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A flow–batch luminometer

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ABSTRACT

The traditional flow chemiluminescence (CL) systems typically use flow cells in spiral shape mounted in front of a photomultiplier tube (PMT). The sample is transported by using carrier fluids, undergoes dispersion, and mixes with reagents and CL reaction occurs before reaching the flow cell. As a consequence, the maximum of the chemiluminescence intensity is not exploited and the sensitivity is significantly diminished. In this work, these drawbacks were overcome by using a flow–batch approach and a large area silicon photodiode instead of PMT in order to build a simple and automatic luminometer for CL measurements. The feasibility of the proposed flow–batch luminometer was demonstrated in the determination of vitamin B_{12} in injection ampoules, yielding limits of detection and quantification (0.11 and 0.36 μg L^{-1}, respectively), recovery rates (between 97.8 and 102.1%), relative standard deviations (RSD<2.2%, n = 3) and sample throughput (about 72 h^{-1}). Thus, it was possible to both project and build a simple, flexible, versatile and automatic luminometer, while keeping the excellent characteristics of the previous flow–batch analyzer such as: low reagent and sample consumption and minimal waste generation.

1. Introduction

Chemiluminescence (CL) analysis has aroused great interest in the scientific community, proving to be an important tool in different areas as clinical, pharmaceutical, environmental and food analyses [1–3]. CL measurements have several advantages over other spectroscopic techniques due to its high sensitivity, wide linear range, low limit of detection and simple instrumentation [1]. Thus, CL procedures are often employed in flow systems [4–13], allowing fast and reproducible analysis with low reagent and sample consumption and minimal waste generation.

Most of these traditional flow chemiluminescence systems use flow cells in spiral shape that are often made in glass or polymer, mounted in front of a photomultiplier tube (PMT) [5–13]. This design has limitations such as: the curved surface of the tube restricts the area in contact with the detector surface and the polymers of the tube are often translucent instead of transparent [14]. Moreover, in these traditional flow systems, the sample is transported by using carrier fluids, undergoes dispersion, and mixes with reagents and CL reaction occurs before reaching the flow cell. As a consequence, the maximum of the chemiluminescence intensity is not exploited and the sensitivity is significantly diminished. This occurs because CL reaction is typically very fast [15], and, in order to increase the sensitivity, it would be better to measure CL signal concomitant with the mixture of reagents and sample.

These inconveniences may be overcome by using the flow–batch analyzer (FBA) [16,17]. FBA is an automated system that uses an instantaneous stop chamber and combines favorable characteristics of both flow and batch analyses by using programmed multi-commutation. The main component is the mixing chamber where the whole analytical process, including fluid addition, sample pretreatment, homogenization, precipitation, extraction, preparation of calibration solutions, and detection, takes place under the total control of the software. The sample is processed seamlessly with less: manipulation, consumption of reagents and samples, waste and chance for human error. Various applications of this system have been reported in the literature, such as: screening analysis [18], titration [19], concentration gradients [20], standard addition [21], multicomponent analysis [22], turbidimetrics [23], slow reaction kinetics [24], and digital image detections [25], for example.

Recently, Grünhut et al. [26] proposed a simple, cheap, flexible, versatile, and highly sensitive FBA with CL detection for determination of dopamine, norepinephrine, and epinephrine in pharmaceutical preparations. The CL measurements were carried out by using a photomultiplier tube (PMT) as a CL detector coupled to the FBA mixing chamber. However, these photodetectors need a very high voltage supply, up to thousands of volts. This fact, together with its bulky size, makes the PMT an inconvenient device for the development of instrumentation [27]. An alternative is the use of photodiodes as CL

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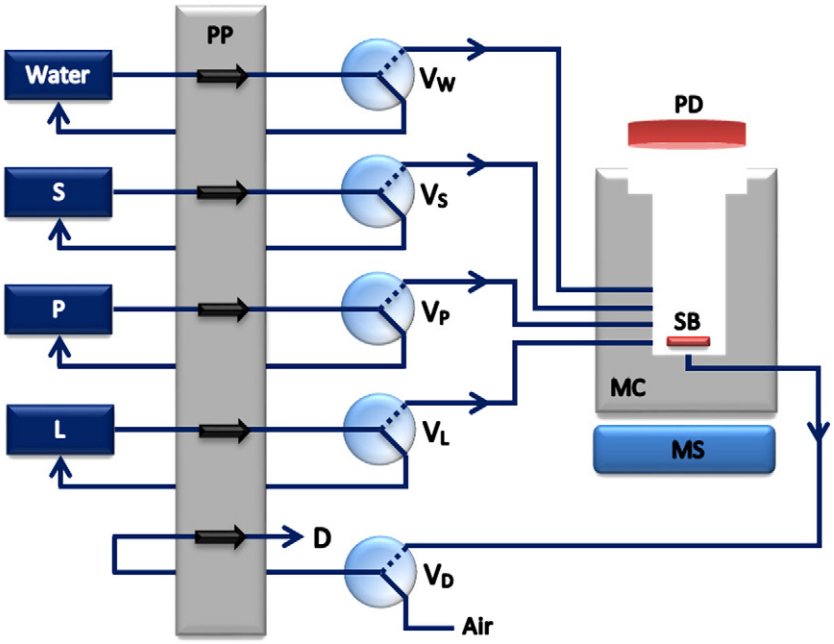

Fig. 1. (a) FBL diagram. Mixing chamber (MC), peristaltic pump (PP), stirring bar (SB), magnetic stirrer (MS), solenoid valves (V_W, V_S, V_P, V_L and V_D), photodiode (PD), sample or standard solutions (2.4 to 12.0 μg L^{-1} of vitamin B_{12}) (S), hydrogen peroxide solution (P), luminol solution (L) and discard (D).

detectors [27–30]; in spite of being less sensitive than PMT, they can be used for detection of radiations, with greater simplicity, lower costs and reduced size.

In this work, we describe a simple, flexible and versatile flow–batch luminometer (FBL) for chemiluminescence measurements. The lab-made automatic luminometer used a large area silicon photodiode as CL detector and a module for amplification of analytical signals. The analytical performance has been evaluated by the determination of vitamin B_{12} in injection ampoules employing the catalytic effect of cobalt(II) ion over luminol oxidation by hydrogen peroxide in alkaline medium [10–13].

2. Experimental

2.1. Reagents and samples

All reagents were of analytical grade and freshly distilled and deionized water (>18 MΩ cm^{-1}) was used to prepare all solutions. Water was obtained from a Millipore Milli-Q purification system for solution preparation and rinsing.

A stock solution of vitamin B_{12} (1.0 mg L^{-1}) was prepared daily by dissolving 1.0 g of crystalline vitamin B_{12} (Sigma) in 1 L of 0.10 mol L^{-1} hydrochloric acid solution (Merck) in a calibrated flask. Standard solutions (2.4 to 12.0 μg L^{-1} of vitamin B_{12}) were prepared by appropriate dilution of the stock solution in 0.10 mol L^{-1} hydrochloric acid solution. The stock and standard of the vitamin B_{12} solution were stored in brown glass bottles. A 1.0×10^{-3} mol L^{-1} luminol solution was prepared by dissolving 0.0913 g of 5-amino-2,3-dihydro-1,4-phthalazinedione (Sigma) in 500 mL of 0.1 mol L^{-1} NaOH solution (Synth). A 1.6×10^{-2} mol L^{-1} hydrogen peroxide solution was prepared daily by diluting 162 μL of H_2O_2 (30% w/v, Merck) in 100 mL of 5.0×10^{-3} mol L^{-1} NaOH solution (Synth). All solutions were stored in brown glass bottles at 4 °C, and all analyses were performed at room temperature.

The vitamin B_{12} injection ampoules having a nominal content of 7.5 mg mL^{-1} were purchased in a local drugstore. Before analysis, these samples were appropriately diluted with 0.10 mol L^{-1} hydrochloric acid solution according to the literature [13].

2.2. Flow–batch system

A schematic diagram of the FBL used for chemiluminescence determination of vitamin B_{12} in injection ampoules is shown in Fig. 1.

The FBL system consists of five three-way solenoid valves (V_W, V_S, V_P, V_L and V_D) model RZ 01540-11 (Cole Parmer); polyethylene tubing connectors with 0.8 mm id; a peristaltic pump (PP) model MCP-Z (Ismatec); and a large area silicon photodiode (PD) as CL detector.

The lab-made mixing chamber (MC) was built in polytetrafluoroethylene (PTFE) with a total internal volume of 2.0 mL, however only 1.5 mL of this volume is used to carry out the measurements inside the MC. The mixture/homogenization of solutions was performed by a stirring bar (SB) located inside the MC driven by a lab-made magnetic

Table 1
System operation schedule.

Valves	V_W	V_S	V_P	V_L	V_D
Flow rate (mL min^{-1})	11.38	11.35	11.88	11.33	11.86
Valve switching on time intervals (s) for:					
Blank	2.6	–	2.5	2.6	–
Sample or standard solutions	1.3	1.3	2.5	2.6	–
Cleaning step [a]	7.9	–	–	–	–
MC emptying [b]	–	–	–	–	8.0

[a] Cleaning step was repeated 2 times.
[b] MC emptying step was performed when necessary.