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A Monolithic Continuous-Flow Microanalyzer with Amperometric Detection Based on the Green Tape Technology

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The development of micro total analysis systems (μTAS) has become a growing research field. Devices that include not only the fluidics and the detection system but also the associated electronics are reported scarcely in the literature because of the complexity and the cost involved for their monolithic integration. Frequently, dedicated devices aimed at solving specific analytical problems are needed. In these cases, low-volume production processes are a better alternative to mass production technologies such as silicon and glass. In this work, the design, fabrication, and evaluation of a continuous-flow amperometric microanalyzer based on the green tape technology is presented. The device includes the microfluidics, a complete amperometric detection system, and the associated electronics. The operational lifetime of the working electrode constitutes a major weak point in electrochemical detection systems, especially when it is integrated in monolithic analytical devices. To increase the overall system reliability and its versatility, it was integrated following an exchangeable configuration. Using this approach, working electrodes can be readily exchanged, according to the analyte to be determined or when their surfaces become passivated or poisoned. Furthermore, the electronics of the system allow applying different voltamperometric techniques and provide four operational working ranges (125, 12.5, 1.25, and 0.375 μA) to do precise determinations at different levels of current intensity.

The growing demand of real-time environmental information to control and manage water resources properly has promoted the development of analytical instrumentation capable of performing online measurements of different chemical parameters and working under autonomous unattended conditions.

In this way, the development of robust continuous-flow chemical analyzers designed for monitoring water quality indicators and contaminants has become an active research field. The main drawbacks of these equipments are maintenance costs and excessive reagents consumption. Their miniaturization would provide operational improvements, including reduced analysis time, reduced reagent and sample consumption, and lower fabrication and maintenance costs. Furthermore, new features such as high portability and low power consumption would make their application in new fields (such as *in situ* monitoring using mobile remote controlled platforms) possible.[1-3] The attainment of a complete micro total analysis system (μTAS) involves the integration of most of the stages associated to an analytical process (preconcentration, detection system, fluidics and electronics, among others).[2-4] This, in turn, increases their complexity when trying to integrate the entire system in the same substrate. The detection system is one of the main parts of a microanalyzer. The high selectivity, sensitivity, and simple implementation of electrochemical methods (mainly voltamperometric), along with how well these devices maintain their analytical features during the scaling-down process compared with the optical methods, have contributed to their application in analytical microsystems. Therefore, miniaturized devices that integrate voltamperometric detectors can be frequently found in the literature.[5-7] Nevertheless, the operational lifetime of the working electrode constitutes a major weak point of this type of monolithic device, because it usually suffers of surface passivation or poisoning. To overcome this problem, the design of microsystems with replaceable electrodes would be desirable.

The integration of electronics and fluidics in the same substrate to obtain a complete microanalyzer still represents a limitation for some well-known microfabrication technologies such as silicon, glass and polymers. All these technologies present well-established fabrication processes that offer high precision and reliability.

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However, to integrate both platforms monolithically, they require extra efforts that imply high costs and long-term fabrication processes. To overcome these limitations, the modular integration of the different platforms is usually done. Under this modular approach, the elements of the system are fabricated separately and later joined originating hybrid devices. There are many microanalyzers based on these microfabrication technologies reported in the bibliography.[8−11] Nevertheless, dedicated microsystems that are designed to accomplish specific requirements to face a particular analytical problem are frequently required. In this case, low-volume production technologies would be the best choice, instead of mass production, to reduce costs and development time.

Some miniaturized electronic circuits for the instrumentation of amperometric[5,12−14] and potentiometric[15,16] detection systems have been developed and applied to miniaturized analyzers. Most of them are based on the printed circuit board (PCB) technology. Although this technology permits the development of highly complex electronic circuits easily, its fabrication processes and material properties do not allow the integration of microfluidic platforms to obtain a complete microanalyzer. To reach a higher integration level and more compact instruments, multilayer techniques are required. The low-temperature co-fired ceramics (LTCC) technology has been widely applied to the production of multilayer electronic circuits, because of its excellent electrical, mechanical, and thermal properties.[17,18] Its compatibility with serigraphic techniques and its fabrication process enable the aggregation of several layers to obtain multilayer circuits, which results in high-density boards of elevated complexity and small size. Recently, this technology has extended its application to the development of complex three-dimensional microsystems that involve fluidics, optics, electronics, and/or mechanics.[19] The integration of electronics, detection systems, and fluidics in the same substrate could provide more autonomous devices that are able not only to detect different analytes but also to apply data acquisition and digital signal processing, all in the same device. In our research group, there is wide experience in the development of chemical analyzers based on the LTCC technology that include microfluidics, detection systems, and/or electronics in the same substrate.[10,20−24] Most of them are applied to environmental monitoring and are based in electrochemical and optical detection systems. Although these microanalyzers offer great versatility and operative features, comparable to their counterparts in the macroscale, they do not integrate the associated microactuators for fluid handling such as micropumps and microvalves. This responds to the complexity involved for such integration and the lack of robustness associated with their small dimensions. Different works regarding the design and fabrication of micropumps and microvalves that can be monolithically integrated to microsystems have been presented.[25,26] Nevertheless, the integration of such microactuators in a monolithic configuration produces devices that lack versatility, operational features, and robustness to be used in real applications.

In this work, the development of a monolithic continuous flow microanalyzer, based on the green tape technology, which allows performing amperometric determinations using an exchangeable working electrode, will be presented.

EXPERIMENTAL SECTION

Materials and Equipment. Dupont 951 green tapes were used as substrate for the fabrication of the microanalyzer, including electronics and fluidics. Electrical conductors were printed using Dupont 6146 for solderable tracks, Dupont 6142D for internal tracks, and Dupont 6141 for via filling.

All the electronic components were carefully selected to reduce noise effects in the amperometric signal. A PIC16F877 microcontroller (Microchip, Inc., USA) was used to control the device operation. Surface mount devices (SMDs) were used to reduce the final dimensions of the microanalyzer. (Details regarding the structure of the microanalyzer are given in the section entitled "Device Layout" in the Supporting Information.)

All reagents used for the evaluation of the microanalyzer were analytical grade and obtained from Fluka. Stock solutions were daily prepared in Milli-Q water. KCl (0.1 M) was always used as reference solution. $K_4Fe(CN)_6$ standard solutions were prepared in KNO_3 (0.1 M) by serial dilution from a 0.1 M stock solution and using KNO_3 (0.1 M) as the carrier solution. Chlorine determination was performed using standards based on NaOCl obtained by serial dilution of a 0.1 M stock solution. In this case, the carrier solution was composed of KH_2PO_4 (0.1 M) and KNO_3 (0.1 M) mixed in a 1:1 ratio (pH 5.5).

The continuous flow system setup consisted of an external peristaltic pump (Minipuls 3, Gilson, Middleton, WI) that was equipped with 1.02-mm-internal-diameter silicon tubing (Ismatec, Zürich, Switzerland) and a six-port injection valve (Hamilton MVP, Reno, NV). Teflon tubing (with an internal diameter of 0.8 mm) (Scharlab, S.L., Cambridge, England) was used to connect the external elements with the microsystem.

To validate the experimental results, they were compared with those obtained using a commercial potentiostat (Model LC-4C, Bioanalytical Systems, Inc., West Lafayette, IN).

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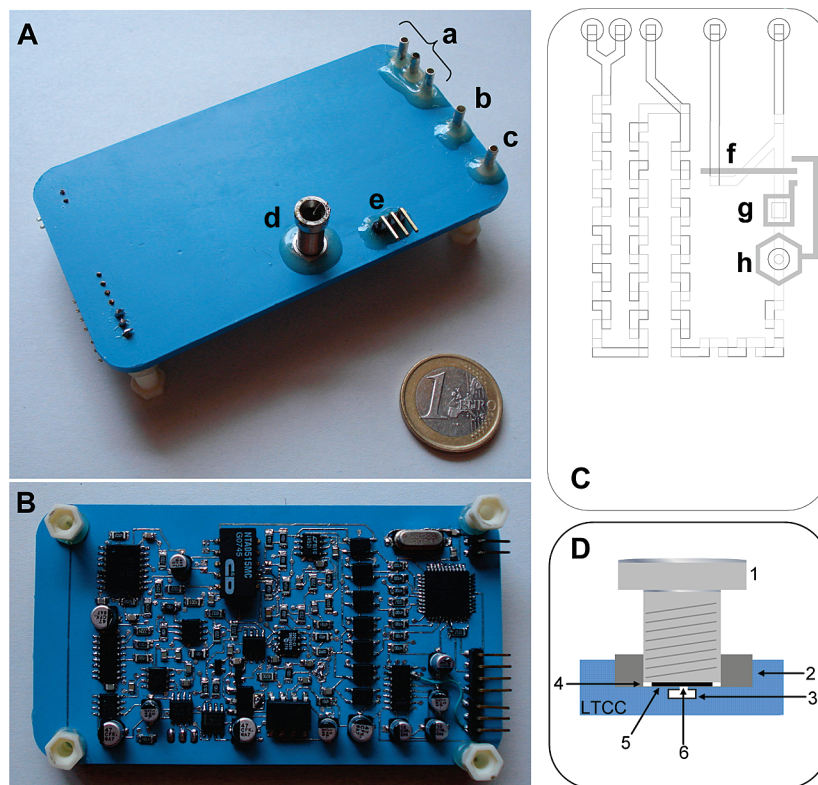(26) Kwang, W. O.; Chong, H. A. *J. Micromech. Microeng.* **2006**, *16*, R13–R39.

Figure 1. Amperometric microanalyzer (5.3 × 10.4 × 0.3 cm). (A) Top view of the fluidics and detection system. (Legend: a, reagent/sample inlets; b, reference solution inlet; c, outlet; d, exchangeable working electrode; and e, electrical connector.) (B) Bottom view of the electronics (potentiostat). (C) Fluidic distribution. (Legend: f, reference electrode; g, counter/auxiliary electrode; and h, working electrode track.) (D) Working electrode setup. (Legend: 1, metallic screw; 2, metallic nut; 3, flow channel; 4, Teflon ring; 5, working electrode (platinum sheet); and 6, channel cavity.)

RESULTS AND DISCUSSION

The main goal of this work was the development of a monolithic device that included not only the microfluidics but also the associated electronics (a programmable potentiostat) and a complete amperometric detection system based on a three-electrode configuration cell (working, reference, and counter electrodes). The reference and counter electrodes were embedded inside the ceramic body using screen-printing during the fabrication process. To increase the overall microsystem reliability and versatility, an exchangeable working electrode that could be released and replaced when needed was included.

To improve the range of possible analytical applications of the microanalyzer and, therefore, its versatility, the electronics of the system were based on a microcontroller, allowing for several voltamperometric techniques (normal and differential pulsed ones) to be easily applied.[27]

Electronics of the Microanalyzer. The complete process was controlled by the PIC (programmable interrupt controller) microcontroller. In general terms, the electronic circuit generates a user-defined waveform voltage that is applied between the working and reference electrodes. Because of the applied potential, reduction/oxidation reactions at the indicator/auxiliary electrode interfaces occur, generating a current that is measured, conditioned, analogue-filtered (second-order Butterworth low-pass filter with a cutoff frequency of 2 Hz), and read by the microcontroller.

The signal is finally sent to a personal computer through its serial port and displayed in the front panel of a virtual instrument. The virtual instrument was developed using Labview (National Instruments, Austin, TX) and permitted the user to configure the instrument operational parameters and to visualize the system response. Details are given in the section entitled "Electronic Circuit" in the Supporting Information.

The electronic design allowed the system to assign different gain values to the signal to do precise measurements at different current intensity levels (working ranges: 125, 12.5, 1.25, and 0.375 μA). The circuit requires only one 5 V power supply and consumes <50 mA; thus, it could be powered using conventional batteries during a specific period of time.

The microcontroller was programmed using specially developed software written in C language. The voltage waveform applied to the electrolytic cell was selected by the user in accordance to the amperometric technique being used. Because amperometric systems are highly sensitive to noise, extra digital filtering was included in the microcontroller code. (Details regarding this software are given in the section entitled "Software" in the Supporting Information.)

Fabrication of the Microanalyzer. The general fabrication process for LTCC devices was described in a previous publication.[20] The continuous flow microanalyzer developed in this work is presented in Figure 1. As illustrated in this picture, electronics were placed on one side of the device while the microfluidics and the detection system were on the other.

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The electronic circuit was designed as two stacked layers interconnected by electrical vias and using SMD components to reduce the final dimensions of the printed board (see Figure 1B). The layout was generated using special software for electronic circuit design.

The design of the microfluidic platform, including the cavities for the electrodes, was developed using computer-aided design (CAD) software that allowed the later incorporation of the electronics. This way, the complete system was included in a single design.

The microanalyzer had two reagent/sample inlets that converged upstream of a three-dimensional mixer. A third inlet, also followed by a three-dimensional mixer, was included in case an additional reagent is required (see Figure 1C). This design renders the analyzer more versatile and makes it adaptable to different analytical methodologies. Microfluidic channels were designed to be 2 mm wide and 0.4 mm high, considering ceramics shrinkage. A confluence point was placed downstream from the working and auxiliary electrodes, to establish a liquid contact between them and the reference electrode, using a KCl solution acting as a flowing liquid junction.

Apart of the microchannels, the fluidic platform included the complete amperometric detection system based on a three-electrode configuration (working, reference, and counter electrodes). The reference electrode, which consists of an embedded screen-printed silver track (Dupont 6142D) that was oxidized in the presence of a Cl^- ion solution, was placed in an auxiliary channel through which a constant KCl (0.1 M) solution flowed continuously. This solution maintained the reference potential at a constant value[10] and acted as a flowing liquid junction. This electrode was placed in an auxiliary channel separated from the main flow, to avoid contact with any other solution that could destabilize the reference signal. This arrangement provided a stable reference signal.

The counter electrode consisted of a 5 mm × 5 mm × 0.125 mm platinum sheet integrated over the main flow channel (after the working electrode) and connected to the electronic circuit by a conductive track and electrical vias. It was placed downstream from the working electrode to prevent that any bubbles formed on its surface could interfere with the performance of the working electrode. To complete the amperometric detection system and increase the system reliability, the working electrode was integrated as an exchangeable device. For this purpose, the device included a 2-mm-diameter cavity over the main channel that allowed the integration (after the fabrication process) of a thin 4-mm-diameter platinum electrode disk. Any metallic or conductive material able to be used as an electrode (i.e., graphite-epoxy composites[28]) can be readily integrated, maintaining the dimensions previously mentioned. The bottom face of this disk was in continuous contact with the liquid flowing through the channel (see Figure 1D). To avoid any liquid leakage that could affect the electrical measurements, a Teflon ring, placed over the electrode top surface, was pushed by a metallic screw that permitted the cavity to be sealed hermetically. The metallic screw also allowed the establishment of electrical contact between the inner surface of the working electrode and the electronics on the opposite side
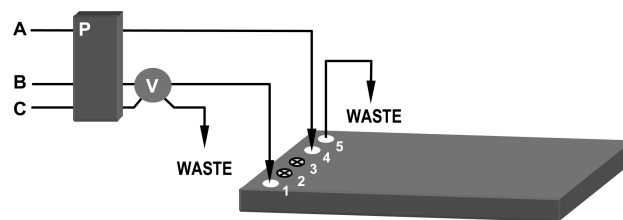
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Figure 2. Experimental setup for the microanalyzer evaluation. (Legend: A, KCl (0.1 M); B, buffer solution; C, sample; P, peristaltic pump; V, six-port injection valve; 1, reagent inlet; 2 and 3, inlets (not used); 4, reference solution inlet; and 5, waste outlet.)

through a metallic nut that supported it. This metallic nut was placed over a conductor track connected to the electronics by means of electrical vias. Using this approach, working electrodes can be easily detached and replaced when their surfaces are passivated or poisoned, or even when a different analyte to be determined requires it.

After the device was sintered, the surface mount electronic elements were soldered to their corresponding pads. To protect the electronic circuit from any leakage from the fluidic platform, it was coated with a proto circuit board varnish.

Before applying any experimental procedure, the electronics of the microanalyzer were calibrated. The calibration consisted on applying well-known potentials to well-known value resistors that emulate the electrolytic cell. This way, the current value measured by the instrument could be compared to its expected value and then corrected modifying the microcontroller code. To validate the electronics (potentiostat) operation, batch analysis using $K_4Fe(CN)_6$ as the analyte and an external electrolytic cell were performed and compared with those obtained using commercial equipment. Results obtained using both methods were compared using the least-squares linear regression approach:

$$Y = (1.01 \pm 0.07)X - (3 \pm 5) \times 10^{-9}$$

$$(n = 5; 95\% \text{ confidence; } r^2 = 0.99)$$

This expression demonstrates that there is no significant difference between the standard method and the proposed method.

Experimental Setup. To evaluate the microanalyzer performance and demonstrate its versatility, experimental tests using the amperometric technique for determining the presence of $K_4Fe(CN)_6$ and free chlorine were conducted. The optimum applied potential for the analyte oxidation was fixed at 250 and 350 mV for each analyte, respectively. The experimental setup is presented in Figure 2.

The carrier solution and the sample were propelled by the peristaltic pump to the microanalyzer, through inlet 1, and to the six-port injection valve, respectively. The baseline signal was established by the carrier solution when the six-port valve was in load position. Simultaneously, the sample solution filled the injection loop. When the six-port valve was shifted, the sample was inserted in the main channel and propelled by the carrier solution to the microsystem. In this case, inlets 2 and 3 were not used.

The reference electrolyte solution (KCl, 0.1 M) was propelled through inlet 4 to flow constantly through the auxiliary channel where the reference electrode channel was placed (marked as
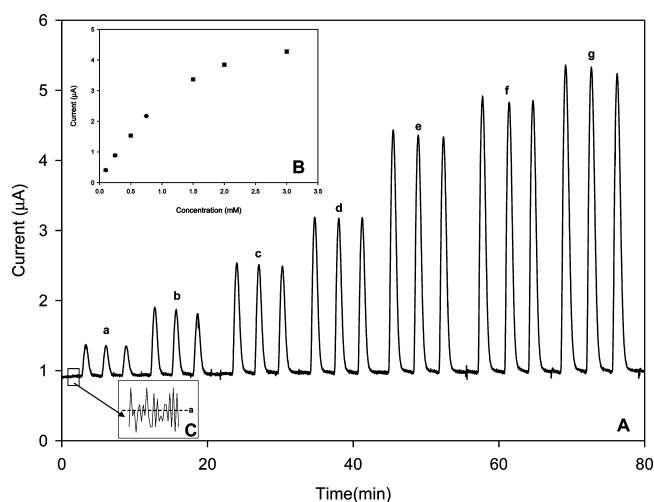
Figure 3. (A) System response to seven different $K_4Fe(CN)_6$ concentrations: 0.1 mM (peak a), 0.25 mM (peak b), 0.5 mM (peak c), 0.75 mM (peak d), 1.5 mM (peak e), 2 mM (peak f), and 3 mM (peak g). (B) Calibration plot. (C) Baseline amplification (the dashed line marked "a" represents a value of $0.9 \pm 0.002 \, \mu A$).
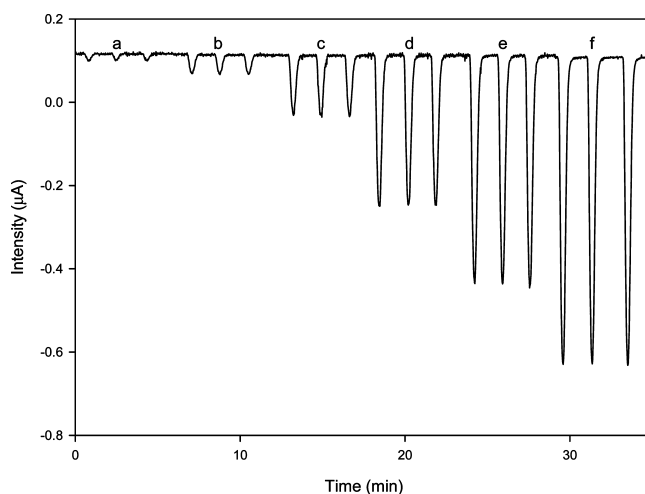

Figure 4. System response to six different chlorine concentrations: 0.35 mM (peak set a), 0.9 mM (peak set b), 1.7 mM (peak set c), 3.5 mM (peak set d), 5.3 mM (peak set e), and 7.1 mM (peak set f).

item "b" in Figure 1A). This way, the reference potential was maintained constant. When the sample reached the detector, a transient signal was obtained. Measurements were done by injecting standard solutions with different concentrations.

As shown in Figure 1C, the main and auxiliary channels converge at a confluence point downstream from the electrolytic cell, allowing the closing of the electrical pathway between the electrodes through the ionic conductivity of the solutions.

Considering the design of the microfluidic platform, this analyzer could be applied in a continuous flow mode, without the need of sample injection, by mixing constantly the carrier solution and the sample through inlets 1 and 2, respectively. This configuration would provide continuous information of the sample status. In addition, the absence of the six-port valve would reduce the complexity of the experimental setup, increasing its robustness and reducing maintenance costs. These properties would also facilitate the experimental setup miniaturization. Despite all the advantages offered by this approach, this operation mode does not allow one to control the baseline signal stability accurately. If the system is not stable enough, any instrumental variation that is not related to a change of the analyte concentration would affect the signal, which introduces measurement biases.

Determination of the presence of $K_4Fe(CN)_6$ was performed using hydrodynamic parameters that were selected based on a previous optimization procedure. In this way, the flow rate of the carrier solution and the sample injection volume were fixed at 0.7 mL/min and 500 μL, respectively, using KNO_3 (0.1 M) as both the supporting electrolyte and the carrier solution. The flow rate of the reference electrolyte solution (KCl, 0.1 M) was also fixed at 0.7 mL/min. $K_4Fe(CN)_6$ standards between 0.1 mM and 3 mM were prepared in KNO_3 (0.1 M) and injected to the microsystem. The applied potential was fixed in 250 mV.

As shown in Figure 3, the noise reduction techniques, applying both digital and analogue filtering, provided a practically noise-free baseline signal (± 2 nA).

The calibration plot obtained allowed the observation of both the linear and the nonlinear response zones of the system. Least-

squares linear regression applied to the system response at the linear zone (0.1−0.75 mM) of the calibration curve provided the following equation:

$$I = (1.7 \pm 0.5) \times 10^{-7} + (2.7 \pm 0.6) \times 10^{-6}[C]$$
$$(n = 3; \, 95\% \text{ confidence}; \, r^2 = 0.997)$$

where I is the measured current (in units of nA) and $[C]$ is the analyte concentration. The repeatability of the system was calculated as the relative standard deviation (RSD, %) of replicate injections. For an intermediate concentration (0.75 mM), the RSD obtained was 1.91% ($n = 13$, 95% confidence).

Given the device configuration and the hydrodynamic parameters used, it was possible to obtain a sampling throughput of 38 samples/h.

As presented in Figure 3, a stable baseline signal with a barely visible drift was obtained during the complete experiment. In addition, the absence of long peak tails after each sample injection may indicate that the device does not include dead zones, where sample accumulates.

To demonstrate the system versatility, it was also applied to free chlorine determination. In this case, key hydrodynamic parameters were selected based on an exhaustive optimization procedure that has been previously reported.[29] Flow rate and sample injection volume were fixed at 1.5 mL/min and 500 μL, respectively, using KH_2PO_4 (0.1 M) and KNO_3 (0.1 M), mixed in a 1:1 ratio (pH 5.5), as the carrier solution. Figure 4 shows the system response during the experiment. The flow rate of the reference electrolyte solution (KCl, 0.1 M) was also fixed at 1.5 mL/min. The applied potential, in this case, was 350 mV.

The linear regression at its linear zone was determined to be given as

$$I = (3.7 \pm 0.1) \times 10^{-8} - (1.1 \pm 0.004) \times 10^{-7}[C]$$
$$(n = 3; \, 95\% \text{ confidence}; \, r^2 = 0.998)$$

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where, again, I is the current flowing in the cell and $[C]$ the analyte concentration. For an intermediate concentration (3.5 mM), the RSD obtained was 1.93% ($n = 15$; 95% confidence).

As demonstrated, the proposed microsystem offers a high versatility and integration level with a performance comparable to conventional macroanalyzers commercially available. Nevertheless, it still presents the need of external actuators such as the pump used to propel the liquids through the microsystem and the six-port valve used to inject the sample solution. At the current state of the art, one way to obtain a complete microanalyzer with an integration level sufficent to be called μTAS is through the hybrid integration of some commercially available external microactuators (micropumps and microvalves).

CONCLUSIONS

The multilayer approach and electrical characteristics of the low-temperature co-fired ceramics (LTCC) technology permit one to fabricate compact and complex electronic circuits, as well as complex three-dimensional fluidic structures. This approach has allowed the development of a robust continuous-flow microanalyzer, based on an amperometric detection system that is able to work autonomously, and, therefore, more similar to a micro total analysis system (μTAS) for real applications. The amperometric detection system included an exchangeable working electrode, increasing the microanalyzer reliability and avoiding its dependence on the lifetime of the working electrode. Results showed a high signal-to-noise ratio that was comparable to those obtained with conventional instrumentation. In addition, because the electronics are based on a microcontroller, its operation mode can be adapted to different amperometric techniques, rendering it highly versatile. The microanalyzer versatility was also demonstrated by its application to the determination of two different analytes—$K_4Fe(CN)_6$ and free chlorine—although it could be adapted to determine many others. One step further in the achievement of a μTAS would be the hybrid integration of external microactuators such as micropumps and microvalves, to increase the system reliability. Work following this approach is currently under development.

SUPPORTING INFORMATION AVAILABLE

Description of the device layout and electronic circuit involved in creating the microanalyzer, as well as the software used to operate the microcontroller. This material is available free of charge via the Internet at http://pubs.acs.org.