Sensors and Actuators B 213 (2015) 215–221

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Chemiluminescence detection in urethane-acrylate microfluidic devices

Thiago Rosa Sampaio, Alexandre Fonseca*

Instituto de Química, Universidade de Brasília, Cx Postal 4478, CEP 70910-900 Brasília-DF, Brazil

ARTICLE INFO

Article history:
Received 15 October 2014
Received in revised form 13 January 2015
Accepted 20 February 2015
Available online 28 February 2015

Keywords:
Microfluidics
Flow analysis
Chemiluminescence
Rapid prototyping

ABSTRACT

This work reports on the use of a urethane-acrylate (UA)-based resin in the construction of micro-flow injection analyzers (μFIA) with integrated chemiluminescence (CL) cells for application in ordinary analytical determinations. The proposed devices, manufactured by a rapid and low-cost prototyping procedure, offered suitable transparency to visible electromagnetic radiation and supported flow rates over 2.0 mL min^{-1} without leakages or damages to the structures of the CL cell. By using a pair of photodiodes as detectors, the determination of hypochlorite ions (2.0–20 mg L^{-1}) based on its reaction with 3-aminophthalhydrazide (luminol) provided a linear response (R^2 = 0.998) with a limit of detection (LOD) of 0.5 mg L^{-1} and repeatability of 0.4% for successive injections (n = 4) of a 2.0 mg L^{-1} standard solution. The determination of nitrite ions (10–80 μg L^{-1}), carried out using a photomultiplier to detect the quenching of luminescence for the hypochlorite (ClO^-)/luminol reaction in the presence of the analyte, demonstrated an adequate linearity (R^2 = 0.998), LOD of 5.0 μg L^{-1} and precision of 0.6% for successive injections (n = 4) of a 10 μg L^{-1} solution. The results obtained for the determination of hypochlorite in household bleaches and of nitrite in commercial pâtés did not differ significantly from the reference methods at a confidence level of 95%, demonstrating a suitable accuracy for the miniaturized methods. In addition, only 24 mL of residues were produced per hour in both procedures, so that the proposed analyzer fits one of the green chemistry requirements.

1. Introduction

Methods of analysis based on microfluidic devices demonstrate excellent performance in terms of reagent/sample consumption, residue generation, and analytical throughput [1], representing a "green chemistry" [2] alternative for determinations in clinical [3] and environmental analysis [4]. Considering this, studies about developing and applying miniaturized flow analyzers have been performed to facilitate the procedures of construction and to demonstrate new uses for microfluidic devices. In fact, the low cost and rapid prototyping procedures of microfabrication [5–7] play an important role in this area, because the manufacturing of different microfluidic structures can be carried out more easily when these approaches are used.

Recently, a deep ultraviolet photolithographic process using a UA resin [8,9] has been applied for the rapid and efficient construction of micro-flow analyzers for different purposes [10,11]. This microfabrication technique was adapted from an approach used for the production of elastomeric stamps, so that a complete miniaturized analyzer is fabricated in approximately one hour at costs of about US$5.00 per chip. In addition, the inherent characteristics of the polymerized substrate have resulted in the successful integration of electrochemical/optical detectors and of units for on-line sample pre-treatment [9–11]. Despite this versatility, UA microflow analyzers for CL determinations have not yet been proposed, although considering the number of analytical methods based on chemiluminescent reactions could expand the analytical applications of these systems.

CL detection presents high sensitivity and good selectivity by using a very simple instrumentation for signal acquisition [12,13]. Because radiation is generated from chemical reactions, there is no need for light sources to perform measurements, so that only photo transducers are used in the detector. Therefore, CL has become a good alternative for determinations with microfluidic analytical devices because handling external light beams through small chip channels—as required in fluorimetric or photometric detection—is not necessary in this technique. Considering this, recent studies about CL and microfluidics have been performed aiming at the quantitative determination of species. Kamruzzaman et al. [14] described the use of a polydimethyl siloxane miniaturized device

* Corresponding author. Tel.: +55 61 3107 3842.
E-mail address: afonseca@unb.br (A. Fonseca).

http://dx.doi.org/10.1016/j.snb.2015.02.096
0925-4005/

for determining vitamin B1 in pharmaceutical tablets using the oxidation of 3-aminophthalhydrazide (luminol) with $AgNO_3$ in the presence of platinum nanoparticles and of analyte to obtain the analytical CL signal. Al Lawati and co-authors [15] demonstrated the use of a commercial borosilicate glass chip for the determination of tranexamic acid in pharmaceutical preparations by using a peroxyoxalate CL system. Yu et al. [16] reported on the use of a microfluidic biosensor fabricated in paper to perform the determination of uric acid in urine samples based on the CL produced by reacting a rhodamine derivative with hydrogen peroxide (H_2O_2) in acid medium.

In the present work, we propose the use of a UA resin to fabricate microfluidic analyzers with integrated chemiluminescence flow-cells. Classical oxidation of luminol with hypochlorite or H_2O_2 was used to evaluate the performance of the proposed devices using detection with a pair of photodiodes (PD) or a photomultiplier (PMT). In addition, determination of nitrite in pâtés based on the quenching of hypochlorite (ClO^-)/luminol CL system was performed and used to estimate the accuracy of analytical measurements.

2. Experimental

2.1. Apparatus

Photoresist polymerization was performed with a lab-made photo-exposer machine equipped with two ultraviolet (UV) lamps (Philips Actinic-BL, TL-D 15W, 380 nm). The photomasks were designed with AutoCad-2002 software (Autodesk) and printed on an overhead transparency (Abezeta, PLT A4) at a resolution of 1200 dpi using a laser printer (HP LaserJet P2055dn). An ultrasonic bath (Unique, UltraCleaner 1400) was used to develop the photolithographed structures.

CL detection in the proposed microfluidic devices was performed by using a compact PMT (Hamamatsu, H7468-03) or a pair of circular (1.0 cm diameter) PD (Centronic, OSD50-E). The PMT is equipped with a 12 bit analog/digital (A/D) converter and a RS-232 interface, permitting communication with a personal computer to set the required parameters (voltage and integration time) and for data transfer. To use the PD, the summing amplifier circuit proposed by Borges et al. [17] was constructed and the output signal was transferred to a personal computer using a lab-made digital interface based on a microcontroller (PIC 16F819) with an 8 bit A/D converter.

A pH meter (Hanna pH-21) was used for pH measurements of the working solutions. For comparison with the results obtained with the proposed microfluidic device, a UV–visible (UV/vis) spectrophotometer (Agilent 8453) was employed to determine nitrite in commercial meat pâtés.

A peristaltic pump (Ismatec-Reglo Analog) equipped with Tygon tubes (internal diameters of 0.19 mm and 0.38 mm) was used to propel the reagent solutions and to aspirate the standards/samples solutions. Three-port solenoid valves (Cole Parmer, 01540-11) were used to control the flow directions. Software to manage all operations of the micro-analyzer and for data acquisition was written in Microsoft VisualBasic 6.0.

2.2. Reagents and solutions

All solutions were prepared in reverse osmosis-purified water. A carbonate/bicarbonate buffer (0.5 mol/L, pH 10.5) was prepared by dissolving the appropriate weight of sodium carbonate (Dinâmica, 99.5%) in water, adjusting the pH with additions of HCl 50% (v/v). Similarly, a borate buffer (0.5 mol/L, pH 9.0) was prepared by dissolving the appropriate mass of sodium tetraborate (Vetec, 99.5%) in water, adjusting the pH with HCl 50% (v/v). Solutions of luminol (0.045 mol/L) were prepared by dissolving the appropriate weight of the reagent (Acros, 98%) in carbonate buffer (pH 10.5) or in borate buffer (pH 9). The catalyst solution of potassium ferricyanide (0.035 mol/L) was prepared by dissolving the salt (Synth, 99%) in water. Standard solutions of H_2O_2 were prepared ranging from 0.05 to 0.2 mmol/L. For this purpose, a stock solution of H_2O_2 (Bold, 30%) was properly standardized by titration with potassium permanganate before the suitable dilutions were generated.

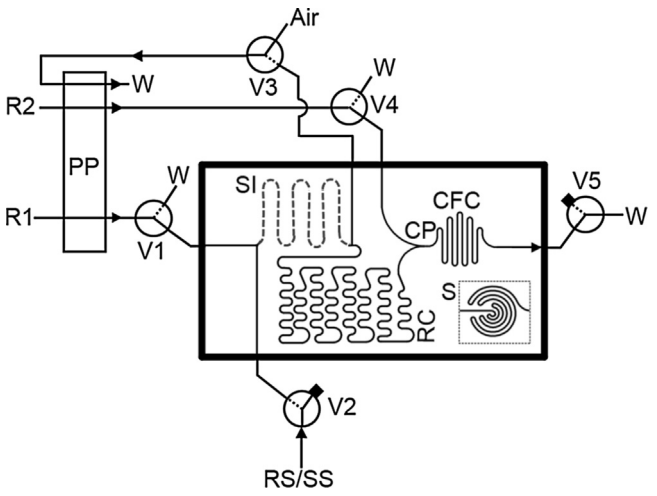

Fig. 1. General flow diagram for evaluation of the proposed microfluidic devices. Peristaltic pump (PP), solenoid valves (V1–V5) switched off, reagent solutions (R1 and R2, see Table 1), reference solutions (RS), sample solutions (SS), channels for sample/standard solutions hydrodynamic injection (SI), reaction coil (RC), confluence point (CP) serpentine chemiluminescence flow cell (CFC) and detail of spiral flow cell (S).

A solution containing sulfuric acid (0.5 mmol/L) and H_2O_2 (0.5 mol/L) was prepared by the appropriate dilution of aliquots of concentrated acid (Quimex, 96–99%) and standardized H_2O_2 solution (Bold, 30%). Standard hypochlorite solutions were prepared ranging from 2.0 to 20.0 mg L^{-1} for detection by a pair of PD, and from 10 to 100 μg L^{-1} for detection by a PMT. In this case, a hypochlorite stock solution (Vetec, 4–6%) was previously standardized by iodimetric titration and aliquots of this solution were properly diluted in water.

A stock solution of sodium nitrite (500 mg NO_2^-/L) was prepared by dissolving the appropriate weight of the anhydrous salt (Vetec, 99%) in water. Standard nitrite solutions were prepared in the concentration range between 10 and 80 μg L^{-1} by diluting aliquots of the stock solution in sodium hypochlorite (ClO^-, 100 μg L^{-1}).

Samples of household bleaches were properly diluted with purified water for determinations in μFIA. Samples of pâtés were properly prepared using the procedures proposed by Borsato et al. [18].

2.3. Construction of the micro-analyzers

A simple, low-cost photolithographic procedure [9] was used to construct the microfluidic devices by employing a UA-based photoresist. The typical layout depicted in Fig. 1 was applied to engrave the microfluidic structures in a 2.0 mm thick plate of UA resin before sealing the device with another sheet of substrate containing no channels. As indicated, the proposed layout included the channels for hydrodynamic insertion [19] of sample/standard solutions (SI), a reaction coil (RC) to carry out the mixture of fluids, a confluence point (CP) for the addition of reagent solutions and the CL flow cell (CFC), which was designed using serpentine or spiral (detail in Fig. 1) shapes. Depending on the application or study, different