

Determination of Hydrogen Peroxide and Cobalt(II) by Flow Injection Analysis with Chemiluminescence Detection

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Summary: Chemiluminescence detection has been used in conjunction with flow injection analysis for the determination of hydrogen peroxide and cobalt(II). In general, the limit of detection is at the femtomole level. The relative standard deviation over the range 1×10^{-11} - 1×10^{-4} M is 0-9% (n=10) with a sample throughput of 350 h^{-1} .

Chemiluminescence (CL) is the phenomenon observed when an electronically excited state in one of the reactants or products of a chemical reaction reverts to its ground state with the emission of photons [1]. CL reactions are oxidative, involving a range of organic molecules [2], the best known of these being luminol (5-amino-2,3-dihydrophthaline-1,4-dione), which was discovered by Albrecht in 1928 [3]. Luminol is oxidized by alkaline hydrogen peroxide in the presence of a catalyst. The catalyst may be one of many species, extending from simple transition metal cations to macromolecules such as peroxidase [4]. The chemiluminescent reaction of luminol is characterized by a rapid rise in emission intensity followed by a gradual decay as the excited state becomes depopulated.

CL reactions can be exploited as a highly sensitive method for measuring substances of biological interest [4]. Quantitative techniques based on the use of chemiluminescent molecules rely on reproducible measurement of low levels of emitted light because of the transient nature of the emission. This has been successfully achieved by flow injection analysis. For example Bur-

guera et al. [6] reported a flow injection CL method for the determination of cobalt(II) and sulphide. The sample was injected directly into a reagent stream, which passed through a short length of tubing to detector (Cecil CE202 spectrophotometer). Detection limits for cobalt(II) were 0.1 and 0.6 pg with 10 and 100 l sample loops, respectively. The relative standard deviation for 1 ng of cobalt(II) was 1.5% (n=5).

Olsson used a microperoxidase-catalyzed luminol reaction to determine hydrogen peroxide [7]. The limit of detection of 3×10^{-9} M was slightly lower than with hexacyanoferrate(III) catalysis. The response was linear up to 1×10^{-5} M H_2O_2 for a plot of log intensity versus log concentration. The relative standard deviation was 0.3% (n=3) for 1×10^{-6} M H_2O_2 and the sample throughput was 120 h^{-1} .

Hydrogen peroxide has also been determined by flow injection analysis using hexacyanoferrate(III) catalyzed luminol CL [8]. The system responded linearly to hydrogen peroxide and was adapted to glucose analysis by incorporating a column of immobilized glucose oxidase.

This paper describes a purpose-built flow injection analyzer for the rapid and sensitive determination of hydrogen peroxide and cobalt(II) using a chemiluminescent reaction, the oxidation of luminol (5-amino-2,3-dihydrophthaline-1,4-dione) in the presence of hydrogen peroxide and cobalt(II).

Experimental

Reagents

Distilled deionized water was used for the preparation of all solutions. An aqueous stock solution of luminol (1 mM) was prepared by dissolving 0.6177 g of luminol (BDH) in 100 ml of carbonate buffer (0.1 M).

Carbonate buffer (0.1M) was prepared by dissolving 10.6 g of sodium carbonate (AnalaR; BDH) in one litre of water and the pH was adjusted to 10.0 with hydrochloric acid (2 M).

A stock (1 mM) solution of cobalt(II) was prepared by dissolving 0.03 g of cobalt nitrate (AnalaR; BDH) in 100 ml of water. Standards covering the range 1×10^{-10} – 1×10^{-4} M were prepared by serial dilution of the stock solution with carbonate buffer (0.1M).

A stock solution of hydrogen peroxide (0.1M) was prepared by dissolving 1.12 ml of 27.2% hydrogen peroxide (Fisons SLR grade) in 100 ml of water. Standards covering the range 1×10^{-10} – 1×10^{-4} M were prepared by serial dilution in carbonate buffer (0.1M).

Instrumentation and Procedures.

The design of a flow-through bioluminescence detector has been described previously [9]. For the CL determination of hydrogen peroxide and cobalt

(II), the single-coil glass flow cell was replaced by a six-coil glass flow cell as described by Abbott et al [10]. The flow injection manifold used for the determination of cobalt(II) is shown in Fig. 1.

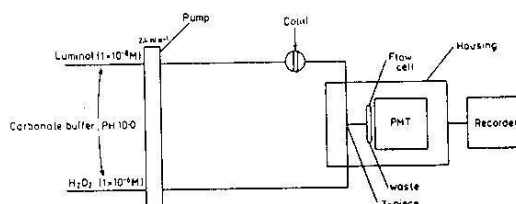


Fig.1: Flow injection manifold for the determination of cobalt(II).

Carrier streams of luminol (1×10^{-8} M) and hydrogen peroxide (1×10^{-6} M) were pumped at the same flow rate (1.25 ml min^{-1}) using a peristaltic pump (Ismatec Mini-S840). The catalyzed reaction was initiated by injecting cobalt standards (30 l) into a stream of luminol via a rotary injection valve (Rheodyne 5020). Both carrier streams contained carbonate buffer (0.1M) at pH 10.0. CL emission occurred when the sample in the luminol stream was mixed with the hydrogen peroxide stream at a perspex T-piece and allowed to pass through a glass coil positioned in front of the photomultiplier. The detector output, which was dependent on the concentration of cobalt(II) injected, was fed to a strip chart recorder. For the determination of hydrogen peroxide standards were injected into a stream of luminol (1×10^{-8} M).

Results and Discussion

Optimization of the flow system

The sensitivity for the determination of cobalt(II) and hydrogen peroxide

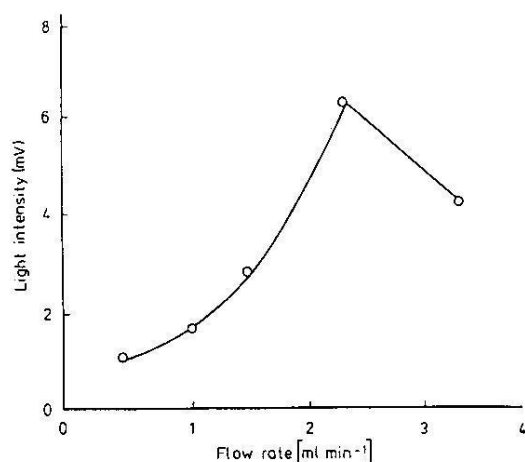


Fig.2: Effect of flow rate on light intensity.

was largely dependent on the flow rate, because the rate of reaction is very rapid. Therefore, the effect of flow rate was investigated in order to get maximum CL intensity in front of the detector using a fixed amount of cobalt(II) ($1 \times 10^{-7} \text{M}$). The peak height increased with increasing flow rate up to 2.5 ml; at this flow rate the maximum CL emission was coincident with the passage of the sample zone through the glass coil. Further increase in the flow rate resulted in a decrease in the emission, as shown in Fig. 2, which means that maximum emission occurred after the sample has passed through the flow cell. Therefore a flow rate of 1.25 ml for both channels was used for subsequent

experiments.

The effect of luminol concentration on the emission signal was studied by injecting varying amounts of luminol, over the range 1×10^{-10} - $1 \times 10^{-4} \text{M}$, into a fixed concentration of cobalt(II) ($1 \times 10^{-7} \text{M}$). The effect of luminol on the CL intensity is shown in Fig.

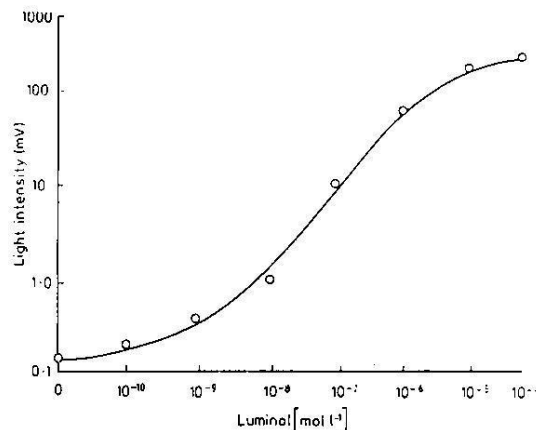


Fig.3: Effect of luminol concentration on light intensity.

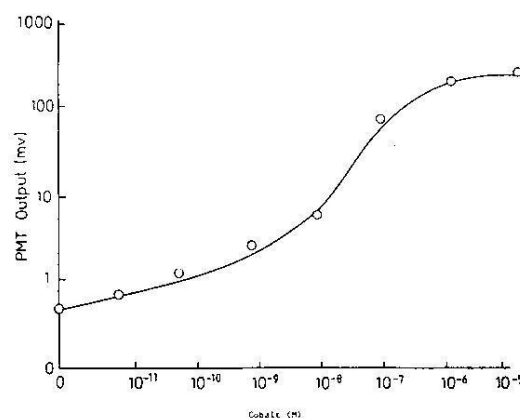


Fig.4: Log-log calibration graphs for the determination of cobalt(II).

$3. 1 \times 10^{-8} \text{M}$ luminol was used for the cobalt(II) and hydrogen peroxide determinations.

Calibration of cobalt(II)

Cobalt(II) standards (30 l) over the range (1×10^{-11} - $1 \times 10^{-5} \text{M}$) were injected into a stream of luminol. Fig. 4 shows a log-log calibration graph of light output in millivolts versus cobalt(II) concentration. A background signal was obtained below

Table-1: Calibration data for the chemiluminescent determination of Cobalt(II) and Hydrogen Peroxide.

Conc. (mol l ⁻¹)	Cobalt(II) Output Voltage (mV)	RSD (%)	Conc. (mol l ⁻¹)	Hydrogen peroxide Output Voltage (mV)	RSD (%)
0	0.40	-	0	2.00	-
1 x 10 ⁻¹¹	0.76	8.9	1 x 10 ⁻¹⁰	2.45	9.4
1 x 10 ⁻¹⁰	1.90	7.4	1 x 10 ⁻⁹	4.25	9.0
1 x 10 ⁻⁹	3.80	6.0	1 x 10 ⁻⁸	6.28	6.9
1 x 10 ⁻⁸	7.80	3.6	1 x 10 ⁻⁷	18.7	4.6
1 x 10 ⁻⁷	54.3	3.5	1 x 10 ⁻⁶	313.6	3.8
1 x 10 ⁻⁶	312.5	3.7	1 x 10 ⁻⁵	460.4	4.3
1 x 10 ⁻⁵	443.3	2.1			
1 x 10 ⁻⁴	468.6	2.0			

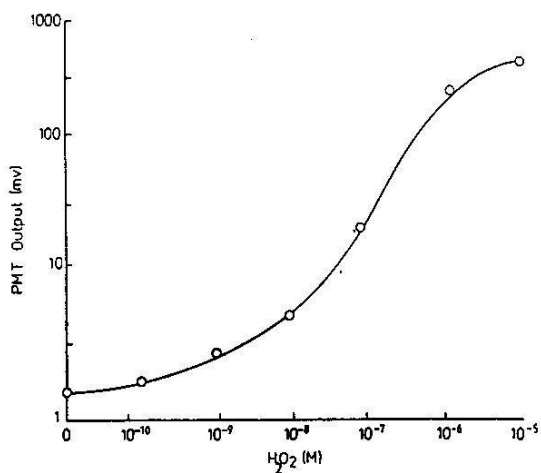


Fig.5: Log-log calibration graph for the determination of hydrogen peroxide.

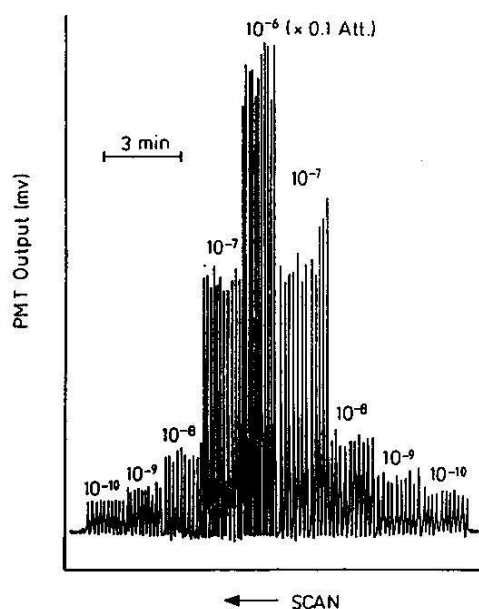


Fig.6: Recorder output for the chemiluminescent determination of hydrogen peroxide by flow injection analysis.

1×10^{-11} M cobalt due to traces of metal ions present in the water. The signal levelled off above 1×10^{-6} M due to the saturation of luminol with respect of cobalt(II) concentration. The sensitivity and reproducibility is given in Table 1.

Calibration for Hydrogen peroxide

Hydrogen peroxide standards over the range 1×10^{-10} - 1×10^{-5} M were injected into a stream of luminol. Fig. 4 shows a log-log calibration graph of hydrogen peroxide concentration versus light intensity over the range 1×10^{-10} - 1×10^{-5} M. The sensitivity and reproducibility for the ten injections over the range 1×10^{-11} - 1×10^{-4} M is given in Table 1. A typical flow injection trace for a range of hydrogen peroxide standards is shown in Fig. 5.

Conclusions

A sample throughput of 350 h^{-1} and a limit of detection in the femtomole range for cobalt(II), hydrogen peroxide and luminol shows that the detector and the flow system provide a sensitive and rapid technique for monitoring chemiluminescent reactions. The sensitivity may be increased by removing trace levels of metal ions such as cobalt from water. Furthermore, the increasing use of chemiluminescent labels for monitoring immunological interactions and the sensi-

vity obtained for luminol and hydrogen peroxide encourage the development of homogenous luminescence immunoassays using a flow-through detector.

References

1. J.S. Woodhead and I. Weeks, *Pure and Appl. Chem.* 57, 523 (1985).
2. J.S. Woodhead, I. Weeks, A.K. Cambell, M.E.T. Ryall, R. Hart, A. Richardson, and F. McCapra, M. Serio, and M. Pazzagli, Editors, *Luminescent Assays, Perspectives in Endocrinology and Clinical Chemistry*, Raven Press, New York, p. 79 (1982).
3. H.O. Albrecht, *Z. Phys. Chem.*, 136, 321 (1928).
4. H.R. Schroeder, F.M. Yeager, *Anal. Chem.*, 50, 1114 (1978).
5. M. DeLuca, Editor, *Bioluminescence and Chemiluminescence Methods in Enzymology*, vol. 57, Academic Press, New York (1978).
6. J.L. Burguera, A. Townshend, and S. Greenfield, *Anal. Chim. Acta.* 114, 209 (1980).
7. B. Olsson, *Anal. Chim. Acta*, 136, 113 (1982).
8. M.L. Grayeski, J. Mullin, W.R. Seitz and E. Zygowics in *Int. Symp. Anal. Appl. Biolumin. Chemilumin.*, M. DeLuca and W. McElroy, Eds., Academic Press, New York, p. 623 (1981).
9. P.J. Worsfold and A. Nabi, *Anal. Chim. Acta.* 179, 307 (1986).
10. R.W. Abbott, A. Townshend, and R. Gill, *Analyst*, 111, 635 (1986).