

Determination of H₂O₂ Content of Various Water Samples Using a Chemiluminescence Technique

Tuğba TÜĞSÜZ, Elmas GÖK, Serdar ATEŞ

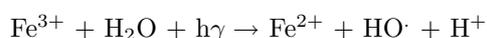
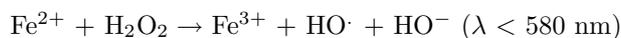
Hacettepe University, Chemistry Department, Beytepe 06532 Ankara-TURKEY

Received 14.11.2002

Hydrogen peroxide (H₂O₂) plays an important role in natural water samples. In this study H₂O₂ concentrations were quantified in different water samples by chemiluminescence detection. This method was chosen because of its high sensitivity and suitability for determining low concentrations of H₂O₂. H₂O₂ is introduced to the oxidation reaction of alkaline luminol solutions in the presence of Co²⁺ ion catalyst. When these components are mixed, blue light ($\lambda = 440$ nm) is emitted. Maximum chemiluminescence intensity occurs within 2 s after mixing and is continuous for up to a few minutes, permitting accurate measurements at selected delay times. H₂O₂ concentrations being very low in water samples made us use the standard addition method. Thus the sample luminescences were measured by the addition of 25 μ L 0.075 M H₂O₂ standard solutions. The H₂O₂ content of water samples was between 0.13 mM (in snow water) and 1.51 mM (in Gölbaşı Lake water). The results indicated that H₂O₂ concentrations in surface waters are representative of aquatic life.

Introduction

Hydrogen peroxide (H₂O₂) is ubiquitous in the hydrosphere and is an important specie in many marine chemical processes. It is believed to play a vital role in redox reactions in sea water, e.g. the “photo-Fenton reaction” (1) involving iron(II), organic matter and hydroxyl radicals:



It is particularly influential in the top 10 m, where it can be present at concentrations of up to 1 μ M. Elevated levels of H₂O₂ are indicative of higher photochemically active sea water (2) and measurement of it has been used to trace areas of reactive water (3). H₂O₂ is primarily produced by photochemical (via free-radical) reactions between dissolved organic chromophores and oxygen (4) but minor (< 5%) sources include biological production from certain species of phytoplankton and wet/dry deposition of photochemically produced H₂O₂ in the atmosphere (5). Decomposition has been found to occur by both biological (6) and photochemical (7) pathways. H₂O₂ is very reactive and plays a transient role in many reactions. The analysis of it in natural water must be carried out immediately after sample collection.

Experimental

Reagents and standards

The stock solution of luminol (5-amino-2,3-dihydro-1,2-phthalazinedione) (2.26×10^{-3} M) was prepared in H₃BO₃ buffered to 9.5 by the addition of 0.1 M NaOH. The stock solution of Co²⁺ (1.00×10^{-2} M) was prepared from Co(NO₃)₂·6H₂O. These solutions were kept refrigerated (4°C) to minimize decomposition.

The stock solution of H₂O₂ acidified with nitric, sulphuric or hydrochloric acids was titrated with standard cerium(IV) sulphate (0.01 M). The stock solution of H₂O₂ was then diluted using boiled pure water to an accurately known final concentration of 0.075 M.

Measurement of chemiluminescence

A Beckman Spectrophotometer was adapted for the measurement of CL intensity (Figure 1). The spectrophotometer was preferred because its detector is directly in the optical line of the cell compartment. The shutter can be fully opened so that the detector can sense all the photons falling on it.

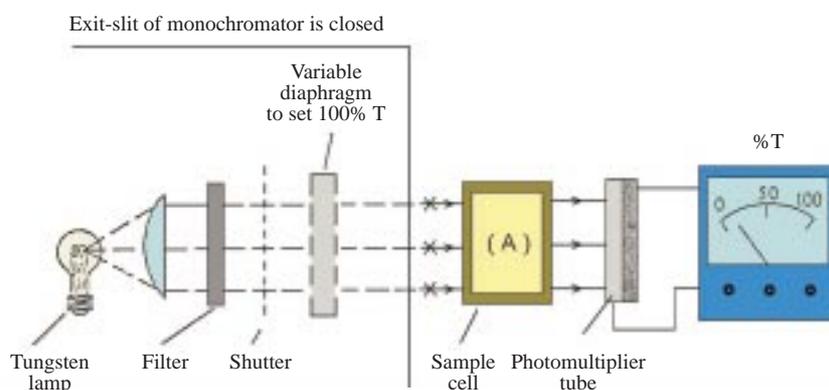


Figure 1. Spectrophotometer adapted for chemiluminescence measurements.

As shown in Figure 1, the optical path of the spectrophotometer is closed at the exit-slit of the monochromator, and a continuous flow cell (Figure 2) is placed into the cell compartment.

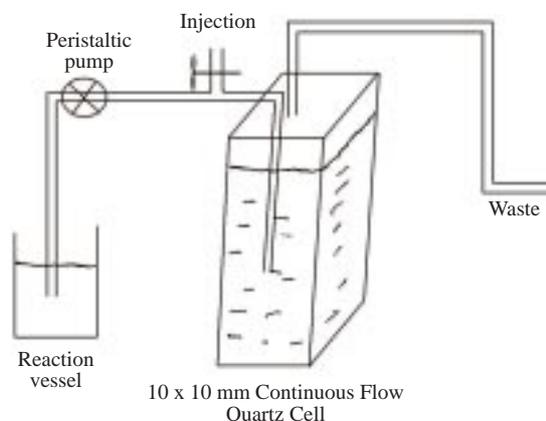


Figure 2. Sample flow system.

The response of the detector is controlled by a “sensitivity” knob x1, x10, x100 at a fixed voltage applied to it. The normal phototube of the system is also replaced with an RC-photomultiplier tube, which is much more sensitive. The A-B-C-D-E control knob of the detector is effective in controlling the DC-voltage applied to the cathode, which gives a range of sensitivity in the detector. Thus we achieved a very sensitive measuring system for all such CL-emissions.

Flow-injection analysis

In a continuous-flow system the sample becomes a part of a flowing stream, as the sample is carried from the injection point to a flow-through measuring device (a photometer in this case) and finally to a waste pipe. A continuous-flow system such as the flow-injection (FI) method is employed. The reagents and a known value of water samples are initially added to a 50 mL glass vessel and the CL-reaction is immediately started by the final injection of a Co²⁺ ion catalyst. Peristaltic pumping is used to optimize the flow rate.

An IBM PS/2 computer with an A/D converter-card connected to the PM-tube signal output measured light-intensity versus time data at 10 μ s intervals. The maximum of the signal at different flow-rates showed a delay time of 60-120 s, which is enough for reasonable measurements. Optimization of the system is carried out as given below.

Optimization

The FI manifold is optimized for rapid determinations due to the instability of the analyte. The key variables for maximizing the sensitivity of CL emission are considered to be the flow rate of the solutions, total volume, pH, the PMT output, luminol and the Co²⁺ ion formal concentrations. The starting point for the optimization is taken from a previously reported manifold for H₂O₂ determination in synthetic media (8). Optimum conditions found in this study are shown in Table 1.

Table 1. Optimization of the FI-CL method for H₂O₂ in boiled water.

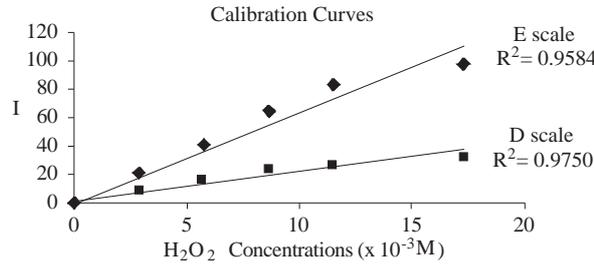
Variable	Optimum Conditions
Flow rate	3.0 mL/min
Total volume	6.5 mL
pH	9.5
PMT output	E (sensitivity \times 1)
Luminol	2.26×10^{-3} M
Co ²⁺	1.00×10^{-2} M

Calibration

To measure the H₂O₂ concentration in the water samples, the calibration curves were prepared for two PMT sensitivities (on scales D and E). Calibration data and graphs are given below in Table 2 and Figure 3. The limit of detection of H₂O₂ is 3.19×10^{-4} M and 2.89×10^{-4} M respectively (S/N = 3). Yet the signals from the real water samples are usually very weak. To compensate for this problem, standard additions of 25 μ L H₂O₂ were carried out for all the samples.

Table 2. Calibration data for H₂O₂ solutions at two different CL intensities (E and D).

0.075 M (mL)	[H ₂ O ₂] added (M)	CL Intensity	
		I (E)	I (D)
0.000	0	0	0
0.025	2.89×10^{-4}	21	8
0.050	5.77×10^{-4}	41	15
0.075	8.65×10^{-4}	65	23
0.100	1.15×10^{-3}	83	26
0.150	1.73×10^{-3}	98	33


Figure 3. Calibration curves for H₂O₂ in two different PM sensitivities (D and E scales).

Results and Discussion

In measuring the CL-intensity of the various water samples (rain water, snow, local Beytepe water sources and Gölbaşı Lake water), the H₂O₂ molar concentrations were evaluated from calibration lines. Mean values are given at 95% confidence level in Tables 3, 4 and 5.

Table 3. H₂O₂ concentration in Gölbaşı Lake water.

Place	mean (\times)	Standard Deviation (s)	[H ₂ O ₂] for 95% C.L. $z = 1.96 s \rightarrow \sigma$
Cağlar Restaurant	1.55×10^{-3}	1.29×10^{-4}	$(1.55 \pm 0.15) \times 10^{-3}$
Chez Le Belge	1.20×10^{-3}	1.23×10^{-4}	$(1.20 \pm 0.14) \times 10^{-3}$
Gulum Aile Cay Bahçesi	1.51×10^{-3}	1.23×10^{-4}	$(1.51 \pm 0.14) \times 10^{-3}$
Rent a Boat	1.76×10^{-3}	1.00×10^{-4}	$(1.76 \pm 0.11) \times 10^{-3}$
Total Lake	1.51×10^{-3}	1.02×10^{-4}	$(1.51 \pm 0.12) \times 10^{-3}$

Table 4. H₂O₂ concentration in aquarium water sample.

Date	mean (x)	Standard Deviation (s)	[H ₂ O ₂] for 95% C.L. $z = 1.96 s \rightarrow \sigma$
17 th February	6.57×10^{-4}	7.75×10^{-6}	$(6.57 \pm 0.10) \times 10^{-4}$
19 th February	5.74×10^{-4}	8.09×10^{-6}	$(5.74 \pm 0.09) \times 10^{-4}$
20 th February	4.82×10^{-4}	1.35×10^{-5}	$(4.82 \pm 0.15) \times 10^{-4}$

Table 5. H₂O₂ concentrations in other water samples.

	mean (x)	Standard Deviation (s)	[H ₂ O ₂] for 95% C.L. $z = 1.96 s \rightarrow \sigma$
Rain water at 5 th December	1.08×10^{-3}	1.58×10^{-4}	$(1.08 \pm 0.18) \times 10^{-3}$
Beytepe Campus gate	9.74×10^{-4}	4.22×10^{-5}	$(9.74 \pm 0.48) \times 10^{-4}$
Department of Chemistry	5.97×10^{-4}	2.09×10^{-5}	$(5.97 \pm 0.24) \times 10^{-4}$
Snow water on 12 th March	1.33×10^{-4}	4.18×10^{-5}	$(1.33 \pm 0.47) \times 10^{-4}$

The experimental limit of detection (S/N = 3) for H₂O₂ is 3.04×10^{-4} M (in D and E scales). Overall reproducibility in terms of standard deviation is 6.25×10^{-5} M. This varies according to the origin of water samples collected; being low in snow and aquarium water (4.22×10^{-5} M and 9.78×10^{-5} M), and higher in lake water (1.02×10^{-4} M).

The data in Tables 3, 4 and 5 are given as mean values. The confidence limits for the mean values of N replicate measurements are derived from t. Note that $t \rightarrow z$ as the number of degrees of freedom becomes infinite. The equation is

$$\text{CL for } \mu = x \pm \frac{z\sigma}{N^{1/2}}$$

The 1.51 mM H₂O₂ content of Gölbaşı Lake water is greater than the other water samples. The H₂O₂ content of other water samples decreases in the following order: rain water (1.08 nM) > Beytepe Campus gate (0.97 mM) > aquarium water (0.66 mM) > tap water from our department (0.60 mM) > snow water (0.13 mM).

H₂O₂ concentrations being higher in Gölbaşı Lake samples clearly shows that natural waters containing life (plant and fish) accumulate H₂O₂ and that it is concentrated on the surface waters of sea or lakes. All the samples analysed are from the surface waters of Gölbaşı Lake.

Also, the H₂O₂ concentrations in the rain water are high because H₂O₂ may come from the atmosphere. H₂O₂ is produced in the atmosphere by photochemical reactions. Another reason for the higher H₂O₂ concentrations in rain water is cold air. H₂O₂ decomposes with heat, and therefore it is more stable in cold air causing elevated figures in the rain samples.

Beytepe Campus gate water, being underground water, cold and having little contact with the atmosphere, gave lower H₂O₂ values. Snow water showed the lowest H₂O₂ accumulation because of its crystalline form.

Conclusion

The chemiluminescence technique is suitable for millimolar or at most micromolar H₂O₂ determinations and is simple to apply, easy to perform and cheap. Very simple chemiluminescence measuring devices can be constructed, which can be carried to the sampling locations. In-situ H₂O₂ analysis is certainly more valuable and reliable than laboratory analysis for the determination of H₂O₂. This CL technique offers such a possibility. The value of H₂O₂ concentrations is directly related to the aqua-life in water, the purity of the water, as in rain, and its long-time equilibrium with the atmosphere.

This article was presented at the XI National Chemistry Congress, İstanbul, Turkey, September 4-7.2001

References

1. R.G. Zika, N.V. Blough and R.G. Zeep, "Effects of Solar Ultraviolet Radiation on Biogeochemical Dynamics in Aquatic Environments" Woods Hole Oceanogr. Inst. Tech. Rep., WHOI-90-09, 1990 B.C.
2. C.A. Moore, C.T. Farmer and R.G. Zika, **J. Geophys. Res.**, **98-C2**, 2289 (1993).
3. R.G. Zika, P.J. Milne and O.C. Zafriou, **J. Geophys. Res.**, **98-C2**, 2223 (1993).
4. W.J. Cooper, R.G. Zika, R.G. Petasne and J.M.C. Plane, **Environ. Sci. Technol.**, **22**, 1156 (1988).
5. W.J. Cooper, E.S. Saltzman and R.G. Zika, **J. Geophys. Res.**, **92-C3**, 2970 (1993).
6. W.J. Cooper and R.G. Zeep, **Can. J. Fish Aquat. Sci.**, **47**, 888 (1990).
7. J.W. Moffet and O.C. Zafriou, **J. Geophys. Res.**, **98-C2**, 2307 (1993).
8. A.I. Vogel, "A Text-Book of Quantitative Inorganic Analysis", 3rd Edition, Longmans, (1962).