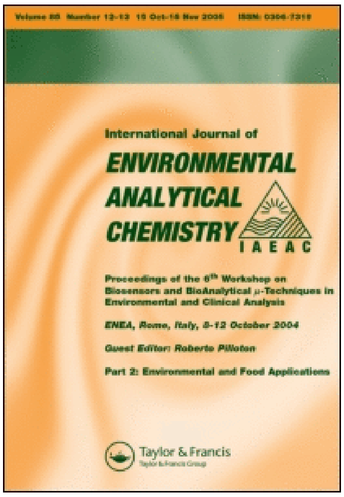

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CHEMILUMINESCENCE FLOW SENSOR FOR THE MONITORING OF HYDROGEN PEROXIDE IN RAINWATER

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A novel chemiluminescence(CL) sensor has been developed for determining hydrogen peroxide in rainwater. The analytical reagents involved in the CL reaction, including luminol and copper ion were immobilized on anion exchange resin and cation exchange resin, respectively. While a volume of sodium sulphate was passed through a column with immobilized reagents, these two reagents were eluted from the resins and then reacted with hydrogen peroxide in alkaline aqueous solution to produce CL. The CL emission intensity was correlated with the hydrogen peroxide concentration in the range 8×10^{-8} to 1×10^{-4} M, and the detection limit was 3.5×10^{-8} M hydrogen peroxide. Interfering ions present in rainwater were effectively separated on-line by an upstream ion exchanger. A complete analysis, including sampling and washing, could be performed in 1 min with a relative standard deviation of less than 5%. The system was stable for over 200 analyses and has been applied successfully to the determination of hydrogen peroxide in rainwater samples.

Keywords: Chemiluminescence; flow sensor; immobilized reagents; ion exchanger; hydrogen peroxide; rainwater

INTRODUCTION

Hydrogen peroxide has an important function in heterogeneous processes as an oxidant, producing sulfuric acid in the atmosphere and rain. For the investigations regarding acid rain, it is necessary to monitor the concentration of hydrogen peroxide in rainwater. So far many methods have been described in literature for such measurement. Spectrophotometric methods have been generally used, based on the formation of intensely absorbing coloured compounds by the

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peroxidase-catalysed oxidations[1,2] or the formation of titanium(IV) complexes using porphyrins[3,4] and pyridylazo compounds[5–7] as the ligands. Chemiluminescence(CL) detection with high sensitivity has been applied successfully to the determination of hydrogen peroxide in rainwater, commonly based on the oxidation reaction of luminol by hydrogen peroxide in the presence of a catalyst such as copper[8] or cobalt.[9] Fluorimetric methods for hydrogen peroxide employed the catalytic fluorescence reaction of peroxidase[10] or its mimetic enzyme.[11] Electrochemical methods including amperometric[12,13] and voltammetric[14] techniques have also been reported for hydrogen peroxide in rainwater.

Among the new analytical tools under development, chemical sensors with the ability of on-line analysis appear to be very attractive for on-site analysis in industrial processes, biomedical engineering and environmental control. For hydrogen peroxide analysis, many sensors with immobilized enzymes such as peroxidase and catalase have been extensively investigated, based on spectrophotometry,[15] fluorimetry,[16,17] chemiluminescence,[18–22] acoustic emission[23] and electrochemical[24–26] techniques. In recent years, systems with immobilized analytical reagents have also been used for hydrogen peroxide, most of them based on chemiluminescence. Ding et al reported a flow injection system including a reactor packed with a solid-state peroxyoxalate and a flow cell containing immobilized fluorophores, located in front of the photomultiplier tube.[27] Nieman and his group evaluated several solid supports and immobilization procedures for use in containing luminol on particles in flow-through columns for use in determination of hydrogen peroxide.[28] Various immolilization procedures for 3-aminofluoranthene have been discussed[29] and a two-bed CL reactor with solid [bis(2,4,6-trichlorophenyl)oxalate]TCPO and 3-aminofluoranthene immobilized on controlled pore glass beads has since been developed for hydrogen peroxide analysis in rainwater.[30]

In this paper, a flow-injection CL sensor for the determination of hydrogen peroxide is presented. It was based on the copper-catalyzed luminol CL system. The two analytical reagents involved in the CL reaction, including copper ion and luminol, were immobilized electrostatically on cation-exchang resin and anion-exchange resin, respectively. Hydrogen peroxide is determined by means of the reaction with copper ion and luminol, which are eluted by sodium sulphate from the ion-exchang resin column with immobilized CL reagents. Interferences from ions co-existing in rainwater are effectively eliminated on-line by an upstream ion exchanger, thus allowing the determination of hydrogen peroxide without any preliminary treatment of the sample. The sensor responded linearly in the concentration range of 8×10^{-8} to 1×10^{-4} M with a relative standard deviation of less than 5%. The detection limit was 3.5×10^{-8} M. A complete analysis could be performed in 1 min including sampling and washing, giving a throughput of about 60 h^{-1}. The column with immobilized CL reagents and the ion exchanger were readily prepared and can be used for 200 and 500 times respectively for the determination of hydrogen peroxide in rainwater. The sensor is suitable for in situ measurements in view of the simple set-up and the rapid procedure and has been applied successfully to monitoring hydrogen peroxide in rainwater.

EXPERIMENTAL SECTION

Reagents

All the reagents were of analytical grade; doubly distilled water was used for the preparation of solutions. Stock solution of hydrogen peroxide (0.100 M) was prepared by diluting 5.5 ml of 30% v/v hydrogen peroxide to 500 ml with water. The solution was standardized by titration with potassium permanganate. Testing standards solutions were prepared daily by appropriate dilution of the stock solution. A 0.25 M luminol solution was prepared by dissolving 44.3 g of luminol in 1 l of 0.5 M NaOH solution. Other solutions were Na_2SO_4 (6×10^{-3} M), $Cu(NO_3)_2$ (0.2 g ml^{-1}) and NaOH(0.01 M). D201 anion exchange resin purchased from Nankai University was used for luminol immobilization. 732 sodium type cation exchange resin was obtained from Shanghai Resin Co. for copper ion immobilization and for cation interference separation, respectively. 717 chloride type anion exchange resin was also obtained from Shanghai Resin Co. for anion interferece seperation.

Apparatus

The flow system used in this work (Figure 1) consisted of two peristaltic pumps. One delivered all reagent streams and a carrier of water at a flow rate(per tube) of 3.5 ml min^{-1}. The other delivered a sample stream at a relatively low flow rate of 1.0 ml min^{-1} for efficient removal of interfering ions in an ion exchanger. PTFE tubing (0.8mm i.d.) was used to connect all components in the flow system. 160 μl sodium sulphate solution was injected by a six-way injection valve through the immobilized ion exchange column. The eluted luminol and copper ion merged with the sample and sodium hydroxide streams just prior to reaching a spiral flow cell(200 μl). The CL was detected by an R456 photomultiplier tube placed close to the flow cell and was recorded with an XWT-204 recorder (Shanghai Dahua Instrument and Meter Plant).

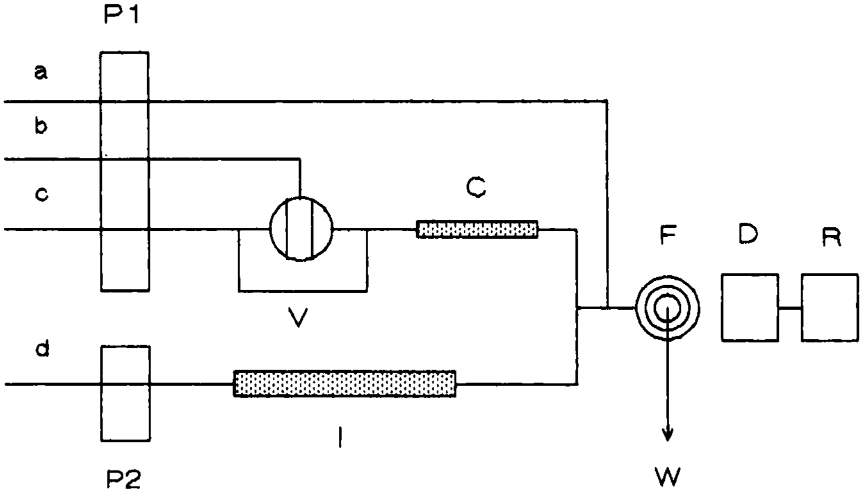

FIGURE 1 Schematic diagram of the flow system for hydrogen peroxide determination: (a) NaOH; (b) Na_2SO_4; (c) H_2O; (d) sample; (P1) pump1; (P2) pump2; (V) valve; (C) column with immobilized reagents; (I) ion exchanger; (F) flow cell; (W) waste; (D) detector; (R) recorder.

Absorbance monitoring was done using a UV-spectrophotometer Model-752 (Shanghai Third Analytical Instrument Plant).

Preparation of Immobilized Reagent Column

0.5 gram D201 or 732 type ion-exchange resin was stirred with 25 ml of 0.25 M luminol or 0.2 g ml^{-1} $Cu(NO_3)_2$ for 12 hours, then the resin was filtered, washed with bidistilled water and dried for storage. The most convenient method to determine the amounts of luminol and Cu^{2+} immobilized is to measure the change of their concentrations in the immobilization solutions. This was done by UV-Vis absorbance. The concentrations were monitored at 360 nm for luminol and at 790 nm for Cu^{2+}. The amounts of luminol and Cu^{2+} immobilized were 2.05 mmol and 1.12 mmol per gram resin, respectively.

To prepare a column with immobilized reagents, 0.2 g of the 1:1(w/w) substrate mixture immobilized with luminol and Cu^{2+}, respectively, was packed into a glass column with an internal diameter of 3.0 mm and a total volume of about 0.6 ml, and equipped with glass wool at both ends to prevent loss of the resins.

Preparation of Ion Exchanger for Sample Separation

1.0 gram of 717 type anion exchange resin and that of 732 type cation exchange resin were mixed together, packed in a 120-cm length of glass column(5.0 mm i.d.) and furnished with glass wool at both ends to retain the resins.

Procedures

Reagents and samples were pumped at a constant speed until a stable baseline was recorded. Then 160 μl of eluant containing 6×10^{-3} M sodium sulphate was injected into the carrier stream and luminol and Cu^{2+} were released quantitatively. The concentration of hydrogen peroxide was quantified by the CL intensity.

RESULTS AND DISCUSSION

Selection of the Eluant

Various eluents with different affinities to the resin were injected through the resin column and released different amounts of luminol and copper ion thus affecting the CL intensity. Since basic eluents such as NaOH, Na_2CO_3 and Na_3PO_4 are unsuitable for the elution of copper ion due to the formation of copper hydrate covering the resin surface, the characteristics of several neutral eluents including Na_2SO_4, NaCl, $NaNO_3$ and NaAc were evaluated for the present sensor. The results are shown in Table I. It was found that sodium sulphate was the best eluant with the highest CL intensity, which is not a surprise as sulphate is strongly retained by the resin. Therefore, sodium sulphate was chosen for subsequent work.

TABLE I Characteristics of eluants for hydrogen peroxide determination

Eluant	*NaCl*	*NaAc*	*$NaNO_3$*	*Na_2SO_4*
Relative CL intensity	32	20	55	100

The concentration of each eluant was 5×10^{-3} M. The relative CL intensity corresponds to the normalized maximum light intensity.

Effect of Mixing Ratio Between Resins with Immobilized Luminol and Copper Ion

To examine the influence of the mixing ratio, 0.20 g resins with different mixing ratios were packed into the glass column. By injection of sodium sulphate at a fixed concentration of 6×10^{-3} M, different amounts of luminol and copper ion were eluted from the resins and caused an effect on CL intensity, which is shown in Table II. The mixing ratio 1:1 between the amount of luminol resin and that of copper resin was selected in the present work for the highest CL light signal.

Effect of Eluant Concentration

The release of luminol and copper ion was determined by the concentration of sodium sulphate injected. The eluent concentration was varied in order to maximize the CL signal, the results are shown in Figure 2. It can be seen that increasing the eluant concentration gives increasing CL intensity. Considering a compromise between higher CL intensity and longer lifetime of the column, 6×10^{-3} M sodium sulphate was used for the present system.

Effect of Sodium Hydroxide Concentration

The copper-catalyzed luminol CL reaction occures in basic solution. Therefore, sodium hydroxide was added in flow line to improve the sensitivity of the system. Since the concentration of sodium hydroxide versus CL intensity plot reached a maximum with sodium hydroxide around 1×10^{-2} M, this concentration was used in subsequent experiments (Figure 3).

Performance of the System for Hydrogen Peroxide Measurements.

Under the selected conditions given above, response to hydrogen peroxide concentration was linear in the range of 8×10^{-8}–1×10^{-4} M with a detection limit of 3.5×10^{-8} M (3σ). The regression equation was $I = 0.93 + 2.07 \times 10^{-6}[H_2O_2](M)$. The correlation coefficient was 0.996. The determination of

TABLE II Effect of mixing ratio between resins with immobilized luminol and Cu^{2+}

Mixing mass ratio (luminol:Cu^{2+})	*4:1*	*2:1*	*1:1*	*1:2*	*1:4*
Relative CL intensity	56	75	100	36	20

CL conditions: 1×10^{-6} M hydrogen peroxide standard; 6×10^{-3} M sodium sulphate; 1×10^{-2} M NaOH.

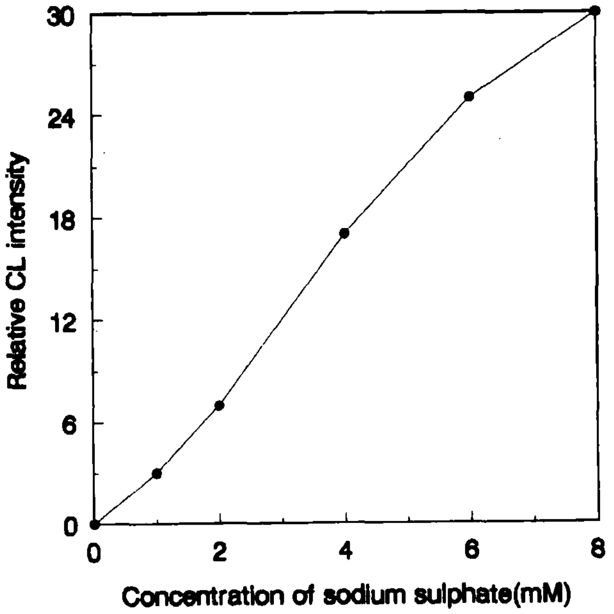

FIGURE 2 Effect of eluant concentration on CL intensity in the presence of 1 × 10^{-6} M hydrogen peroxide.

hydrogen peroxide could be performed in 1 min including sampling and washing, giving a throughput of about 60 h^{-1}. The relative standard deviation was less than 5% for 1.0×10^{-6} M H_2O_2 (n = 7).

Release of Chemiluminescence Reagent from Resin and Lifetime of Column

While sodium sulphate at the fixed concentration of 6×10^{-3} M was injected through the column with immobilized luminol and copper ion, the downstream solution was collected. The amounts of luminol and copper ion released were 3.5×10^{-8} mol measured by UV-Vis absorbance and 1.3×10^{-8} mol measured by the CL method, respectively. In this case, the column with immobilized CL reagents could be used over 200 times.

Interference Study

Without the ion exchanger for sample separation, the effect of foreign ions was tested by analysing a standard solution of hydrogen peroxide (1×10^{-6} M) to which increasing amounts of interfering ions were added. The tolerable concen-

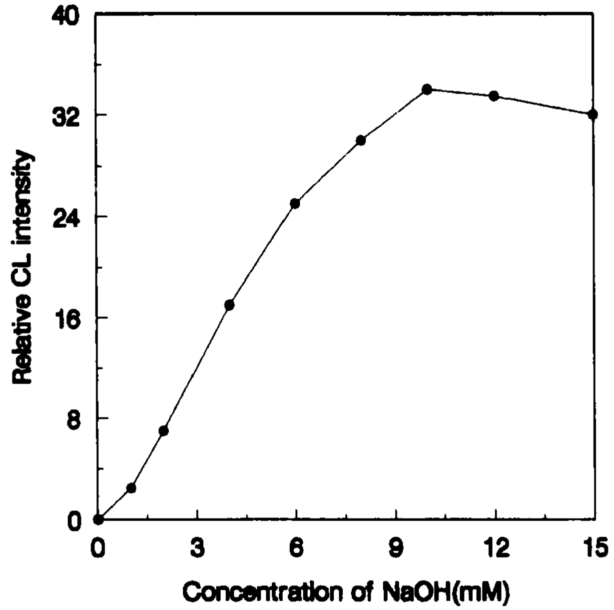

FIGURE 3 Effect of NaOH concentration on CL intensity of 1×10^{-6} M hydrogen peroxide.

tration ratios with respect to 1×10^{-6} M hydrogen peroxide for interference at 5% level were over 1000 for Na^+, K^+, Al^{3+}, Br^-, NO_3^-, Ac^-, SO_4^{2-}, Cl^-, F^- and HCO_3^-, 200 for HPO_4^{2-} and Ba^{2+}, 100 for Ca^{2+} and NH_4^+, 50 for Zn^{2+}, NO_2^- and I^-, 20 for Mg^{2+} and HSO_3^-, 10 for Cd^{2+}, 5 for Sn^{2+}, Ni^{2+}, Pd^{2+} Fe^{3+} and S^{2-}, respectively. Equal amounts of Mn^{2+}, Cr^{3+}, Fe^{2+} and Co^{2+} interfered with the determination of hydrogen peroxide. In the presence of the ion exchanger column, none of these ions at a concentration of 1×10^{-4} M showed any effect on CL signal compared to the 1×10^{-6} M hydrogen peroxide standard, thus confirming the efficacy of the ion exchanger in removing the ion interferences. As reported by the manufacturer, the theoretical capacities of the cation exchange resin and anion exchange resin are 4.5 mEq g^{-1} resin and 3.0 mEq g^{-1} resin, respectively. In this case, it would be estimated that the ion exchanger would be reused over 500 analyses for most rainwater samples.

Analysis of Rainwater Samples

The rainwater samples collected on different days were analyzed for hydrogen peroxide by both the present CL system and the cobalt-catalyzed luminol CL method.[9] The results are given in Table III. It is shown that the results obtained by the system agreed well with those obtained by the CL method.

TABLE III Results of analysis of hydrogen peroxide in rainwater samples

Sample	*Proposed method (M)*	*Recovery (%)*	*CL method (M)*
Rainwater 1	6.2×10^{-7}	92	5.9×10^{-7}
Rainwater 2	8.5×10^{-7}	95	8.7×10^{-7}
Rainwater 3	4.2×10^{-7}	90	4.5×10^{-7}
Rainwater 4	9.4×10^{-7}	92	9.0×10^{-7}
Rainwater 5	1.3×10^{-6}	110	1.0×10^{-6}
Rainwater 6	2.0×10^{-6}	106	1.6×10^{-6}

Average of four measures

Acknowledgement

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