Chemiluminescence Sensor for the Determination of Hydroxylamine by Electrostatically Immobilizing Luminol and Periodate*

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A novel chemiluminescence(CL) sensor, which can be used for hydroxylamine determination in combination with flow injection analysis, was developed by electrostatically immobilizing luminol and periodate on anion-exchange resin respectively. Hydroxylamine was sensed by its enhancing effect on the weak CL reaction between luminol and periodate, which were eluted from the ion exchange column. The response of the sensor to hydroxylamine was linear in the concentration range of 8.0×10^{-8}—2.0×10^{-6} mol/L with a detection limit of 4.0×10^{-8} mol/L hydroxylamine(3σ). The relative standard deviation(RSD) was 2.0% for 9 repetitive determinations at a hydroxylamine concentration of 5.0×10^{-7} mol/L. The sensor could be reused for over 400 times with a good reproducibility and was used to determine hydroxylamine in wastewater.

Keywords Chemical sensor, Chemiluminescence, Luminol, Periodate, Hydroxylamine

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Introduction

Analytical methods based on chemiluminescence(CL) reactions have received considerable attention for their application in various fields, owing to their extremely high sensitivity along with other advantages such as wide linear dynamic range, simple instrumentation, easy miniaturization and coupling to various separation techniques[1—6]. CL sensors have been developed for being used as the continuous and real-time monitors of inorganic, organic, biological and pharmaceutical compounds[7—10] since Freeman and Seitz[8] reported the first CL sensor in 1978 for hydrogen peroxide analysis. Hobo *et al.*[9] reported a CL sensor to detect free chlorine in tap water by immobilizing uranin on an anion exchange resin. This sensor could provide a constant signal within 1 min. Qin *et al.*[10] developed a reagentless flow sensor for sulfite determination by electrostatically immobilizing the oxidant and the sensitiser on an anion exchange column. A type of so-called bleeding CL sensors, prepared by electrostatically immobilizing luminol and some metal ions on an anion/cation exchange column, has been extensively studied for the determination of various analytes[11—13]. Recently, Song *et al.*[13] reported a sensor by immobilizing luminol and periodate on an anion exchange resin for the determination of riboflavin[14]. All those systems involve bleeding the immobilized reagents from the resins at a remote site followed by being mixed with the sample in front of a flow cell. This procedure leads to an unnecessary dilution of the eluted reagents and the sample, therefore reducing its detection ability. Furthermore, the need to merge two streams prior to detection makes it difficult to miniaturize the configuration. In our previous work[15], a flow injection CL sensor for isoniazide detection was developed by electrostatically immobilizing luminol and periodate on an anion exchange resin, which was packed into a piece of glass tube, and served as a flow cell in front of the detection window of a photomultiplier tube.

Hydroxylamine is not only an important reducing agent in industrial production but also an intermediate of many biological processes. Many analytical methods have been reported for their detection including titration[16], gas chromatography[17], spectrophotometric[18, 19] and electrochemical method[20, 21], liquid chromatography[22, 23] and capillary electrophoresis[24]. But some of those methods require complex preparation and suffer from low sensitivity.

In this work we prepared a sensor by mixing

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luminol and periodate immobilized anion exchange resins, and presented a method to detect hydroxylamine concentration and observed a significant enhancing effect of hydroxylamine on the CL reaction between luminol and periodate in basic solutions, which was used to sense the hydroxylamine by mixing the sample in a suitable eluate through the sensor with immobilized luminol and periodate. Compared with other CL methods for the determination of hydroxylamine, the sensor was of economy and conservancy, in addition to the simplicity, rapidity and high sensitivity. The proposed method has been applied successfully to the determination of hydroxylamine in water samples.

Experimental

1 Reagents

All the reagents were of analytical grade and all the solutions were prepared with doubly distilled water. A 1.0×10^{-2} mol/L stock solution of hydroxylamine was prepared by dissolving 0.1097 g of hydroxylamine hydrochloride (Xi' an Chmical Reagent Plant, Xi 'an, China) in 1000 mL of bidistilled water. The standard test solutions were prepared by appropriate dilution of the stock solution. Luminol was purchased from the Department of Chemistry, Shaanxi Normal University (Xi 'an, China). A luminol solution of 0.025 mol/L was prepared with a 0.1 mol/L NaOH solution. The KIO_4 solution (0.01 mol/L) was stored in a brown bottle to avoid photochemical decomposition. D201$\times$7 and other anion exchange resins, purchased from Nankai University (Tianjin, China), were used for the immobilization of luminol and KIO_4.

2 Apparatus

Fig. 1 shows the flow injection analysis system. One of the pumps of the Luminescence Analyzer (IFFM-D, Remex Electronic Instrument Limited Co., Xi 'an, China) was used to deliver flow streams at a flow rate of 2.0 mL/min. A PTFE tube (0.8 mm i. d.) was used to connect all the components in the flow system. The sample was injected into the carrier stream (water) *via* a six-way injection valve. The sensor, served as the flow cell, was made of a mini glass column (50 mm $\times$ 3 mm i. d.) packed with the resin. It was placed in front of the detection window of PMT. The CL emission was converted by PMT to current signal and the output was fed to a computer *via* an A/D convert card and special software. The determination of hydroxylamine was carried out by continuously pumping carrier water and injecting 30 μL of samples into the sensor samples at a frequency of 60 h^{-1}.

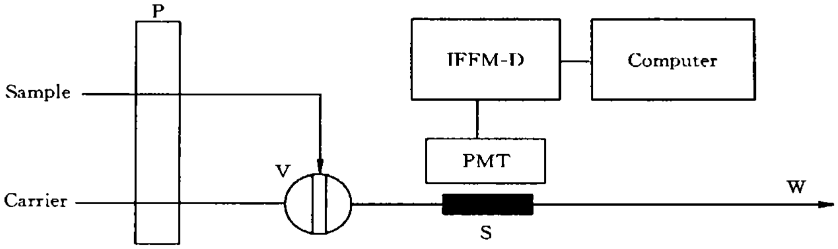

Fig. 1 Schematic diagram of flow injection system with a sensor for the determination of hydroxylamine.

P: peristaltic pump; V: six-way valve; IFFM-D: IFFM-D Luminescence Analyzer; S: sensor; W: waste.

The UV-Vis absorbance determination was carried out with a S2000 miniature fiber optic spectrometer (Ocean Optics Inc., Florida, USA).

3 Preparation of Sensor

D201 $\times$ 7 ion-exchange resin of 1.0 g was dipped in 1.0 mol/L HCl for 1 h and then rinsed with water for six times. The resin was divided into two equal shares that were stirred in 50 mL of 0.025 mol/L luminol and 0.01 mol/L KIO_4 solution for 24 h, respectively. Then the resins were filtered and washed with water for six times. About 0.1 g of the mixture of luminol and IO_4^- immobilized resins (mass ratio 1 : 2) was packed into the glass tube. The amounts of immobilized luminol and IO_4^- on the resins were determined by the changes of the UV-Vis absorbance of the immobilization solutions at 360 nm for luminol and at 222 nm for IO_4^-, respectively[9,23]. The amounts of immobilized reagents were 2.05 mmol per gram resin for luminol and 0.94 mmol per gram resin for IO_4^-.

Results and Discussion

1 Preliminary Study of Luminol-KIO_4-hydroxylamine CL System

The reaction between luminol and periodate shows a weak CL emission and has been used for the determination of H_2O_2, Co(Ⅱ), Mn(Ⅱ) and Fe(Ⅱ)[24—26]. The mixed solution of luminol and periodate has a very low background emission even with an extremely sensitive instrument. A strong CL emission was observed upon the injection of hydroxylamine into the mixed solution. The peak height of the CL emission is proportional to the concentration of hydroxylamine.

2 Condition Optimization for Sensor Preparation

2.1 *Substrate Selection*

Since both luminol and IO_4^- are anions, anion exchange resin has to be selected as the substrate for their immobilization. Six styrene-PVB anion exchange resins were compared and the signals of the CL sensors based on these resins are shown in Table 1. It indicates that the strongest CL signal was obtained when the sensor was prepared with D201×7 anion resin.

Table 1 Comparison of different ion-exchange resins for the CL sensor[a]

Resin type	Signal intensity[b]
D201×7 Styrene-DVB(strong basic)	265
D290 Styrene-DVB(strong basic)	182
D296 Styrene-DVB(strong basic)	207
D301R Styrene-DVB(weakly basic)	104
D396 Styrene-DVB(weakly basic)	96
D370 Styrene-DVB(weakly basic)	87

a. Conditions: sample hydroxylamine in 1.0×10^{-3} mol/L NaOH; carrier water; flow rate 2.0 mL/min. *b*. Mean of three times with RSD less than 5%.

2.2 *Immobilization Method*

Two immobilization methods were used for the sensor preparation. First, luminol and IO_4^- were separately immobilized on the resins and then their mixture was packed into a glass tube. Another method was to treat the resin with the solution containing 2.5×10^{-2} mol/L luminol and 1.0×10^{-2} mol/L KIO_4. The sensors prepared with both the methods gave a strong CL emission when the sample was injected. The sensor prepared with the first method was accompanied with a high background emission. The sensor prepared with the second method had a lower background emission and showed a shorter lifetime than that obtained with the first method and could only be used to detect sample for about 100 determinations. Since the background emission from the sensor prepared with the first method was very steady and could be seen as the baseline of the detector, the first immobilization method was used for preparing the sensor.

2.3 *Effect of KIO_4 Concentration in Immobilization Solution*

The results indicate that the CL intensity upon the sample injection and the lifetime of the sensor were related to the concentration of KIO_4 in the immobilization solution. The higher concentration of KIO_4 gave a longer lifetime of the sensor and a stronger CL intensity. In consideration of the solubility of KIO_4 in water, a 0.01 mol/L KIO_4 solution was used for immobilizing IO_4^-.

2.4 *Effect of Mixing Ratio Between Resins Immobilized with Luminol and IO_4^-*

The effect of the ratio of the resins immobilized with luminol and periodate in the sensor was also investigated. CL signal intensity was not sensitive to the ratio of the resins immobilized with luminol to IO_4^- within a limited range. In the view of the stability and durability of the sensor, a mixing ratio of 1∶1 of the resins immobilzed with luminol to IO_4^- was selected for all the experiments.

3 Selection of Determination Conditions

3.1 *Eluant Selection*

The immobilized luminol and periodate can be eluted from the resin by various anions injected through the anion exchange column. The characteristics of some eluants such as NaCl, $NaNO_3$, Na_2SO_4, NaAc, NaOH, Na_2CO_3 and Na_3PO_4 solutions were evaluated. Although NaCl, Na_2SO_4 and $NaNO_3$ solutions could be used as the eluants of a freshly prepared sensor, they showed a gradually decreasing signal and baseline. It was caused by the reducing concentration of OH^- in the micro circumstance of the sensor. Thus the basic eluants were investigated and the results are listed in Table 2. It could be seen that NaOH was the best eluant with the highest CL intensity. Moreover, the baseline was steady when NaOH was used as the eluant.

Table 2 The effect of eluants on the emission intensity[a]

Base	$c/(\mathrm{mol\cdot L^{-1}})$	Relative Emission Intensity[b]
NaOH	5.0×10^{-3}	264
NaAc	5.0×10^{-3}	227
Na_2CO_3	5.0×10^{-3}	115
Na_3PO_4	5.0×10^{-3}	232

a. Conditions: flow rate 2.0 mL/min. Sample: 5.0×10^{-7} mol/L hydroxylamine. *b*. Mean of determination for three times.

3.2 *Effect of Eluant Concentration*

The release of luminol and that of periodate were determined by the concentration of NaOH injected through the sensor. A 1.0×10^{-2} mol/L NaOH was tested first since it was the most suitable medium for this CL system. Although this solution gave a high signal, it caused a decrease in baseline during a long interval. A lower concentration of NaOH gave a steady baseline but a lower CL intensity. Considering the CL signal and the stability of the baseline, a solution of 5.0×10^{-3} mol/L NaOH was selected as the eluant. In this case, the concentration of released luminol, monitored with the UV-Vis absorbance method, was about 1.0×10^{-7} mol/L.

3.3 *Flow Rate*

The CL signal intensity was affected by the flow rate of carrier. The flow rate impacted the rate of the eluant molecules contacting with the resins and the mixing of eluted reactants. The low flow rate caused a lower CL signal as shown in Fig. 2, slowing down the sampling rate. But the flow rate higher than 2.0 mL/min could be recommended since a high flow rate leads to an eccentric signal and shortens the sensor lifetime. Thus the flow rate of carrier was set at 2.0 mL/min.

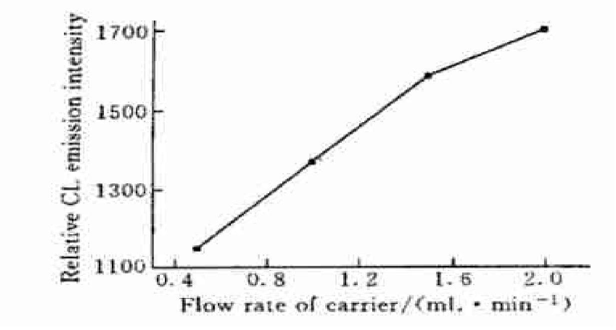

Fig. 2 Effect of flow rate on CL intensity.

Conditions: sample 1.0×10^{-7} mol/L hydroxylamine in 1.0×10^{-3} mol/L NaOH; carrier water.

4 Interference Study

In order to assess the analytical applicability of the sensor, the effects of coexisting compounds were studied by analyzing synthetic samples containing 5.0×10^{-7} mol/L hydroxylamine and various additives. The recovery results are given in Table 3. The results show that Na^+, K^+, Ca^{2+}, Mg^{2+}, SO_4^{2-}, NO_3^-, Cl^-, glucose, lactose and sugar did not affect the determination of hydroxylamine. But Cr^{3+}, Mn^{2+} and Co^{2+} showed a strong effect on the determination of hydroxylamine even at 5.0×10^{-8} mol/L level. Fortunately, the content of those ions in the samples was very low and the interferences of them could be eliminated by the addition of EDTA.

Table 3 Tolerable concentration ratios with respect to hydroxylamine*

Substance	Tolerable concentration ratio
Na^+, K^+, Ca^{2+}, Mg^{2+}, SO_4^{2-}, NO_3^-, Cl^-	1000
Lactose, Sugar, EDTA, Nicotinic acid, Al^{3+}	1000
Nicotinamide, Sodium citrate, Starch, Zn^{2+}	100
Fe^{3+}, Cu^{2+}, Ascorbic acid, Thiamine	10
Fe^{2+}, Hydrazine sulphate	1
Cr^{3+}, Mn^{2+}, Co^{2+}	0.1

* 5.0×10^{-7} mol/L hydroxylamine for interfering species(< 5% error).

5 Performance for the Determination of Hydroxylamine

Under the selected conditions the response of the sensor to hydroxylamine was linear in the concentration range of 8.0×10^{-8}—2.0×10^{-6} mol/L with a detection limit of 4.0×10^{-8} mol/L hydroxylamine(3σ). The regression equation was $I = -8 + 45.5c$ (where c is the concentration of hydroxylamine in 10^{-7} mol/L). The relative standard deviation for nine determinations of 5.0×10^{-7} mol/L hydroxylamine was less than 2.0%. A complete analysis, including sampling and washing, could be performed within 1 min.

The accuracy of the proposed sensor was evaluated by analysing hydroxylamine in wastewater. Sample solutions equivalent to approximate 2.0×10^{-7} and 4.0×10^{-7} mol/L of hydroxylamine were prepared in the presence of 1.0×10^{-3} mol/L EDTA and the recovery results are given in Table 4.

Table 4 Results of the determination of hydroxylamine in wastewater

Sample	Added/ (10^{-7} mol/L)	Found*/ (10^{-7} mol/L)	Recovery(%)
1	2.00	1.97(±2.8)	98.0
2	4.00	3.92(±2.5)	98.8

* Mean of determinations(±RSD%) for three times.

6 Stability of Sensor

Under the selected conditions, the typical CL signals from the sensor are shown in Fig. 3. The peak height of 23 times repetitive injections of 30 μL of 6.0×10^{-7} mol/L hydroxylamine in 5.0×10^{-3} mol/L NaOH had a relative standard deviation of 2.1%. As more as 400 times repetitive injections were performed and the results showed a good durability of the sensor(signal deviation was less than 5%). The CL intensity was decreased gradually after 400 times determination and a new sensor was needed when the signal deviation was larger than 5%. The sensor was stored in the flow injection system after being flushed with water during the experiment.

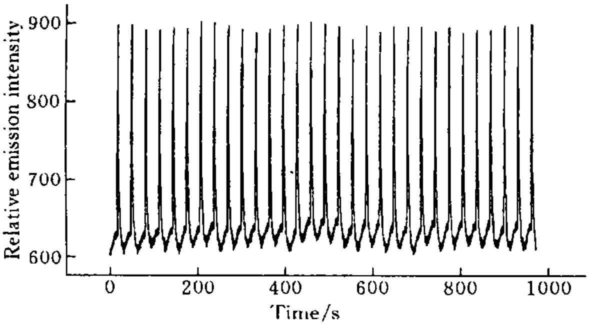

Fig. 3 Typical signals of the CL sensor.
Flow rate of carrier: 2.0 mL/min; sample 30 μL 6.0×10^{-7} mol/L hydroxylamine hydrochloride in 1.0×10^{-3} mol/L NaOH solution.

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