl and left capped for two days. When needed for new permanganate solutions, they were rinsed ten times with water. This treatment ensures the removal of MnO₂ formed in old permanganate
### Table VI. Major Stock Solutions

<table>
<thead>
<tr>
<th>Solution</th>
<th>Source</th>
<th>Preparation Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1.0 \times 10^{-2} \text{ M KMnO}_4$</td>
<td>Baker analyzed Rgt. (3228)</td>
<td>0.395 g of KMnO$_4$ diluted to 250 mL</td>
</tr>
<tr>
<td>95% (w/v) HNO$_3$</td>
<td>Baker analyzed Rgt. (9602-3)</td>
<td>94.2 mL concentrated HNO$_3$ diluted to 100 mL. Cooled to room temperature before raised to mark</td>
</tr>
<tr>
<td>Pyrogallol 500 mg/L</td>
<td></td>
<td>0.100 g diluted to 200 mL</td>
</tr>
<tr>
<td>Catechol 500 mg/L</td>
<td>Aldrich 99 + % (13, 501-1)</td>
<td>0.100 g diluted to 200 mL</td>
</tr>
<tr>
<td>0.02 M Hydrogen Peroxide</td>
<td>Mallinckrodt Analytical Rgt. (5239)</td>
<td>0.204 mL of 30% (w/w) solution diluted to 100 mL Refrigerated</td>
</tr>
<tr>
<td>Ethyl Alcohol 200 proof, 100%</td>
<td>U.S. Industrial Chemicals Co.</td>
<td>Refrigerated</td>
</tr>
<tr>
<td>BHA 100 mg/L</td>
<td>Fluka AG(20021) &gt; 98%</td>
<td>0.50 g diluted to 500 mL 20% (w/v) acetone</td>
</tr>
<tr>
<td>BHT 100 mg/L</td>
<td>Aldrich 99% (D4, 740-4)</td>
<td>0.050 g diluted to 500 mL with reagent grade acetone</td>
</tr>
<tr>
<td>Propyl Gallate</td>
<td>Aldrich (P5,330-6)</td>
<td>0.080 g diluted to 500 mL</td>
</tr>
<tr>
<td>Mandelic Acid</td>
<td>MCB (MX 0170)</td>
<td>0.5 g diluted to 100 mL</td>
</tr>
<tr>
<td>0.1 M Fe(SO$_4$)$_2$·7H$_2$O</td>
<td>Mallinckrodt Analytical Rgt. (5056)</td>
<td>2.781 g diluted to 100 mL acidified to prevent iron oxidation</td>
</tr>
<tr>
<td>0.1 M NaF</td>
<td>Baker Analyzed Rgt. (3688)</td>
<td>0.42 g diluted to 100 mL</td>
</tr>
<tr>
<td>0.2 M NaNO$_3$</td>
<td>Amachem Rgt. Grade (SS415)</td>
<td>1.700 g diluted to 100 mL</td>
</tr>
<tr>
<td>0.2 M Na$_3$P$_4$·12H$_2$O</td>
<td>Baker Analyzed Grade (5-3836)</td>
<td>7.6024 g diluted to 100 mL</td>
</tr>
<tr>
<td>0.2 M NaCH$_3$COO·3H$_2$O</td>
<td>Baker Analyzed Grade (5-3460)</td>
<td>2.7216 g diluted to 100 mL</td>
</tr>
</tbody>
</table>
### Table VI. Major Stock Solutions (cont.)

<table>
<thead>
<tr>
<th>Solution</th>
<th>Source</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 M NaCl</td>
<td>Baker Analyzed Grade (3624)</td>
<td>1.1688 g diluted to 100 mL</td>
</tr>
<tr>
<td>0.2 M Na₂SO₄</td>
<td>Baker Analyzed Grade (3891-1)</td>
<td>2.8408 g diluted to 100 mL</td>
</tr>
<tr>
<td>0.2 M NaClO₄</td>
<td>MCB Rgt. Grade (SX 0692)</td>
<td>2.4492 g to 100 mL</td>
</tr>
<tr>
<td>1.0 x 10⁻² M K₃[Fe(CN)₆]·3H₂O</td>
<td>Mallinckrodt Rgt. Grade (6912)</td>
<td>0.1646 g diluted to 50 mL</td>
</tr>
<tr>
<td>1.0 x 10⁻² M Mn(CH₃COO)₃·2H₂O</td>
<td>Alpha (10724)</td>
<td>0.1165 g diluted to 50 mL, with glacial Acetic Acid</td>
</tr>
<tr>
<td>0.2 M Mn(NO₃)₂</td>
<td>Baker Analyzed Rgt. (1-2544)</td>
<td>7.16 mL of 50% (w/v) solution diluted to 100 mL</td>
</tr>
<tr>
<td>2.5% (w/v) Cyclobutanol</td>
<td>Aldrich 99 + % (15,643-4)</td>
<td>0.250 g diluted to 10 mL</td>
</tr>
<tr>
<td>Vanillin 100 mg/L</td>
<td>Fisher Scientific (V10-100)</td>
<td>0.050 g diluted to 500 mL</td>
</tr>
<tr>
<td>Acetone</td>
<td>Baker Analyzed Rgt. (9006)</td>
<td></td>
</tr>
<tr>
<td>4 M Potassium hydroxide</td>
<td>Baker (5-3140)</td>
<td>22.4 g in 100 mL</td>
</tr>
<tr>
<td>100 mg/L Co(NO₃)₂·6H₂O</td>
<td>Mallinckrodt Rgt. Grade (4544)</td>
<td>0.1 g to 1.0 L</td>
</tr>
<tr>
<td>Ferric tartrate rgt.</td>
<td>EM Science</td>
<td>1.0 g of NaK tartrate (Rochelle salt) and 0.200 g FeSO₄·7H₂O were dissolved in water and diluted to 100 mL. This solution was freshly prepared and used immediately.</td>
</tr>
<tr>
<td>Sodium Acetate solution</td>
<td>EM Science</td>
<td>15.00 g of NaOAc.3H₂O dissolved in water and diluted to 100 mL.</td>
</tr>
<tr>
<td>Vanillin 100 mg/L</td>
<td>Fisher Scientific</td>
<td>25 mg of vanillin were dissolved in 250 mL</td>
</tr>
<tr>
<td>Vanillic acid 200 mg/L</td>
<td>Aldrich H3, 600-1</td>
<td>50 mg of vanillic acid were dissolved in 250 mL</td>
</tr>
</tbody>
</table>
Table VI. Major Stock Solutions (cont.)

<table>
<thead>
<tr>
<th>Stock Solution</th>
<th>Source or Formula</th>
<th>Preparation Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.0 x 10^{-4} M DBA in 50% (v/v) ethanol or isopropanol</td>
<td>Aldrich 98% D3,885-5</td>
<td>30.2440 mg were dissolved in 50 mL of ethanol or isopropanol and diluted to 100 mL with Cl₃CH</td>
</tr>
<tr>
<td>0.01 M Biacetyl</td>
<td>Aldrich</td>
<td>0.22 mL of biacetyl in 250 mL</td>
</tr>
<tr>
<td>0.2 M NaNO₃</td>
<td>Amachem Rgt. Grade</td>
<td>1.700 g in 100 mL</td>
</tr>
<tr>
<td>0.2 M Na₃PO₄·12H₂O</td>
<td>Baker Rgt.</td>
<td>7.602 g in 100 mL</td>
</tr>
<tr>
<td>0.2 M NaHC₃COO·3H₂O</td>
<td>Baker Rgt.</td>
<td>2.722 g in 100 mL</td>
</tr>
<tr>
<td>0.2 M NaCl</td>
<td>Baker Rgt.</td>
<td>1.169 g in 100 mL</td>
</tr>
<tr>
<td>0.2 M Na₂SO₄</td>
<td>Baker Rgt.</td>
<td>2.841 g in 100 mL</td>
</tr>
<tr>
<td>0.2 M NaClO₄</td>
<td>MCB Rgt.</td>
<td>2.449 g in 100 mL</td>
</tr>
<tr>
<td>0.1 M Na₄P₂O₇·10H₂O</td>
<td>Baker Rgt.</td>
<td>4.461 g in 100 mL</td>
</tr>
<tr>
<td>0.01 M NaIO₄</td>
<td>Mallinckrodt Analytical Rgt.</td>
<td>0.2139 g in 100 mL</td>
</tr>
<tr>
<td>0.01 M K₂Cr₂O₇</td>
<td>Mallinckrodt Analytical Grade</td>
<td>0.147 g in 50 mL</td>
</tr>
<tr>
<td>0.01 M K₃[Fe(CN)₆]·3H₂O</td>
<td>Mallinckrodt Rgt. Grade</td>
<td>0.1646 g in 50 mL</td>
</tr>
<tr>
<td>0.01 M H₂O₂</td>
<td>Mallinckrodt Analytical Rgt.</td>
<td>0.10 mL of 30% (w/w) H₂O₂ in 100 mL</td>
</tr>
<tr>
<td>0.5% (w/v) Caffeic acid</td>
<td>Sigma C0625</td>
<td>0.5 g in 100 mL 4 M KOH</td>
</tr>
<tr>
<td>55 mg/L Guaiacol</td>
<td>Aldrich G1-090-3</td>
<td>50 µL in 1 L</td>
</tr>
</tbody>
</table>
solutions from the bottle walls. A sufficient quantity of permanganate solution was always freshly prepared for a given experiment.

All polyphenol solutions (e.g., pyrogallol, catechol, etc.) were prepared just prior to analysis. The volumetric flasks containing the solutions were covered with aluminum foil to prevent photodecomposition caused by light. If solutions were not used within one hour, they were refrigerated until needed. Solutions were generally used within two hours of preparation and completely discarded after use.

Ethyl alcohol solutions were prepared as % (v/v) solutions by delivering the appropriate amount of 100% alcohol needed with volumetric pipets into volumetric flasks and diluting to volume with solvent. The flask stoppers were tightly covered with parafilm to prevent ethanol evaporation. All dilute solutions were used within one hour of preparation or were refrigerated until needed. Refrigerated solutions were allowed to warm up to room temperature before analysis.

Commercial products were purchased at different stores. They included Dominican rum, gin, vanilla, coffee, hair dye, lard, tea, beer, and wine. The preparation of these samples for analysis is discussed in detail later in this section.

Methodology for Chemiluminescence Analysis

The general procedure followed for CL analysis involved the addition of 1.0 mL of sample to the sample cell followed by addition of 0.5 mL of acid. Solution and reagents were delivered to the sample cell by Eppendorf pipets. Plastic tips were disposed after use. With the reaction vessel lid closed and the PMT shutter open, 0.81 mL of the
final CL reagent (oxidant) was injected into the sample cell using the Hamilton pneumatic injector. For some studies, different conditions (e.g., reagents, volumes, order of addition) were employed as will be noted. Blank runs were performed by addition of 1 mL of water to the cell instead of sample. CL peaks for the blank were negligible unless otherwise stated. Between runs the reaction cell was cleaned by adding HCl 50% (v/v), letting it stand for 2-3 minutes, and then rinsing ten times with water. Reaction mixtures and rinsing solutions were removed by vacuum aspiration.

Peaks for the sample were recorded and their peak heights taken as the analytical signal. All CL signals are reported as the photoanodic voltage in mV or V. The mean and the standard deviations of the CL signal for repetitive runs were calculated.

The instrumental conditions employed during analysis were as follows:

PMT type: RCA 1P28
Bias voltage: 820 V
I/V gain: 10 V/A
Noise filter cut-off frequency: 0.5 - 1.0 Hz
Gain of filter: 2
Cell temperature: 25°C

The noise filter cut-off frequency was set to the lowest value that did not attenuate the CL signal. This setting was optimized prior to analysis for each chemical system. The chart recorder sensitivity was adjusted to keep all CL peak signals on scale.
Preliminary Studies

Coffee

Initial studies were based on coffee as a model sample for the CL reaction. Colombian coffee grounds, MJB instant coffee, and Taster’s Choice decaffeinated instant coffee were purchased from a local store. A 0.50 g coffee sample was weighed, transferred to a 100 mL volumetric flask and diluted to volume with water to yield a 0.5% (w/v) stock solution. For the Colombian coffee, the solution was vigorously mixed and then filtered through Whatman No. 1 filter paper.

The first experiments were carried out by dispensing 1.0 mL of a coffee sample or blank and 0.5 mL of oxidant into the sample cell. The reaction was initiated by injecting 1 mL of KOH with the pneumatic syringe. Both potassium permanganate and H₂O₂ were used as oxidants. The oxidant and KOH concentrations were varied.

Model compounds

A few model compounds were tested for their ability to produce CL when oxidized by KMnO₄ which proved to be a better oxidant than H₂O₂. Because of the potential danger in injecting concentrated KOH, it was decided to switch to injecting 0.5 mL of the oxidant. This also allows the pH of the reaction mixture to be easily adjusted. Generally a KMnO₄ concentration of 1.0 x 10⁻³ M was used. Before injection of the oxidant, 1.0 mL of sample and 0.5 mL of KOH were added to the cell. In some cases, the KOH solution was omitted.
After it was discovered that a CL signal was only observed without the base added and that the CL signal increased when acidic compounds (e.g., citric acid, quinic acid) were added to the reaction mixture, it was decided to study the dependence of the CL intensity on sample pH. Nitric acid was used instead of the organic acids already tested as the acidifying agent. The pH of a relatively large volume sample solution (e.g., 50-75 mL) was adjusted to the desired pH with a small volume of 50% (w/v) nitric acid or 4 M KOH prior to addition in the reaction cell. In this case 2 mL of the pH adjusted solution were introduced in the cell followed by injection of 0.5 mL of oxidant.

Once it was realized that low pH yielded the largest CL signal, the following procedure was used to evaluate the CL response from a large number of model compounds: to 1 mL of sample, 0.5 mL of 50% (w/v) acid was added, followed by injection of 0.5 mL of 1.0 x 10^{-3} M KMnO_4. For each compound, the CL signal from a stock solution (generally 500 mg/L) was first tested. Lower concentration solutions were also analyzed until no CL signal was recorded. For most compounds, the reaction was also run in basic solution (0.5 mL of 4 M KOH added instead of HNO_3). In some cases, the effect of KMnO_4 concentration was briefly studied.

**Other samples**

The final CL procedure and conditions that produced the highest CL signals for the model compounds were also applied to coffee (0.5% (w/v)), humic acid (500 mg/L), rum (1:10 dilution), beer (1:10
dilution), wine (1:10 dilution) and tea (1 envelope of Lipton tea immersed in 80 mL of dw at room temperature and filtered).

Pyrogallol Chemiluminescence

The study of pyrogallol (Py) CL and the determination of pyrogallol in hair dye consisted of the following parts: 1) preliminary studies of Py CL reaction (peak shape and spectra), 2) optimization, 3) extraction and separation of Py from hair dye, 4) determination of Py in hair dye, and 5) effect of Mn(II) and Fe(II) on CL intensity.

Spectral studies

Absorption spectra of a 25 mg/L solution of Py in dw and 50% (w/v) HNO₃ were obtained with water as a blank. Additionally, absorption spectra of a 1:20 dilution of the reaction mixture (1.0 mL of the Py solution, 0.5 mL of 50% (w/v) nitric acid and 0.810 mL of 0.01 M potassium permanganate), and of a 1:20 dilution of a blank reaction mixture (1.0 mL of water substituted for Py) were taken.

The settings on the UV-VIS spectrophotometer (Cary 118C) were as follows:

- Gain: Auto
- Period: 1 s
- Slit: 0.2 mm
- Wavelength scan rate: 1 nm/s
- Chart scan rate: 50 nm/in.

Fluorescence excitation and emission spectra were obtained from 25
and 500 mg/L Py solutions and from a 1:20 dilution of the reaction mixture solution. The settings on the spectrofluorometer (SF-30) were as follows:

- **Readout mode:** 0.25 s
- **Response time:** 0.25 s
- **Sensitivity:** 1
- **Selector:** X10
- **Variable:** 0.00
- **Excitation slit:** 10 nm
- **Emission slit:** 20 nm
- **Scan speed:** 50 nm/min
- **Chart speed:** 4 cm/min

**Optimization**

Reagent concentrations (e.g., acid and oxidant) for the CL determination of Py were optimized by a simplex technique. A simplex of seven dimensions was used in which the values of the following variables were varied: concentration and volume of KMnO₄, concentration and volume of HNO₃, concentration and volume of Py, and filter cutoff frequency. The values for the starting vertex were based on the final concentrations and volumes used in the model compound studies and 500 mg/L Py. Seven other vertex were calculated, their responses were obtained, and the movement was initiated from then on.

The effect of acid and permanganate concentrations was further studied by varying the concentration of one reagent while holding the concentration of the other reagent constant. A 25 mg/L Py solution was
prepared, and using a $1.0 \times 10^{-3}$ M KMnO$_4$ solution, the concentration of nitric acid was varied from 10 to 95% (w/v). The concentration of acid which gave the best response was used in conjunction with the Py solution and the concentration of KMnO$_4$ was varied from $1.0 \times 10^{-4}$ to $1.0 \times 10^{-2}$ M.

Other studies

The effect on the CL signal of addition of cobalt (II), manganese (II), and iron (II) to a standard reaction mixture containing Py was also studied. Solutions of hydrogen peroxide, potassium ferricyanate, potassium dichromate, and sodium periodate were tested and compared to potassium permanganate solutions to determine the best oxidant to inject. For the first three oxidants 0.02 M solutions were prepared and 0.4000 mL was injected so that the number of moles of the oxidant was equivalent to the number of moles injected when potassium permanganate (reference) was used. For potassium periodate, a 0.01 M solution was prepared and 0.7931 mL of oxidant was injected to the reaction cell. When dichromate was used as the injected oxidant, the reaction was run with and without addition of Mn(II) before injection.

Extraction and separation of pyrogallol from hair dye

Salon Formula Colorsilk, 7A medium ash blonde, was purchased from a local store in October 1985. The Py in this sample was determined by an accepted spectrophotometric method of the AOAC$^{28}$ and by the new CL
method. The extraction method used to separate Py from the matrix is first described.

The AOAC method specifies that 10 mL of sample be pipetted into a 125 mL separatory funnel containing approximately 0.3 g of solid NaHSO₃ and extracted with 30 mL portions of ether. The hair dye is so viscous that measuring volumes accurately is difficult. When the ether (extracting solvent) was added, no phase separation was observed after 20 min.

To solve the problem of no phase separation, the extraction scheme was modified as follows. First, 5 mL of colorant were dissolved in 10 mL of ether. Next, 5 mL of this solution were pipetted into a 125 mL separatory funnel containing approximately 0.3 g of solid NaHSO₃, about 10 mL of water were added, and the dye was extracted six times with 30 mL ether portions. Solutions were shaken vigorously for one minute. The ether layers were filtered successively through cotton wet with ether. The combined ether extractions were evaporated using a rotary evaporator where the temperature was maintained at all times to be less that 40°C. Evaporation was carried out until the ether odor was completely gone. The residue was dissolved in 20 mL of water, washed completely into a 50 mL volumetric flask, and diluted to volume with water. The solution was then filtered through dry paper (Whatman qualitative No. 1) discarding approximately the first 20 mL of filtrate. The aqueous filtrate was used in both the spectrophotometric and CL analysis procedures.

Throughout the analysis, exposure of pyrogallol solutions to room light was minimized by covering all volumetric flasks with aluminum foil. All solutions were refrigerated unless used within ten minutes.
Before analysis, all refrigerated solutions were allowed to warm up to room temperature.

**Analysis of hair dye**

*Spectrophotometric determination.* The spectrophotometric determination was carried out as follows. The preparation of the NaOAc and Fe tartrate reagent solutions is specified in Table VI. To prepare the standards, 2.50, 5.00, 7.50, 10.00, 12.50, and 15.00 mL of the stock 200 mg/L Py solution were added with a buret to six 100 mL volumetric flasks. Next, 10 mL of the stock 15 g/100 mL NaOAc solution and 10 mL of the Fe tartrate reagent solution were pipetted into the flasks and followed by dilution to volume with water. For the hair dye sample, 5.00 and 26.50 mL of the aqueous filtrate obtained after the solvent extraction procedure were treated identically. With 1 cm pathlength plastic cells, the absorbance of solutions were measured at 540 nm within 15 minutes after the color was developed against a freshly prepared solution blank consisting of 10 mL of the NaOAc reagent solution and 10 mL of the Fe tartrate reagent solution diluted to volume with water in a 100 mL flask. Settings for the Cary 118 spectrophotometer were as follows: gain, 4; period, 1 s; slit, 0.085 mm (spectral bandpass, 1.8 nm). The standard curve was constructed and used to calculate the concentration of Py in the unknown.

*Chemiluminescence determination.* To prepare the standards for CL analysis, 2.50, 5.00, 10.00, 20.00, and 40.00 mL of a 200 mg/L stock solution of Py were delivered with a buret into six 100 mL volumetric
flasks diluted to volume with dw. For the hair dye samples the aqueous filtrate from the extraction procedure was analyzed directly and after 1:5 and 3:10 dilutions in dw. The CL procedure already presented was followed. From the analytical signals obtained, the standard calibration curve was constructed and used to calculate the concentration of Py in the unknowns.

BHA and Propyl Gallate Chemiluminescence

The steps followed for determination of the antioxidants, propyl gallate (PG) and butylated hydroxianisole (BHA) were as follows: 1) optimization of conditions, 2) extraction and separation of the antioxidants from the fat or oil, 3) CL and HPLC analysis of lard for the antioxidants, 4) interference study.

Optimization

To prepare the standards, 1.00, 2.00, 5.00, 10.00, 20.00, and 40.00 mL of a 500 mg/L PG stock solution (see Table VI) were delivered with a buret into six 100 mL volumetric flasks followed by dilution with H2O. With the PG stock solution, the best concentrations of nitric acid and potassium permanganate were found. The best concentrations found by the simplex technique for the Py determination were used as a starting point.

Nitric acid solutions were prepared to be 10, 25, 50, 75, and 95% (w/v) by dilution of the concentrated nitric acid (100.2% (w/v)). Potassium permanganate solutions were prepared by dilution of a
Concentrations included $1.0 \times 10^{-2}$, $5.0 \times 10^{-3}$, $1.0 \times 10^{-3}$, $5.0 \times 10^{-4}$, and $1.0 \times 10^{-4}$ M. After the best set of conditions was found, a calibration curve was constructed in order to find the linear range.

The 100 mg/L BHA stock solution in 20% (w/v) acetone was prepared as specified in Table VI. BHA is insoluble in water and in 1.0, 5.0, and 10.0% (w/v) acetone in water. Pure acetone, when used as a blank with 50.0% (w/v) nitric acid does not produce a CL signal. However, a significant background signal is obtained when pure acetone is used as a blank with 95.0% (w/v) nitric acid.

To prepare BHA standards, 3.0, 6.0, 12.5, 25.0, and 50.0 mL of the 100 mg/L stock solution were delivered with a buret to five 100 mL volumetric flasks and raised to mark with 20% (w/v) acetone. With the stock solution, the best concentrations of nitric acid and potassium permanganate were found with the same procedure used for PG. A calibration curve was constructed using the optimized conditions.

**Extraction and separation of the antioxidants from lard**

*Initial studies.* The procedure developed for extraction of PG in oils and fats by Sastry, Rao and Prasad\textsuperscript{75} was initially followed in this work and is shown in Figure 12.

To evaluate the extraction efficiency, exactly 50 mg of PG were weighed and added to 9.9964 g of peanut oil. This results in a final theoretical concentration of PG in the final ethanol fraction from the oil of 500 mg PG/L. The Planters peanut oil was purchased from a local store in February 1984.
An extraction of peanut oil without addition of PG was carried out at the same time and used as a reagent blank. A 500 mg/L standard solution of PG in 50% (v/v) EtOH was also prepared. Its CL signal was compared to that of the extracted oil sample to evaluate the extraction efficiency.

![Procedure for extraction of PG in oils and fats.](image)

Figure 12. Procedure for extraction of PG in oils and fats.
HPLC extraction procedure. The standard method for determination of antioxidants in fats and oils is based on the following principle. The sample is dissolved in hexane and the antioxidants are extracted into acetonitrile. After solvent evaporation, the sample is diluted with an equal volume of isopropanol and injected into a liquid chromatograph to separate the antioxidants and detect them with UV absorption detection at 280 nm.28

All glassware was rinsed with CHCl₃, acetone and MeOH, in that order, and then blow dry with nitrogen. The acetonitrile was HPLC grade glass distilled. The hexane and acetonitrile were saturated with the other solvent by shaking the solvents for two minutes in a separatory funnel and withdrawing the hexane.

First 10.032 g of lard were weighed into a 150 mL beaker. Armour hydrogenated lard, which contains BHA, PG and citric acid to protect flavor, was purchased at a local grocery store in September 1985. This product has been inspected and passed by the U.S. Department of Agriculture. Second, 60 mL of hexane were added and the sample was dissolved by heating. The 30 mL of hexane specified in the standard procedure did not dissolve the lard. This mixture was quantitatively transferred to 100 mL volumetric flask by rinsing with hexane and diluted to the mark to obtain a transparent, colorless solution. Then 25 mL of this solution were pipetted into a 125 mL separatory funnel and extracted with three 50 mL portions of acetonitrile. These portions were collected in a 250 mL separatory funnel and allowed to drain slowly into a 250 mL round bottom flask to aid in the removal of hexane oil droplets. The acetonitrile was completely removed in less than 10 min. with a rotary evaporator. To avoid overheating the residue,
2 mL of dw were added with the temperature held to less than 40° C. To decrease the evaporation time, an efficient vacuum source and ice-water cooling was used and the adaptor between the round bottom flask and the distillation column in the rotary evaporator was heated with a heat gun.

With a disposable pipet, the majority of the acetonitrile oil droplet mixture was transferred to a 10 mL glass graduated cylinder which was modified so it could be stoppered with a ground glass stopper. The flask was rinsed with small portions of non-saturated acetonitrile. These rinsings were transferred to the graduated cylinder with a disposable pipet until 5 mL were collected. The disposable pipet was rinsed with acetonitrile. The flask was then further rinsed with small portions of 2-propanol. All rinsings were transferred to the graduated cylinder until exactly 10 mL were collected. The contents of graduated cylinder were mixed and immediately analyzed.

At the same time, a reagent blank extraction was carried out with the above procedure by substituting 25 mL of hexane for the hexane-oil mixture. Both extraction solutions were covered with aluminum foil to prevent oxidation by direct light and refrigerated. At time of analysis, they were allowed to warm up to room temperature.

**Chemiluminescence extraction procedure.** According to Sastry, Rao and Prasad, the simultaneous determination of BHA and PG in food requires that they be separated before determination. The procedure developed by Schwien and Conroy is suggested. This extraction procedure was modified and summarized in Figure 13 shown below.
30.0272 g lard + 60 mL hexane (to dissolve lard)

15 mL water  

Water phase  Hexane phase

PG + others  BHA + others

7 x 15 mL water

Water phase  Hexane phase

PG + others  BHA + others

20 mL ether

Water phase  Ether phase

PG  others

10 x 20 mL ether

Water phase  Combined

PG  Discarded

Combined

20 mL Mehtanol

Acetonitrile

Two approaches

20 mL Methanol

Methanol phase  Hexane phase

BHA  others

7 x 20 mL Methanol

Methanol

Combined  BHA

Hexane

Discarded

a Extraction with acetonitrile and solvent evaporation produced an oily yellow precipitate which was discarded.

Figure 13. Modification of extraction procedure for BHA and PG given by Schwien and Conroy.
First, 30.0272 g of lard were weighed, instead of the 10 g recommended by the authors, dissolved in approximately 60 mL of hexane, and transferred to 250 mL separatory funnel. The original procedure recommends less sample and petroleum ether as the solvent. This solvent did not dissolve the lard. Hexane was used instead of petroleum ether since the lard was slightly soluble in hexane. Next 15 mL of water were added, and the mixture was gently shaken for one minute. After separation of the phases, the aqueous phase was drained into 125 mL separatory funnel leaving any emulsion in organic phase. Extraction of the hexane layer containing the BHA was repeated with seven additional 15 mL portions of water. The hexane solution was reserved for further extraction with methanol. The water fractions were combined and extracted with one 20 mL portion of ether and shaken for one minute. After separation, the aqueous phase was transferred to a 125 mL separatory funnel and extracted with ten additional 20 mL portions of ether. The final aqueous solution was discarded. The ether fractions were all combined and transferred to 250 mL round bottom flask. This solution was evaporated in a rotary evaporator until the ether odor was all gone with the temperature held below 40° C. To prevent decomposition of PG, 5 mL of water were added to the flask before evaporation. An ice water bath was used to reduce evaporation time. With a disposable pipet, the residue was collected with water and transferred to a 10 mL volumetric flask and diluted to volume with H₂O. This solution was analyzed by CL without dilution.

The hexane solution was extracted with a 20 mL portion of CH₃OH and shaken for two minutes. The original procedure specifies acetonitrile as the extracting solvent. However, this solvent was found to
quench the CL from the oxidation of BHA almost completely. After separation, the methanol phase was transferred to a 250 mL round bottom flask. The hexane phase was extracted with seven additional 20 mL portions of methanol.

The final hexane phase was discarded. All methanol fractions were combined and evaporated to about 5 mL in a rotary evaporator using conditions identical to that used for the ether extraction. The remaining solvent was evaporated overnight under vacuum. The white solid was dissolved in 20% (w/v) acetone and transferred to 10 mL volumetric flask. This solution was analyzed by CL without dilution and after 1 to 5 and 1 to 10 dilutions in 20% (w/v) acetone.

At the same time, a blank extraction was carried out using 25 mL or petroleum ether instead of lard and is denoted the reagent blank.

Analysis of lard sample

HPLC analysis. For HPLC analysis, a stock solution 1 mg/mL of BHA and PG was prepared in a 50 mL volumetric flask with a 1 to 1 mixture of 2-propanol-acetonitrile as the solvent. This solution was diluted with the same solvent to produce a standard solution that was 0.01 mg/mL in both PG and BHA.

The chromatographic column was pre-conditioned and the system was run following the instructions given in reference 77. The HPLC mobile phases were distilled water with 5% (v/v) acetic acid added (A) and acetonitrile (HPLC grade) with 5% acetic acid added (B). A linear gradient was used from 30% B/70% A to 100% B over 10 minutes with a four minute hold period at 100% B at 1 mL/min. The sample loop size
was 20 µL. For sample runs, the flow rate was increased to 6 mL/min at 100% B for five minutes after the hold period to clean the column. For samples and standards, the solvent composition was returned to 30% B/70% A at 2 mL/min. Baseline, pressure and mobile phase composition was allowed to stabilize for approximately 10 minutes.

All runs were made in duplicate; the sequence for analysis was as follows: blank gradient - reagent blank - standard solution (10 µg/mL) - sample - standard solution - sample - standard solution - reagent blank. The blank gradient is a run with no injection, while a reagent blank run was made by injecting 20 µL of a 1:1 mixture of 2-propanol and acetonitrile.

Peaks in the sample were identified by comparison with retention times of PG and BHA in the standard solution. The blank gradient chromatogram was used to establish the baseline.

The average areas of antioxidants peaks in the sample were determined from duplicate injections and corrected for the reagent and gradient blanks. The areas of antioxidants peaks for the standards injected before and after the sample were averaged and corrected for the gradient blank.

The concentration of the antioxidants in lard was calculated with the following formula

\[
\text{Antioxidant (µg/g)} = \frac{(A/A')}{(C/V/w)}
\]  

where \( A \) = peak area for sample, \( A' \) = peak area for standard, \( C \) = concentration of the antioxidant in the standard in µg/mL, \( w \) = weight of sample extracted and \( V \) is the final extraction volume (10 mL).
Chemiluminescence determination. As previously stated, the Code of Federal regulations allows addition of PG and BHA to fats and oils to an amount not to exceed 0.02% (w/w) (total antioxidants added) of the fat or oil content. This was taken into consideration when developing the CL method.

For CL analysis, standard solutions of 5.0, 10.0, 20.0, 40.0 and 80.0 mg/L of PG were prepared from a 180 mg/L stock solution with water as the solvent. Standard solutions of BHA were prepared identically except 20% (w/v) acetone in water was used as the solvent. The CL determination has already been described.

Interference study

It has long been known that PG is synergic with citric acid. The lard contains an antioxidant mixture which includes citric acid. To test potential interference from this acid, the CL signal from a 36 mg/L PG solution was compared to the CL signal from solutions containing 36 mg/L PG and 18 mg/L citric acid and 36 mg/L PG and 40 mg/L citric acid.

Vanillin Chemiluminescence

The study of the CL of vanillin and its determination in vanilla extract was divided into the following four parts: 1) optimization of conditions, 2) extraction of the vanillin from the vanilla extract for the CL technique, 3) CL and spectrophotometric determination of vanillin in vanilla, 4) interference study.
Optimization

To prepare standard solutions, 6.0, 12.0, 25.0, and 50.0 mL of a 100 mg/L stock vanillin solution were delivered with a buret to four 100 mL volumetric flasks and were diluted with water. The stock solution was used to find the best concentrations of nitric acid and potassium permanganate. The starting values were those used for Py. Then, a calibration curve was constructed to find the linear range.

Extraction of vanillin from the vanilla extract

Two mL of House of McCormick vanilla purchased in November 1985 were pipeted into 125 mL separatory funnel. The sample was extracted seven times with 20 mL portions of ether. The ether layers were collected and combined in a 250 mL round bottom flask. The ether was evaporated with a rotary evaporator until the ether odor was completely gone. The water bath temperature was kept below 40°C at all times and an efficient vacuum source was used to decrease evaporation time to about 11 min.

The residue was quantitatively transferred into a 10 mL volumetric flask with a disposable pipet and water rinsing. After dilution to volume, 2.0 mL of this solution were pipeted into a 50 mL volumetric flask and diluted to volume.

Analysis of vanilla extract

Spectrophotometric method. The standard method for determination of
vanillin is based on dilution of the sample down to the proper range and determination of the absorbance of an alkaline solution of the diluted sample at 348 nm. A neutral solution of the diluted sample is used as the reference blank.

To prepare standard and blank solutions, three intermediate stock solutions were prepared by diluting 5, 10 and 15 mL of a stock solution of 1 g/L vanillin with 5% (v/v) ethanol in water in three 250 mL volumetric flasks with water. To prepare the three standard solutions, 10 mL of each one of the intermediate stock solutions were pipetted into 100 mL volumetric flasks. Approximately 80 mL of water plus 2 mL of a 0.1 M sodium hydroxide solution were added to each flask before dilution to volume with water. Three blank solutions were prepared by diluting 10 mL of each of the intermediate stock solutions to 100 mL with H₂O.

The best working level for the determination of vanillin, specified by the Association of Official Analytical Chemists, lies below 0.3 g vanillin/100 mL sample. The vanilla extract was first diluted 1:10 with 35% (v/v) ethanol. Next, 10 mL of this solution were pipetted into a 100 mL volumetric flask and diluted to volume with water. From this last solution, 2 mL were pipetted into each of two 100 mL volumetric flasks. One of them was diluted to volume with water and the other was diluted to volume after addition of approximately 80 mL of water and 2 mL of the 0.1 M sodium hydroxide. The same dilution scheme was performed on an undiluted sample (i.e., the first 1:10 dilution with 35% (v/v) ethanol was omitted).

The absorbance of the alkaline standard and sample solutions were determined at 348 nm, using the corresponding neutral solution as the
blank. The vanillin content was determined from the standard curve.

Chemiluminescence analysis. Standard solutions of 5, 10, 40, and 80 mg/L solutions were prepared from a 160 mg/L stock solution of vanillin. The sample was analyzed after the several types of dilutions. These included a 1:25 and a 2:125 dilution with water of the 10 mL aqueous solution from the extraction procedure, and a 1:50 and a 1:250 dilution with water of the original vanilla extract.

Interference study

Vanillic acid, also present in vanilla extract, may cause the emitted signal to increase or decrease depending on the role it plays on the CL reaction. To access the potential interference from vanillic acid, solutions were prepared from a stock 160 mg/L vanillin solution and a 200 mg/L vanillic acid solution to be 16 mg/L vanillin and 2, 4, and 10 mg/L in vanillic acid. The CL signals from all four solutions were obtained and compared.

Ethanol Chemiluminescence

As discussed in the historical section, the mechanism and kinetics of the oxidation of ethanol by potassium permanganate have been studied. The chemiluminescence accompanying the oxidation has not been reported.

The CL of ethanol and the determination of ethanol in gin is divided in the following parts: 1) optimization of type of acid and
Reagent concentrations, 2) CL and absorption spectra, 3) kinetics and mechanistic studies, 4) determination of ethanol in gin, and 5) interference study.

**Optimization**

The effect of the type of acid was studied by using 0.5 mL of 50% (w/v) nitric, phosphoric, acetic, perchloric, and sulfuric acid and concentrated HCl in the reaction mixture for the ethanol CL reaction. The potassium permanganate concentration was 0.01 M throughout these studies. Calibration curves over the 1 to 50.0% (v/v) ethanol concentration range were obtained and the best acid was chosen based on best linear range, highest signal to noise ratio (S/N), and lowest detection limit.

Calibration curves were obtained with potassium permanganate concentrations of 1.0 x 10^{-3}, 3.0 x 10^{-3}, 1.0 x 10^{-2}, and 3.0 x 10^{-2} M with ethanol concentrations of 1.0, 12.5, 25.0, and 50.0% (v/v). The concentration of nitric acid was 50.0% (w/v) for this study.

The dependence of the CL signal on HNO₃ concentration was obtained by varying the concentration of acid (10.0, 55.0, 77.5, and 95.0% (w/v)) with different KMnO₄ concentrations. The ethanol concentration was 12.5% (v/v) for this study.

A three dimensional simplex optimization was performed for a 1.0% (v/v) ethanol solution. The initial simplex vertex was chosen from the optimum reagent concentrations obtained for other substrates (e.g., PG, Py, BHA).
CL and absorption spectra

To obtain a crude CL spectra, the CL signal was recorded with different cut-on filters between the reaction cell and the photomultiplier tube in the CL photometer sample compartment. The filters used have cut-on wavelengths of 530, 570, 600, 640, 660, 720, and 780 nm.

Further studies were performed using the multiple signal spectrophotometer already described to obtain CL spectra. For this study, 2.0 mL of absolute ethanol and 0.5 mL of 95% (w/v) nitric acid were pipeted into the reaction cell. The CL reaction was initiated by injection of 0.8 mL of 0.01 M KMnO₄. Spectral information about the effect of Fe(II) on the CL reaction was obtained by adding 1 mL of 0.01 M Fe(II) to the reaction cell before injection of 0.8 mL of 0.01 M KMnO₄.

Absorption spectra were gathered with a diode array spectrophotometer with a minimum integration time of 0.1 s. Collection of information was started as soon as solutions were mixed in the reaction cell. Mixing was accomplished with a stirring rod for approximately 3 s. In this study, the effect of cations such as Fe(II) and Mn(II) on the reaction was also investigated. Due to the high absorption of HNO₃ in the UV region, absorption spectra were also gathered with HClO₄ instead of HNO₃ as the acid in the reaction mixture.

Reaction product studies

GC-MS served as a major tool for detection of the reaction products. Initially the reaction between mandelic acid and potassium
permanganate in acidic medium was used as a model system for the CL oxidation of hydroxy-containing compounds. Benzoic acid is a known end product and CO$_2$ is evolved as shown in the following reaction.

\[
\begin{align*}
\text{OH} & \quad \text{MnO}_4^- \\
\text{H-C-COOH} & \quad \text{H}^+ \quad -\text{CO}_2 \\
\text{C-O-COOH} & \quad \text{MnO}_4^-
\end{align*}
\]

From the stoichiometry, 1 g of benzoic acid is produced from 1.2476 g (8.2 x 10^{-3} mol) of mandelic acid and 2.5919 g (2 x 8.2 x 10^{-3} mol) of potassium permanganate. These amounts were separately dissolved in minimum amounts of water. In a 500 mL beaker, the mandelic acid was mixed with 40 mL of a 95.0\% (w/v) nitric acid solution. The permanganate solution was then added slowly. The solution was then filtered, and the filtrate extracted four times with 50 mL portions of ether. The solvent was evaporated with a rotary evaporator and the yellow precipitate was recrystallized in hot water. This white solid was characterized by a $^{13}$C NMR, TLC, and its melting point. For the NMR spectrum, the solid was dissolved in chloroform.

The TLC analysis was conducted by spotting a given silica plate in three locations with three different samples using capillary tubes. On the left side of the plate, the reaction product solid dissolved in chloroform was spotted three times. In the center, pure benzoic acid and reaction product were co-applied, and on the right side, pure benzoic acid was spotted. The plates were developed with ethyl acetate or a 20:80 mixture of ethyl acetate and CH$_2$Cl$_2$. For observation of the eluted compounds, the plates were sprayed with
Ce⁴⁺ in sulfuric acid and observed under UV light.

In the above experiment, the amounts of reagents were adjusted to ensure complete oxidation of mandelic acid to benzoic acid. However, when performing CL experiments, the analyte is often in excess relative to the KMnO₄ such that it cannot be completely oxidized.

In order to measure the amount of mandelic acid oxidized under these conditions, the procedure outlined above was repeated for a mixture of 1.2476 g of mandelic acid and 0.3161 g of potassium permanganate. The molar ratio of mandelic acid to permanganate is equivalent to that in a solution of 1 mL of a 5.0 \times 10^{3} \text{ mg/L} substrate solution (usually used) and 0.8 mL of a 0.01 M KMnO₄ solution (3.29 \times 10^{-5} \text{ moles of substrate} - 8.0 \times 10^{-6} \text{ moles of oxidant}).

The final residue was characterized by ¹H NMR and TLC. For the TLC analysis, the residue was dissolved in acetone. The plate was spotted with pure benzaldehyde on the left side of the plate, the reaction product in the center, and pure benzoic acid on the right side. A 20:80 mixture of ether and C₂H₆ was used as the mobile phase because it was found to yield a good separation of benzaldehyde and benzoic acid. Again, the plates were sprayed with Ce⁴⁺ in sulfuric acid and observed under UV light.

After the above experiments, the oxidation of isopropanol by potassium permanganate was studied. Isopropanol is oxidized to acetone which is much more stable to permanganate oxidation than acetaldehyde which is produced in the oxidation of ethanol.

First, 0.1 mL of isopropanol 99.6% (v/v) was mixed with 0.4 mL of D₂O. Next, 0.25 mL of nitric acid 95% was rapidly added to the
mixture followed by 0.4 mL of 1.0 x 10^{-2} M KMnO_4. This reaction mixture was introduced in an NMR tube and a \textsuperscript{1}H NMR spectrum was obtained. The time from the addition of the oxidant to the first collected spectrum was 32 s.

For the GC-MS analysis, a standard vacuum rack and Hg diffusion pump\textsuperscript{79} apparatus was used to prepare the sample. In a round bottom flask, 2 mL of 9.96\% (v/v) isopropanol, 1.0 mL of 95.0\% (w/v) nitric acid and 1.62 mL of 0.01 M potassium permanganate were mixed. The reaction mixture was allowed to react for ten minutes in a closed environment until precipitation of MnO_2 was observed. Next the products were vacuum distilled into a U-tube (immersed in liquid nitrogen) for one hour. Then, the system was closed, and the U tube where reaction products accumulated was dismantled. Under a clean and dust free environment, the frozen residue was allowed to warm up to room temperature. The U tube was opened and the liquid residue was dispensed into a glass vial. The product was labeled and analyzed by GC-MS.

The oxidation of ethanol was also studied in a similar manner. It was necessary to optimize the ratio of the unreacted ethanol to the acetaldehyde and/or acetic acid formed. Since potassium permanganate is the limiting reagent, the total number of moles of the products is independent of the initial concentration of ethanol. This number of moles (for 8.0 x 10^{-6} mol of oxidant) is equivalent to 0.05\% (v/v) acetaldehyde or acetic acid.

Following Benerjee and Sengupta's\textsuperscript{38} oxidation reaction model, the mole to mole ratio of ethanol to oxidant is 1:0.4. From this stoichiometry, the amount of unreacted ethanol can be calculated as shown in Table VII.
Table VII. Dependence of Unreacted Ethanol on Its Initial Concentration

<table>
<thead>
<tr>
<th>EtOH conc. (% (v/v))</th>
<th>EtOH conc. (mol/1 mL)</th>
<th>Unreacted Moles of EtOH (A)</th>
<th>Conc. of A (%) (v/v))</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5</td>
<td>2.14 x 10^{-3}</td>
<td>2.12 x 10^{-3}</td>
<td>5.38</td>
</tr>
<tr>
<td>5.0</td>
<td>8.57 x 10^{-4}</td>
<td>8.36 x 10^{-4}</td>
<td>2.12</td>
</tr>
<tr>
<td>3.0</td>
<td>5.14 x 10^{-4}</td>
<td>4.94 x 10^{-4}</td>
<td>1.25</td>
</tr>
</tbody>
</table>

a 1 mL of EtOH + 0.8 mL of 10^{-2} M KMnO₄ or 2.025 x 10^{-5} mol EtOH, 8.1 x 10^{-6} mol KMnO₄.
b 2.3 mL total volume in cell

As a compromise so that unreacted EtOH would not mask the detection of the reaction products in this study, 6.0% (v/v) ethanol was used for the GC-MS experiments. To 2.0 mL of 6.0% (v/v) ethanol in a round bottom flask was added 1.0 mL of 95% (v/v) nitric acid followed by 1.62 mL of 0.01 M potassium permanganate. This reaction mixture was treated identically to the isopropanol reaction mixture. This time, the liquid sample product was divided into two glass vials, and 0.1 M NaOH was added to one vial until the pH was approximately 8. These vials were kept in the freezer until ready to use. A sample of pure ethanol was first analyzed by GC-MS to evaluate if it was contaminated with acetaldehyde or acetic acid. A solution of 0.1% (v/v) acetaldehyde and 1.0% (v/v) ethanol in water was also analyzed to determine if excess ethanol would mask the detection of acetaldehyde.

All the above samples were run under the same GC-MC conditions. The NMR spectrum was analyzed to higher m/e ratios to check the possibility of formation of condensation products such as shown below.
Effect of other species and deaeration on the chemiluminescence intensity

The effect of other species on the CL reaction of ethanol was studied by adding them to the reaction mixture. These species included MnO₂, various cations (Mn²⁺, Mn³⁺, Fe²⁺), different anions (NO₃⁻, PO₄³⁻, CH₃COO⁻, Cl⁻, SO₄²⁻, ClO₄⁻, F⁻), CH₃OH and H₂O₂. The effect of the type of oxidant, the solvents for EtOH, and deaeration with helium was also investigated. Additionally, DBA and diacetyl were added to test the possibility of sensitized CL.

Cations and manganese dioxide. For most of these experiments, the effect of other species was evaluated by adding the test species to the standard CL reaction mixture consisting of 1.0 mL of EtOH, 0.5 mL of HNO₃ and 0.8 mL of KMnO₄. First MnO₂ was tested by comparing the CL signal observed with and without a small amount of solid MnO₂.
introduced. All other species were added as solutions. In this case, the CL signal obtained with addition of x mL of the test solution was compared with addition of x mL of water.

To test the effect of Mn$^{2+}$ calibration curves were constructed with ethanol standards ranging from 3 to 100% (v/v) with addition of 0.5 mL of 0.2 M Mn$^{2+}$ or 0.5 mL of water.

Most experiments to test the effect of Fe(II) were carried out in which the standard reaction was run with 6% (v/v) ethanol. However, a second injection of 0.81 mL of KMnO$_4$ was made after a suitable delay time from the first injection. Before injection of permanganate, 0.5 mL of H$_2$O or a Fe(II) solution was added to the reaction mixture. The concentration of the Fe(II) solution added was varied over the range shown in Table VIII. Table VIII also shows the number of moles of Fe(II) added and the molar ratio (MR) of the moles of Fe(II) added to the reaction mixture to the moles of MnO$_4^-$ injected in different experiments.

Several additional experiments were conducted with Fe(II). In one experiment, 0.5 mL of Fe(II) solutions varying in concentration from $1 \times 10^{-5}$ to $1 \times 10^{-1}$ M was added approximately two minutes after injection of KMnO$_4$ into the standard reaction mixture with 3% (v/v) ethanol. Calibration curves over the 3 to 100% (v/v) ethanol range were also obtained with addition of 0.5 mL of $1 \times 10^{-5}$ and $1 \times 10^{-3}$ M Fe(II) to the standard reaction mixture. Finally the effect of adding Mn(II) and Fe(II) individually and in combination to the standard reaction mixture with 6% (v/v) ethanol was tested.

According to Rocek and Radkowsky,$^{53}$ a solution of Mn$^{3+}$ is obtained by adding excess Mn$^{2+}$ to a potassium permanganate solution
Table VIII. Study of Effect of Fe(II) Concentration

<table>
<thead>
<tr>
<th>[Fe$^{2+}$] (M)</th>
<th>No. of Moles in Solution</th>
<th>MR$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 x 10$^{-1}$</td>
<td>5.0 x 10$^{-5}$</td>
<td>6.17</td>
</tr>
<tr>
<td>8 x 10$^{-2}$</td>
<td>4.0 x 10$^{-5}$</td>
<td>4.94</td>
</tr>
<tr>
<td>5 x 10$^{-2}$</td>
<td>2.5 x 10$^{-5}$</td>
<td>3.09</td>
</tr>
<tr>
<td>4.86 x 10$^{-2}$</td>
<td>2.43 x 10$^{-5}$</td>
<td>3.00</td>
</tr>
<tr>
<td>1 x 10$^{-2}$</td>
<td>5.0 x 10$^{-6}$</td>
<td>0.62</td>
</tr>
<tr>
<td>1 x 10$^{-3}$</td>
<td>5.0 x 10$^{-7}$</td>
<td>0.062</td>
</tr>
<tr>
<td>1 x 10$^{-4}$</td>
<td>5.0 x 10$^{-8}$</td>
<td>0.0062</td>
</tr>
<tr>
<td>1 x 10$^{-5}$</td>
<td>5.0 x 10$^{-9}$</td>
<td>0.00062</td>
</tr>
</tbody>
</table>

$^a$ MR = molar ratio = mol of Fe(II)/mol of MnO$_4^-$

in acid. Hence to test the effect of Mn(III), a pink solution consisting of 0.01 M of KMnO$_4$ in glacial acetic acid and 1.0 mL of a 50% (w/v) Mn$^{2+}$ solution was prepared. A 0.01 M solution was also prepared from Mn(CH$_3$COO)$_3$·2H$_2$O as described in Table VI. These Mn$^{3+}$ solutions were used as the oxidant in the reaction by injecting them into the standard reaction mixture instead of MnO$_4^-$. Additional experiments were conducted by adding 0.8 mL of the Mn$^{3+}$ solutions and glacial acetic acid to the standard reaction mixture with an ethanol concentration of 12.0% (v/v) before injection of MnO$_4^-$. Anions. To study the effect of anions on the CL reaction, 0.5 mL of the 0.2 M solutions of the sodium salts of nitrate, phosphate, acetate, chloride, sulfate, perchlorate, fluoride, and 0.1 M sodium pyrophosphate were added to the standard reaction mixture with ethanol concentrations in the 3 to 100% (v/v) range.
Methanol and hydrogen peroxide. The effect of methanol was studied by adding 0.5 mL of pure methanol to the standard reaction mixture (EtOH = 100%). Hydrogen peroxide was used instead of Fe(II) to act as a reducing species by adding 0.5 mL of 0.01 M H₂O₂ to the reaction mixture.

Other oxidants. Oxidants other than KMnO₄ were studied by injecting 0.81 mL of 0.01 M solutions of potassium periodate, chromic acid, potassium dichromate, hydrogen peroxide, and potassium ferricyanate into the standard reaction with an ethanol concentration of 100%.

Other solvents. The effect of the solvent for ethanol was evaluated by comparing the CL signal obtained from the standard reaction mixture with 50% (v/v) ethanol in H₂O to 50% (v/v) solutions of ethanol in DMSO, DMF, THF, CH₂Cl₂, hexane, ether and chloroform.

Activators. In order to test the possibility of sensitized CL, the CL signal from the standard reaction mixture with 50% (v/v) ethanol in chloroform was compared to that from a solution of 50% (v/v) ethanol containing 9.0 × 10⁻⁴ M 9,10-dibromoanthracene (DBA) in chloroform. This concentration is suggested in most published literature for similar fluorescent compounds. DBA is a good fluorophore and an efficient acceptor of energy from excited species.

It is known that when isopropanol is oxidized to acetone, the latter is promoted to a triplet by thermolysis. Energy transfer from the acetone to DBA has been reported to enhance the fluorescence by approximately a factor of 10². To evaluate if DBA could
enhance the CL from the oxidation of isopropanol, the CL signals from 50% (v/v) isopropanol in chloroform with and without $9.0 \times 10^{-4}$ M DBA added were evaluated.

Biacetyl phosphoresces naturally. The natural lifetime of this compound in water is 10 ms. To evaluate the potential enhancement of the CL by biacetyl in the oxidation of ethanol, the CL signal of a 20% (v/v) solution of ethanol in water was compared with the CL signal of reaction mixtures of 20% (v/v) ethanol to which 0.5 mL of $1 \times 10^{-4}$ or $1 \times 10^{-3}$ M of a biacetyl solution was added instead of 0.5 mL of water.

Deaeration. To evaluate if the presence of oxygen from the air is important in the CL reaction, the standard acidic ethanol reaction mixture was deaerated for 1 min by bubbling helium through the solution. The He (1 psi) was brought into the photometer with tubing attached to a Pasteur pipet. The pipet was lowered to the bottom of the sample cell. After deaeration the tubing was raised and left suspended about 1/2 inch above the reaction mixture and the oxidant was immediately injected. Calibration curves were obtained over the 3.0 to 100% (v/v) ethanol range with and without deaeration.

Cyclobutanol study

The CL oxidation of cyclobutanol was briefly studied and compared to the reaction with ethanol as the substrate. The reaction was run with 2.5% (v/v) cyclobutanol with and without Mn(II) added to the reaction mixture before injection of KMnO$_4$. 
Ethanol determination in gin

Three different brands of gin (Gordons Distilled Dry Gin, Tanqueray Distilled English Gin and Beefeater Distilled Dry Gin) were purchased at a local store in October 1984 and the ethanol content in these liquors was determined by CL. Each sample was analyzed by means of the bracketing technique (the concentration in the unknown is between two standards), a three point calibration curve, and standard additions. The data for the first two techniques were obtained with standards of concentrations 35.0, 40.0, 45.0, and 50.0% (v/v). Samples were analyzed without dilution.

For standard additions, it was necessary to work at lower ethanol concentrations where the calibration curve is linear with a zero intercept (0.3 - 10.0% (v/v)). All samples were diluted so that the concentration of EtOH in the samples would be approximately 2.0% (v/v). Table IX shows the initial ethanol concentration calculated from alcohol proof written on the label and the dilution factor and procedure used for each type of gin. To carry out three different standard additions, 5 mL portions of each one of the diluted samples were pipeted into three different beakers. Next 0.1, 0.2, and 0.3 mL of a 100% (v/v) ethanol solution were added to each beaker, respectively. The additions increase the effective concentration of alcohol in the sample by approximately factors of 2, 3, and 4, respectively, as shown in Table X.
Table IX. Dilution of Gin Samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial EtOH Conc. (% (v/v))</th>
<th>Dilution Factor</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gordon's</td>
<td>40.0</td>
<td>1:40</td>
<td>5.0 mL of gin diluted to volume in a 100 mL volumetric flask</td>
</tr>
<tr>
<td>Tanqueray</td>
<td>47.3</td>
<td>1:23.25</td>
<td>4.3 mL of gin diluted to volume in a 100 mL volumetric flask</td>
</tr>
<tr>
<td>Beefeater</td>
<td>47.0</td>
<td>1:23.25</td>
<td>Same as Tanqueray gin</td>
</tr>
</tbody>
</table>

Table X. Effective Concentrations of Ethanol Before and After Standard Addition

<table>
<thead>
<tr>
<th>Sample</th>
<th>Volume of 100% (v/v) Ethanol Added (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Gordons</td>
<td>2.00</td>
</tr>
<tr>
<td>Tanqueray</td>
<td>2.034</td>
</tr>
<tr>
<td>Beefeater</td>
<td>2.021</td>
</tr>
</tbody>
</table>

a in % (v/v) with correction for dilution

Interference study

Because glucose was considered a potential interferent in the determination of alcohol, the CL signals from the pure ethanol solutions and ethanol solutions containing glucose were compared. The test solutions were 45% (v/v) ethanol with 6% (v/v) glucose, and 10% (v/v)
ethanol with glucose concentrations of 0.01, 0.02, 0.05, and 0.1% (w/v). The optimization of reagent concentrations and the calibration curve characteristics for the CL determination of glucose were also briefly studied.
RESULTS AND DISCUSSION

Preliminary Studies

Coffee

This research began by confirming that basic coffee solutions chemiluminesce when oxidized by H₂O₂ as noted by Marino. A typical CL peak is shown in Figure 14. A filter cut-off frequency of 1.0 Hz was chosen for these studies as lower cut-off frequencies attenuate the CL signal significantly.

Scouting experiments were conducted to determine the effect of the oxidant (KMnO₄ vs. H₂O₂) and the concentration of the reagents (oxidant and KOH) on the CL reaction. Figure 15 shows that coffee chemiluminesces in highly basic solutions even without an oxidant. The largest CL signal with or without H₂O₂ is obtained with a KOH concentration of about 4 M. Using this KOH concentration, the effect of the type and concentration of oxidant was studied and the results are given in Figure 16. At this point, potassium permanganate was chosen as the oxidant for further studies because it gave higher intensities than H₂O₂ and also produced very low or negligible background signals. The peak shape and duration are similar to that observed at high pH (see Figure 14).

It was suspected that the CL might arise during the oxidation of phenols in the coffee. Chemiluminescence from oxidation of phenols such as gallic acid and pyrogallol in basic solutions with H₂O₂ is documented in the literature. Coffee is known to contain a large variety of
Figure 14. Typical CL peak for coffee.

Reaction mixture: 1 mL of 50 mg/L Colombian coffee, 0.5 mL of $1.0 \times 10^{-5}$ M $\text{H}_2\text{O}_2$, 0.5 mL of 4 M KOH (injected).
Figure 15. Dependence of CL signal for coffee on KOH concentration.

Reaction mixture: 1 mL of 0.5% (w/v) MJB instant coffee, 0.5 mL KOH (injected).
Figure 16. Dependence of CL intensity for coffee on oxidant concentration.

* KMnO₄, □ H₂O₂.

Reaction mixture: 1 mL of 0.5% (w/v) coffee, 0.5 mL of oxidant, 0.5 mL of 4 M KOH (injected).
phenols and polyphenols which all together amount to about 8.0% of its total composition. Figure 17 illustrates some of these compounds and their abundance in coffee.

At this point caffeine free instant coffee was also tested and found to yield approximately the same CL signal as regular instant coffee. Thus caffeine, a compound which does not contain -OH, is not primarily responsible for the CL observed from coffee.

Model compounds

To obtain more information about what types of compounds chemiluminesce when oxidized by KMnO₄, solutions of a number of model compounds were tested. These included species known to be in coffee such as phenols (see figure 17) and some other compounds that do not contain an -OH group (e.g., caffeine, DL-alanine, glutamic acid, oxalic acid). Additionally, solutions of other phenols, aliphatic alcohols, aldehydes, ketones, acids and amines were tested. The first studies were conducted with a small group of model compounds (e.g., catechol, pyrogallol, hydroquinone, tannic acid, gallic acid, DL-malic acid and a few more). With 0.5 mL of 4 M KOH in the reaction mixture, no CL signal was observed. However, without KOH, a small CL signal was recorded. The pH of the test solution was in the range of 4.0 to 5.0. When organic acids (e.g., citric acid, quinic acid) or mineral acids (e.g., nitric acid, hydrochloric acid) were added to the reaction mixture, the CL signal increased.

Because of these results, the effect of the pH of the sample solution on the CL signal was studied in more detail. The results for
Figure 17. Phenols, diphenols, and triphenols in roasted coffees. The numbers indicate the typical concentration of the compound in µg/g. a) arabusta; b) robusta; c) arabica. From reference 88.
the initial set of model compounds is shown in Figure 18. Note that the maximum CL intensity for all the tested compounds is observed for a sample pH in the 0.5 to 2.5 range. No CL signal was observed for a sample pH in the 6 to 11 range. The pH yielding maximum CL intensity varies from sample to sample.

For convenience in further studies, the pH of the reaction mixture was adjusted by mixing 1.0 mL of the test solution (pH not adjusted) with 0.5 mL of 50% (w/v) HNO₃. In this case, the added acid controls the pH of the reaction mixture to a calculated value of -0.2.

Table XI shows the model compounds tested, and their respective analytical signals and approximate detection limits. Most phenolic compounds exhibited CL and the CL signals are generally greater than those for other types of compounds. The CL intensity is greater for polyphenols than that for phenol. The results indicate that electron donating substituents in the aromatic ring (e.g., -OH, -OCH₃, -CH₃) of phenols enhance the CL intensity, whereas electron withdrawing substituents (e.g., -NO₂) decrease the CL emission signal. Note that the nitro group in either the ortho or para positions inhibits the reaction completely.

The CL signal appears to be correlated to the relative ease of oxidation of the phenol. As shown from the half-wave potentials in Table XII, the polyphenols are more readily oxidized than phenol. From these data, it is expected that the rate of oxidation follows the following trend: hydroquinones > pyrocatechols > resorcinols > phenols. It is interesting to notice that the CL intensity decreases in the above order although the CL signals for catechol and resorcinol are approximately equal. The oxidation rate for phenols and hydroquinones
Figure 18. Dependence of CL intensity on pH for some model compounds.

* 4-methyl catechol (100 mg/L)  • DL malic acid (0.5% (w/v))  ▲ 3,4 dihydroxybenzoic acid (0.1% (w/v))
□ 1,2,4 trihydroxybenzene (40 mg/L)  ○ catechol (0.1% (w/v))
<table>
<thead>
<tr>
<th>Compounds</th>
<th>Conc.(^b) (mg/L)</th>
<th>Structure</th>
<th>CL signal (mV)</th>
<th>DL(^c) (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenols</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenol</td>
<td>120</td>
<td><img src="image" alt="PhenolStructure" /></td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Guaiacol</td>
<td>55</td>
<td><img src="image" alt="GuaiacolStructure" /></td>
<td>66</td>
<td>---</td>
</tr>
<tr>
<td>Catechol</td>
<td>50</td>
<td><img src="image" alt="CatecholStructure" /></td>
<td>86</td>
<td>1</td>
</tr>
<tr>
<td>Resorcinol</td>
<td>50</td>
<td><img src="image" alt="ResorcinolStructure" /></td>
<td>89</td>
<td>1</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>50</td>
<td><img src="image" alt="HydroquinoneStructure" /></td>
<td>137</td>
<td>10</td>
</tr>
<tr>
<td>2,4-dinitrophenol</td>
<td>100</td>
<td><img src="image" alt="2,4-dinitrophenolStructure" /></td>
<td>ND</td>
<td>---</td>
</tr>
<tr>
<td>2,4,6-trinitrophenol</td>
<td>100</td>
<td><img src="image" alt="2,4,6-trinitrophenolStructure" /></td>
<td>ND</td>
<td>---</td>
</tr>
<tr>
<td>Compound</td>
<td>Signal 1</td>
<td>Signal 2</td>
<td>Signal 3</td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td></td>
</tr>
<tr>
<td>2-aminophenol</td>
<td>80</td>
<td>52</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>4-methyl catechol</td>
<td>50</td>
<td>245</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>50</td>
<td>30</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3,4-dihydroxybenzoic acid</td>
<td>50</td>
<td>88</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>500</td>
<td>94</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Pyrogallol</td>
<td>500</td>
<td>76</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Gallic acid</td>
<td>500</td>
<td>70</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>1,2,4-trihydroxybenzene</td>
<td>40</td>
<td>186</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>
Table XI. CL Signals for Model Compounds (continued)

<table>
<thead>
<tr>
<th>Compound</th>
<th>CL Signal</th>
<th>Structure</th>
<th>Relative Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3-dihydroxynaphthalene</td>
<td>100</td>
<td><img src="image" alt="Structure" /></td>
<td>176</td>
</tr>
<tr>
<td>Purpurogallinquinone</td>
<td>2 x 10^3</td>
<td><img src="image" alt="Structure" /></td>
<td>266</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Aliphatic Alcohols</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>99%</td>
<td>CH\textsubscript{3}OH</td>
<td>1.5</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Absolute</td>
<td>C\textsubscript{2}H\textsubscript{5}OH</td>
<td>24</td>
</tr>
<tr>
<td>Propanol</td>
<td>98%</td>
<td>C\textsubscript{3}H\textsubscript{7}OH</td>
<td>28</td>
</tr>
<tr>
<td>2-propanol</td>
<td>98%</td>
<td>CH\textsubscript{3}-CH-CH\textsubscript{3}</td>
<td>33</td>
</tr>
<tr>
<td>Sec-butanol</td>
<td>99%</td>
<td>C\textsubscript{2}H\textsubscript{5}CH-OH</td>
<td>232</td>
</tr>
<tr>
<td>Glycol</td>
<td>99%</td>
<td>CH\textsubscript{3}-CH-CH-CH\textsubscript{3}</td>
<td>12</td>
</tr>
</tbody>
</table>
Table XI. CL Signals for Model Compounds (continued)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>Chemical Structure</th>
<th>CL Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>t-butanol</td>
<td>99.5%</td>
<td>![t-butanol structure]</td>
<td>ND</td>
</tr>
<tr>
<td>Cyclohexanol</td>
<td>99%</td>
<td>![cyclohexanol structure]</td>
<td>45</td>
</tr>
</tbody>
</table>

**Aldehydes and Ketones**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>Chemical Structure</th>
<th>CL Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formaldehyde</td>
<td>37%</td>
<td>![formaldehyde structure]</td>
<td>19</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>99%</td>
<td>![acetaldehyde structure]</td>
<td>872</td>
</tr>
<tr>
<td>Acetone</td>
<td>99.9%</td>
<td>![acetone structure]</td>
<td>ND</td>
</tr>
</tbody>
</table>

**Acids**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>Chemical Structure</th>
<th>CL Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>DL-Malic acid</td>
<td>0.5% (w/v)</td>
<td>![DL-Malic acid structure]</td>
<td>19</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (w/v)</th>
<th>Structure</th>
<th>Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinic acid</td>
<td>0.5% (w/v)</td>
<td><img src="image" alt="Structure of Quinic Acid" /></td>
<td>4</td>
</tr>
<tr>
<td>Tartaric acid</td>
<td>0.5% (w/v)</td>
<td><img src="image" alt="Structure of Tartaric Acid" /></td>
<td>0</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>Glacial</td>
<td><img src="image" alt="Structure of Acetic Acid" /></td>
<td>0</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.1% (w/v)</td>
<td><img src="image" alt="Structure of Citric Acid" /></td>
<td>ND</td>
</tr>
<tr>
<td>Oxalic acid</td>
<td>0.5% (w/v)</td>
<td><img src="image" alt="Structure of Oxalic Acid" /></td>
<td>ND</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>0.5% (w/v)</td>
<td><img src="image" alt="Structure of Glutamic Acid" /></td>
<td>ND</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>0.5% (w/v)</td>
<td><img src="image" alt="Structure of Benzoic Acid" /></td>
<td>ND</td>
</tr>
</tbody>
</table>
Table XI. CL Signals for Model Compounds (continued)

<table>
<thead>
<tr>
<th>Other Compounds</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzyl alcohol</td>
<td>99%</td>
<td>![Chemical Structure]</td>
<td>16</td>
</tr>
<tr>
<td>Benzene</td>
<td>99%</td>
<td>![Chemical Structure]</td>
<td>ND</td>
</tr>
<tr>
<td>Aniline</td>
<td>99%</td>
<td>![Chemical Structure]</td>
<td>ND</td>
</tr>
<tr>
<td>DL-Alanine</td>
<td>100%</td>
<td>CH₃-CH(NH₂)-COOH</td>
<td>ND</td>
</tr>
<tr>
<td>Mercaptoethane</td>
<td>2.0%</td>
<td>C₂H₅SH</td>
<td>180</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other Samples</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Humic acid</td>
<td>50</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Dominican Rum</td>
<td>620</td>
<td>1:100&lt;sub&gt;d&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>Lipton Tea</td>
<td>92</td>
<td>1:100&lt;sub&gt;d&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>Beer</td>
<td>820</td>
<td>1:100&lt;sub&gt;d&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>Wine</td>
<td>96</td>
<td>1:100&lt;sub&gt;d&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>Coffee</td>
<td>0.5% (w/v)</td>
<td>284</td>
<td>50 mg/L</td>
</tr>
</tbody>
</table>
ND = no detected signal.

a Reaction mixture: 1 mL of sample, 0.5 mL of 50% (w/v) HNO$_3$, 0.81 mL of 1.0 x 10$^{-3}$ M KMnO$_4$

b Unless otherwise stated.

c Defined as minimum concentration tested that gave a detectable signal.

d Maximum dilution of sample for which CL signal is detected.
increases with the number of alkyl substituents. Note that the CL signal for 4-methyl catechol is about three times greater than that for catechol.

Table XII. Relative Oxidation Potentials.

<table>
<thead>
<tr>
<th>Phenol</th>
<th>$E_{1/2}^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>1004</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>560</td>
</tr>
<tr>
<td>Catechol</td>
<td>600</td>
</tr>
<tr>
<td>Resorcinol</td>
<td>800</td>
</tr>
</tbody>
</table>

$^a$ Half-wave potential vs. SCE in mV

Aliphatic alcohols also chemiluminesce when oxidized by potassium permanganate in highly acidic medium. In this case, a CL signal is observed only at a much higher concentration (typically 1.0% (w/v)) than the concentration for which CL is observed for polyphenols (down to ~1 mg/L). The peak width is also much greater than that for phenols because the reaction rate for oxidation in aliphatics is much slower. The CL signal for secondary alcohols is greater than that for primary alcohols. Tertiary alcohols do not chemiluminesce as they cannot be oxidized to ketones. The CL intensity appears to be correlated to the rate of oxidation (see Table III).

Aldehydes also chemiluminesce at relatively high concentrations and the peak width is in the order of seconds. Acetone does not chemiluminesce with 50.0% (w/v) acid; however, at higher acidities (95.0% (w/v) acid), it does weakly chemiluminesce.
Some acids with -OH groups (DL-malic and quinic) do chemiluminesce but tartaric acid and citric acid do not. Citric acid chemiluminesces only in the presence of Fe(II). Possibly the presence of Fe(II) increases the rate of oxidation. These four acids mentioned above are in coffee and thus do not contribute significantly to the CL observed from coffee.

No CL is observed from benzene, naphthalene, or alanine. However, CL is observed from mercaptoethane.

These preliminary results seem to indicate that any compound capable of being oxidized by potassium permanganate would emit light during the reaction. The CL signal is related to the rate of oxidation. Thus a larger CL signal of shorter duration is expected for compounds that are easily oxidized.

Other samples

Real samples, such as tea, coffee, beer, rum and wine gave relatively large CL signals. This would be expected since polyphenols, aside from aliphatic alcohols, are present in these samples. From Figures 19 and 20 it is observed that acidic conditions favor considerably the chemiluminescence reaction. However, for some samples (e.g., beer, wine) a small signal is recorded at basic pH.

Humic acid represents the exception to this work (see Figure 21). Chemiluminescence is much more efficient in basic solutions. Due to the complex structure of this group of compounds, it is hard to derive conclusions. However, the solubility of humic acid is much greater in basic solutions.
Figure 19. Dependence of CL intensity of some real samples on pH.

- □ 1:10 dilution wine
- △ 1:10 dilution beer
- ○ tea
- * 1:10 dilution dominican rum.
Figure 20. Dependence of CL signal of coffee on pH.

Reaction mixture: 2 mL of 0.5% (w/v) Colombian coffee, 0.5 mL of $1.0 \times 10^{-3}$ M KMnO$_4$ (injected).
Figure 21. Dependence of CL intensity of humic acid on pH.

Reaction mixture: 2 mL 500 mg/L humic acid, 0.5 mL $1.0 \times 10^{-3}$ M KMnO$_4$ (injected).
Pyrogallol Chemiluminescence

Initial studies

The CL reaction of pyrogallol, when oxidized by KMnO$_4$ in highly acidic medium, occurs very fast as illustrated in Figure 22. The peak shape is limited by the response time of the recorder. The insert in Figure 22 shows the peak shape when monitored by an oscilloscope when a 500 mg/L Py solution is analyzed by the CL technique. Here the rise time of the current-to-voltage converter was adjusted from 300 to 0.3 ms. Clearly the reaction time is on the order of milliseconds. The duration of the peak appears to be controlled by the time necessary to inject and mix the oxidant. Within the time resolution of the system, there appears to be no induction period as CL is observed immediately when the first part of MnO$_4^-$ solution enters the reaction cell.

Due to the short duration of the peak, spectral information is indeed difficult to gather. However, a crude CL spectrum was obtained by using cut-on filters as shown in Figure 23. Clearly most of the emission is at wavelengths above 600 nm, with a maximum between 680 and 700 nm. The decrease in observed intensity at higher wavelengths may be due to the decreasing responsivity of the PMT photocathode.

Absorption spectra

Figure 24 shows the spectra of 25 mg/L Py in neutral and acidic medium. Py has a strong absorption band below 250 nm and a weaker band
Figure 22. Typical CL peak for pyrogallol (Py).

Reaction mixture: 1 mL of Py, 0.5 mL of 50% (w/v) HNO$_3$, 0.800 mL of KMnO$_4$. For the insert, the Py concentration is 500 mg/L.
Figure 23. Dependence of the CL signal on cut-on wavelength for Py.

Reaction mixture: 1 mL of 500 mg/L Py, 0.5 mL of 50% (w/v) HNO₃, 1.0 x 10⁻² M KMnO₄ (injected).
Figure 24. Absorption spectrum for 25 mg/L Py.

--- Py in neutral solution vs dw

--- Py in HNO₃ vs dw
with a wavelength of maximum absorption ($\lambda_m$) at 266 nm. The spectrum of Py in 50% (w/v) nitric acid shows two absorption bands above 250 nm with $\lambda_m$'s of 270 and 300 nm. The band at 300 nm is due to HNO$_3$ absorption as shown by the spectrum of 1.0% (w/v) HNO$_3$ versus dw in Figure 25. This strong absorption of nitric acid makes it difficult to monitor the UV absorption of Py.

Figure 26 shows the spectrum of a 1:20 dilution of blank versus dw and the spectrum of a 1:20 dilution of reaction mixture versus dw. In the reaction mixture, the Py absorption band around 270 nm is not observed. This may be due to consumption of Py in the reaction and masking by the absorption of the acid. The HNO$_3$ bands (300 nm and below 240 nm) are observed, as expected, after the reaction, and the structured band near 550 nm (due to KMnO$_4$) disappears due to complete use of the oxidant by the substrate.

Fluorescence spectra

With 268 and 275 nm as excitation wavelengths, fluorescence emission maxima were observed at 335 and 625 nm with 500 mg/L of Py. In HNO$_3$, Py shows an emission maximum at 340 nm. No emission was observed for the reaction mixture, the blank, or a 25 mg/L Py solution with excitation wavelengths of 302, 350, 540, 600, 625 and 710 nm. Therefore fluorescence cannot be conveniently used to monitor the reaction.
Figure 25. Absorption spectrum for 1% (w/v) nitric acid vs dw.
Figure 26. Absorption spectra of Py CL reaction mixture and blank.

--- 1:20 dil blank vs. dw
--- 1:20 dil reaction mixture vs. dw

Reaction mixture: 1 mL of 500 mg/L Py, 0.5 mL of 50% (w/v) HNO₃, 0.8 mL of 1.0 x 10⁻² M KMnO₄ (injected).
Optimization

A simplex optimization procedure was performed to find the best reaction conditions. Table XIII shows the results. The first vertex is based on the final conditions finally used to test the model compounds. It is obvious that a large improvement (a factor of 18 increase in the CL signal) was obtained from this optimization. The KMnO₄ concentration is shown to be a critical variable. Based on these results, the final conditions for all further studies of Py CL were chosen to be 1 mL of Py, 0.5 mL of 55.0% (w/v) HNO₃, and 0.8 mL of 1 x 10⁻² M KMnO₄.

The dependence of CL signal on HNO₃ and KMnO₄ concentrations is shown in Figures 27 and 28. The test solution was 25 mg/L of Py. Note that the best conditions are different than those found by simplex optimization which was conducted with a much higher Py concentration. It is clear that 10% (w/v) HNO₃ and 5.0 x 10⁻⁴ M KMnO₄ yield the highest CL signals.

It is obvious that the optimum conditions vary with Py concentration (e.g., 500 mg/L vs. 25 mg/L). This is expected since at higher substrate concentrations more KMnO₄ is needed. The mole ratio of permanganate to Py in the reaction mixture with 1 x 10⁻² M KMnO₄ for these two cases is 2.02 and 40.5, respectively. Note also that there is some overlap between the CL emission and absorption spectrum of permanganate. For lower Py concentrations, the permanganate is in great excess such that most of it remains after the reaction. The remaining permanganate may reduce the CL signal because of absorption of the CL photons.
Table XIII. Movement of Seven-Dimensional Simplex for Pyrogallol (Py)\(^a\)

<table>
<thead>
<tr>
<th>Vertex No.</th>
<th>Vertices retained from previous simplex(^b)</th>
<th>Variables</th>
<th>CL signal (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 [\text{[KMnO}_4]} (M)</td>
<td>2 vol. [\text{KMnO}_4] (mL)</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>1.0 x 10(^{-3})</td>
<td>0.5000</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>1.0 x 10(^{-2})</td>
<td>0.6000</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>3.0 x 10(^{-3})</td>
<td>0.7446</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>3.0 x 10(^{-3})</td>
<td>0.6000</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>3.0 x 10(^{-3})</td>
<td>0.6000</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>3.0 x 10(^{-3})</td>
<td>0.6000</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>3.0 x 10(^{-3})</td>
<td>0.6000</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>3.0 x 10(^{-3})</td>
<td>0.6000</td>
</tr>
<tr>
<td>9</td>
<td>2,3,4,5,6,7,8</td>
<td>7.0 x 10(^{-3})</td>
<td>0.7419</td>
</tr>
<tr>
<td>10</td>
<td>2,3,4,6,7,8,9</td>
<td>6.0 x 10(^{-3})</td>
<td>0.6845</td>
</tr>
<tr>
<td>11</td>
<td>2,3,6,7,8,9,10</td>
<td>7.0 x 10(^{-3})</td>
<td>0.7035</td>
</tr>
<tr>
<td>12</td>
<td>2,3,7,8,9,10,11</td>
<td>8.0 x 10(^{-3})</td>
<td>0.7367</td>
</tr>
<tr>
<td>13</td>
<td>Pr &gt; B so P exp. 2 Pr &lt;c</td>
<td>1.0 x 10(^{-2})</td>
<td>0.8000</td>
</tr>
<tr>
<td>14</td>
<td>2,3,7,9,10,11,13</td>
<td>1.0 x 10(^{-2})</td>
<td>0.7927</td>
</tr>
<tr>
<td>15</td>
<td>Pr &gt; B so P exp.</td>
<td>1.3 x 10(^{-2})</td>
<td>0.889</td>
</tr>
<tr>
<td>16</td>
<td>2,3,9,10,11,13,14</td>
<td>1.2 x 10(^{-2})</td>
<td>0.8478</td>
</tr>
</tbody>
</table>
Table XIII. Movement of Seven-Dimensional Simplex for Pyrogallol (Py) (continued)

a Values on this table are reported as values used and not as calculated.
b Volumes delivered by automatic syringe. Amounts calculated by weight of water.
c Only 1 and 5 Hz available as minimum filtering. If the simplex value was higher than 2.5 Hz, it was taken as 5 Hz. If less than or equal to 2.5 Hz, it was taken as 1 Hz.
d boundary violation

e Pr is reflection of worst point, B is the best response for all the vertices, P_{exp} is expansion of last reflected point.
Figure 27. Dependence of CL intensity of Py on nitric acid concentration.

Reaction mixture: 1 mL of 25 mg/L Py, 0.5 mL of HNO₃, 0.8 mL 1.0 x 10⁻³ M KMnO₄ (injected).
Figure 28. Dependence of CL intensity of Py on concentration of KMnO₄.

Reaction mixture: 1 mL of 25 mg/L Py, 0.5 mL of 10% (w/v) HNO₃, 0.8 mL of KMnO₄ (injected).
Effect of cations

The effect of adding several cations to the reaction mixture is shown in Table XIV. The cations tested have some effect on the CL signal. Some of the metal ion concentrations tested are higher than expected in most real samples. With 150 mg/L Py, Mn\(^{2+}\) enhances the CL signal while Fe\(^{2+}\) depresses the signal. With 7.5 mg/L Py, Mn\(^{2+}\) depresses the signal. Co\(^{2+}\) depresses the signal by a factor of 4. These cations probably affect the CL reaction by changing the concentrations and rates of production and disappearance of intermediate oxidation states of Mn. Further studies are required to understand these effects.

Table XIV. Effect of cations on the CL signal for the reaction of Py

<table>
<thead>
<tr>
<th>Added species</th>
<th>Conc. of added species (M)</th>
<th>Py conc. (mg/L)</th>
<th>CL signal (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>—</td>
<td>500</td>
<td>891</td>
</tr>
<tr>
<td>none</td>
<td>—</td>
<td>150</td>
<td>261</td>
</tr>
<tr>
<td>none</td>
<td>—</td>
<td>7.5</td>
<td>10.5</td>
</tr>
<tr>
<td>Co(^{2+}) a</td>
<td>0.0002</td>
<td>500</td>
<td>198</td>
</tr>
<tr>
<td>Mn(^{2+}) b</td>
<td>0.01</td>
<td>150</td>
<td>369</td>
</tr>
<tr>
<td>Mn(^{2+}) b</td>
<td>0.01</td>
<td>7.5</td>
<td>8.8</td>
</tr>
<tr>
<td>Fe(^{2+}) b</td>
<td>0.01</td>
<td>150</td>
<td>241</td>
</tr>
</tbody>
</table>

a 0.5 mL added
b 100 µL added

Reaction mixture: 1.0 mL of Py, 0.5 mL of 50% (w/v) H\(_2\)NO\(_3\), and 1.0 x 10\(^{-2}\) M KMnO\(_4\).
Other oxidants

The oxidants, dichromate, peroxide, ferricyanate were injected instead of MnO₄⁻ and no CL was observed at any concentration of Py. Since the following reaction scheme is known to occur43,90,

\[
\begin{align*}
\text{Cr(VI) + Substrate} & \rightarrow \text{Cr(IV) + Product} \\
\text{Cr(IV) + Mn(II)} & \rightarrow \text{Mn(III) + Cr(III)} \\
2 \text{Mn(III)} & \rightarrow \text{Mn(II) + MnO₂}
\end{align*}
\]

100 μL of a 50% (w/v) solution of Mn(II) were added to the reaction mixture before injection of dichromate. No CL was observed. This would suggest that the CL pathway does not include oxidation of the Py by Mn(III).

Determination of Py in hair dye

In this study and other studies reported later in this section, the analyte concentration in samples was found by using calibration curves for all analytical techniques except HPLC. Where appropriate, the data were fit by a linear least-squares fitting procedure. Often for such fitting procedures, the variance in the y value (signal) is assumed to be constant or independent of the analyte concentration. In analytical chemistry, this assumption is not always valid;91-94 unless the concentration range is very small, the variance often varies with analyte concentration. Use of an unweighted least-squares procedure for such data may lead to improper fitting of the data.

Because of the above, both non-weighted (constant variance) and weighted (non-constant variance) were applied to calibration curve
data. The weighting factors were based on the standard deviation from repetitive measurements on each standard solution.

The BASIC program for fitting the data and statistical analysis is listed in Appendix II. It calculates the best fit slope and intercept, the standard error, the analyte concentration in the sample and a confidence interval chosen by the operator (95% in this case) for the sample analyte concentration. In addition, confidence bands for the calibration curves are found.92

The results for the determination of Py in hair dye by the spectrophotometric and CL procedures are summarized in Tables XV and XVI. The fitted calibration curves and confidence bands are shown in Figures 29 through 32. The difference between the weighted and unweighted values fits is mainly the shape of the confidence bands.

For the spectrophotometric determination, the Py concentration determined from the 5 to 100 dilution is in error because the absorbance is significantly below that of the lowest standard and is in the nonlinear region of the calibration curve. The Py value obtained with the 25.5 to 100 dilution is taken as more valid. Even here, the sample absorbance is in a region where some nonlinearity is obvious. If the Py concentration is determined from a line drawn between the two lowest concentration standards, a value of 0.065% (w/v) is obtained.

Analysis of the extracted hair dye by the CL technique yielded different results depending on the degree of dilution as shown in Table XVI. This would suggest that an interferent is present and its effect on the CL signal is not proportional to its concentration. The CL signal for the 1:5 dilution is below that of the lowest standard. The value determined from the 3:10 dilution (0.056% (w/v)) is
Table XV. Spectrophotometric Determination of Py

<table>
<thead>
<tr>
<th>Standard Py conc. (mg/L)</th>
<th>Mean absorbance and SD</th>
<th>Conc. in test solution&lt;sup&gt;a&lt;/sup&gt; (µg/mL)</th>
<th>Conc. in hair dye&lt;sup&gt;a&lt;/sup&gt; (% (w/v))</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>0.0926 (0.0011)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td>0.197 (0.0011)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.0</td>
<td>0.291 (0.0008)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.0</td>
<td>0.381 (0.0016)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sample dilution<sup>b</sup>

<table>
<thead>
<tr>
<th>Sample dilution</th>
<th>Mean absorbance and SD</th>
<th>1.05</th>
<th>0.063 ± 0.088</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 to 100</td>
<td>0.0236 (0.00017)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.11</td>
<td>0.066 ± 0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26.5 to 100</td>
<td>0.109 (0.00013)</td>
<td>5.56</td>
<td>0.063 ± 0.012</td>
</tr>
<tr>
<td>5.58</td>
<td>0.063 ± 0.018</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The first value is from unweighted data; the second is from weighted data; the error interval is based on a 95% confidence level

<sup>b</sup> Dilution of aqueous solution after ether extraction and evaporation.
Table XVI. CL Determination of Py

<table>
<thead>
<tr>
<th>Standard Py conc. (mg/L)</th>
<th>Mean absorbance and SD</th>
<th>Conc. in test solution(^a) (µg/mL)</th>
<th>Conc. in hair dye(^a) (% w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>8.57 (0.15)</td>
<td>10.0</td>
<td>16.0 (0.20)</td>
</tr>
<tr>
<td>10.0</td>
<td></td>
<td>20.0</td>
<td>31.2 (0.74)</td>
</tr>
<tr>
<td>20.0</td>
<td></td>
<td>40.0</td>
<td>61.7 (0.80)</td>
</tr>
<tr>
<td>40.0</td>
<td></td>
<td>40.0</td>
<td>61.7 (0.80)</td>
</tr>
</tbody>
</table>

Sample dilution\(^b\)

<table>
<thead>
<tr>
<th>Sample dilution</th>
<th>Mean absorbance and SD</th>
<th>Conc. in test solution</th>
<th>Conc. in hair dye</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>50.4 (1.5)</td>
<td>32.6</td>
<td>0.098 ± 0.0006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32.7</td>
<td>0.098 ± 0.0058</td>
</tr>
<tr>
<td>1:5</td>
<td>6.00 (0.35)</td>
<td>3.37</td>
<td>0.050 ± 0.0069</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.33</td>
<td>0.050 ± 0.0069</td>
</tr>
<tr>
<td>3:10</td>
<td>9.37 (0.35)</td>
<td>5.58</td>
<td>0.056 ± 0.0024</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.56</td>
<td>0.056 ± 0.0046</td>
</tr>
</tbody>
</table>

\(^a\) The first value is from unweighted data; the second is from weighted data; the error interval is based on the 95% confidence level.

\(^b\) Dilution of aqueous solution after ether extraction and evaporation.

Reaction mixture: 1 mL of Py or sample, 0.5 mL of 50% (w/v) HNO\(_3\), 0.8 mL of 1.0 \times 10^{-2} M KMnO\(_4\).
Figure 29. Unweighted calibration curve and confidence bands for the determination of Py by spectrophotometry.

- Experimental points. Equation of the line: $Y = 1.92X + 0.0096$. Standard error = $2.8 \times 10^{-5}$. 
Figure 30. Weighted calibration curve and confidence bands for the determination of Py by spectrophotometry.

- Experimental points. Equation of the line: $Y = 1.93X - 0.0048$. 

PYROGALLOL CONC. (mg/L)
Figure 31. Unweighted calibration curve and confidence bands for the determination of Py by CL.

Experimental points. Equation of the line: \( Y = 1.52X + 0.880 \). Standard error = \( 8.8 \times 10^{-3} \).
Figure 32. Weighted calibration curve and confidence bands for the determination of Py by CL.

- Experimental points. Equation of the line: \( Y = 1.51X + 0.957 \). Standard error = 0.18.
considered more valid.

It is difficult to evaluate the accuracy of the CL procedure. The value of Py in the hair dye is not specified or available from the manufacturer. The spectrophotometer method could be in error due to other species in the hair dye that absorb or react with the reagents to form a compound that absorbs at the monitored wavelengths. The spectrophotometer value for Py from the 26.5:100 dilution and the CL value from the 3:10 dilution differ by 12% with weighted fitting and 11% with unweighted fitting. The t statistic was used to evaluate the difference between the Py concentration determined by the two techniques. The difference is significant at the 95% confidence level.

The linearity of the CL procedure is excellent over the 5 to 40 mg/L Py range. Reasonable linearity extends up to 150 mg/L Py. The detection limit is 0.7 mg/L.

BHA and Propyl Gallate Chemiluminescence

Optimization

The dependence of the CL signal of PG and BHA on HNO₃ and KMnO₄ concentration is shown in Figures 33 and 34. From these data, the reagent concentrations for determination of both PG and BHA were chosen to be 50.0% (w/v) nitric acid and 1.0 x 10⁻³ M potassium permanganate.

The efficiency of the ethanol extraction of PG from peanut oil was evaluated and the data are shown in Table XVII. These results indicate that the PG extraction was greater than 99.9% efficient and that other
Figure 33. Dependence of antioxidant CL signals on acid concentration. ○ PG, * BHA

Reaction mixture: 1 mL of 500 mg/L PG or 1 mL of 100 mg/L BHA, 0.5 mL of HNO₃, 0.8 mL of 1.0 x 10⁻² M KMnO₄ (injected).
Figure 34. Dependence of antioxidant CL signals on KMnO₄ concentration. * PG, ○ BHA

Reaction mixture: 1 mL of 500 mg/L PG or 1 mL of 100 mg/L BHA, 0.5 mL of 50% (w/v) HNO₃, 0.8 mL of KMnO₄ (injected).
species in pure peanut oil that are extracted do not produce a blank CL signal. No PG was detected in the peanut oil sample. It appears that this extraction procedure and CL analysis could be used to detect PG in peanut oil.

Table XVII. Determination of PG in Peanut Oil

<table>
<thead>
<tr>
<th>Sample</th>
<th>Signal Type</th>
<th>CL Signal (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtOH 50% (v/v)</td>
<td>Blank A (B1kA)</td>
<td>0.056</td>
</tr>
<tr>
<td>500 mg/L PGA in 50% (v/v) EtOH</td>
<td>Standard (Std)</td>
<td>1.200</td>
</tr>
<tr>
<td>Extract from peanut oil</td>
<td>Blank B (B1kB)</td>
<td>0.032</td>
</tr>
<tr>
<td>Extract from peanut oil Spiked</td>
<td>Sample (Smp)</td>
<td>1.180</td>
</tr>
<tr>
<td>to 500 mg/L PG</td>
<td>Net Std Signal</td>
<td>1.149</td>
</tr>
<tr>
<td></td>
<td>(Std-B1kA)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Net Smp Signal</td>
<td>1.148</td>
</tr>
<tr>
<td></td>
<td>(Smp-B1kB)</td>
<td></td>
</tr>
</tbody>
</table>

Determination of PG and BHA in lard

HPLC analysis. Table XVIII shows the results found by HPLC analysis of lard for PG and BHA. The amount of PG determined is within 2.7% of the expected value (0.0012% (w/w)) and the amount of BHA is within 2.1% of the expected value (0.002% (w/v)). These expected results were obtained from direct contact with the Armour and Company in Phoenix, Arizona.
Table XVIII. Determination of PG and BHA in Lard by HPLC.

<table>
<thead>
<tr>
<th></th>
<th>Solvent blank</th>
<th>Extraction blank</th>
<th>Sample</th>
<th>Corrected Std.</th>
<th>Corrected sample</th>
<th>Conc. in Lard (%) (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG</td>
<td>17.6 (0.3)</td>
<td>0.1</td>
<td>0.069 (0.009)</td>
<td>5.2 (3.0)</td>
<td>17.5</td>
<td>5.10</td>
</tr>
<tr>
<td>BHA</td>
<td>4.84 (0.2)</td>
<td>0.0</td>
<td>0.070 (0.01)</td>
<td>2.38 (0.4)</td>
<td>4.84</td>
<td>2.37</td>
</tr>
</tbody>
</table>

a area of peak; standard deviation in parenthesis

b For PG, \((5.10/17.5) \times (10^{-2}/(100 \times 2.500))\)

For BHA, \((2.37/4.84) \times (10^{-2}/(100 \times 2.500))\)
Chemiluminescence analysis. For the BHA extraction, two solvents, acetonitrile and methanol, were used to extract BHA from the hexane layer. Since acetonitrile was found to quench the CL reaction, an attempt was made to evaporate completely this solvent by extending the heating time in the rotary evaporator. The yellowish solid obtained was dissolved in 20% acetone for the final CL analysis. This sample gave no CL signal. Therefore the sample extracted with methanol was used for all further studies.

The aqueous phase remaining after extraction of PG with ether was also analyzed by CL. No signal was recorded. This indicates a complete recovery of the PG from the aqueous phase.

The results of the CL analyses are summarized in Tables XIX and XX and calibration curves are shown in Figures 35 through 38. For the CL technique, the value determined for PG is 42% higher than the expected value (0.0012% w/v) and 44% higher than the value determined by HPLC for the determination based on a non-weighted fit. For the weighted calibration data, the values are 45% and 49% higher, respectively. Because the concentration of PG determined by the CL technique is so much higher than the expected one or the value determined by HPLC, an interferent is probably present.

The value determined for BHA by CL is close to the expected value (0.002% w/v), within 4% for the determination based on non-weighted data and within 2.5% for a weighted calibration curve. The concentration found by CL is also 2% within the one found by HPLC for the non-weighted case and 0.4% from the HPLC value for the weighted case.
Table XIX. Determination of BHA in Lard by CL.

<table>
<thead>
<tr>
<th>Standard BHA conc. (mg/L)</th>
<th>Mean CL signal and SD (mV)</th>
<th>Conc. in test solution (µg/mL)</th>
<th>Conc. in lard (% (w/w))</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>7.43 (0.61)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>13.8 (0.40)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>26.2 (0.17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>53.9 (0.48)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>108.8 (1.3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Sample Dilutionb          | 78.3 (0.33)               | 57.7                           | 0.00192 ± 0.000050c     |
|                          | 78.3                      | 58.6                           | 0.00195 ± 0.00010       |
| 1:5                      | 14.6 (0.35)               | 10.8                           | 0.00180 ± 0.00023       |
|                          | 14.6                      | 11.2                           | 0.00186 ± 0.00039       |
| 1:10                     | 7.36 (0.12)               | 5.43                           | 0.00180 ± 0.00048       |
|                          | 7.36                      | 5.75                           | 0.00192 ± 0.00045       |

- a The first value is from unweighted data; the second is from weighted data.
- b Dilution of aqueous solution after methanol extraction and evaporation.
- c Confidence interval for 95% confidence level

For PG, linearity is reasonably good in the range 5 to 80 mg/L and up to 160 mg/L there is still reasonable linearity. The detection limit for this analyte is 0.3 mg/L. For BHA, linearity is excellent in the range of 5 mg/L to 80 mg/L. Again, reasonable linearity is observed up to 160 mg/L. The detection limit for this analyte is 0.2 mg/L.
Figure 35. Unweighted calibration curve and confidence bands for the determination of BHA by CL.

Equation of the line: $Y = 1.36X - 0.00295$. Standard error = 0.52.
Figure 36. Weighted calibration curve and confidence bands for the determination of BHA by CL.
Equation of the line: \( Y = 1.35X - 0.503 \). Standard error = 4.4.
Figure 37. Unweighted calibration curve and confidence bands for the determination of propyl gallate by CL. Equation of the line: \( Y = 1.10X - 2.37 \). Standard error = 1.7.
Figure 38. Weighted calibration curve and confidence bands for the determination of propyl gallate by CL. Equation of the line: \( Y = 1.06X - 1.40 \). Standard error = 1.3.
Table XX. Determination of PG in Lard by CL.

<table>
<thead>
<tr>
<th>Standard PG conc. (mg/L)</th>
<th>Mean CL signal and SD (mV)</th>
<th>Conc. in test solution ((\mu g/mL))</th>
<th>Conc. in lard (% (w/v))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4.11 (0.096)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>9.17 (0.17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>18.9 (0.19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>40.2 (0.93)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>86.7 (0.76)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sample</td>
<td>53.9 (0.51)</td>
<td>51.1 0.00170 ± 0.000099(^c)</td>
<td>0.00174 ± 0.00025</td>
</tr>
</tbody>
</table>

\(\text{a} \quad \text{The first value is from unweighted data; the second is from weighted data.}\)

\(\text{b} \quad \text{Dilution of aqueous solution after ether extraction and evaporation.}\)

\(\text{c} \quad \text{Confidence interval for 95% confidence level}\)

**Interference study**

Citric acid (CA) is expected in lard at about the 0.0006% (w/w) level. Table XXI summarizes the results of a study to determine the interference effect of this acid on the CL determination of PG. The CL signal from PG is too high by 51% when the PG and CA concentrations are about equal. This obviously indicates the synergic effect of this compound with PG. However, when the CA concentration is about one half of the PG concentration, as expected in the lard sample, the CL signal from PG is slightly depressed. Thus the interferent in lard for the PG determination may be some other species than CA.
Citric acid by itself does not chemiluminesce when oxidized by potassium permanganate in acidic medium unless Fe(II) is present. A calibration curve of citric acid with concentrations ranging from 10 mg/L to 1.0% (w/v) with the addition of 0.5 mL of 1.0 x 10^{-2} M Fe(II), was constructed. The detection limit is 10 mg/L and the linear range extended up to about 100 mg/L. The CL signal at 1000 mg/L was 7% of the value expected by linear extrapolation. Above this concentration, nonlinearity was severe.

Table XXI. Interference Study for PG

<table>
<thead>
<tr>
<th>Conc. (mg/L)</th>
<th>CL Signal (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG</td>
<td>CA</td>
</tr>
<tr>
<td>36</td>
<td>0</td>
</tr>
<tr>
<td>36</td>
<td>18</td>
</tr>
<tr>
<td>36</td>
<td>40</td>
</tr>
</tbody>
</table>

Vanillin Chemiluminescence

Optimization

The dependence of the CL signal from vanillin on HNO₃ and KMnO₄ concentrations is shown in Figures 39 and 40. From these data, the working conditions for the vanillin assay were chosen to be 95.0% (w/v) nitric acid and 1.0 x 10^{-3} M potassium permanganate.
Figure 39. Dependence of CL intensity of vanillin on HNO$_3$ concentration.
Reaction mixture: 1 mL of 100 mg/L vanillin, 0.5 mL of HNO$_3$, 0.8 mL of $1.0 \times 10^{-2}$ M KMnO$_4$ (injected).
Figure 40. Dependence of CL intensity of vanillin on KMnO₄ concentration.

Reaction mixture: 1 mL of 100 mg/L vanillin, 0.5 mL of 95% (w/v) HNO₃, 0.8 mL of KMnO₄ (injected).
Determination of vanillin in vanilla extract

Tables XXII and XXIII summarize the results of the spectrophotometric and CL analyses of vanilla extract. Calibration curves are presented in Figures 41, 42, and 43.

**Table XXII. Spectrophotometric Determination of Vanillin in Vanilla Extract**

<table>
<thead>
<tr>
<th>Standard vanillin conc. (mg/L)</th>
<th>Mean absorbance and SD</th>
<th>Conc. in test solution$^a$ (mg/L)</th>
<th>Conc. in vanilla (% (w/v))</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.307 (0.0020)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.640 (0.0032)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.972 (0.0024)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Sample Dilution**

<table>
<thead>
<tr>
<th>Sample Dilution</th>
<th>Mean absorbance and SD</th>
<th>Conc. in test solution</th>
<th>Conc. in vanilla (% (w/v))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:500</td>
<td>0.597 (0.0028)</td>
<td>3.75</td>
<td>0.189 ± 0.0013$^b$</td>
</tr>
</tbody>
</table>

$^a$ Only the results for unweighted data are presented. The weighted least squares fit resulted in a calculation yielding the square root of a negative number, which is mathematically illegal.

$^b$ Confidence interval for 95% confidence level
Table XXIII. Chemiluminescence Determination of Vanillin in Vanilla

<table>
<thead>
<tr>
<th>Standard vanillin conc. (mg/L)</th>
<th>Mean CL signal and SD (mV)</th>
<th>Conc. in test solution(^a) (mg/L)</th>
<th>Conc. in vanilla (% (w/v))</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2.20 (0.35)</td>
<td>26.9</td>
<td>0.34 ± 0.041(^c)</td>
</tr>
<tr>
<td>10</td>
<td>4.87 (0.46)</td>
<td>25.7</td>
<td>0.32 ± 0.095</td>
</tr>
<tr>
<td>20</td>
<td>11.4 (0.32)</td>
<td>9.96</td>
<td>0.31 ± 0.12</td>
</tr>
<tr>
<td>40</td>
<td>26.8 (0.058)</td>
<td>9.45</td>
<td>0.30 ± 0.15</td>
</tr>
<tr>
<td>80</td>
<td>51.9 (0.70)</td>
<td>137</td>
<td>0.66 ± 0.19</td>
</tr>
</tbody>
</table>

**Sample Dilution**

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Mean CL signal and SD (mV)</th>
<th>Conc. in test solution (mg/L)</th>
<th>Conc. in vanilla (% (w/v))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:25(^b)</td>
<td>16.7 (0.46)</td>
<td>26.9</td>
<td>0.34 ± 0.041(^c)</td>
</tr>
<tr>
<td>2:125(^b)</td>
<td>5.37 (0.21)</td>
<td>9.96</td>
<td>0.31 ± 0.12</td>
</tr>
<tr>
<td>1:50</td>
<td>90.5 (2.3)</td>
<td>137</td>
<td>0.69 ± 0.042</td>
</tr>
<tr>
<td>1:250</td>
<td>18.3 (1.3)</td>
<td>29.3</td>
<td>0.73 ± 0.081</td>
</tr>
</tbody>
</table>

\(a\) The first value is from unweighted data; the second is from weighted data.

\(b\) Dilution of aqueous solution after ether extraction and evaporation

\(c\) Confidence interval for 95% confidence level

The expected concentration of vanillin in vanilla extract is 0.2% (w/v), according to reference 96. The spectrophotometric determination yields a value within 5.5% of the expected value. The closest value found by CL is that of the extracted vanilla with a 2:125 dilution. However, the value determined by the CL procedure is 65% higher than the concentration found by spectrophotometry. The
Figure 41. Unweighted calibration curve and confidence bands for the determination of vanillin by spectrophotometry. Equation of the line: $Y = 0.166X - 2.50$. Standard error = $2.2 \times 10^{-7}$. 
Figure 42. Unweighted calibration curve and confidence bands for the determination of vanillin by CL. Equation of the line: $Y = 0.672X - 1.40$. Standard error = 0.93.
Figure 43. Weighted calibration curve and confidence bands for the determination of vanillin by CL. Equation of the line: \( Y = 0.708X - 1.57 \). Standard error = 13.2.
weighted CL value is within 56% of the spectrophotometric value. The vanillin concentration determined by diluting the extracted sample by 1:25 is slightly higher. The values determined by the spectrophotometric and CL techniques are significantly different at the 95% confidence level (t-test).

The vanillin concentrations found by analyzing the sample without the ether extraction (1:50, 1:250) are about a factor of 2 higher than the concentrations found after extraction. Without extraction, the more dilution, the greater the difference from the value determined with the official technique. However, the CL signal for the 1:50 dilution is outside of the range of the calibration data.

From Table XXIII, it is interesting to observe that, for all dilutions, the values determined with a weighted calibration curve are smaller than those of the concentrations found by calibration curves assuming constant variance. For this determination, linearity only holds to about 20 mg/L vanillin. The detection limit for vanillin is 0.5 mg/L.

The value determined by CL (0.30% (w/v)) that is closest to the expected value is so high that an interferent is expected. Vanillic acid, which is also present in the vanilla extract, is a likely candidate. The structures of vanillin (a) and vanillic acid (b) are shown below.

![Vanillin and Vanillic Acid](image-url)
The concentration of vanillic acid expected in vanilla is 0.014 g/100 mL or about 7% of the vanillin concentration.96

Interference study

Table XXIV summarizes the results of the interference study for vanillic acid. Clearly vanillic acid causes a significant blank interference. However, it does not fully account for the error in the analysis of vanilla extract. The error expected when the vanillic acid concentration is 7% of the vanillin concentration is estimated to be about 10%.

Table XXIV. Interference Study for Vanillic Acid

<table>
<thead>
<tr>
<th>Conc. of vanillic acid (mg/L)</th>
<th>CL signal (b) (mV)</th>
<th>error (c) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10.6 (0.1)</td>
<td>---</td>
</tr>
<tr>
<td>2</td>
<td>12.5 (0.3)</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>14.8 (0.5)</td>
<td>40</td>
</tr>
<tr>
<td>10</td>
<td>21.7 (0.5)</td>
<td>105</td>
</tr>
</tbody>
</table>

\(a\) Concentration of vanillin was 16 mg/L for all solutions

\(b\) SD in parentheses

\(c\) Percent increase in CL signal relative to no vanillic acid.
Adjustment of the pH could be a very important factor in the extraction of vanillin. With low pH it would be expected that vanillic acid is also extracted. However, high pH would result in a partial extraction of vanillin into the ether phase since the molecule would be ionized and remain in the water phase. Possibly an intermediate pH could be found at which the vanillic acid is completely ionized and the vanillin is not ionized.

Ethanol Chemiluminescence

Optimization

To choose the optimum conditions for CL analysis, the effect of the type of acid was first studied and the results are summarized in Table XXV and Figure 44. No CL was observed with phosphoric or acetic acid. Apparently the pH of reaction mixture is too high with these acids for the reaction to proceed at a significant rate. Hydrochloric acid has the advantage of dissolving the MnO₂ formed during the reaction. However, the precision is not good. The detection limit with sulfuric acid is worse than with some other acids. Also the reaction rate is slow and there is a potential explosion hazard of this acid when combined with potassium permanganate. Perchloric acid has two disadvantages: an imprecise blank signal and a potential explosion hazard when mixed with organic compounds. Nitric acid was finally chosen as the best acid because it yields a relatively high CL
Figure 44. Effect of type of acid on CL intensity of ethanol.

- □ 50% sulfuric acid
- + 50% perchloric acid
- △ 50% nitric acid
- ◆ conc. hydrochloric acid

Reaction mixture: 1 mL of ethanol, 0.5 mL of acid, 0.81 mL of $1.0 \times 10^{-2}$ M KMnO$_4$. 
intensity, a relatively fast reaction rate, a good detection limit, good precision, and good linearity at lower EtOH concentrations.

Table XXV. Effect of Different Mineral Acids on CL Intensity.

<table>
<thead>
<tr>
<th>Acid</th>
<th>E&lt;sub&gt;CL&lt;/sub&gt; &lt;sup&gt;b&lt;/sup&gt; (mV)</th>
<th>RSD &lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>t&lt;sub&gt;R&lt;/sub&gt; &lt;sup&gt;c&lt;/sup&gt; (min)</th>
<th>DL &lt;sup&gt;d&lt;/sup&gt; (% (w/v))</th>
<th>Fit&lt;sup&gt;e&lt;/sup&gt; (order)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>132</td>
<td>0.79</td>
<td>1.25</td>
<td>1</td>
<td>2nd</td>
</tr>
<tr>
<td>HCl</td>
<td>315</td>
<td>28</td>
<td>2.50</td>
<td>1</td>
<td>2nd</td>
</tr>
<tr>
<td>HClO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>212</td>
<td>2.8</td>
<td>0.94</td>
<td>1</td>
<td>2nd</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>33.3</td>
<td>0.87</td>
<td>4.38</td>
<td>3</td>
<td>No correlation</td>
</tr>
<tr>
<td>H&lt;sub&gt;3&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt; and HAc</td>
<td>Not detected</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

a All 50% (w/v) except HCl was conc., 1.0 x 10<sup>-2</sup> M MnO<sub>4</sub><sup>-</sup>

b for 50% (w/v) ethanol
c duration of CL (base width of CL peak)
d smallest concentration detected
e the order of the polynomial yielding the best fit

The effect of HNO<sub>3</sub> concentration on the CL signal for 12.5% (v/v) ethanol for different KMnO<sub>4</sub> concentrations is shown in Figure 45. For all KMnO<sub>4</sub> concentrations, higher acid concentrations increase the CL signal. The dependence of CL signal on KMnO<sub>4</sub> concentration is more complex. The KMnO<sub>4</sub> concentration yielding the highest CL signal depends upon the HNO<sub>3</sub> concentration. Only for the case of a KMnO<sub>4</sub> concentration of 1.0 x 10<sup>-3</sup> M, the dependence of the CL signal on the nitric acid concentration is approximately linear.
Figure 45. Dependence of the CL intensity of 12.5% (v/v) ethanol on the concentration of HNO₃ for different KMnO₄ concentrations.

- □ 1.0 x 10⁻³ M + 3.3 x 10⁻³ M
- ○ 1.0 x 10⁻² M
- △ 3.0 x 10⁻² M
(pseudo-first order). However, at higher concentrations of KMnO₄, a nonlinear dependence is observed. This is most obvious at the highest KMnO₄ concentration used in this study (3.0 x 10⁻² M). In this case, at low HNO₃ concentrations, the CL signal for a given ethanol concentration falls below that observed with lower KMnO₄ concentrations.

Figure 46 shows calibration curves with different permanganate concentrations at a fixed HNO₃ concentration of 50% (w/v). The largest calibration slope is obtained with 0.01 M KMnO₄. With 3 x 10⁻² M MnO₄⁻, nonlinearity is quite obvious and the calibration slope is less than those obtained with 1.0 x 10⁻² and 3 x 10⁻³ M MnO₄⁻.

For ethanol concentrations of 0.1% (v/v) and above, the ethanol is in molar excess relative to permanganate in the reaction mixture. This is clearly shown in Table XXVI. If the oxidation proceeds to acetic acid (see equation 19 in the Historical section), it takes 0.8 mol of permanganate to oxidize 1 mol of ethanol. If acetaldehyde is the oxidation product as shown in the reaction below,

$$5C₂H₅OH + 2MnO₄⁻ + 6H^+ \rightarrow 5CH₃CHO + 2Mn^{2+} + 8H₂O$$

(30)

it takes 0.4 mol of permanganate to oxidize 1 mol of ethanol.

Under the above conditions where ethanol is in excess, the rate of oxidation is proportional to the permanganate and ethanol concentrations. Since the CL signal is proportional to the rate of oxidation, it might be expected that the CL signal is proportional to the ethanol and permanganate concentrations.

In Figure 45, the slope of the calibration curve increases as the permanganate concentration is increased from 1 x 10⁻³ to 1 x 10⁻² M. However, the slope decreases when the permanganate concentration is further increased to 3 x 10⁻² M. As discussed later in this section,
Figure 46. Effect of the concentration of KMnO₄ on the shape of calibration curves for ethanol.

- □ 1.0 x 10⁻³ M
- + 3.3 x 10⁻³ M
- ◇ 1.0 x 10⁻² M
- △ 3.0 x 10⁻² M

Nitric acid conc., 50% (w/v).
Table XXVI. Dependence of Molar Ratio of Ethanol on the Ethanol Concentration

<table>
<thead>
<tr>
<th>EtOH conc. (% (v/v))</th>
<th>ethanol conc. (% (w/v))</th>
<th>ethanol conc. (^a)</th>
<th>molar ratio (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>78.9</td>
<td>1.71 x 10(^{-2})</td>
<td>4.73 x 10(^{-4})</td>
</tr>
<tr>
<td>50.0</td>
<td>39.5</td>
<td>8.57 x 10(^{-3})</td>
<td>9.45 x 10(^{-4})</td>
</tr>
<tr>
<td>25.0</td>
<td>19.7</td>
<td>4.28 x 10(^{-3})</td>
<td>1.89 x 10(^{-3})</td>
</tr>
<tr>
<td>12.5</td>
<td>9.87</td>
<td>2.14 x 10(^{-3})</td>
<td>3.78 x 10(^{-3})</td>
</tr>
<tr>
<td>6.25</td>
<td>4.93</td>
<td>1.07 x 10(^{-3})</td>
<td>7.56 x 10(^{-3})</td>
</tr>
<tr>
<td>3.13</td>
<td>2.47</td>
<td>5.35 x 10(^{-4})</td>
<td>1.51 x 10(^{-2})</td>
</tr>
<tr>
<td>1.56</td>
<td>1.23</td>
<td>2.68 x 10(^{-4})</td>
<td>3.03 x 10(^{-2})</td>
</tr>
<tr>
<td>1.00</td>
<td>0.789</td>
<td>1.71 x 10(^{-4})</td>
<td>4.73 x 10(^{-2})</td>
</tr>
<tr>
<td>0.1</td>
<td>0.0789</td>
<td>1.71 x 10(^{-5})</td>
<td>4.73 x 10(^{-1})</td>
</tr>
</tbody>
</table>

\(^a\) per mL of the reaction mixture of total volume 2.3 mL
\(^b\) mol KMnO\(_4\)/mL to mol EtOH/mL, mol/mL of MnO\(_4\)\(^-\) for injection
of 0.81 mL of 0.01 M KMnO\(_4\) equals 8.1 x 10\(^{-6}\) moles

There is some overlap between the CL spectrum and MnO\(_4\)\(^-\) absorption spectrum. Hence the reduced CL signal at the highest MnO\(_4\)\(^-\) concentration observed in both Figures 45 and 46 may be due to absorption of the CL photons by permanganate. In other words, at high permanganate concentrations, the rate of oxidation and number of CL photons generated may be greater, but the observed CL signal is less.

At low ethanol concentrations, this attenuation effect is greater because the rate of disappearance of MnO\(_4\)\(^-\) is less. For example, for the lowest ethanol concentration tested in Figure 46 (1% (v/v)), the CL signal with 1 x 10\(^{-2}\) MnO\(_4\)\(^-\) is less than that observed with 1 x 10\(^{-3}\) M MnO\(_4\)\(^-\). At higher ethanol concentrations, the opposite is true. Note also in Figure 45, that the CL signal is largest with 3 x 10\(^{-2}\) M
MnO$_4^-$ only with 95% (w/v) HNO$_3$ where the rate of reaction and the rate of disappearance of MnO$_4^-$ is greatest.

The detection limit for ethanol for the CL technique with 0.01 M KMnO$_4$ and 50% (w/v) HNO$_3$ is about 1.0% (v/v). With these reagent concentrations as a starting point, a simplex optimization of the HNO$_3$ and KMnO$_4$ concentration was conducted with 1% (v/v) ethanol. The movement of the simplex is illustrated in Figure 47. The optimum concentrations were found to be 1.0 x 10$^{-2}$ M KMnO$_4$ and 95.0% (w/v) nitric acid. These conditions yield an improvement of a factor of 12 for the CL signal of ethanol. The detection limit is about 0.3% (v/v).

Figure 48 shows a typical ethanol calibration curve under the optimized conditions. Least squares fitting of the data with first, second, and third order polynomials revealed that the second order fit was the best (i.e., minimum standard error).

A calibration curve for lower ethanol concentrations is shown in Figure 49. The curve is reasonably linear over the 0.3 to 10% (v/v) ethanol range.

Figure 50 shows the typical peaks obtained from the ethanol CL reaction. The greater the ethanol concentration, the faster the CL reaction and the higher the peak maximum.

Absorption spectra

Figure 51 shows the absorption spectra of the reactants, ethanol, nitric acid, and potassium permanganate, in the CL reaction versus water as the blank. Ethanol only absorbs below 200 nm. Nitric acid absorbs very strongly in the UV region, while MnO$_4^-$ absorbs
Figure 47. Simplex optimization of reagent concentrations for the determination of ethanol. Response (CL signal in mV) in parentheses by vertex.

W = worst
N = next to worst
B = best
R_x = reflexion; x = 1, 2, 3
P_T = contraction
Figure 48. Typical ethanol calibration curve. Reaction mixture: 1 mL of ethanol, 0.5 mL of 95% (w/v) HNO$_3$, 0.8 mL of $1.0 \times 10^{-2}$ M KMnO$_4$. 
Figure 49. Typical ethanol calibration curve at low concentrations. Reaction mixture: 1 mL of ethanol, 0.5 mL of 95% (w/v) HNO₃, 0.8 mL of 1.0 x 10⁻² M KMnO₄. Filter gain 2.
Figure 50. Typical peak shapes for the CL signals obtained from the oxidation reaction of ethanol by
KMnO$_4$ in acidic medium. Percents are (v/v). Reaction mixture: 1 mL of ethanol, 0.5 mL of 95% (w/v)
HNO$_3$, 0.8 mL of 1.0 x 10$^{-2}$ M KMnO$_4$ (injected).
Figure 51. Absorption spectra of reagents.

a 6.0% (v/v) ethanol vs. dw
b 95% (w/v) HNO₃ vs. dw
c 1.0 x 10⁻² M KMnO₄ vs. dw
strongly from 200 to 400 nm and from 420 to 800 nm.

Figure 52 shows a time sequence of the absorption spectra of a standard reaction mixture. Clearly, most of the potassium permanganate is used up in 30 s.

If perchloric acid is used instead of HNO₃, (Figure 53), the oxidation rate of the alcohol and the rate of disappearance of potassium permanganate is slower by about a factor of three than if nitric acid is used. Under these conditions, a cherry pink color characteristic of Mn(III) complexes is visually observed. The appearance and disappearance of the absorption band due to the colored complex is not obvious in the spectra. The Mn³⁺ absorption band with a maximum at about 466 nm is apparently masked by the side of the absorption band of MnO₄⁻.

Figure 54 shows the spectra obtained when Mn(II) is included in the reaction mixture. In this case, the permanganate is used up in about 10 s, after which the absorbance in the 425-625 nm region increases. This may be due to the formation of another absorbing species such as Mn(III) and Mn(IV) or scattering by MnO₂.

Figure 55 shows spectra taken with and without Fe(II) added. With Fe(II), a fraction of the potassium permanganate is reduced rapidly due to reduction of permanganate by this species such that the absorbance due to MnO₄⁻ is less at a given time than that without Fe(II) added.

Chemiluminescence spectra

The spectral distribution of the CL emission from the oxidation of ethanol was first studied with cut-on filters and the results are shown
Figure 52. Absorption spectra taken during the oxidation reaction of ethanol by KMnO₄ in acidic medium. Integration time \( t_i \) = 0.1 s. Time between spectrum \( t_b \) = 5 s. Reaction mixture: 1 mL of 6.0% (v/v) ethanol, 0.5 mL of 95% (w/v) HNO₃, 0.8 mL of \( 1.0 \times 10^{-2} \) M KMnO₄. Order of spectra: decreasing absorbance.
Figure 53. Absorption spectra taken during the oxidation reaction of ethanol by KMnO₄ with different acids. $t_1 = 0.1 \text{ s}$, $t_b = 15 \text{ s}$. Reaction mixture: 1 mL of 6.0% (v/v) ethanol, 0.5 mL of 95% (w/v) (A) HNO₃, (B) HClO₄, 0.8 mL of 1.0 x $10^{-2} \text{ M KMnO}_4$. Order of spectra: decreasing absorbance.
Figure 54. Absorption spectra taken during the oxidation reaction of ethanol by KMnO₄ in acidic medium with and without the presence of Mn(II). tᵢ = 0.1 s, tᵦ = 10 s. Reaction mixture: 1 mL of 6.0% (v/v) ethanol, 0.5 mL of 95% (w/v) HNO₃, 0.1 mL of (A) H₂O, (B) 50% (w/v) Mn(II), 0.8 mL of 1.0 x 10⁻² M KMnO₄. Numbers refer to order of spectra.
Figure 55. Absorption spectra taken during the oxidation reaction of ethanol by KMnO₄ with and without Fe(II) added. \( t_i = 1 \) s, \( t_b = 10 \) s. Reaction mixture: 1 mL of 6.0% (v/v) ethanol, 0.5 mL of (A) H₂O, (B) \( 1.0 \times 10^{-2} \) M Fe(II), 0.5 mL of 95% (w/v) HNO₃, 0.8 mL of \( 1.0 \times 10^{-2} \) M KMnO₄. Order of spectra: decreasing absorbance.
in Figure 56. The broad emission band begins at about 530 nm with an apparent maximum at about 660 nm.

For these fast CL reactions, the intensified diode array provides a better means to obtain spectral information. Figure 57 shows successive CL spectra taken with this detector. It takes about four seconds for the reaction to reach its maximum intensity. Note that the wavelength of maximum CL intensity ($\lambda_m$) shifts to lower wavelengths as the reaction proceeds. After 1 s, $\lambda_m \approx 650$ nm, while $\lambda_m \approx 580$ nm after 4 s. At 4 s, the CL emission occurs over the 450 to 750 nm region. This apparent shift may be due to the absorption of a fraction of the CL photons by permanganate. The permanganate absorption spectrum (see Figure 51) overlaps the CL spectrum. The strongest absorption occurs in the lower wavelength region of the CL spectrum. As the reaction proceeds, the permanganate is consumed and the degree of absorption of the lower wavelength CL photons decreases. This phenomenon causes an apparent blue shift in the wavelength of maximum CL emission as the reaction proceeds.

In the presence of Fe(II), the CL reaction proceeds about three times faster (i.e., 1.5 s to reach maximum intensity) as shown in Figure 58. Note that $\lambda_m$ is about 530 nm and emission occurs from about 380 to 800 nm. This spectrum probably represents the truest picture of the CL spectrum. In this case, the Fe(II) reduces almost immediately all the MnO$_4^-$ to lower Mn oxidation states which do not significantly absorb the CL radiation.
Figure 56. Dependence of the transmitted CL signal for ethanol on the filter cut-on wavelength.

Reaction mixture: 1 mL of absolute ethanol, 0.5 mL of 95% (w/v) HNO₃, 0.8 mL of 1.0 x 10⁻² M KMnO₄ (injected).
Figure 57. CL spectra of the oxidation reaction of ethanol by KMnO₄. Each spectrum was taken 1 s apart. Reaction mixture: 2 mL of absolute ethanol, 0.5 mL of 95% (w/v) HNO₃, 0.8 mL of 1.0 x 10⁻² M KMnO₄. Numbers indicate the order of the spectra.
Figure 58. CL spectra of the oxidation reaction of ethanol by KMnO$_4$ in the presence of Fe(II).

Reaction mixture: 1 mL of absolute ethanol, 1 mL of $1.0 \times 10^{-1}$ M Fe(II), 0.5 mL of 95% (w/v) HNO$_3$, 0.8 mL of $1.0 \times 10^{-2}$ M KMnO$_4$ (injected).

- **a** spectrum taken 1.5 s after injection
- **b** after 2.5 s
- **c** 3.5 s
- **d** 4.5 s
Reaction products studies

In the first experiment designed to observe the reaction products produced by oxidation of hydroxy-containing compounds with permanganate in acidic solutions, mandelic acid was the model compound. With stoichiometric amounts of mandelic acid and permanganate, decoloration of permanganate was the first apparent sign of reaction. A sharp odor of benzaldehyde was recorded and evolution of CO$_2$ from the reaction was observed. Production of MnO$_2$ was noticed.

Figure 59 shows the $^{13}$C NMR spectrum of the solid obtained from the reaction after extraction with ether, evaporation of the solvent and recrystallization. This spectrum matches the $^{13}$C NMR spectrum of benzoic acid. In the TLC experiment, the $R_f$ of the only eluted spot from the reaction mixture corresponded to benzoic acid. The TLC plate was sprayed with Ce$^{4+}$ solution and no oxidation for any of the spots was observed. The melting point of the reaction product was 122.0 °C; the melting point of benzoic acid is 122.4 °C.$^{76}$ These results suggest that, with sufficient oxidant, complete oxidation of mandelic acid, including decarboxylation, occurs.

For the second experiment with an excess of mandelic acid, an instant decoloration of the oxidant and the strong odor of benzaldehyde was noted. However, evolution of CO$_2$ was not visible. After solvent extraction and evaporation, a yellowish liquid plus a white precipitate were obtained. The presence of benzaldehyde, benzoic acid and excess mandelic acid were obvious from TLC experiments. Figure 60 shows the $^1$H NMR spectrum of the reaction mixture. The mandelic acid and benzaldehyde peaks are very obvious. These results suggest that under
Figure 59. $^{13}$C NMR spectrum of the solid obtained after reaction of stoichiometric amounts of mandelic acid and KMnO$_4$ in acidic medium. This NMR spectrum corresponds to the $^{13}$C NMR of benzoic acid.
Figure 60. $^1$H NMR spectrum of reaction products after reaction of an excess of mandelic acid with KMnO$_4$ in acidic medium.

a mandelic acid ($\text{-CH} - \text{C} \text{-OH}$)

b benzaldehyde ($\text{-C} - \text{H}$)
normal CL reaction conditions, the major product is benzaldehyde and that decarboxylation process is not obvious.

The next set of experiments involved identification of reaction products from oxidation of isopropanol. Acetone is the expected oxidation product. No acetone was detected in the $^1$H NMR spectrum of the reaction mixture and only isopropanol was detected. Figure 61 shows the GC chromatograph of the reaction products recovered from vacuum distillation of the reaction mixture. The two peaks shown were identified by MS to be isopropanol and acetone.

The last reaction product study involved ethanol as the starting compound. No acetaldehyde was detected in the GC-MS spectrum of absolute ethanol. It was also confirmed that acetaldehyde and ethanol could be separated and detected by GC-MS with a standard composed of 0.1% (v/v) acetaldehyde and 1% (v/v) ethanol in water. The GC chromatogram of the vacuum distilled reaction mixture is shown in Figure 62. Similar results were obtained with a sample neutralized to pH 8. MS analysis showed that the three GC peaks correspond to ethanol, acetic acid, and acetaldehyde. The ethanol peak is the largest as there is an excess of ethanol. Thus the CL reaction produces the products expected for the oxidation reaction of alcohol. No condensation products were detected.

Effect of other species and deaeration on the chemiluminescence intensity

Effect of Mn(II). Figure 63 shows the effect of Mn(II) on the CL intensity. It is obvious that a relatively large concentration of
Figure 61. GC chromatogram of reaction products recovered from vacuum distillation in the oxidation of isopropanol by KMnO₄ in acidic medium.

a Isopropanol

b Acetone
Figure 62. GC chromatogram of the reaction products recovered from vacuum distillation in the oxidation of ethanol by KMnO₄ in acidic medium.

a acetaldehyde
b ethanol
c acetic acid
Figure 63. Effect of Mn(II) on the CL intensity of ethanol.  + Mn(II) added  □ water added
Reaction mixture: 1 mL of ethanol, 0.5 mL of H₂O or 0.2 M Mn(II), 0.5 mL of 95% (w/v) HNO₃, 0.8 mL of 1.0 x 10⁻² M KMnO₄.
Mn(II) decreases the CL signal significantly. As discussed in the Historical section, it would be expected that Mn(II) would increase the rate of reduction of MnO$_4^-$ to Mn(III) or Mn(IV) (see equations 21 and 22). The more rapid reduction of MnO$_4^-$ was also noted in the absorption spectra shown in Figure 54B. Also 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) (see equation 23). The effect of Mn(II) on the CL signal is consistent with the theory that Mn(IV) is responsible for the CL oxidation of ethanol. The CL signal is decreased with Mn(II) added because the concentration of the oxidizing species is decreased.

It was also visually noted that the appearance of the cherry red color characteristic of Mn(III) appears about 11 s after addition of the permanganate when Mn(II) was added. Without Mn(II), the appearance of Mn(III) takes longer ($\approx$ 16 s) as expected. With Mn(II) added, the formation of brown MnO$_2$ is also quite obvious and occurs faster than if no Mn(II) is present in the reaction mixture.

**Effect of Fe(II).** When Fe(II) is added to the reaction mixture, there are several possibilities for the reduction of Mn(VII) by Fe(II). Since there was no indication of the green Mn(VI) anion (manganate) in acidic medium, the formation of this intermediate was ruled out. Also anions of Mn(V) are unknown. Therefore, only the following reductions were considered to be possible in the initial stages of the reaction.

$$\text{MnO}_4^- + 3 \text{Fe}^{2+} + 8\text{H}^+ \rightarrow \text{Mn}^{4+} + 3 \text{Fe}^{3+} + 4\text{H}_2\text{O}$$ \hspace{1cm} (31)

$$\text{MnO}_4^- + 4 \text{Fe}^{2+} + 8\text{H}^+ \rightarrow \text{Mn}^{3+} + 4 \text{Fe}^{3+} + 4\text{H}_2\text{O}$$ \hspace{1cm} (32)

If equation 31 represents the dominant mechanism for reduction, it is
expected that the Fe(II) reacts rapidly with MnO$_4^-$ to produce Mn(IV) and eventually Mn(III) through reaction with Mn(II). Visually it was noted that the cherry red color due to Mn(III) appeared much sooner (≈ 5 s) after addition of MnO$_4^-$ when Fe(II) was added than without Fe(II) (≈ 16 s). If equation 31 represents the dominant reduction mechanism, there is quantitative reduction of Mn(VII) to Mn(IV) when the molar ratio of Fe(II) added to permanganate injected (MR) equals 3 (e.g., 2.43 x 10$^{-5}$ moles of Fe$^{2+}$ are needed to quantitatively reduce the 8.1 x 10$^{-6}$ moles of MnO$_4^-$ added to the reaction mixture by injection of 0.8 mL of 1.0 x 10$^{-2}$ M KMnO$_4$). If equation 32 represents the dominant reduction mechanism, a MR of 4 (3.24 x 10$^{-5}$ moles of Fe$^{2+}$) would be required for quantitative reduction of Mn(VII).

The results of experiments involving double injections of potassium permanganate with addition of Fe(II) are shown in Figure 64. For these experiments the MR was varied from 0 to above 6. Without any Fe(II) added (Figure 64a), the second injection produced a CL peak of long duration very similar to that from the first injection. This is expected as the ethanol is in excess. The second peak has a lower peak intensity because of dilution effects. When Fe(II) is added to the reaction mixture, a rapid CL peak precedes the normal broad CL peak (Figures 64b to h). This is believed to be due to a rapid production of Mn(IV) which reacts with the ethanol to produce CL. Note that the height of the rapid peak from the first injection (Figures 64b to f) increases up to the point that the MR is 3 at which point all the MnO$_4^-$ is rapidly reduced. For MR's between 0 and 3, the broad CL peak is still observed as some permanganate still remains after the
Figure 64. CL peak shapes for the reaction of 6% (v/v) ethanol with KMnO₄ in acidic medium with two injections of KMnO₄ in the presence of Fe(II). The arrows indicate the point of the second injection of the oxidant. Here the value of y in mV is given in parentheses. If two values are given the first value is for the fast peak and the second value is for the slow peak. a, no Fe(II) added (50); the second time, 0.5 mL of acid was added before the second injection; b, MR = 0.0006 (50); c, MR = 0.006 (100, 50); d, MR = 0.06 (500, 50); e, MR = 0.6 (500, 50); f, MR = 3 (500, 50); g, MR = 5 (50); h, MR = 6 (50).
initial rapid reduction by Fe(II). However, the height of the broad peak decreases as the MR increases. When the MR is 3 (Figure 64f), only the fast CL peak is observed for the first injection because no MnO$_4^-$ remains after the initial fast reaction.

The CL behavior observed with the second injection is consistent with the above results. When the MR $\leq$ 3, only a broad peak is observed after the second injection. However, when the Fe(II) concentration is greater such as that MR $>$ 3 (Figures 64g and h), a fast CL peak also precedes the slow peak for the second injection. This occurs because some Fe(II) remains after the first injection to react with the MnO$_4^-$ from the second injection. Note in Figure 64h that the broad peak from the second injection disappears when MR $>$ 6 because there is enough Fe(II) left to reduce all the MnO$_4^-$ from the first and second injections.

If Fe(II) reduces the Mn(VII) to Mn(III) instead of Mn(IV), there would still be a broad peak after the fast CL peak, with a MR of 3 because some MnO$_4^-$ would remain after the first injection. In other words, the peak shapes as shown in Figure 64e instead of Figure 64f would be observed.

These results again support the hypothesis that the oxidant does not directly attack the alcohol. If Mn(VII) was directly responsible for oxidation, the addition of Fe(II) would decrease the CL intensity since it rapidly decreases the initial concentration of MnO$_4^-$. Hence it appears that Fe(II) increases the oxidation rate by rapidly reducing Mn(VII) to an intermediate oxidation state of Mn which then attacks the alcohol. This rapid reduction was also noted in the absorption spectra shown in Figure 55 and the CL spectra shown in
Figure 58. The dependence of the peak shapes on the MR suggest that Mn(IV) is the species responsible for oxidation of the alcohol.

In another experiment, the CL reaction was run without addition of Fe(II) for approximately two minutes, and then 0.5 mL of H₂O or Fe(II) solutions of different concentrations was injected. For 1.0 x 10⁻¹ and 1.0 x 10⁻² M Fe(II), (Figures 65b and c), the CL signal rapidly drops to zero. This appears to indicate that Fe(II) reduces the concentration of the Mn intermediate which is already involved in the oxidative reaction. In other words, all the Mn(VII) is already reduced to Mn(IV) or other species, and the presence of Fe(II) interrupts the oxidation of ethanol (CL reaction) by reducing Mn(IV) to Mn(II). With 1.0 x 10⁻³ M Fe(II), (Figure 65a), the CL intensity is only decreased slightly. Finally, with 1.0 x 10⁻⁴ and 1.0 x 10⁻⁵ M Fe(II), the CL intensity is enhanced by about 60% for approximately 36 s after which the emission signal returned to normal. Apparently with lower Fe(II) concentrations, the Fe(II) causes an increase in the concentration of intermediate oxidation states of Mn.

Figure 66 shows calibration curves for ethanol with and without Fe(II) added before injection of MnO₄⁻. When Fe(II) is added, the detection limit for the determination of alcohol drops from 0.3 to 0.001% (v/v). For concentrations of ethanol of 25% (v/v) and higher in the presence of Fe(II), only a fast CL signal is observed. Here, the ethanol is in such excess that the reaction between the Mn(IV) produced by reaction with Fe(II) and that normally produced without Fe(II) cannot be distinguished. On the other hand, for concentrations of ethanol of 20% (v/v) and lower, first a fast CL signal is obtained followed by a broad CL signal as shown in Figure 64. The height of
Figure 65. CL peak shapes for the reaction of 3% (v/v) ethanol with KMnO₄ in acidic medium. After 2 min, different solutions were injected. Arrows indicate the point of oxidant injection, triangles indicate the point of injection of H₂O or Fe(II), and asterisks indicate the point the shutter was closed intentionally. Reference: 1 mL of 3% (v/v) ethanol, 0.5 mL of 95% (w/v) HNO₃, 0.8 mL of 1.0 x 10⁻² M KMnO₄. a, injection of 0.5 mL H₂O; b, 1.0 x 10⁻¹ M Fe(II); c, 1.0 x 10⁻² M Fe(II); d, 1.0 x 10⁻³ M Fe(II); e, 1.0 x 10⁻⁴ M Fe(II); f, 1.0 x 10⁻⁵ M Fe(II).
Figure 66. CL calibration curves for ethanol with and without Fe(II) added. ○ H₂O, □ 1.0 x 10⁻³ M Fe(II). Reaction mixture: 1 mL of ethanol, 0.5 mL of H₂O or Fe(II); 0.5 mL of 95% (w/v) HNO₃, 0.81 mL of 1.0 x 10⁻² M KMnO₄ (injected).
both peaks decreases with decreasing ethanol concentration. In this case, the reaction between the remaining $\text{MnO}_4^-$ and ethanol is slower with a lower ethanol concentration. In the calibration plots, the peak height for the fast first peak is used where there is also a slow second peak.

Table XXVII compares the results obtained with Fe(II) and Mn(II) added individually and in combination to the reaction mixture with 6.0% (v/v) ethanol. As previously shown, these cations affect the CL reaction in opposite ways. Together, the CL signal is intermediate between that obtained with each ion individually.

Table XXVII. Effect of Fe(II) and Mn(II) on the CL Intensity of Ethanol

<table>
<thead>
<tr>
<th>Voi$^a$ (mL)</th>
<th>Solution</th>
<th>CL signal and SD (mV)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>water</td>
<td>5.1 (0.1)</td>
<td>Reference</td>
</tr>
<tr>
<td>0.5</td>
<td>water</td>
<td>578 (13)</td>
<td>Enhances CL signal (sharp peak) by factor of 113</td>
</tr>
<tr>
<td>0.5</td>
<td>0.01 M Fe(II)</td>
<td>2.0 (0.01)</td>
<td>Reduces CL signal by a factor of 2.5</td>
</tr>
<tr>
<td>0.5</td>
<td>0.01 M Mn(II)</td>
<td>86.6 (2.7)</td>
<td>Reduces the effect of Fe(II) by a factor of 6.7</td>
</tr>
</tbody>
</table>

$^a$ Volume added to reaction mixture of 1.0 mL of 6% (v/v) ethanol, 0.5 mL of 95% (w/v) HNO$_3$, 0.8 mL of 1.0 x $10^{-2}$ M KMnO$_4$ (injected).

Effect of Mn(III). The effect of Mn(III) on the CL reaction was evaluated with two solutions as discussed in the Experimental section. Solution A was prepared from Mn(CH$_3$COO)$_3$·2H$_2$O from Alpha.
Solution B was prepared by mixing MnO$_4^-$ and an excess of Mn(II). In both cases the solvent was glacial acetic acid to stabilize the Mn(III). Both solutions were injected with a disposable syringe through the sample module septum. The reaction mixture was as follows: 1 mL of ethanol 12% (v/v), 1 mL of water, 0.5 mL of HNO$_3$, 0.810 mL Mn(III).

When solution A was injected no CL signal was obtained. This suggests that all the Mn(III) is stabilized as the acetate complex and does not react. When solution B was injected, a very small (1.6 mV) signal was observed. This small peak may be due to some Mn(VII) used still present that was not reduced by the Mn(II). These results suggest that Mn(III) is not responsible for the CL oxidation of ethanol.

Table XXVIII shows the results obtained when 0.8 mL of different solutions are added to the reaction mixture. When glacial acetic acid

<table>
<thead>
<tr>
<th>Species added$^a$ [x]</th>
<th>Signal and SD (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>water</td>
<td>11 (0.06)</td>
</tr>
<tr>
<td>glacial acetic acid</td>
<td>7.1 (0.5)</td>
</tr>
<tr>
<td>0.01 M Mn(II) in water</td>
<td>2.8 (0.2)</td>
</tr>
<tr>
<td>0.01 M Mn(III) A</td>
<td>5.7 (0.5)</td>
</tr>
<tr>
<td>0.01 M Mn(III) B</td>
<td>6.4 (0.04)</td>
</tr>
</tbody>
</table>

$^a$ Reaction mixture: 1 mL of ethanol 12% (v/v), 0.5 mL of 95% (w/v) HNO$_3$, 0.81 mL of x, 0.810 mL 0.01 M KMnO$_4$ (in water).
acid is added, the CL peak height is reduced about 35% and the peak duration is reduced by a factor of about 15. This suggests that the production of emitting species is faster than in water. When Mn(II) is added, the CL signal is depressed as already discussed. Mn(III) affects both the CL intensity and the CL peak duration. It appears that most of this effect is due to the glacial acetic acid in the Mn(III) solutions added. Even when Mn(II) is also present, Mn(III) does not appear to be involved in the CL pathway.

Effect of Anions. Figures 67 and 68 show the effect of various anions on the CL intensity for different ethanol concentrations. All the anions depress the CL to some degree in the following order: $P_2O_7^{4-} > PO_4^{3-} > SO_4^{2-} > CH_3COO^- > CIO_4^- > F^- > Cl^- > NO_3^-$. These anions would be expected to decrease the CL intensity due to complexation of Mn(III) or Mn(IV). If Mn(IV) is the attacking species, complexation of Mn(IV) could reduce its oxidation power or complexation of Mn(III) could shift the equilibrium (Mn(II) + Mn(IV) $\rightleftharpoons$ 2 Mn(III)) towards Mn(III). As expected, pyrophosphate has the greatest effect as it is known to form strong manganic complexes under acidic conditions. Other anions which also form relatively strong Mn(III) complexes are phosphate and acetate.

Effect of CH$_3$OH and H$_2$O$_2$. Methanol and hydrogen peroxide were also added to the ethanol reaction mixture. Methanol had little effect on the CL intensity. This is expected since methanol reacts slowly with potassium permanganate. Hydrogen peroxide, however, increased the CL signal by a factor of three. It appears to produce the same effect at
Figure 67. Effect of anions on the CL intensity of ethanol.

- $\text{PO}_4^{3-}$
- $\text{CH}_3\text{COO}^-$
- $\text{ClO}_4^-$
- $\text{H}_2\text{O}$

Reaction mixture: 1 mL of EtOH, 0.5 mL of 95% (w/v) HNO$_3$, 0.5 mL of water or anion, 0.81 mL of 1.0 x 10$^{-2}$ M KMnO$_4$ (injected).
Figure 68. Effect of other anions on the CL intensity for ethanol.

\[ + \text{SO}_4^{2-} \quad \triangle \text{F}^{-1} \quad \times \text{ClO}_4^- \quad \diamond \text{NO}_3^- \quad \square \text{H}_2\text{O} \]

Reaction mixture: 1 mL of EtOH, 0.5 mL of 95% (w/v) HNO_3, 0.5 mL of water or anion, 0.81 mL of 1.0 \times 10^{-2} \text{ M KMnO}_4 (injected).
observed with Fe(II). If potassium permanganate and hydrogen peroxide are mixed before injection and then added to the acidic ethanol reaction mixture, the CL signal is decreased by a factor of five. It is obvious that hydrogen peroxide reduces permanganate to Mn(II) and only a fraction of the original MnO₄⁻ is left to react with the ethanol.

**Effect of other oxidants.** No CL signal was observed when the following oxidants were injected instead of KMnO₄: K₂Cr₂O₇, H₂O₂, K₃[Fe(CN)₆], and Ce(IV). Potassium dichromate was also used with Mn(II) added to the reaction mixture. It is expected that the Mn(III) is produced (Cr⁶⁺ + S ⇌ Cr⁴⁺ + P; Cr⁴⁺ + Mn²⁺ ⇌ Cr³⁺ + Mn⁴⁺) in this case. A very small CL signal was recorded (0.56 mV) relative to 1.5 V obtained with the concentration of ethanol with permanganate as oxidant. This seems to indicate a very slow reaction of Mn(III) towards the ethanol.

**Effect of solvent.** The effect of the solvent for ethanol on the CL reaction was studied and the results are shown in Table XXIX. The CL signal with all solvents is within a factor of two of that observed with water except for acetonitrile for which the CL signal is severely depressed.

**Effect of activators.** Table XXX shows the results obtained when 9,10-dibromoanthracene (DBA) was added to the CL reaction mixture to serve as an activator. Clearly DBA has little effect on the CL reaction. It depresses the ethanol CL signal by 6% and enhances the isopropanol CL signal by 12%. As stated before the ethanol emission
### Table XXIX. Effect of the Solvent on CL Intensity for Ethanol

<table>
<thead>
<tr>
<th>Solvent</th>
<th>CL signal and SD (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$O</td>
<td>141 (9)</td>
</tr>
<tr>
<td>DMSO</td>
<td>115 (4)</td>
</tr>
<tr>
<td>DMF</td>
<td>75 (4)</td>
</tr>
<tr>
<td>CH$_3$C≡N</td>
<td>0.5 (0.7)</td>
</tr>
<tr>
<td>THF</td>
<td>161 (4)</td>
</tr>
<tr>
<td>CH$_2$Cl$_2$</td>
<td>154 (10)</td>
</tr>
<tr>
<td>Hexane</td>
<td>194 (5)</td>
</tr>
<tr>
<td>Ether</td>
<td>176 (9)</td>
</tr>
<tr>
<td>CHCl$_3$</td>
<td>175 (8)</td>
</tr>
</tbody>
</table>

**Reaction mixture:**
- 1 mL of 50% (v/v) ethanol
- 0.5 mL of 95% (w/v) HNO$_3$
- 0.8 mL of $1.0 \times 10^{-2}$ M KMnO$_4$

### Table XXX. Effect of DBA on the CL Intensity of Ethanol and Isopropanol$^a$

<table>
<thead>
<tr>
<th>Sample</th>
<th>[DBA] (M)</th>
<th>CL signal and SD (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>---</td>
<td>266 (3)</td>
</tr>
<tr>
<td>Ethanol</td>
<td>$9 \times 10^{-4}$</td>
<td>250 (12)</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>---</td>
<td>53 (3)</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>$9 \times 10^{-4}$</td>
<td>61 (6)</td>
</tr>
</tbody>
</table>

$^a$ 1.0 mL of 50% (v/v) Ethanol or isopropanol
- 0.5 mL of 95% (w/v) HNO$_3$
- 0.8 mL of $1.0 \times 10^{-2}$ M MnO$_4^-$
occurs in the visible (450 - 750 nm), the wavelength of maximum phosphorescence for DBA in CS$_2$ is 711 nm.

Table XXXI shows the results obtained when diacetyl was used as activator with the ethanol and propyl gallate CL reactions. Diacetyl appears to be a moderately good activator for the CL reaction of ethanol. The fluorescence and phosphorescence wavelengths of maximum emission of biacetyl in water are 462 and 514 nm, respectively. It

Table XXXI. Effect of Diacetyl on the CL Intensity for Ethanol and PG Reactions$^a$

<table>
<thead>
<tr>
<th>Sample</th>
<th>[Diacetyl] (M)</th>
<th>CL signal (mV)</th>
<th>Enhancement of CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtOH</td>
<td>---</td>
<td>9.7</td>
<td></td>
</tr>
<tr>
<td>EtOH</td>
<td>$1.0 \times 10^{-3}$</td>
<td>39</td>
<td>Factor of 3</td>
</tr>
<tr>
<td>EtOH</td>
<td>$1.0 \times 10^{-4}$</td>
<td>13</td>
<td>37%</td>
</tr>
<tr>
<td>PG</td>
<td>---</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>PG</td>
<td>$1.0 \times 10^{-3}$</td>
<td>39</td>
<td>7%</td>
</tr>
<tr>
<td>PG</td>
<td>$1.0 \times 10^{-4}$</td>
<td>38</td>
<td>6%</td>
</tr>
</tbody>
</table>

$^a$ 1 mL of 20% (v/v) ethanol or 1 mL of 500 mg/L PG
0.5 mL of 95% (w/v) HNO$_3$ for ethanol
0.5 mL of 50% (w/v) HNO$_3$ for PG
0.5 mL of diacetyl or H$_2$O
0.8 mL of $1.0 \times 10^{-3}$ M KMnO$_4$

seems more likely for energy transfer to occur between the excited intermediate or product of the oxidation reaction of ethanol to the
triplet state of the diacetyl. The efficient triplet transition 
\( T_1 \rightarrow S_0 + h\nu \) for diacetyl has already been discussed in the 
Historical section.

With PG, energy transfer and emission is not very efficient. 
Possibly the excited state of the primary emitter lies at a different 
energy level (e.g., slightly lower) than that of the acceptor making 
the energy transfer inefficient.

**Deaeration.** Figure 69 shows the effect of helium on the CL intensity 
of ethanol. At low ethanol concentrations the effect is not obvious. 
However from 25% (v/v) ethanol to higher concentrations the effect is 
more obvious. Oxygen in the air is a very good catalyst in some 
oxidation reactions. Possibly the deaeration procedure causes 
volatileization of the ethanol.

**Cyclobutanol studies**

In order to learn more about the reaction mechanism, a 2.5% (w/v) 
cyclobutanol solution was run under the conditions used for ethanol. A 
very fast CL peak was obtained of high intensity (138 mV as compared 
with 8 mV for a solution of 2.5 (% (v/v)) ethanol). When 0.5 mL of 
0.01 M Mn(II) is included in the reaction mixture, the CL intensity 
decreases only by 8.4% and the peak shape is unchanged. Moreover, in 
the oxidation of cyclobutanol with or without Mn(II) present, neither 
precipitation of colloidal MnO\(_2\) nor the transient appearance of the 
cherry red color of Mn(III) is clearly noticeable as they were in the 
experiments with ethanol.
Figure 69. Effect of deaeration on the CL intensity of ethanol.

+ He bubbled through solution

□ no He
These data suggest that the mechanism for oxidation of cyclobutanol is different than that for ethanol. It appears the Mn(III) rather than Mn(IV) is the species responsible for oxidation. According to Rocek and Rackowski, cyclobutanol is very reactive towards Mn(III) which is a one electron oxidant. In this case, the series of reactions proposed by these authors as shown below are consistent with experimental observations.

\[ \text{Mn(VII)} + \text{Mn(II)} \rightleftharpoons 3 \text{Mn(III)} \]

\[ \text{Mn(III)} + \text{OH} \stackrel{\text{rds}}{\rightarrow} \text{Mn(II)} + \text{HO-(CH}_2)_3\text{-CHO} \]

**Determination of ethanol in gin**

Table XXXII summarizes the results for the CL determination of ethanol in three types of gin. The amount of alcohol was determined by a bracketing technique, a three point calibration curve, and by three separate standard additions. Table XXXIII summarizes the results and Figures 70 and 71 show the three point calibration curves used for the determination.

The bracketing technique yielded excellent results as did the unweighted calibration curve technique. The results with weighted calibration curves were more in error than the unweighted ones. The standard additions results were generally poor except for the Tanqueray sample. The standard additions procedure probably did not work well since the calibration curve is not perfectly linear.
Table XXXII. Results in the Determination of Ethanol in Gin

<table>
<thead>
<tr>
<th>Sample</th>
<th>[Standard] (% (v/v))</th>
<th>Calibration curve CL signal and SD (mV)</th>
<th>Standard Additions CL signal and SD (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gordon's</td>
<td>35</td>
<td>126 (3)</td>
<td>1)(^a) 6 (0.006) 12 (0.3)</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>162 (2)</td>
<td>2)</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>196 (4)</td>
<td>3)</td>
</tr>
<tr>
<td>Sample</td>
<td></td>
<td>159 (4)</td>
<td></td>
</tr>
<tr>
<td>Beefeater (1)</td>
<td>45</td>
<td>201 (8)</td>
<td>1)(^a) 5.7 (0.15) 12 (0.7)</td>
</tr>
<tr>
<td>and</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tanqueray (2)</td>
<td>50</td>
<td>240 (3)</td>
<td>2)</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>293 (6)</td>
<td>3)</td>
</tr>
<tr>
<td>Sample (1)</td>
<td>217 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample (2)</td>
<td>220 (3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Numbers refer to order of addition
Table XXXIII. Determination of Ethanol in Gin

<table>
<thead>
<tr>
<th>Sample</th>
<th>Expected Value (% (v/v))</th>
<th>Conc. and RSD</th>
<th>Error</th>
<th>Conc.</th>
<th>Error</th>
<th>Conc.</th>
<th>Error</th>
<th>Conc.</th>
<th>Error</th>
<th>Conc.</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gordon's</td>
<td>40.0</td>
<td>39.58 (0.013)</td>
<td>1.05</td>
<td>39.63 ± 1.3</td>
<td>0.93</td>
<td>38.56</td>
<td>3.6</td>
<td>35.34</td>
<td>12</td>
<td>35.53</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>39.61 ± 2.5</td>
<td>0.98</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beefeater</td>
<td>47.0</td>
<td>47.07 (0.012)</td>
<td>0.15</td>
<td>47.00</td>
<td>0.00</td>
<td>42.82</td>
<td>8.9</td>
<td>36.42</td>
<td>22.5</td>
<td>35.66</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>47.49</td>
<td>1.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tanqueray</td>
<td>47.3</td>
<td>47.40 (0.0077)</td>
<td>0.21</td>
<td>47.30</td>
<td>0.00</td>
<td>45.72</td>
<td>3.3</td>
<td>46.03</td>
<td>2.7</td>
<td>41.81</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>47.77</td>
<td>0.99</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A = bracketing technique; B = 3 point calibration curve; C = first standard addition (0.1 mL); D = second standard addition (0.2 mL); E = third standard addition (0.3 mL).

First value without weights followed by value with weights plus 95% confidence interval.
Figure 70. Three point calibration curve for the determination of ethanol in gin (Gordon's).
Figure 71. Three point calibration curve for the determination of ethanol in gin (Beefeater and Tanqueray).
Wine and beer were briefly studied and their alcohol content determined. The alcohol concentrations found by CL were approximately two fold that of the expected ones. Apparently other species in these samples were oxidized and contributed to the CL signal.

**Interference study**

A study was conducted to analyze the potential interference of glucose in the determination of ethanol in gin or other alcoholic beverages. The results in Table XXXIV indicate that sugar would be a serious interferent for the determination of ethanol.

**Table XXXIV. Effect of Glucose on the CL Determination of Alcohol**

<table>
<thead>
<tr>
<th>Glucose Conc. (% (w/v))</th>
<th>Effect on Signal (%)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>1.9 Decrease</td>
</tr>
<tr>
<td>0.02</td>
<td>1.9 Decrease</td>
</tr>
<tr>
<td>0.05</td>
<td>30 Increase</td>
</tr>
<tr>
<td>0.1</td>
<td>71 Increase</td>
</tr>
<tr>
<td>6.0$^b$</td>
<td>651 Increase</td>
</tr>
</tbody>
</table>

$^a$ Relative to signal of 10% (v/v) ethanol without interferent

$^b$ Relative to signal of 45% (v/v) ethanol without interferent

The determination of glucose by CL was briefly studied. With 25% (v/v) glucose as the test solution, the optimum reagent concentrations were determined to be 10% (w/v) nitric acid and 1.0 x 10^{-2} M
KMnO₄. However, not even 5% (w/v) glucose could be detected. If the nitric acid concentration is raised to 95% (w/v), the detection limit is about 0.5% (w/v). However, the calibration curve is quite nonlinear and suitable for glucose determination only above about 8% (w/v). The peak shape of the CL reaction of this compound resembles the ones for ethanol. For up to 10% (w/v) glucose, a slow reaction (6 min average duration) is recorded. Above this concentration (20 and 30% (w/v) glucose), a sharper peak is observed.
CONCLUSIONS

In this research, it was demonstrated for the first time that chemiluminescence (CL) is produced during the oxidation of hydroxy-containing compounds by permanganate at low pH. This phenomenon appears to be universal for aliphatic alcohols and phenols.

This new CL reaction was used as the basis to develop several analytical methods for specific hydroxy-containing compounds. These CL methods are particularly unique because the analyte is a necessary reagent for the CL reaction. For most previously developed CL analytical methods, the CL precursor is not the analyte. Rather, the analyte is an activator or acceptor that generally enhances the CL signal observed without the analyte.

Although this CL technique provides selectivity for compounds with a particular functional group, it does not discriminate well among compounds that have hydroxy groups. Therefore, an attempt was made to choose samples in which the hydroxy-containing compound of interest is in sufficient excess with respect to other hydroxy-containing compounds. In this way, the CL signal is ideally due essentially to the analyte.

The CL determination of ethanol in gin proved to be a near ideal application of the technique. The accuracy was 1% or better with no sample preparation, even dilution, required. The method is rapid because the CL signal is obtained within 2 s of adding the sample to the sample cell.

The CL procedures developed for other analytes are less ideal. First, a solvent extraction procedure was necessary to isolate the analyte from interferents. This considerably decreases the sample
throughput. Results accurate to 0.1% were obtained in the CL determination of propyl gallate when added to peanut oil. Reasonably accurate results were realized for the CL determination of BHA in lard (2.5% error) and pyrogallol in hair dye (11% error). The accuracy for the CL determination of propyl gallate in lard (42% error) and vanillin in vanilla extract (48% error) are unacceptable.

These results clearly indicate that in many complex samples better separation procedures (e.g., solid phase extraction) are needed to obtain accurate results with the CL method. Ultimately, the CL reaction might find its greatest use in an HPLC CL detector. Here the eluate of an HPLC would be mixed with acid and permanganate solutions in a post-column reactor and the resulting CL would be detected. Because of the functional group specificity of the reaction, it would only be necessary to separate hydroxy-containing compounds from each other rather than from all other types of compounds as is necessary with more universal detectors (e.g., refractive index, UV-absorption).

For special cases, where separations are not required, the same type of system without a column could be used for flow injection analysis. This would increase the sample throughput considerably.

Different studies revealed much about the nature of the CL reaction although much more study is required. The intensity and duration of CL signals produced is correlated to the rate of oxidation by permanganate. In general, aliphatic alcohols are oxidized slowly and the duration of the CL is 4 s to 6 min, depending on the alcohol concentration. The detection limit is in the 0.3 to 1.0% (v/v) range. In contrast, phenols are oxidized rapidly such that a sharp CL peak with a duration of about 1 s is observed. The detection limit for
polyphenols or phenols with electron-donating groups is typically in the 0.1 to 1.0 mg/L range.

The HNO₃ and KMnO₄ concentrations yielding the maximum CL signal do vary with the analyte structure and the analyte concentration. However, as a general rule (that could be applied to other compounds not tested), the HNO₃ concentration should be varied over the 10 to 100% (w/v) range and the KMnO₄ concentration over the $5 \times 10^{-4}$ to $1 \times 10^{-2}$ M range for optimization. The high limit for KMnO₄ appears to be controlled by absorption of CL photons by the permanganate before they reach the detector. The absorption effect by permanganate also appears to cause nonlinear calibration curves in some cases and distortion of the CL spectrum.

For polyphenols, it is difficult to gather data giving information about the mechanism of the reaction. First, the reaction rate is so fast that peak shape information can not be obtained. Here stopped-flow mixing might allow monitoring of the reaction on a millisecond time scale. Second, many of the tests which would give experimental evidence of reaction pathways produced colored solutions at the end. In this case, the MSS (multiple signal spectrophotometer) would be of invaluable help.

For aliphatic alcohols, much more data about the nature of the reaction were obtained. Some of the key observations are summarized below.

1) Maximum CL intensity is achieved at high acidity.
2) Potassium permanganate is the only oxidant able to produce CL in acidic media.
3) An induction period occurs before the maximum CL intensity is
reached.

4) After some time, the reaction mixture turns a cherry red color for a while which is characteristic of Mn(III) complexes.

5) At the end of the reaction, formation of MnO₂ is observed.

6) The higher the substrate concentration (e.g., ethanol), the higher the CL intensity, the faster the oxidation rate, and the shorter the induction period and duration of the CL signal.

7) When Mn(II) is added to the reaction mixture, the CL intensity decreases for all substrate concentrations, the formation of MnO₂ is more obvious, the rate of disappearance of permanganate is faster, and the cherry red color is still visible but for less time.

8) When Fe(II) is present, the CL intensity increases for all substrate concentrations, and both a sharp CL peak with no observable induction period and the normal slow CL peak (reduced in intensity) are 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.

9) When both Mn(II) and Fe(II) are added to the reaction mixture, their individual effects compromise.

10) Most anions, in particular pyrophosphate, added to the reaction mixture decrease the CL intensity to some extent.

11) Biacetyl increases the CL intensity by three fold.

12) Acetonitrile quenches the CL emission by about 95%, but the reaction apparently goes on.

13) Reaction of cyclobutanol with potassium permanganate does not produce an observable cherry red intermediate, is very fast (< 1 s
duration as compared to 6 min for the same ethanol concentration), and produces no observable MnO₂.

14) When Mn(III) is injected into the reaction mixture instead of permanganate, no CL signal is observed.

15) If aqueous Fe(II) is added to a solution of Mn(III), the solution turns colorless almost immediately.

Two important questions to be answered are: 1) what is the attacking species?, and 2) what is the emitting species?

The attacking species could be Mn(VII), Mn(IV), or Mn(III). 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.

The production and disappearance of Mn species can involve the following reactions.

\[
\text{MnO}_4^- + 4\text{Mn(II)} + 8H^+ \longrightarrow 5\text{Mn(III)} + 4H_2O \quad (1)
\]

\[
2\text{MnO}_4^- + 3\text{Mn(II)} + 16H^+ \longrightarrow 5\text{Mn(IV)} + 8H_2O \quad (2)
\]

\[
\text{Mn(IV)} + \text{Mn(II)} \iff 2\text{Mn(III)} \text{ cherry red} \quad (3)
\]

\[
2\text{Mn(III)} + 2H_2O \iff \text{Mn(II)} + \text{MnO}_2 + 4H^+ \quad (4)
\]

\[
\text{Mn(IV)} + 2H_2O \iff \text{MnO}_2 + 4H^+ \quad (5)
\]

Note that Mn(III) and Mn(IV) can be produced directly from MnO₄⁻ by reactions 1 and 2, respectively, or indirectly from the other species with reaction 3.
The following three schemes are possible if Mn(VII), Mn(III), or Mn(IV) is the attacking species.

Scheme I (Mn(VII))

\[
\text{Mn(VII)} + \text{CH}_3\text{CH}_2\text{OH} \rightarrow \text{Mn}^{V_04H^2-} + \text{CH}_3\text{HC}=\text{OH} \tag{6}
\]

\[
\text{CH}_3\text{HC}=\text{OH} \rightarrow \text{CH}_3\text{CHO} + \text{H}^+ \tag{7}
\]

\[
\text{Mn}^{V_04H^2-} \rightarrow \text{Mn(II)} + \text{Mn(VII)} \tag{8}
\]

Scheme II (Mn(IV))

\[
\text{CH}_3\text{CH}_2\text{OH} + \text{Mn(IV)} \rightarrow \text{CH}_3\text{CH}_2^+\text{Mn(III)} \rightarrow \text{CH}_3\text{CHO} + \text{Mn(II)} + 2\text{H}^+ \tag{9}
\]

Scheme III (Mn(III))

\[
\text{Mn(III)} + \text{CH}_3\text{CH}_2\text{OH} \rightarrow \text{CH}_3\text{CH}_2\text{O}^\text{H-Mn(III)} \tag{10}
\]

\[
\text{CH}_3\text{CH}_2\text{O}^\text{H-Mn(III)} \rightarrow \text{CH}_3\text{CH}_2\text{O}^\text{H} + \text{Mn(II)} + \text{H}^+ \tag{11}
\]

\[
\text{CH}_3\text{CH}_2\text{O}^\text{H} + \text{Mn(III)} \rightarrow \text{CH}_3\text{CHO} + \text{Mn(II)} + \text{H}^+ \tag{12}
\]
Observation 1 is consistent with all the mechanisms. In the case of scheme I, the stronger oxidant, permanganic acid (HMnO₄) is formed with permanganate in very acidic medium. According to Benerji the linear dependence of the oxidation rate of alcohol on acidity, may be due to the formation of this acid. Likewise, Mn(III) and Mn(IV) would be stabilized at high acidities.

Although observation 7 agrees with scheme I (Mn(II) would compete with the alcohol to reduce Mn(VII)), observations 3, 8, 9, and 10 suggest that Mn(VII) is not the attacking species. If the attacking species is indeed Mn(VII), with the rate determining step being the hydrogen transfer, there should be no induction period. Also, Fe(II) being a good reducing agent, would react competitively with the alcohol for the attacking ion. Instead, Fe(II) increases the reaction rate and CL intensity and reduced the induction period rate. Finally, anions should not affect the oxidation rate.

If we rule out scheme I at this point, then Mn(III) or Mn(IV) is the attacking species. According to Waters the reduction of Mn(VII) to Mn(III) in acidic medium is more probable than reduction of Mn(VII) to Mn(IV). The induction period (observation 3) might be due to the slow reduction of Mn(VII) to Mn(III) or reaction 3 being slow. Since the cherry red is not visible (observation 4) for some time after mixing, the former is suggested. According to Wells and Davies, the conversion of Mn(VII) to Mn(III) should be slow because of the change from tetrahedral to octahedral symmetry. After reaction of Mn(IV) or Mn(III) with the substrate or at the same time, a very slow formation of MnO₂ (observation 5) is noticed.

Observation 7 agrees better with scheme II. Mn(II) shifts the
equilibrium between Mn(III) and Mn(IV) towards Mn(III). This should decrease the concentration of Mn(IV) (equation 3) and therefore the CL intensity if Mn(IV) is the oxidant. The CL signal should increase if Mn(III) is the oxidant.

Observation 8 is very important. Fe(II) causes a rapid reduction of Mn(VII) to Mn(III) or Mn(IV) such that the the induction period is essentially eliminated and the CL intensity is increased substantially. The second part of observation 8 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. It could be postulated that Mn(III) is still the attacking species formed from equation 3 after Mn(IV) is produced by reduction with Fe(II). However, observation 7 suggests that concentration of Mn(III) builds up as the reaction proceeds. If Mn(III) was the attacking species, the reaction would still be slow in the presence of Fe(II).

Most anions (observation 10) complex Mn(III) to some extent. This should shift the disproportionation equilibrium in equation 3 away from Mn(IV) so that CL intensity would decrease. It is known that pyrophosphate is one of the most stable complexes of Mn(III) at low acidities. Both observations 7 and 10 suggest that the equilibrium between Mn(III) and Mn(IV) is important.

Observation 13 needs further discussion here. Cyclobutanol is very reactive towards Mn(III), in which case neither the cherry red nor the MnO₂ precipitation is observed. It might be postulated that ethanol reacts so slowly towards Mn(III) that the distinctive color is visible and MnO₂ is formed.

Observation 14 also suggests that Mn(III) is not the oxidant in
the CL reaction. It could be argued that the acetic acid present to stabilize the Mn(III) reduces its oxidizing power. However, CL is still observed when permanganate is injected into a reaction mixture containing acetic acid.

In conclusion, the experimental results suggest that Mn(VII) does not directly attack the alcohol, and that Mn(IV) is the attacking species. It appears that Mn(IV) is produced by disproportionation of Mn(III), and the rate of formation of Mn(III) the rate limiting step.

The broad CL spectrum, between 430 and 750 nm, suggests that the emitting species is not a reaction product such as acetic acid or acetaldehyde. These species fluoresce in the UV region. Rather the data suggest that some excited Mn-alcohol intermediate is responsible for the luminescence observed. If Scheme II applies, the intermediate in reaction 9 would have the following octahedral structure:

```
  OH2
 /   \
 H2O -> Mn -> H2O
 /    \
OH2
```

This d⁴ species has a ground electronic structure of t⁡₂g⁴e⁡₉. If this species is produced in the excited state (t⁡₂g⁴e⁡₉), an e⁡₉→t⁡₂g transition could be responsible for the CL.
BIBLIOGRAPHY


27. Ingle, Jr., J. D.; *Chemistry 520 notes*, Oregon State University, 1980.

29. *Title 27*, Department of the Treasury, Bureau of Alcohol, Tobacco and Firearms.


   (1939).
57. Alabaster, Oliver, M. D., ed., *What You Can Do To Prevent Cancer*,
60. Commerce Clearing House, Inc., *Food Drug Cosmetic Law report New


77. Hawkes, S. J.; *Chemistry 234 notes*; Oregon State University, 1986.

78. Code of Federal Regulations - Food and Drugs, Chemical Preservatives Food and Drugs, April 1, 1985, 21, part 500-599, 582.2122, page 656.


95. Discussion with Glenn Harless of Armour and Co. in October, 1985.

APPENDIX
APPENDIX I

BASIC and Machine Language Programs
for Chemiluminescence Data Acquisition
10 R EN BASIC CLDATA
11 PRINT "USEFUL INFORMATION."
12 PRINT "AFTER INJECTION OF"
13 PRINT "RT. THE N.L."
14 PRINT "SUROUTINE TAXES"
15 PRINT "MICROSECONDS"
16 PRINT "BEFORE ACQUISITION"
17 PRINT "OF FIRST DATA POINT."
18 PRINT "ENTER NUMBER OF POINTS"
19 PRINT "TO GATHER FROM 60"
20 PRINT "TO 512."
21 INPUT N1
22 IF N1=0 GOTO 80
23 IF N1=0 GOTO 791
24 PRINT "ENTER ACQUISITION TIME"
25 PRINT "IN S FROM 1.1 TO 30."
26 INPUT T1
27 LET T1=I
28 PRINT "ENTER NUMBER OF POINTS"
29 PRINT "TO GATHER FROM 60"
30 PRINT "TO 512."
31 INPUT N2
32 PRINT "MS. IN DETE UEN EACH"
33 PRINT "DATA POINT.":I1=T*1E61/N1)
34 PRINT "P OKE 22186.16"
35 PRINT "POKE 58.5 INT(T/256)+2*U1)
36 PRINT "POKE 584.(N1-1)*INT(T/256)+256"
37 PRINT "POKE 585.INT((1-1)/256)
38 PRINT "ENTER GAIN"
39 INPUT F1
40 POKE 11.11
41 GOTO 12
42 X=USR(Y)
43 PRINT "ENTER NUMBER OF RUNS"
44 INPUT N3
45 FOR N3=0 TO N3-1
46 PRINT "READY"
47 INPUT AM1
48 IF AM1="Y" GOTO 47
49 POKE 11.29
50 POKE 12.86
51 X=USR(Y)
52 PRINT "ENTER TOTAL ACQUISITION TIME IN S FROM 1.1 TO 30."
53 INPUT T2
54 LET T2=I
55 PRINT "ENTER NO. OF POINTS"
56 PRINT "TO GATHER FROM 75"
57 INPUT N4
58 T=INT(T*1E61/(N4-1))
59 PRINT "AVDS.D.S.D.CL"
APPENDIX II

Statistical Analysis Programs
and Sample Outputs
220 DIM X(100), Y(100), B4(100), P2(100), P4(100), B1(100), S7(100)
230 PRINT "ENTER NUMBER OF POINTS N=3."
240 INPUT N
250 SUMF = 0
260 SUMG = 0
270 SUMH = 0
280 PRINT "ENTER 4 VALUES, X=VALUES"
290 FOR I = 1 TO N
300 INPUT X(I), Y(I)
310 SUMF = SUMF + X(I)
320 SUMG = SUMG + Y(I)
330 NEXT I
340 SUMH = SUMH / N
350 SUMF = SUMF / N
360 SUMG = SUMG / N
370 SUMH = SUMH / N
380 SUMF2 = SUMF2 + X(I) * Y(I)
390 NEXT I
400 SUMF2 = SUMF2 / N
410 SUMG2 = SUMG2 + Y(I) * Y(I)
420 NEXT I
430 SUMG2 = SUMG2 / N
440 SUMF3 = SUMF3 + X(I) * X(I)
450 NEXT I
460 SUMF3 = SUMF3 / N
470 SUMG3 = SUMG3 + Y(I) * Y(I)
480 NEXT I
490 SUMG3 = SUMG3 / N
500 PRINT "SLOPE M=1/SUMF2-SUMF3/SUMG3-SUMG2"
10 PRINT "BASIC PROGRAM FOR CALCULATION OF BEST LINE THROUGH POINTS WITH"
20 PRINT "NON UNIFORM VARIANCE: x VALUES ARE TRUE VALUES."
30 PRINT "WRITTEN BY SOCORRO MONTALVO, O.S.U., 1986."
40 DIM x(100), y(100), p(100), s1(100), s7(100), s4(100), p2(100)
50 PRINT "ENTER N=NUMBER OF POINTS.N>3."
60 INPUT n
70 SUMP=0
80 SUMP2=0
90 SUMP4=0
100 SUMS7=0
120 PRINT "ENTER x-values and 1ST STANDARD DEVIATION IN x, y-values and 1ST STANDARD DEVIATION IN y."
140 FOR I=1 TO n
150 INPUT x(I), y(I), z(I)
160 SUMP=SUMP+x(I)
170 SUMP2=SUMP2+x(I)*x(I)
180 SUMP4=SUMP4+x(I)*x(I)*x(I)
190 SUMS7=SUMS7+x(I)*x(I)*x(I)*x(I)
200 NEXT I
210 P1=(SUMP2/SUMP)*P2
220 S1=SUMP*P1
230 S4=SUMP4/SUMP
240 S7=SUMS7/SUMP
270 NEXT I
290 P3=(SUMP4/SUMP2)*P1
300 S4=SUMP4/SUMP
350 S7=SUMP7/SUMP
400 SUMS=SUM(SQUARES OF RESIDUALS) "SIS15
420 S1=S15/(n-2)
430 PRINT "STANDARD ERROR OF ESTIMATE " SIS16
440 S17=S16*S1
450 PRINT "STANDARD DEVIATION OF SIGNAL, y= " S17
460 S18=S16/S1
470 P1=P1^2/SUMP
480 P4=P1/P4
490 P5=P1/P5
500 INPUT "DO YOU WANT TO OBTAIN THE INTERVAL ESTIMATE OF THE x VALUE?"
510 IF A=0 THEN GOTO 500
520 IF A=1 THEN GOTO 500
530 PRINT "OBSERVING A NEW VALUES OF Y (WITH AVERAGE Y') OBTAIN THE INTERVAL"
540 PRINT "DO YOU WANT TO OBTAIN THE INTERVAL ESTIMATE OF THE y VALUE?"
550 IF A=0 THEN GOTO 500
560 IF A=1 THEN GOTO 500
570 PRINT "ENTER A DESIRED CONFIDENCE LEVEL.%"
580 INPUT A
590 A1=A/100
600 PRINT "FOR A CONFIDENCE INTERVAL FOR THE LINE AS A WHOLE, LOOK UP FB FOR (2,n-2)"
610 PRINT "DEGREES OF FREEDOM IN TABLE A-5, PAGE T-5 TO T-9"
620 INPUT S
630 PRINT "ENTER A=MEAN OF NEW VALUES OF Y."
640 INPUT M
650 PRINT "ENTER STANDARD DEVIATION OF NEW VALUE OF Y."
660 INPUT S
670 INPUT A10
680 IF A10=1 THEN GOTO 500
690 IF A10=0 THEN GOTO 500
700 PRINT "DO YOU HAVE ANOTHER SET OF DATA TO WHICH YOU WANT TO OBTAIN A NON-CONSTANT VARIANCE FIT?"
710 IF A=0 THEN GOTO 500
720 IF A=1 THEN GOTO 500
730 END
RUN
BASIC PROGRAM FOR CALCULATION OF BEST LINES THROUGH POINTS WITH UNIFORM VARIANCE
WRITTEN BY SOCORRO MONTALVO, O.S.U., 1986
ENTER N=NUMBER OF POINTS. N= 1.
N= 4
ENTER X= VALUES, Y= VALUES
X= 5, 8, 6
Y= 10.16
X= 20.31
Y= 40.62
SUM OF SQUARES OF RESIDUALS = .157959
STANDARD ERROR OF ESTIMATE = .0789795
STANDARD DEVIATION OF SIGNAL, S_Y = .2810329
EQUATION OF THE LINE
SLOPE = 1.527652
INTERCEPT = .7565213
ESTIMATED VARIANCE OF THE SLOPE = 1.098845E-04
ESTIMATED VARIANCE OF THE INTERCEPT = 5.837615E-02
DO YOU WANT TO OBTAIN THE INTERVAL ESTIMATE OF THE X VALUE? Y OR N
Y
OBSERVING A NEW VALUES OF Y (WITH AVERAGE Y') OBTAIN THE INTERVAL
ESTIMATE OF THE VALUE X THAT PRODUCED THESE VALUES OF Y
ENTER DESIRED CONFIDENCE LEVEL = A%
A = .95
D = .975
LOOK UP TD FOR N-2 DEGREES OF FREEDOM
TD = 4.303
ENTER E = MEAN OF NEW VALUE OF Y
E = 9.4
ENTER M = NUMBER OF TIMES FOR OBTAINING NEW VALUE Y
M = 3
UNKNOWN = 5.84659
A% CONFIDENCE INTERVAL ESTIMATE FOR THE UNKNOWN IS = .718013
DO YOU WANT TO CALCULATE A% CONFIDENCE BAND FOR THE LINE AS A WHOLE? Y OR N
Y
FB = .95
FOR A% CONFIDENCE BAND FOR THE LINE AS A WHOLE, LOOK UP FB FOR (2, N-2)
DEGREES OF FREEDOM
FB = 19
S10(I) = .3994783
W(I) = 1.24087
S20(I) = 16.03304
W(I) = 1.034408
S30(I) = 11.30957
W(I) = .8699596
S40(I) = 61.88261
W(I) = 1.623952
DO YOU HAVE ANOTHER SET OF DATA? Y OR N
N
OK
RUN

BASIC PROGRAM FOR CALCULATION OF BEST LINE THROUGH POINTS WITH NON UNIFORM VARIANCE. X VALUES ARE TRUE VALUES.
WRITTEN BY SOCORRO MONTALVO, O.S.U., 1986

ENTER N=NUMBER OF POINTS. N=3.

ENTER X=VALUES; Y=VALUES AND S=STANDARD DEVIATION IN Y.

5.8, 6.0, 2
10.16, 0.2
20.31, 0.7
40.62, 0.8

SUM OF SQUARES OF RESIDUALS = 0.8125
STANDARD ERROR OF ESTIMATE = 0.40625
STANDARD DEVIATION OF SIGNAL, Sy = 0.677775
EQUATION OF THE LINE
SLOPE = 1.517831
INTERCEPT = 0.9166817
ESTIMATED VARIANCE OF THE SLOPE = 1.869375E-04
ESTIMATED VARIANCE OF THE INTERCEPT = 2.246364E-02

DO YOU WANT TO OBTAIN THE INTERVAL ESTIMATE OF THE X VALUE?
Y OR N

? Y

OBSERVING n NEW VALUES OF Y (WITH AVERAGE Y') OBTAIN THE INTERVAL ESTIMATE OF THE VALUE X THAT PRODUCED THESE VALUES OF Y

ENTER DESIRED CONFIDENCE LEVEL=A%

? 95

LOOK UP TD FOR N-2 DEGREES OF FREEDOM. FOR EXAMPLE, IN NATRELLA'S NBS HANDBOOK, TABLE A-4 PAGE T-5

? 4.303

ENTER E=MEN OF NEW VALUES OF Y

? 9.4

ENTER M=STANDARD DEVIATION OF NEW VALUE OF Y

? 0.4

UNKNOWN = 5.58409
A% CONFIDENCE INTERVAL ESTIMATE FOR THE UNKNOWN IS = 0.775208

DO YOU WANT TO CALCULATE A% CONFIDENCE BAND FOR THE LINE AS A WHOLE?
Y OR N

? Y

FB = 0.95

FOR A% CONFIDENCE BAND FOR THE LINE AS A WHOLE, LOOK UP FR FOR (2, N-2) DEGREES OF FREEDOM IN TABLE A-5, PAGES T-6 TO T-9

? 19

S30(I) = 8.505834
W1(I) = 6303444
S30(I) = 16.09499
W1(I) = 544271
S30(I) = 31.27329
W1(I) = 1.076823
S20(I) = 61.6299
W1(I) = 2.675257

DO YOU HAVE ANOTHER SET OF DATA TO WHICH YOU WANT TO OBTAIN A NON-CONSTANT VARIANCE FIT?
Y OR N

? N

OK