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Talanta 67 (2005) 673–677
Talanta
www.elsevier.com/locate/talanta

Short communication

A portable and low cost equipment for flow injection chemiluminescence measurements

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Received 3 December 2004; received in revised form 21 February 2005; accepted 23 March 2005
Available online 3 May 2005

Abstract

A compact, reliable and low cost flow injection chemiluminescence system is described. The flow system consists of a set of solenoid micropumps that can dispense reproductive micro-volumes of solutions. The luminometer was based on a coiled cell constructed from polyethylene tubing that was sandwiched between two large area photodiodes. The whole equipment costs about US$ 750 and weights ca. 3 kg. Equipment performance was evaluated by measuring low concentrations of hydrogen peroxide by oxidation of luminol and for the determination of ammonium, based on its inhibition of the luminescence provided by the reaction of luminol and sodium hypochlorite. Linear responses were achieved within 1.0–80 μmol L^{-1} H_2O_2 and 0.6–60 μmol L^{-1} NH_4^+ with detection limits estimated as 400 nmol L^{-1} H_2O_2 and 60 nmol L^{-1} NH_4^+ at the 99.7% confidence level. Coefficients of variation were 1.0 and 1.8%, estimated for 20 μmol L^{-1} H_2O_2 and 15 μmol L^{-1} NH_4^+ ($n = 20$), respectively. Reagent consumption of 55 μg luminol, effluent volume of 950 μL per determination and sampling rate of 120 samples per hour were also achieved.

Keywords: Chemiluminescence; Flow injection; Multicommutation; Solenoid pumps; Luminol

1. Introduction

Analytical procedures based on chemiluminescence (CL) usually employ a spiral quartz flow cell placed as close as possible to the detector, which is in general a photomultiplier tube [1]. As the involved reactions are typically fast, precision and sensitivity are highly dependent on the ability of mixing the solutions and measuring the emitted radiation. Thus, CL procedures are often carried out in continuous flow systems, with solutions flowing at relatively high flow rates [1,2]. This design provides attractive characteristics such as high sensitivity, low detection limits and high sampling rates, but also some drawbacks like limited robustness, costs relatively high (instrumentation and reagent consumptions) and high generation of wastes. The usual flow cell geometry also limits the amount of radiation detected to lower than 50%. Some alternatives have been proposed in order to overcome these hindrances, such as the employment of photodiodes [3–6], specially designed cells [2,7,8] and immobilization of the luminogenic reagents [9].

Multicommutation is an alternative to increase versatility of flow systems, by employing discrete commuting devices for solution handling [10,11]. This process has also the advantage of minimizing both reagent consumption and production of wastes [12,13]. One recent proposal, also related to the employment of discrete devices, is the use of solenoid micropumps that can reproductively dispense micro-volumes of solutions [14,15]. In contrast to conventional flow injection systems, these devices can replace the injection and

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doi:10.1016/j.talanta.2005.03.021

propulsion units, yielding compact manifolds that provide low reagent consumption and minimize the production of wastes. An additional advantage is the lower power requirement of the solenoid micro-pumps as compared with the conventional flow injection devices.

In this work, we describe a compact and low cost flow injection chemiluminescence system that involves solenoid micro-pumps for solution handling and a lab-made luminometer based on a simple coiled polyethylene cell sandwiched between two large area photodiodes. The analytical performance has been evaluated by means of measurements of hydrogen peroxide and ammonium employing luminol as luminogenic reagent.

2. Experimental

2.1. Apparatus

The flow system comprised four solenoid micro-pumps Bio-Chem. 090SP with a nominal volume of 8 μL per pulse (Boonton, USA), flow lines of 0.8 mm i.d. PTFE tubing and two confluence connectors. A Pentium 133 MHz microcomputer equipped with an electronic interface Advantech, PCL-711S was employed for system controlling and data acquisition by means of a software written in Microsoft Visual Basic. A lab-made electronic interface, analogous to the previously described [10], was used to generate the electric potential and current required to switch the solenoid pumps (12 V, ca. 100 mA).

The flow cell was made from polyethylene tubing (20 cm length, 0.8 mm i.d.) coiled around a transparent and rectangular acrylic piece (10 mm wide and 2.0 mm thickness). This configuration provided a mechanical support and two large observation surfaces with a cell with 100 μL internal volume. The cell was inserted between two 100 mm^2 photodiodes Hamamatsu, 12337-1010BR and directly connected to the flow system. The whole detection system was placed into a dark box to protect it from ambient light.

2.2. Reagents and solutions

All solutions were prepared with deionized water (18.2 $M\Omega\,cm^{-1}$) and analytical grade chemicals. Stock sodium hypochlorite solution was previously standardized by iodometric titration and hydrogen peroxide stock solution was valorated with potassium permanganate. Ammonium stock solution (0.1 $mol\,L^{-1}$) was prepared from ammonium chloride previously dried at 110 °C for 2 h. Ammonium and hydrogen peroxide work solutions were daily prepared by dilution of the stocks.

Luminol solution (4.5 $mmol\,L^{-1}$) was prepared by dissolving 5-amino-2,3-dihydro-1,4-phthalazinedione in 0.2 $mol\,L^{-1}$ sodium carbonate with pH adjusted to 9.8 with hydrochloric acid. This solution was employed preferably after 48 h, according to literature recommendations [5,16].

For ammonium determination, a 1.0×10^{-2} $mol\,L^{-1}$ NaClO solution was daily prepared. A 5.0×10^{-2} $mol\,L^{-1}$ $K_3[Fe(CN)_6]$ solution was employed as catalyst in the luminol-hydrogen peroxide reaction.

2.3. Flow injection chemiluminescence system

The chemiluminescence detector comprised two photodiodes coupled to operational amplifiers. The output voltage of the two operational amplifiers showed a direct relation to the light intensity from the chemiluminescence cell. A third operational amplifier was assembled as a voltage summing, thus the output signal was directly proportional to the sum of the signals from both devices. A variable resistor was assembled to set up the reference voltage, thus permitting baseline adjustment that was carried out with the flow cell filled with carrier and photodiodes in the dark.

The solenoid micro-pumps were arranged as shown in Fig. 1, employing one device for each solution handled. The devices were operated at 2 Hz (840 μL min^{-1}). The polyethylene cell acted as both, reactor coil and detection cell. The confluence point *y* was placed as close as possible (ca. 5 mm) of the flow cell.

For hydrogen peroxide determination, the system was operated as described in Table 1. In the first step, hydrogen peroxide (S) and luminol solution (R_1) were simultaneously mixed, merged at confluence point *x* and inserted in reactor B. Then, the catalyst (R_2, hexacyanoferrate (III)) was added in the sample zone in the confluence point *y* and data acquisition was started (step 2). Finally, removal of the sample zone and washing was performed by using the carrier solution (step 3).

For ammonium determination, the system was analogously operated (see Table 1). Ammonium (S) and sodium hypochlorite (R_1) were simultaneously inserted in reactor B, merging at confluence point *x* (step 1) and, in the next step, luminol solution (R_2) was added to the sample zone in

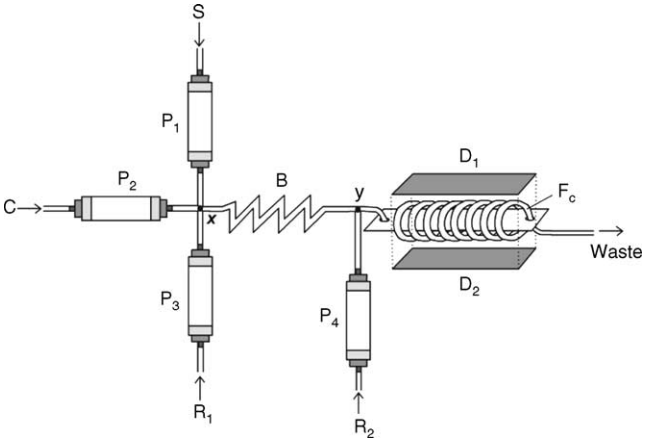


Fig. 1. Flow injection chemiluminescence system. P_i, solenoid micro-pumps; D_i, 100 mm^2 active area photodiodes; FC, 20 cm-coiled polyethylene flow cell; B, 25 cm mixing tube; *x*, *y*, confluence points; S, sample; R_i, reagents; C, carrier (water).

Table 1
Solenoid micro-pumps switching course for CL peroxide and ammonium determination[a]

Step	Description	P_1	P_2	P_3	P_4	Pulses H_2O_2[b]	Pulses NH_4^+[c]
1	Mixing of S and R_1	1/0	0	1/0	0	10	25
2	R_2 addition and reading	0	1/0	0	1/0	10	10
		0	1/0	0	0	–	
3	Sample zone removal and reading	0	1/0	0	0	150	150

[a] Numbers 1/0 indicate a pulse of the solenoid micro-pump.
[b] Hydride peroxide determination.
[c] Ammonium determination.

the confluence point *y* and data acquisition was immediately started. The binary sampling approach [10] was exploited to add luminol aliquots, in order to save the reagent and improving its distribution into the sample zone. As above described, transport of the sample zone and washing was performed by the carrier (step 3).

3. Results and discussion

3.1. General characteristics

Commercial solenoid micro-pumps can dispense volumes within 3 and 50 μL and can be operated in frequency of up to 5 Hz [15]. The employed devices have nominal volume of 8 ± 2 μL per pulse. It was verified that the mean volume was 7 μL for pumps P_1–P_3 and 6 μL for pump P_4 (see Fig. 1). The proposed flow system operates at the pulsed-flow mode that shows advantages of improving sample-reagent mixing [15,17]. This is an important characteristic, mainly for chemiluminogenic reactions usually characterized by fast and short-lived emissions [18].

Polyethylene is reasonably transparent to electromagnetic radiation emitted by luminol chemiluminescence (λ_{max} 420–440 nm) and can thus be employed to construct a robust and low cost cell for CL measurements. The flow cell geometry and the large area of the photodiodes increase the amount of radiation detected. In view of the low cost of this kind of detector, additionally more than one device can be employed for maximizing sensitivity. With the electronic circuit employed, baseline oscillation was estimated as 20 mV (0.4% of the full scale). Baseline drift was lower than 35 mV h^{-1}.

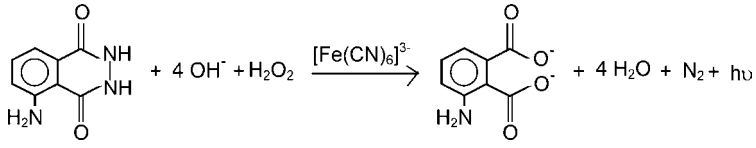
Excluding the microcomputer, the whole equipment weighs about 3 kg and costs about US$ 750 (US$ 650 for the flow system and US$ 100 for the lab made luminometer). The whole system can be conditioned in a box with 30 cm × 10 cm × 25 cm and a notebook can be employed for system controlling and data acquisition. A 12 V car battery can be employed as power supply to drive the micro-pumps. As the OP07 operational amplifier can work with voltages ranging from ±3 to ±18 V, the luminometer can be energized using two 9 V alkaline batteries configured to supply ±9 V.

To enhance the performance of the system, it was evaluated the effect of the flow cell coil length from 5 to 100 cm and that of the coil B from 5 to 100 cm, being selected coils of 20 and 25 cm, respectively, as the most appropriate to obtain a high sensitivity.

On the other hand, it was evaluated the best position of detection in front of the coiled polyethylene flow cell.

3.2. Hydrogen peroxide determination

The classical oxidation of luminol by hydrogen peroxide catalyzed by hexacyanoferrate(III) was initially exploited to evaluate analytical features of the proposed equipment. Reaction occurs slowly in the absence of the catalyst but become very fast after its addition (see reaction scheme (1)). The flow system was then operated for adding the catalyst at the confluence point *y* (see Fig. 1), placed as close as possible of the chemiluminescence cell.



(1)

To establish the best conditions for hydrogen peroxide determination, it was evaluated the effect of the luminol concentration from 0.75 to 9 mmol L^{-1}, being selected a 4.5 mmol L^{-1} value. It were studied the pH range (from 9.4 to 10.6), being chosen a pH of 9.8, and the number of pulses of the luminol solution (from 5 to 20), being selected 10 pulses. The number of pulses of the H_2O_2 solution was varied from 2 to 20, being selected 10 pulses.

Analytical features attained by the proposed equipment and those reported in previously published procedures for hydrogen peroxide determination by CL with photodiodes as detector are presented in Table 2. The detection limit was better than those previously reported and sampling rate is comparable or higher than those found in the literature. Low reagent consumption (55 μg per determination) and waste generation (900 μL per determination) were also observed. Linear response range was comparable to that found in previous

Table 2
Analytical features of flow procedures for hydrogen peroxide determination by luminol chemiluminescence and using photodiode as detector

Analytical characteristics	Proposed system	Preuschoff et al. [4]	Borges et al. [6]	Leite et al. [8]
Linear range (μmol L^{-1})	1.0–80	10–1000	2.5–500	2.5–315
Detection limit (nmol L^{-1})	400	1000	800	1000
Sampling rate (determination h^{-1})	120	65	150	70
CV (%), 20 μmol L^{-1} H_2O_2 ($n = 20$)	1.0	2.5	0.9	1.2
Waste volume (mL/determination)	0.9	0.9	3.0	3.9
Luminol (μg/determination)	55	41	478	500

procedures, being described by the equation:

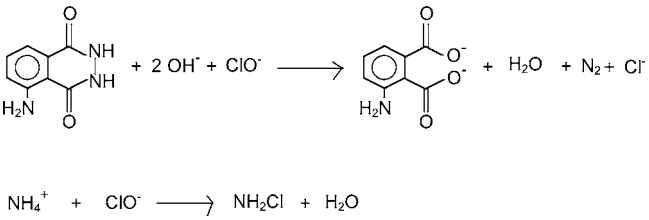
$$\text{CL intensity (mV)} = 155 + 35.5C_{H_2O_2}\ (\mu g\,L^{-1}),$$
$$r = 0.999 \qquad (2)$$

Analytical signals obtained for different hydrogen peroxide solutions are shown in Fig. 2a. In view of the transient characteristics of the flow system and the kinetics of the luminogenic reaction, good precision is only attained with highly reproductive solution volumes, mixing and timing. Coefficient of variation of 1.0% is then a clear indication that these conditions were attained.

Some analytical features compare unfavorably with those attained by Hayashi et al. [5]—detection limit of 3 nmol L^{-1}, linear response up to concentrations 3000-fold higher than LD and sampling rate of 180 determinations per hour. However, these authors employed a more expensive catalyst (peroxidase from *Artheromyces ramosus*) that according to their own descriptions intensify the emission of radiation in comparison with the previously reported ones.

3.3. Ammonium determination

The performance of the equipment was also evaluated with an indirect procedure based on the inhibition of luminol chemiluminescence. Hypochlorite reacts very fast with luminol even in the absence of catalysts. CL intensity reaches a maximum in about 800 ms [19]. Ammonia reacts with hypochlorite in alkaline media to form chloramines that do not react with luminol. Thus, ammonium can be indirectly determined by the consumption of hypochlorite [2,9,20] (see reaction scheme (3)). However, for obtaining reliable data, the reference signal related to the initial concentration of the oxidant need to be highly reproducible.



(3)

For ammonium ion determination, the conditions fixed for H_2O_2 determination were retained and, additionally, it was evaluated the effect of the ionic strength for 0.1–1.0 mol L^{-1} K_2CO_3 and the number of pulses of ammonium solution (from 10 to 40), being selected a K_2CO_3 concentration of 0.2 mol L^{-1} and 25 pulses.

As it can be seen in Table 3, analytical features for ammonium obtained with the developed device compare favorably with those attained by literature procedures, all those employing photomultiplier tubes as detector. The lowest detection limit and highest sampling rate was attained, with good precision and minimized generation of wastes. The reagent consumption was lower than that reported by Kraus and Crouch [20], but ca. two and four times higher than that attained by Li and Dasgupta [2] and Quin et al. [9], respectively. In these works, low reagent consumption was attained due to the low flow rate (100 μL min^{-1}) [2] or to the immobilization of the luminogenic reagent [9].

Sensitivity found for ammonium determination was very dependent on the hypochlorite concentration, and best results

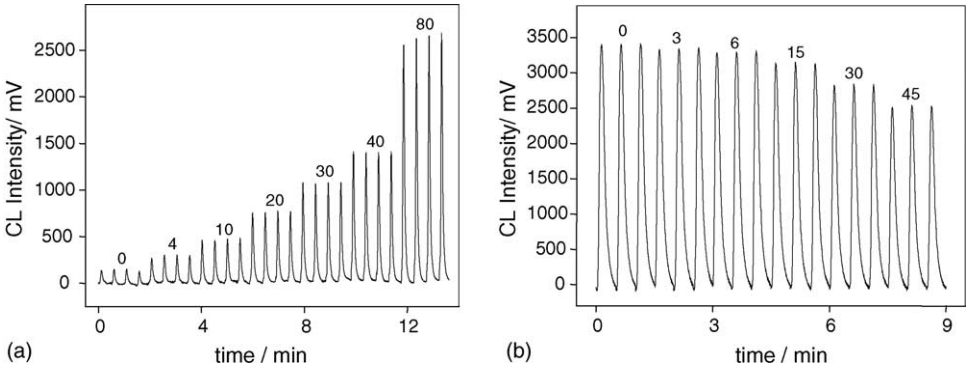


Fig. 2. Transient signals obtained for (a) hydrogen peroxide and (b) ammonium. Numbers indicate concentrations in μmol L^{-1}.

Table 3
Apparatus and analytical features of flow procedures for ammonium determination by chemiluminescence

Analytical characteristics	Proposed system	Li and Dasgupta [2]	Qin et al. [9]	Kraus and Crouch [20]
Detector	Photodiode	Photomultiplier	Photomultiplier	Photomultiplier
Flow cell	Coiled poliethylene tubing	Teflon AF-2400 liquid-core waveguide	Spiral quartz tubing	Coiled
Linear range (μmol L^{-1})	0.6–60.0	<120	1.0–100	250–1900
Detection limit (nmol L^{-1})	60	120	400	100000
Sampling rate (determination h^{-1})	120	42	60	30
CV (%), 15 μmol L^{-1} NH_4^+ ($n = 20$)	1.9	–	<6.0	–
Waste volume (mL/determination)	0.95	0.9	17.5	11.4
Luminol (μg/determination)	55	25	12	2800

were observed for 1.0×10^{-2} mol L^{-1} NaClO. This concentration is considerably higher than the employed in previous works, because reagent dilution is implemented in situ in the proposed flow system. In view of the instability of very diluted hypochlorite solutions [16], previous works exploited the electrochemical generation of the reagent [2,9]. However, in the present work, satisfactory performance was observed by preparing the oxidant freshly for each working day.

Linear response was also comparable to the previous procedures that employ a photomultiplier tube as detector, showing that the limitation should be imposed by the hypochlorite concentration, magnitude and stability of the reference signal. By considering the analytical signal as the difference between the reference signal and that obtained in the presence of ammonium, the calibration curve can be described by the equation:

$$\text{CL intensity (mV)} = 91.9 + 28.3C_{NH_4^+}\ (\mu\text{g L}^{-1}),$$
$$r = 0.999 \quad (4)$$

Analytical signals obtained for ammonium solutions of different concentrations are shown in Fig. 2b, which also evidences the high repeatability and sampling throughput of the developed system.

4. Conclusions

The association of a flow system constructed with solenoid micro-pumps and a lab-made photodiode luminometer yields a compact equipment with profitable characteristics: portability (small size and weight), robustness (high precision in volumes of reagent dispensed), low consumption of reagent (discrete sampling of micro-volumes) and energy as well as minimized generation of effluents. These features make the equipment attractive for measurements out of laboratory, in which conventional flow systems and luminometers are difficult to use. The low cost of the photodiode make feasible the use of more than one detector to improve the amount of radiation detected. In addition, the proposed flow cell is simple, robust, easy to construct and as cheaper as possible.

Acknowledgements

The authors acknowledge the financial support from The Ministerio de Educación, Cultura y Deporte (Spain), ref. PHB2002-0054-PC and from CAPES/MECD (Brazil), processo 042/03 and grants supported by Generalitat Valenciana (CTESIN/2004/051) and “CINC SEGLES” from the Universitat de València (Spain).

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