Environmental Laboratory Exercise

Analysis of Hydrogen Peroxide by Fluorescence Spectroscopy

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Gaseous hydrogen peroxide is found in parts-per-billion by volume concentrations in clean and polluted atmospheres. Studies show that H_2O_2 and organic peroxides are deleterious to plant tissues (1, 2), and may play a part in forest decline in Europe (3). A number of analytical methods have been developed for determining atmospheric peroxides; these methods and their applications in the field are the subject of two recent reviews (4, 5).

Episodes of high concentrations of ozone, hydrocarbons, particulate matter and oxides of nitrogen that occur in urban areas during the summer, sometimes referred to as "Los Angeles smog", are complex chemical events initiated by sunlight and involving the free radicals OH• and HO₂• as intermediates. Figure 1 illustrates the key chemical reactions involved in the production of photochemical air pollution. In spite of the central role played by free radicals, low ambient concentration makes their measurement in the field difficult. One of the ways we verify our understanding of photochemical air pollution is by comparing field measurements to the results of computer models (e.g., 6, 7) that predict the time evolution of key chemical species under specified meteorological conditions. Products such as O_3, NO_2, HNO_3, and H_2O_2 are easier to measure than their highly reactive free-radical precursors. Such measurements in conjunction with model calculations provide information about free radical concentrations.

The effect of acidic precipitation on streams and lakes in the Northeastern United States and Canada was well-documented during the 1980's (8, 9), as were the consequences to building materials and the ecosystem (9–11). It has been demonstrated that precipitation in these areas contains sulfuric acid, the oxidation product of SO_2 generated during the combustion of sulfur-rich coal. The principal reactions involved in acid rain formation are illustrated in Figure 1. Although gas-phase oxidation of SO_2 by OH• radical contributes to acid formation, aqueous-phase oxidation pathways are believed to be more important, as illustrated by the substantial increase in SO_2 oxidation rates in power plant plumes that impact fog banks (12, 13). Principal aqueous-phase oxidants for SO_2 are H_2O_2, O_3, and, in the presence of transition metal ions, O_2, with H_2O_2 playing the most important role in the Northeast (14). Its large Henry's Law coefficient, 7.4×10^4 M/atm, ensures substantial partitioning of H_2O_2 into the aqueous phase (15). Field studies have demonstrated that cloud droplets typically contain either dissolved SO_2 or H_2O_2, but not both, suggesting that the reaction between them goes to completion during a typical cloud lifetime (16), and laboratory studies have elucidated the mechanism of the H_2O_2–S(IV) reaction, which exhibits pH-dependent kinetics (17, 18). Because H_2O_2 may be the limiting reagent in the vicinity of SO_2 emitters, predicting the extent of SO_2 oxidation requires accurate determination of H_2O_2 in the atmosphere (19). Recent evidence has emerged suggesting that organic peroxides are present in the atmosphere and also play a role in S(IV) oxidation (20, 21).

In this experiment, students analyze precipitation samples for trace concentrations of H_2O_2 using a newly developed fluorescence technique (22). Analysis is based on the production of OH• radical by the reaction between H_2O_2 and ferrous ion (Fenton's reagent) with subsequent radical scavenging by benzoic acid:

$$Fe(II) + H_2O_2 \rightarrow OH\bullet + OH^- + Fe(III) \tag{1}$$
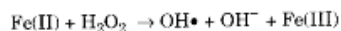
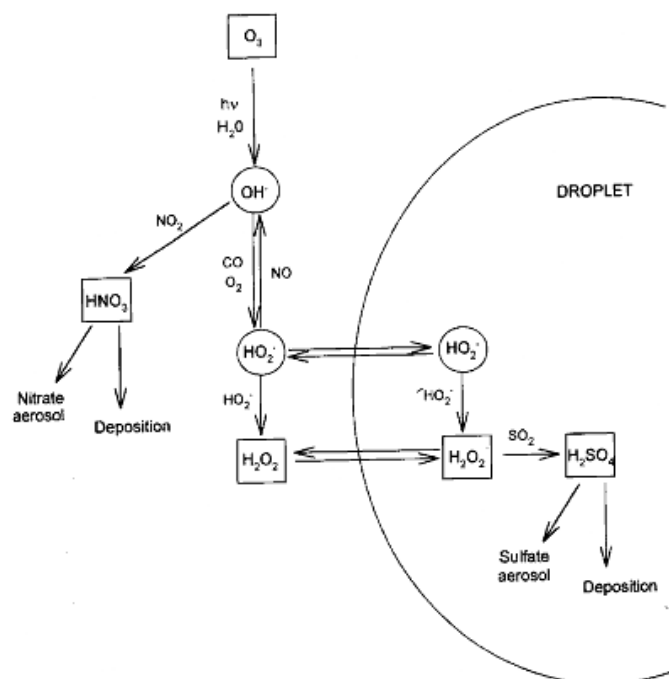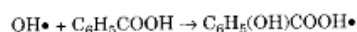$$OH\bullet + C_6H_5COOH \rightarrow C_6H_5(OH)COOH\bullet \tag{2}$$

Figure 1. Principal reactions of gas-phase species implicated in the formation of acid rain and photochemical pollution.

In the presence of O_2 and dissolved iron, the cyclohexadienyl radical formed in reaction 2 produces fluorescent hydroxybenzoic acid (OHBA) isomers (23). The substantial increase in fluorescence intensity of OHBA upon complexation with Al(III) ion is exploited to increase sensitivity (24). Unlike standard spectroscopic and titrimetric techniques, Fenton–OHBA analysis is sensitive enough for the determination of ambient peroxide in precipitation, which typically varies from 10^{-7} to 10^{-4} M. And, in contrast to a more widely used, highly sensitive enzyme-based method (25), the reagents are inexpensive and easily available, and reagent solutions are stable, making this experiment well-suited for the undergraduate laboratory.

Experimental Procedure[1]

Rainwater samples are collected as close to the time of the scheduled experiment as possible, and pH measured. To account for matrix effects, samples are spiked with small aliquots of a known hydrogen peroxide standard and analyzed by conventional standard additions methods (26). For good precision, the instructor should make a preliminary determination of the H_2O_2 concentration and use a peroxide standard which, when diluted, will approximate the unknown concentration. Although H_2O_2 can be purchased in assayed 3% solution from Mallinkrodt (St. Louis, MO), the concentration gradually declines, so it is advisable to titrate the 3% solution against standardized $KMnO_4$. We have found that H_2O_2 keeps its titer for several months when refrigerated and protected from contamination. If rainwater collection is impractical, an H_2O_2 unknown can be prepared by the instructor.

A multichannel peristaltic pump is used to deliver the product of reaction between sample, fluorescence reagent (iron(II) sulfate and benzoic acid) and enhancing reagent (Al(III) in acetate buffer) to a fluorometer flow-through cell, as illustrated in Figure 2. The reaction coils in the figure are lengths of Teflon tubing that introduce a two-minute delay to allow the reactions to proceed substantially toward completion. If a pump is unavailable, equal volumes of the sample and fluorescence reagent can be mixed, and a third equal aliquot of enhancing reagent

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[1]A detailed experimental procedure is available from J. W.- L. upon request.

added. The reaction with fluorescence reagent should be timed for three minutes; the Al(III) complexation will be complete in 15 min. Maximum fluorescence intensity of the hydroxybenzoic acid–aluminum complex under the experimental conditions described above is obtained with $\lambda_{ex}=305$ and $\lambda_{em}=410$ nm (10).

The low peroxide concentrations determined in this experiment call for careful analytical work and attention to detail. A few precautions are noted below

- Many common impurities, including detergents, fluoresce. It is important to clean and rinse thoroughly all glassware that will come into contact with peroxide samples. Rinsing with nitric or hydrofluoric acid may be necessary to remove traces of organic impurities.
- Background fluorescence can be minimized by using freshly prepared reagents that have not absorbed H_2O_2 from the air, and protecting solutions from light. Students should be cautioned to prepare all reagents from the same water for consistent results.
- Rainwater should not have impacted trees or other objects prior to collection. A heavy downpour is preferable to a light rain, because collection time, and chances for contamination, are minimized.

Safety Considerations

- Safety glasses and protective clothing should be worn for the procedure.
- Concentrated sulfuric and acetic acids, required for reagent preparation, must be used with caution.
- Concentrated nitric or hydrofluoric acid, which may be needed for rinsing glassware, must be used with care.
- The dilute H_2O_2 reagent used here poses no safety hazard.

Results

A typical graph of fluorescence intensity versus volume of added standard H_2O_2 is shown in Figure 3. For this rain sample, the concentration of H_2O_2, determined from the ratio of intercept to slope, as described in *this Journal* (26), is 1.2×10^{-5} M. On rare occasions, rain samples show no measurable H_2O_2 because it has been titrated by an excess of dissolved SO_2. When this occurs, calibration curves may exhibit nonlinearity due to reaction between SO_2 and added H_2O_2. It should be noted that the Fenton–OHBA method shows limited sensitivity to some organic perox-
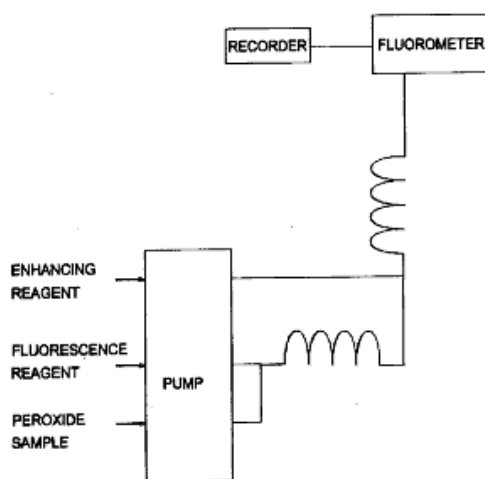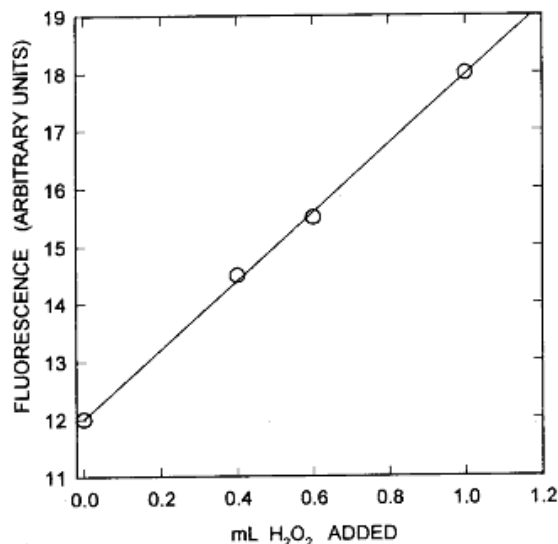
Figure 2. Experimental setup for H_2O_2 measurement. Fluorescence reagent contains 0.8 mM $FeSO_4$ and 2.0 mM benzoic acid in pH 1.9 sulfuric acid; enhancing reagent contains 6.0 mM $Al(NO_3)_3$ in 0.10 M acetate buffer, pH 3.8. Peroxide sample preparation is described in text.

Figure 3. Results of standard addition analysis of H_2O_2 in precipitation. Abscissa shows milliliters standard H_2O_2 (4.5×10^{-5}M) added to 25 mL samples.

ides in addition to H_2O_2. However, its high solubility and greater stability under ambient conditions make H_2O_2 the predominant peroxide observed in precipitation.

Conclusion

This experiment is appropriate for an upper-level instrumental analysis or integrated laboratory course. Although it can be conducted as a simple quantitative procedure using commercial H_2O_2 unknowns, the experience can be broadened by having students collect and handle authentic environmental samples, examine the excitation and emission spectrum of the fluorescent product, or analyze precipitation samples for S(IV) and other inorganic ions.

Blank solutions may exhibit substantial background fluorescence, precluding the use of the most sensitive scales on some fluorescence detectors. This, coupled with low ambient peroxide concentrations may challenge instrument detection limits in this experiment. Unlike most conventional laboratory exercises that specifically are designed to avoid such conditions, this experiment gives students valuable practical experience with some of the otherwise abstract concepts we teach; i.e., noise, detection limit and sensitivity. Because hydrogen peroxide is ubiquitous in the atmosphere and highly soluble, the experiment also illustrates the critical importance of blank measurements.

The Fenton method has been used recently to monitor peroxide concentrations in air, cloudwater, precipitation and surface waters in a number of field missions (27, 28). Our students have carried out this experiment for several years with good results. They are enthusiastic about collecting and analyzing authentic precipitation samples. The experiment also serves as a point of departure for discussions on environmental chemistry and environmental regulation. The 1990 Clean Air Act mandated a 10-million ton per year reduction in SO_2 emissions from 1980 levels through improved scrubber technology and the use of SO_2 emissions trading allowances, a market-based approach toward environmental improvement. Each emitter is provided with a number of emission allowances that can be used to emit SO_2 or can be sold to other emitters by plants that successfully reduce their own emissions. Both H_2O_2 and SO_2 are required for the production of acid rain, as illustrated in Figure 1, but peroxide must be present in excess if the mandated SO_2 reductions are to have the desired effect. Chemistry students who understand the concept of the limiting reagent know that the total amount of product (e.g., H_2SO_4) cannot be decreased by lowering reactant concentration (e.g., SO_2) if H_2O_2 is a limiting reagent. Details such as this illustrate the role of careful scientific work in formulating public policy (29).

Acknowledgment

Literature Cited

1. Kettrup, A. A. F.; Kicinski, H. G.; Masuch, G. Anal. Chem. 1991, 63, 1047A–1056A.
2. Sobel, F. H. Nature 1956, 177, 977–983.
3. Masuch, G.; Kettrup, A.; Mallant, R. K. A. M.; Slanina, J. Int. J. Environ. Anal. Chem. 1986, 27, 183–213.
4. Sakugawa, H.; Kaplan, I. R.; Tsai, W.; Cohen, Y.; Environ. Sci. Technol. 1990, 24, 1452–1462.
5. Gunz, D.; Hoffman, M. R. Atmos. Environ. 1990, 24, 1601–1633.
6. Jacob, D. J.; Gottlieb, E. W.; Prather, M. J.; J. Geophys. Res. 1989, 94, 12975–13002.
7. Chang, J. S.; Brost, R. A.; Isaksen, S. A.; Madronich, S.; Middleton, P.; Stockwell, W. R.; Walcek, C. J. J. Geophys. Res. 1987, 92, 14681–14700.
8. "Interim Assessment: The Causes and Effects of Acid Deposition;" National Acid Precipitation Assessment Program, Government Printing Office: Washington, DC, 1987.
9. Hicks, B. B., Ed. Deposition, Both Wet and Dry; Acid Precipitation Series Volume 4, Butterworth: MA, 1984.
10. Tomlinson, G. H. Effects of Acid Deposition on the Forests of Europe and North America; Lerner Publications: MN, 1990.
11. Allen, R. O.; Allen, C. R.; Sherwood, S. Today's Chemist at Work 1993, 2, 28–38.
12. Eatough, D. J.; Arthurn, R. L.; Etough, N. L.; Hill, M. W.; Mangelson, N. F.; Richter, B. E.; Hansen, L. D. Environ. Sci. Technol. 1984, 18, 855–859.
13. Bizjak, M.; Benner, W. H., Hansen, A. D. A.; Hrcek, D.; Hudnik, V.; Novakov, T. Atmos. Environ. 1988, 22, 2851–2862.
14. Schwartz, S. E. in SO_2, NO and NO_2 Oxidation Mechanisms: Atmospheric Considerations; Calvert, J. G., Ed.; Butterworth: MA, 1984.
15. Lind, J. A.; Kok, G. L. J. Geophys. Res. 1986, 91, 7889–7895.
16. Daum, P. H.; Schwartz, S. E.; Kelly, T. J.; Newman, L. Atmos. Environ. 1984, 18, 2671–2684.
17. Martin, L. R.; Damschen, D. E. Atmos. Environ. 1981, 15, 1615–1621.
18. Lee, Y.-N.; Shen, J.; Klotz, P. J.; Schwartz, S. E.; Newman, L. J. Geophys. Res. 1986, 91, 13264–13274.
19. Kleinman, L. I.; Daum, P. H. Atmos. Environ. 1991, 25A, 2023–2028.
20. Hellpointner, E.; Gab, S. Nature 1989, 337, 631–634. 21. Zhou, X.; Lee, Y.-N. J. Phys. Chem. 1992, 96, 265–272.
22. Lee, J. A.; Tang, I. N.; Weinstein–Lloyd, J. B. Anal. Chem. 1990, 62, 2381–2384.
23. Dorfman, L. M.; Taub, I. A.; Bühler, R. E. J. Chem. Phys. 1962, 36, 3051–3061.
24. Lee, J. H.; Tang, I. N.; Weinstein–Lloyd, J. B.; Halper, E. B. Environ. Sci. Technol. 1994, 28, 1180–1185.
25. Lazrus, A. L.; Kok, G. L.; Gitlin, S.; Lind, J. A. Anal. Chem. 1985, 57, 917–922.
26. Bader, M. J. Chem. Educ. 1980, 57, 703–706.
27. Lee, J. H.; Leahy, D. F.; Tang, I. N.; Newman, L. J. Geophys. Res. 1993, 98, 2911–2915.
28. Weinstein–Lloyd, J. B.; Daum, P. H.; Nunnermacker, L. J.; Lee, J. H.; Kleinman, L. I. J. Geophys. Res. 1995, in press.
29. Schwartz, S. E. Science 1989, 243, 753–763.

letters

Classic Urea Synthesis: Micro and Macro Scale
To the Editor:

The synthesis of urea by Tanski, Petro, and Ball (1) reproduces on a micro scale Friedrich Wöhler's work of 1828 (2), universally recognized as a chemical classic (3). Those interested in the historical background and significance of Wöhler's experiment, its modification for the undergraduate laboratory on a macro scale from commercial silver cyanate or lead cyanate or from silver cyanate prepared by either cation or anion exchange, and tests to differentiate urea from ammonium cyanate as well as additional confirmatory tests for urea may wish to consult our "modern version of a classic experiment" (4) that we developed in 1978 to commemorate the sesquicentennial of Wöhler's synthesis. Whether students perform the micro (1) or macro version (4), they "should gain the satisfaction of having duplicated a classic experiment and should emerge from this experience with a greater appreciation for the experimental and observational skills of a master scientist"(4).

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Literature Cited

1. Tanski, S.; Petro, J.; Ball, D. W. J. Chem. Educ. 1992, 69, A128.
2. Wöhler, F. Ann. Physik und Chem. 1828, [2] 12, 253.
3. Benfey, O. T.; Kauffman, G. B. J. Coll. Sci. Teaching 1979, 8, 148.
4. Kauffman, A.; Chooljian, S. H. J. Chem. Educ. 1979, 56, 197.