A Comparison of the Enzyme Fluorometric and the Peroxyoxalate Chemiluminescence Methods for Measuring $H_2 O_2$

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Abstract. During September 25 to October 28, 1985, the enzyme fluorometric (Lazrus *et al.,* 1985) and the peroxyoxalate chemiluminescence (Klockow and Jacob, 1986; Jaeschke, 1986) techniques for analyzing H_2O_2 were compared in laboratory studies at the National Center for Atmospheric Research in Boulder, Colorado, U.S.A. and under field conditions at the Whiteface Mt. field station of the Atmospheric Sciences Research Center, Albany, New York, U.S.A. Both methods showed excellent agreement, with a maximum deviation of $\pm 5%$. Only at unusually high concentrations of some potential atmospheric species could slight interferences be observed. During the experiments the detection limits were 1.3×10^{-8} mol/l (0.44 ppbm) of H₂O₂ for the fluorometric instrument and 4×10^{-8} mol/1 (1.36 ppbm) of H₂O₂ for the chemiluminescence instrument. For the chemiluminescence technique, the response to methylhydroperoxide was approximately 80-fold less than that to an equivalent concentration of H_2O_2 .

Key words. Enzyme fluorometry, peroxyoxalate chemiluminescence, hydrogen peroxide analysis, comparative study.

1. Introduction

Accurate measurements of H_2O_2 in cloud and fog water are important because $H₂O₂$ plays a dominant role as an oxidant in heterogeneous processes which lead to the formation of sulfuric or nitric acids in the atmosphere, particularly at high insolation and at pH-values below pH 5.

 $H₂O₂$ in precipitation and cloud water has been measured by several groups (Bufalini *et al.,* 1979; Kok, 1980; Roemer *et al.,* 1985) using a luminol chemiluminescence technique. However, there are some interferences in the technique (Ibusuki, 1983; Lazrus *et al.,* 1985) which brought its results into question and led to the development of other analytical techniques. One is the enzyme fluorometric technique (Lazrus *et al.,* 1985), which is applied by several groups in the United States for analyzing precipitation and cloud water samples

(Richards *et al.,* 1983; Kadlecek *et al.,* 1983; McLaren *et al.,* 1985; Kelly *et al.,* 1985). Another technique is the peroxyoxalate chemiluminescence method (Klockow and Jacob, 1986), which is mainly used by different European groups with various modifications (Guebitz *et al.,* 1985; Jaeschke, 1986; Neftel *et al.,* 1984). A study comparing these two methods was conducted from September 25 to October 28, 1985, with laboratory tests at the National Center for Atmospheric Research (NCAR) at Boulder, Colorado and subsequent field measurements at the Whiteface Mt. {Atmospheric Sciences Research Center, Albany, New York).

2. Laboratory Studies

2.1. Experimental

The NCAR fluorometric method is based on the enzyme-catalyzed reaction of $H₂O₂$ with p-hydroxyphenylacetic acid, which forms a fluorescent dimer (Lazrus *et al.,* 1985). The analytical chemical reaction responds both to H_2O_2 and to organic hydroperoxides. With catalase as a discriminating agent for $H₂O₂$, and with a dual channel flow system, separate signals can be obtained for H_2O_2 and the organic peroxides. For each analytical run in our study a 1.5-ml sample was used.

In the chemiluminescence method a reaction of H₂O₂ with *bis*-trichloro phenyloxalate is used (Rauhut *et al.,* 1986). The high-energy dioxetanedione (Stauff and Jaeschke, 1972) that forms transmits its chemiluminescence to perylene as a fluororescent, which relaxes to its ground state with the emission of light (Jaeschke, 1972). Our analysis was performed in a batch process by injection of 0.5 ml of a sample into 1.1 ml of the reagent solution (Beltz, 1987).

In our study a common set of aqueous standards was used to calibrate both instruments daily. The samples to be tested for potential interferences were generated individually. Analyses were performed as quickly as possible after preparation to minimize the chances of reactions between the H_2O_2 and a potential interferent. Analyses of the standards and the test samples (containing an unknown amount of H_2O_2 and a variety of potential interferent compounds) were conducted simultaneously with both instruments.

2.2. Results and Discussion

The results of these interference studies are compiled in Table I. These studies were conducted in the presence of H_2O_2 and a variety of organic and inorganic compounds, typically in concentrations of 1×10^{-4} mol/l. This is much higher than would be seen in ambient precipitation or cloud water samples; however, it provides a rigorous check on the techniques. The test did not include common compounds, such as sulfate, nitrate, or heavy metals, because their role had

already been examined individually in the home laboratories for each technique and could be neglected (Lazrus *et al.,* 1985; Beltz, 1987). A solution containing a mixture of common anions and cations was used which provided a surrogate of ambient precipitation (Kok *et al.,* 1986). Analysis of the synthetic rain mixture gave an excellent agreement between the two techniques.

The reproducibility of the techniques can be seen from the analysis of eight individually prepared standards $(2.15 \times 10^{-7} \text{ mol/1 H}_{2} O_{2})$ incorporated into this series of samples. The fluorometric technique gave 2.31 ± 0.06 mol/l and

Table la. Interference studies.

The samples were prepared freshly to contain 2.15×10^{-7} mol/l H_2O_2 for each analytical cycle.

 $FI =$ fluorescent instrument, $CI =$ chemiluminescent instrument

 $% =$ percentage deviation from x

 $a = peak$ shape distorted, no quantitation possible

 $b =$ same value as the blank water (Milli-Q)

 $c =$ below baseline of blank water.

Table lb. Interference studies

HMSA = Hydroxymethanesulfonic acid

 $TBHP = t$ -butylhydroperoxide

 $PAA =$ peroxyacetic acid

 $[org.]=$ concentration of organic peroxide (analyzed with the fluorometric instrument).

the chemiluminescence instrument 2.09 ± 0.25 mol/1 of H₂O₂. These values include the variation in the sample preparation, and the variation in analysis is less than that.

Table Ia shows a positive interference in the presence of CO_3^- , Ca^{++} , and ethanol in the chemiluminescence technique, but not interference in the fluorometric instrument. In the case of the extremely acidified synthetic rain (pH 2.7), the chemiluminescence signal was destroyed since the buffer capacity of the system was exceeded. This is a recognized problem in the chemiluminescence system; therefore, most of the samples are diluted before analysis.

Table Ia lists detailed studies on amine compounds and some organic acids. At the 1×10^{-4} -mol/l level the chemiluminescence technique had a negative interference from methylamine, hydroxylamine, and ammonia. Only hydroxylamine gave an interference in the fluorescence technique. These negative interferences were still observed at 10^{-5} mol/l. At the 10^{-6} -mol/l level no interferences were seen for either technique. Formaldehyde at a concentration of **10** -4 mol/l did not effect either instrument.

Included in these series (Table Ib) was a sample containing 4.3×10^{-6} mol/l (146 ppbm) of H₂O₂, which was analyzed in the presence of 1×10^{-4} mol/l of hydroxymethanesulfonate (HMSA) and of 1×10^{-3} mol/l of formaldehyde. No interference was noted in either technique. Samples containing t-butylhydroperoxide (TBHP) and peroxyacetic acid (PAA) were also examined. The chemiluminescence technique does not see TBHP or PAA. The fluorometric method responds only to PAA.

The detection limits for the techniques are not directly comparable. The fluorometric instrument uses a continuous-flow system referenced against a base line. In contrast, the chemiluminescence instrument analyzes each sample individually in a batch process, and there is a contributing signal from the background chemiluminescence. Deriving a detection limit for the fluorometric technique based on three times the signal-to-noise yields a value of 1.3×10^{-8} mol/l (0.44 ppbm). Using three times the standard deviation of the blank as detection limit criteria for the chemiluminescence instrument a value of 4×10^{-8} mol/l (1.36 ppbm) is obtained.

The precision of the analytical techniques can be examined by considering the standard deviation in the analysis of a sample containing H_2O_2 . At an H_2O_2 concentration of 4.3×10^{-8} mol/1 (1.46 ppbm), the fluorometric technique has a standard deviation of 0.06×10^{-8} mol/1 (0.02 ppbm). For the chemiluminescence technique the standard deviation is 0.3×10^{-8} mol/1 (0.10 ppbm). Both of these measurements are based on three analysis.

The fluorometric method is known to respond to organic hydroperoxides. The dual-channel flow system employs catalase as a discriminating agent to determine both H_2O_2 and organic hydroperoxides. To examine the response of the chemiluminescence technique to organic hydroperoxides detailed tests were employed. Table II gives the response of the chemiluminescence instru-

Matrix	[H, O, F] 10^{-6} mol/l			
	Fl	Cla	Clb	
MHP 4.57×10^{-7} mol/1	1.09	0.09	0.69	
MHP 1.37×10^{-6} mol/l	0.96	0.23	0.72	6
MHP 4.57×10^{-6} mol/l	0.98	0.12	0.75	38
MHP 1.37×10^{-5} mol/l		0.19	0.87	72
MHP 4.57×10^{-5} mol/l		0.69	1.21	66
MHP 1.37×10^{-4} mol/l		1.96	2.41	70
MHP 4.57×10^{-4} mol/l		7.15	9.06	64

Table 11. Chemiluminescent response to methylhydroperoxide

All samples contained a fixed amount of 0.86×10^{-6} mol/l H_2O_2 . With no added MHP the fluorometric instrument gave 0.8×10^{-6} mol/l and the chemiluminescence instrument 0.68×10^{-6} mol/l.

 $Cla = Cl$ signal for MHP alone with no added H_2O_2

Clb = Cl signal for samples containing MHP and 0.86×10^{-6} mol/l H₂O₂

Response ratio = MHP-concentration/H₂O₂ response Cla.

ment, at a fixed H₂O₂ concentration of 8.59×10^{-7} mol/l (29.2 ppbm), to the presence of methylhydroperoxide (MHP) in the range from 4.57×10^{-4} mol/1 to 4.57×10^{-4} mol/1 and to MHP alone in water (in the same range). These data show an additive response due to the added MHP. At high MHP concentrations ($> 10^{-5}$ mol/l) the response was approximately 60- to 80-fold less than for H_2O_2 in the same molar concentration. At low concentrations of MHP there was a small elevated signal due to the organic hydroperoxide. An exact quantitation is not possible. The signal caused by the added MHP was in the same range as the response of the blank water for this analytical run (0.14 \pm 0.06×10^{-6} mol/l). The samples were also analyzed by the fluorometric instrument up to a MHP concentration of 4.57×10^{-6} mol/l, which was the upper limit in the dynamic range of the instrument configured for these studies.

In Table III the results from a series of samples containing a fixed concentration (4.57 \times 10⁻⁵ mol/1) of MHP and varying concentrations of H₂O₂ are given. This MHP concentration was above the operational range of the fluorometric technique as implemented, and duplicate samples of H_2O_2 without the MHP were prepared for analysis as a check on the sample preparation. With the chemiluminescence instrument the response to the added MHP was independent of the concentration of H_2O_2 . In all cases the average response of the chemiluminescence instrument to MHP was 80-fold less than that of an equivalent H_2O_2 concentration.

There are no accurate measurements of MHP or other organic hydroperoxides in atmospheric liquid samples. Some limited measurements of peroxides

Matrix						
$[H_2O_2]$ 10^{-6} mol/l	[org.] 10^{-5} mol/l	$[H_2O_2]$ 10^{-6} mol/l			Response ratio	
		Fl	C1	Cla		
4.29		4.56	4.15	0.64	71	
4.29	4.57		4.79			
3.32		3.4	2.79	0.87	53	
3.32	4.57		3.66			
2.15		2.29	1.89	0.55	83	
2.15	4.57		2.44			
1.29		1.38	1.22	0.4	114	
1.29	4.57		1.62			
0.43		0.43	0.38	0.49	93	
43	4.57		0.87			
H_2O			0.04	70		
H_2O	4.57		0.69			

Table III. Chemiluminescent response to MHP

 $Cla = Cl$ signal with MHP minus Cl signal without MHP Response ratio = MHP concentration/H₂O₂ response Cla Mean value of response ratio = 80 ± 21 .

in precipitation and fog samples indicate that the residual signal which can be attributed to the organic hydroperoxides is about 10%. The results of the chemiluminescence instruments indicate an influence due to organic peroxides only for low H₂O₂ concentrations (i.e. $10 \times^{-6}$ mol/l) and relatively high MHP concentrations ($10 \times^{-5}$ mol/l). It is highly unlikely that these conditions would occur in ambient samples.

In summary, both the fluorometric and the chemiluminescence techniques for analyzing H_2O_2 were subjected to a wide variety of potentially interfering species. A few minor interferences have been observed; however, none of these species would be expected in ambient water samples at concentrations near the levels which were used in these studies. Direct comparison of the two methods demonstrates excellent agreement. Both techniques performed satisfactorily during the studies. An advantage of the fluorometric method is the possibility to detect both H_2O_2 and organic peroxides. The chemiluminescence technique has the capability to process samples at a faster rate than the fluorometric technique (about 35 samples per hour for the chemiluminescent instrument and 10-12 samples per hour for the fluormetric).

3. Field Measurements

3. I. Experimental

Additional comparisons were conducted under field conditions at the Whiteface Mt. field station of the Atmospheric Sciences Research Center (ASRC), State University New York at Albany. ASRC used its own analytical instrument based on the NCAR fluorometric technology in comparison with the chemiluminescence technique.

The field studies consisted of the common analysis with both instruments of 27 cloud water samples collected at the summit of Whiteface Mt. from October 10 to 28. One sample of snow from Boulder, Colorado, was also included.

The samples were collected by the ASRC string collector mounted above the roof of the observatory at the summit of Whiteface Mt. (Falconer and Falconer, 1980). Cloud water flows from the collector through a Teflon tube to a bottle inside the laboratory. All samples were analyzed immediately after the end of a collection cycle (20-60 minutes). At the measuring site both instruments were calibrated with the same standards, and the analyses of each sample were carried out simultaneously. Some of the samples were used for standard addition procedures or dilution tests.

3.2. Results and Discussion

Table IV shows the results of the analysis of ambient cloud water samples.

Sample	$[H_2O_2]$ 10^{-6} mol/l		Sample	[H, O, 1] 10^{-6} mol/l	
Date/Time	Fl	C1	Date/Time	Fl	Cl
10/10/85			10/14/85		
14:00	a	a	12:00	20.21	22.21
14:25	a	a	12:30	15.62	15.32
14:45	a	0.21	13:00	10.76	10.53
15:22	a	0.21	13:30	7.74	8.12
15:45	a	0.21	14:05	5.91	7.00
10/13/85			14:30	4.59	5.50
11:15	\mathbf{a}	a	15:00	4.32	4.76
12:00	a	0.18	10/24/85		
13:00	a	0.18	14:30	2.18	2.26
14:00	a	0.21	15:30	0.68	0.65
15:00	1.00	1.29	16:30	0.29	0.24
16:00	3.38	3.44	17:30	0.21	0.15
16:55	1.79	1.94	18:30	0.15	0.06
			19:30	0.47	0,41
			20:30	0.29	0.29
			21:30	1.53	1.32

Table IV. lntercomparison of cloud water samples

Time is the end time of a collection cycle.

 $a =$ below detection limit $(Fl = 0.15 \times 10^{-6} \text{ mol/l}, Cl = 0.06 \times 10^{-6} \text{ mol/l}).$

The samples gathered between October 10 and 13 contained very low amounts of H_2O_2 , amounts only slightly above the detection limit of the chemilumi**nescence instrument. At this low concentration the fluorometric instrument showed no signals above the background noise, which was unusually high because HF radiation was present. This interference is an artifact of radio transmitters present at the site. At higher concentrations the data showed good agreement between the two instruments. All data of Table IV are plotted in** Figure 1 for regression analysis. The coefficient of $r = \pm 0.997$ is obtained, **and the regression curve is given by the following equation:**

Fluorometric $[H_2O_2] = 0.944$ Chemiluminescent $[H_2O_2] + 0.024$.

All the measured values fit in the 1:1 line with a deviation of \pm 5% to 10%.

The results of the standard addition procedures are compiled in Table V. In a first run each sample was analyzed in the normal manner. Then three aliquots of 20 ml of each sample were taken and 20 μ l, 40 μ l, or 60 μ l of a 2.94 \times 10⁻⁴mol/l (10000 ppbm) H₂O₂ standard were added before the subsequent analysis. **In the case of the snow sample analyzed at NCAR other amounts of added H202 were used. Again both instruments showed good agreement, even when** the added H_2O_2 obviously reacted with reduced compounds in the sample **(two samples from October 13).**

Fig. 1. Intercomparison between the fluorometric and the chemiluminescent techniques on cloud water samples collected at Whiteface Mt., New York. The straight line represents least-squares fit; the 1:1-line represents perfect agreement and the dashed lines are \pm 5% deviation from this line.

In order to examine the effects of diluting samples, in two experimental runs 1 and 2 ml of sample were added to l0 ml of deionized water. The results of these experiments are presented in Table VI. The results of analysis and the calculated value are in a reasonably good agreement.

4. Summary

Both instruments worked well in laboratory tests and under field conditions. Analytical results show good agreement over a wide range of H_2O_2 concentration. Both methods are also applicable in standard addition and dilution procedures. The result of this comparison underlines the capability of both methods

Sample	$[H_2O_2]$ 10^{-6} mol/l	$[H_2O_2]$ 10^{-6} mol/l			
Date/Time	added	F1		C1	
		total	diff.	total	diff.
9/29/85		2.26		2.29	
$\mathbf b$	0.85	3.18	2.33	2.94	2.09
	1.71	4.00	2.29	3.82	2.11
	2.15	4.44	2.29	4.44	2.29
	2.59	4.82	2.23	4.79	2.20
10/13/85					
13:00		$\bf a$		0.18	
$\mathbf c$	2.94	1.68	-1.26	1.97	-0.97
	5.88	4.56	-1.32	4.85	-1.03
	8.82	7.85	-0.97	8.21	-0.61
14:00		\mathbf{a}		0.21	
$\mathbf c$	2.94	1.94	-1.00	2.21	-0.70
	5.88	5.12	-0.76	5.53	-0.35
	8.82	7.85	-0.97	8.20	-0.62
15:00		1.00		1.29	
$\mathbf c$	2.94	4.00	1.06	3.94	1.00
	5.88	7.29	1.41	6.79	0.91
	8.82	10.32	1.50	10.06	1.24
10/24/85					
16:30		0.29		0.23	
$\mathbf c$	2.94	3.24	0.29	3.24	0.29
	5.88	6.18	0.29	6.00	0.12
	8.82	8.82	θ	9.11	0.29
19:30		0.47		0.42	
$\mathbf c$	2.94	3.53	0.59	3.09	0.15
	5.88	6.47	0.59	6.00	0.12
	8.82	8.47	-0.35	9.29	0.47

Table V. Standard addition on cloud water samples

a = below detection limit.

 $b =$ snow sample collected in Boulder, Colorado.

 $c =$ cloud water samples collected at Whiteface Mt., New York.

for accurate measurements of H_2O_2 in ambient precipitation, cloud, and fog water samples.

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The Atmospheric Sciences Research Center's Whiteface Mountain participa-

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Date/Time	dil. factor	$[H_2O_2]$ 10^{-6} mol/l				
		F1		Cl		
		a	b	a	b	
10/14/85						
12:00		20.21		22.21		
	1:6	3.06	18.35	3.38	20.29	
	1:11	1.79	19.74	2.21	24.26	
12:30		15.62		15.44		
	1:6	2.62	15.71	2.85	17.12	
	1:11	1.29	14,24	1.44	15.85	
13:00		10.76		10.53		
	1:6	1.62	9.71	1.82	10.94	
	1:11	0.88	9.71	0.88	9.71	

Table VI. Dilution tests on cloud water samples

 $a =$ measured [H₂O₂].

 $b =$ calculated initial [H₂O₂].

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