Versatile and automated continuous flow colorimetric microanalyzer for environmental determinations

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Abstract
In this work, an automated colorimetric microanalyzer based on the green tape technology is presented. The microanalyzer includes the fluidics, a complete optical detection system and the associated electronics, all in a unique device. The optical detection system was based on a LED array of seven elements and a highly sensitive and low cost photodetector. The optical flow cell was successfully embedded into the device, defining an optical path of 25 mm. To demonstrate its versatility, the microanalyzer was automated by means of the multicommutation concept and later applied to the determination of different analytes with a significant environmental relevance (PO_4^{3-}, Cr^{6+}, NO_2^- and phenols).

Keywords: colorimetric microanalyzer, continuous flow system, environmental determinations, green tape technology.

INTRODUCTION

The growing demand of simple, rapid and precise chemical analysis in different scientific and technological fields has promoted the development of robust, portable and highly precise analyzers able to undergoing analysis of a large number of samples *in situ* and in time with the minimum user interaction. One of the most interesting and simple alternatives to carry out environmental determinations is the combination of flow techniques and flow-through detectors based on light-emitting diodes (LEDs) and photodetectors. Analyzers resulting from this combination provide high sensitivity, portability, miniaturization possibilities and low cost, among others; promoting their application in routine environmental analysis. Analytical devices from the micrometer to the centimeter scale are usually fabricated using glass, silicon and polymers.[1] Recently, the green tape technology has extended its application from multilayer electronic circuits to the development of highly integrated miniaturized analyzers. This technology does not require clean rooms, allows easily developing three dimensional structures with perfect sealing between layers and involves reduced prototyping times. Moreover, since it is perfectly compatible with screen-printing techniques, electronics and printed electrodes can be easily integrated within the same substrate.[2]

Additionally to the attainment of highly integrated miniaturized analyzers, devices able to work under unattended conditions and the minimum user interaction are

required. Following this approach, there are multiple works in the literature that report the use of multicommutation techniques to determine different parameters involved by routine analysis.[3-5] The application of the multicommutation concept to automate continuous flow systems provides some advantages regarding conventional techniques.[6-8] In this work, a highly integrated continuous flow microanalyzer, based on the green tape technology for colorimetric determinations, was applied to the determination of different analytes with environmental relevance (PO_4^{3-}, Cr^{6+}, NO_2^- and phenols). To simplify its performance and increase its sensitivity and reproducibility, a combination of stopped-flow and multicommutation techniques was applied. This way, a microanalyzer able to work under unattended conditions was obtained. Furthermore, using this approach, small volumes of standard solutions with different concentrations, samples and reagents can be easily handled and on-line prepared.

EXPERIMENTAL

Microanalyzer description. The device was fabricated using Dupont 951 as a substrate and applying the multilayer approach of the green tape technology, as described elsewhere.[2] Figure 1 presents a top view of the microanalyzer developed for this study. Electronics (not shown in the picture) were placed on the opposite to avoid that any possible leakage in the microfluidic platform could affect its performance. The device includes three inlets for reagent and sample insertion, a three-dimensional mixer, an optical pathway in a Z configuration (25 mm), a complete optical detection system and the associated electronics, all in a unique device. The detection system was composed by a LED array with seven ultrabright elements (420, 480, 515, 565, 592, 632 and 700 nm - 0.25x0.25x0.25mm, Cromatek, Brazil) and a highly sensitive photodetector TCS230 (TAOS, USA) both controlled by a microcontroller PIC18F4550 (Microchip Technology Inc., Chandler, USA). The microanalyzer is able to work at different wavelengths according to the analyte to be determined. Additionally, using all the available working wavelengths, it could be applied to emulate a spectrophotometer by the automatic and sequential activation of all the elements in the optical detection system to provide discrete spectra.

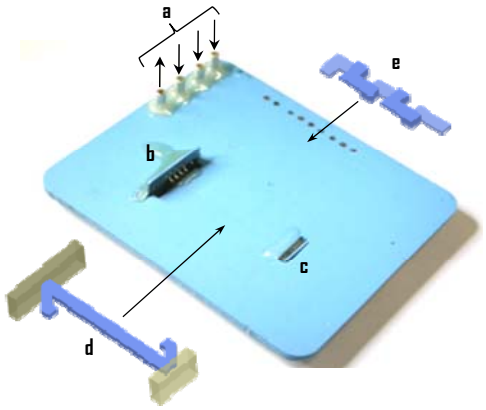

Figure 1. Continuous flow colorimetric microanalyzer based on the green tape technology; **a:** inlets/outlet; **b:** photodetector; **c:** LED array; **d:** embedded optical pathway (25 mm); **e:** embedded three-dimensional mixer.

Multicommutation system. Additionally to high versatility and the possibility to work at different wavelengths, environmental applications require highly confident, robust and stable analytical instruments able to work autonomously during long periods of time under unattended conditions. Following this approach, a multicommutation system was developed and coupled to the microanalyzer. This multicommutation system was implemented by means of a set of solenoid valves (NResearch, USA) electronically controlled by means of a virtual instrument especially developed for this purpose. The system was configured to calibrate itself from standard solutions on-

line prepared using a unique stock solution at the highest concentration. According to the final application, stopped-flow techniques can also be implemented to improve the system sensitivity (i.e phosphate determination).

FIA Manifold. Figure 2 represents the experimental manifold used for the microanalyzer evaluation. The baseline signal was obtained by continuously propelling the corresponding reagent(s) into the microanalyzer. While the solenoid valve was in its normally open position, milliQ water flew into the microsystem thorough its inlet 3 and mixed with the reagent(s) in a convergence point downstream. When the valve was switched, the sample (PO_4^{3-}, Cr^{6+}, NO_2^- or phenols) was propelled during a prefixed time to define its volume and introduced inside the device. The solution resulted from the mixture of the reagent(s) and the sample, reached the flow cell, generating a transient signal registered by the optical detection system and processed by the electronics on the opposite of the microanalyzer.

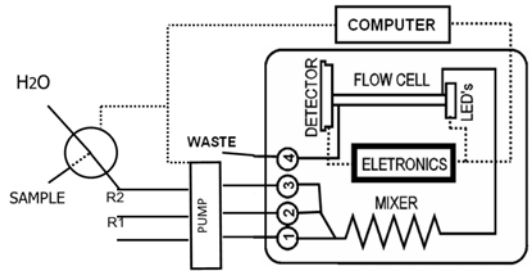

Figure 2. Block diagram of the experimental set-up used for the microanalyzer evaluation for the determination of different analytes (PO_4^{3-}, Cr^{6+}, NO_2^- or phenols).

Standard solutions were on line prepared from a stock solution with the highest concentration for all the analytes evaluated.

RESULTS AND DISCUSSION

The microanalyzer coupled to the multicommutation system was applied to the determination of different parameters with a significant environmental relevance (PO_4^{3-}, Cr^{6+}, NO_2^- and phenols). For this purpose, the elements of the optical detection system, the optimized hydrodynamic parameters and the multicommutation system were configured according to each application.

For phosphate determination the molybdenum blue method was used.[9] This method is based in the chemical reaction of its most bioavailable form, orthophosphate, with ammonium molybdate and ascorbic acid to form heteropolyacid of molybdophosphate. After an optimization procedure flow rate for reagents and sample and sample time were set at 1.5 mL/min, 3.2 mL/min and 12 s (640µL), respectively. The wavelength of the optical detection system was set at 700 nm. The system response for PO_4^{3-} is presented in figure 3.

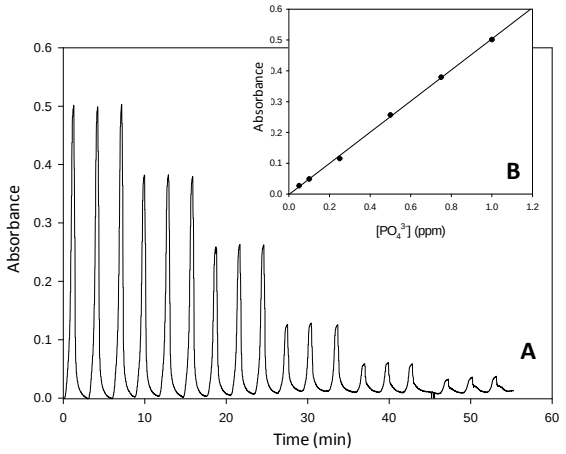

Figure 3. A: System response for the automatic microanalyzer when applied to PO_4^{3-} determination at different concentrations (0.05-1 ppm). **B:** Calibration plot obtained.

Samples ranging from 0.05 to 1 ppm were analyzed. A linear response was obtained for the complete range. The least squares linear regression provided the equation: $A = 0.48 (\pm 0.01)\cdot[PO_4^{3-}] - 0.001(\pm 0.009)$; (n=12; 95% confidence). The repeatability of the system

was calculated as the relative standard deviation (RSD %) of replicated injections. For a concentration of 1 ppm, the RSD obtained was 1.98% (n=14; 95% confidence).

The hexavalent chromium was determined colorimetrically by its reaction with diphenylcarbazide in an acid solution.[10] This reaction forms a red-violet coloured complex that absorbs light at 530 nm. The nearest wavelength included in the LED array was 515nm, so it was the one used for Cr^{6+} determinations. In this case, flow rate for sample/reagents and the sample time were set at 1 mL/min and 12 s (200µL), respectively. Samples with hexavalent chromium concentration ranging from 0.01 to 7.50 mg/L were analyzed. A linear range was obtained from 0.01 to 5.00 mg/L. The equation that describes the linear range of the system response for this analyte was $A=0.339\pm0.007)[Cr^{6+}]+0.008(\pm0.006)$; $r^2=0.998$. For a concentration of 0.5 mg/L, the RSD obtained was 2.07% (n=16; 95% confidence).

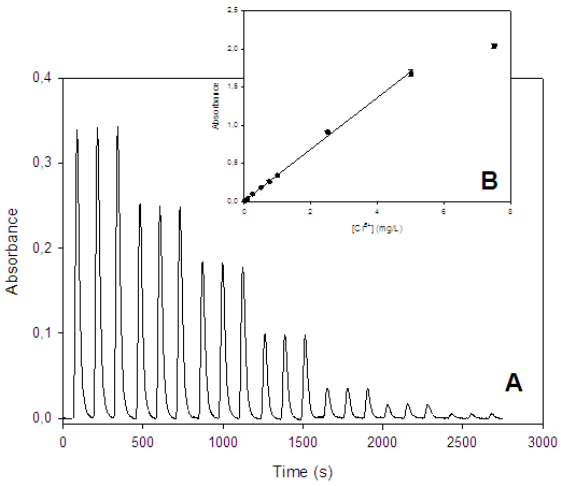

Figure 4. A: System response for a range between 0.01 and 1 mg/L of Cr^{6+}. **B:** Calibration curve for a range from 0.01 to 7.5 mg/L.

Phenols were determined using the 4-aminoantipyrine colorimetric method.[11] In this method, the stream distillable phenols react with 4-aminoantipyrine at a pH=8 in presence of potassium ferricyanide to form a coloured antipyrine dye that can be determined at 500 nm; therefore, the 515 nm LED included in the array was used. The proposed method provides a simple and rapid procedure for the phenol index determination in water. For this evaluation, flow rate for reagents and sample and sample time were set at 1.5 mL/min, 3.2 mL/min and 12 s (640µL), respectively. Phenol standard solutions in the range from 0.01 to 10.0 mg/L were analyzed. The microanalyzer response obtained during this stage of the study is presented in figure 5. As it can be observed, a linear response range between 0.01 and 5 mg/L was obtained. The equation that describes the linear zone is $A=0.106(\pm0.002)[Phenols]+(0.0038\pm0.008)$; $r^2=0.999$. For a concentration of 0.75 mg/L, the RSD obtained was 0.98% (n=16; 95% confidence).

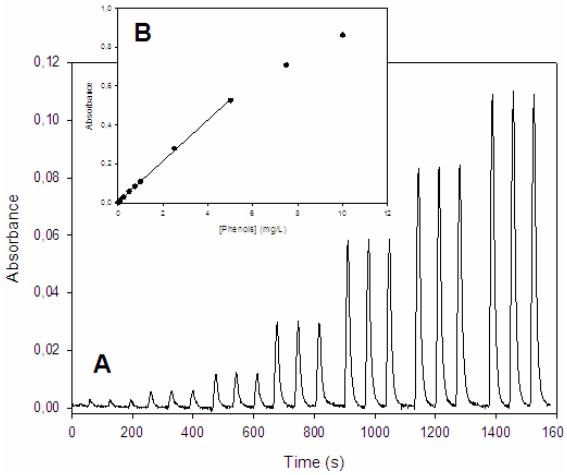

Figure 5. A: System response for a range between 0.01 and 1 mg/L of phenols. **B:** Calibration curve for a range from 0.01 to 10 mg/L.

The last analyte used during the microanalyzer evaluation was nitrite, which can be directly determined by the Griess method.[12] The reaction mechanism for the detection of nitrite employing the Griess reaction method is based on the conversion of sulfanilic acid to a diazonium salt by reaction with nitrite in an acid media. The

diazonium salt is then coupled to N-(1-naphthyl)ethylenediamine (NED), forming a dye that can be determined at 543nm. In this case, the LED used emitted at 545 nm. Flow rate and sampling time were set at 1 mL/min and 12 s (200µL), respectively. Samples ranging from 0.01 to 10 mg/L of nitrite were analyzed. Figure 6 presents the system response. A linear range was obtained from 0.01 to 5 mg/L.

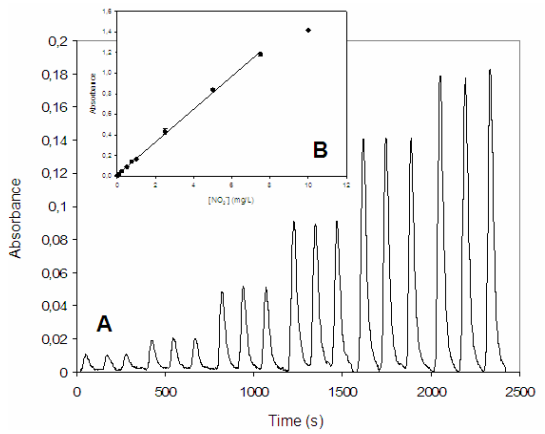

Figure 6. A: System response for a range between 0.05 and 1 mg/L of phenols. **B:** Calibration curve for a range from 0.05 to 10 mg/L.

The equation that describes the linear range of the system response for this analyte was $A=0.160(\pm0.001) \cdot [NO^{2-}]+(0,0114\pm0.002)$; $r^2 = 0.998$. For a concentration of 0.1 mg/L, the RSD obtained was 4.1% (n=13; 95% confidence).

CONCLUSIONS

A highly integrated and automated microanalyzer based on the green tape technology was presented. Due to the microsystem characteristics, it could be operated not only as a simple photometric analyzer but also in a similar way to a spectrophotometer, being able to full fit multiple applications. By adjusting the working wavelength and using the appropriate reagents, the determination of multiple environmental parameters can be performed, even simultaneously if the experimental conditions are correctly set.
Further work is under development to monolithically integrate sample pre-treatment stages that allow determining analytes that currently can not be optically detected.

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