

This is a postprint version of the following published document:

Martínez-Cisneros, C. S., da Rocha, Z., Ferreira, M., Valdés, F., Seabra, A., Góngora-Rubio, M., Alonso-Chamarro, J. (2009). A monolithic continuous-flow microanalyzer with amperometric detection based on the green tape technology. *Analytical chemistry*, 81(17), pp. 7448-7453.

DOI: [10.1021/ac9012418](https://doi.org/10.1021/ac9012418)

**A NOVEL MONOLITHIC CONTINUOUS FLOW
MICROANALYZER WITH AMPEROMETRIC
DETECTION BASED ON THE GREEN TAPE
TECHNOLOGY**

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ABSTRACT

The development of microTotal Analysis Systems (μ TAS) 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 due to 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 with 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 ($125\mu\text{A}$, $12.5\mu\text{A}$, $1.25\mu\text{A}$ and $0.375\mu\text{A}$) to do precise determinations at different current intensity levels.

Keywords: LTCC, miniaturization, potentiostat, μ TAS, monolithic amperometric microanalyzer, exchangeable electrode.

INTRODUCTION

The growing demand of real time environmental information to control and to manage water resources properly has promoted the development of analytical instrumentation capable to perform on-line measurements of different chemical parameters and to work 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 possible their application in new fields such as *in-situ* monitoring using mobile remote controlled platforms¹⁻³. The attainment of a complete micro total analysis system (μ TAS) involves the integration of most of the stages associated to an analytical process (pre-concentration, detection system, fluidics and electronics, among others)²⁻⁴. This in turn increases their complexity when trying to integrate the whole system in the same substrate. The detection system is one of the main parts of a microanalyzer. The high selectivity, sensitivity and the 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 literature⁵⁻⁷. Nevertheless, the operational lifetime of the working electrode constitutes a major weak point of this kind of monolithic devices since 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. 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 bibliography⁸⁻¹¹. Nevertheless, dedicated microsystems, 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 in order 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 Cofired Ceramics (LTCC) technology has been widely applied to the production of multilayer electronic circuits due to 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 mechanics¹⁹. The integration of electronics, detection systems and fluidics in the same substrate could provide more autonomous devices 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 a 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. Even though 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 to 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

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 in order to reduce noise effects in the amperometric signal. A PIC16F877 microcontroller (Microchip Inc., USA) was used to control the device operation. Surface Mount Devices (SMD) were used to reduce the final dimensions of the microanalyzer.

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

The continuous flow system setup consisted of an external peristaltic pump (Minipuls 3, Gilson, Wisconsin, US) equipped with 1.02mm internal diameter silicon tubing (Ismatec, Zürich, Switzerland) and a six-port injection valve (Hamilton MVP, Reno, US). 0.8mm internal diameter Teflon tubing (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 (LC-4C - Bioanalytical Systems Inc., West Lafayette, USA).

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. Due to the applied potential, reduction/oxidation reactions at the indicator/auxiliary electrode interfaces take place generating a current that is measured, conditioned, analogue filtered (2nd order Butterworth low-pass filter with a cut-off frequency of 2Hz) 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, USA) and

permitted the user to configure the instrument operational parameters and to visualize the system response.

The electronic design allowed the system to assign different gain values to the signal in order to do precise measurements at different current intensity levels (working ranges: 125 μ A, 12.5 μ A, 1.25 μ A and 0.375 μ A). The circuit requires only one 5V power supply and consumes less than 50mA, 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. Since amperometric systems are highly sensitive to noise, extra digital filtering was included in the microcontroller code.

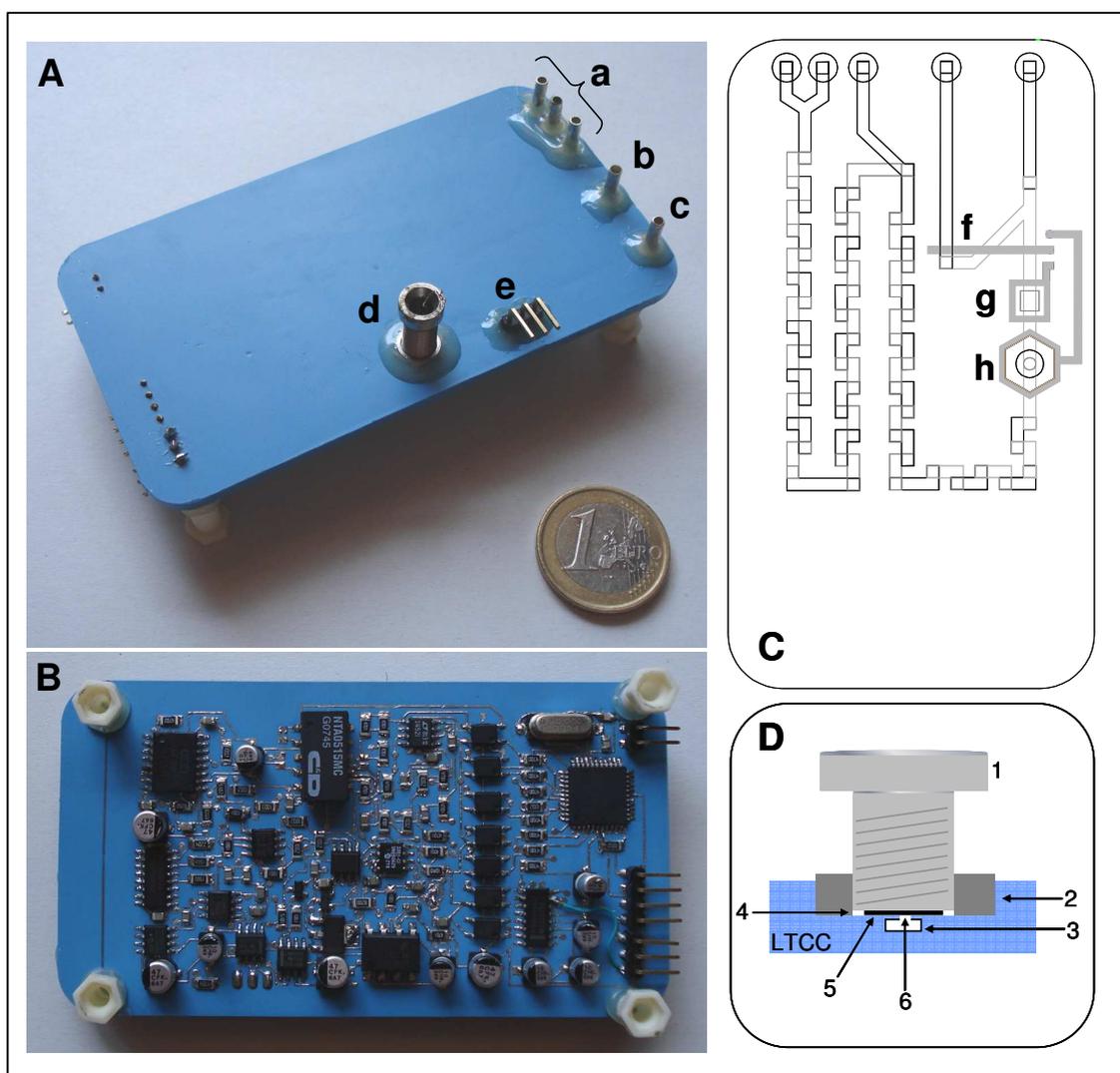
Fabrication of the microanalyzer

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.

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 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 2mm wide and 0.4mm high, considering ceramics shrinkage. A confluence point was placed downstream 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, consisting of an embedded screen-printed silver track (Dupont 6142D) that was oxidized in presence of a chloride ion solution, was placed in an auxiliary channel through which a constant KCl (0.1M) solution flowed continuously. This solution maintained the reference potential at a constant value¹⁰ 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 5x5x0.125mm 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 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 to increase the system reliability, the working electrode was integrated as an exchangeable device. For this purpose, the device included a 2mm diameter cavity over the main channel that allowed the integration (after the fabrication process) of a thin 4mm diameter platinum electrode disc. Any metallic or conductive material able to be used as 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 to seal the cavity hermetically. The metallic screw also

allowed establishing electrical contact between the inner surface of the working electrode and the electronics on the opposite side 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, poisoned or even when a different analyte to be determined requires it.

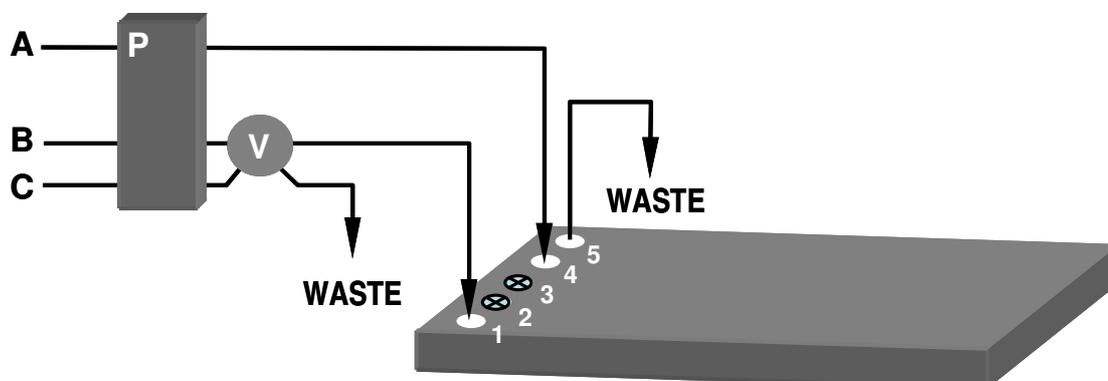
Once the device was sintered, the surface mount electronic elements were soldered to their corresponding pads. To protect the electronic circuit from any leakage from the fluidic platform, it was coated with a proto circuit board varnish.

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 analyte and an external electrolytic cell were performed and compared with those obtained using the commercial equipment. Results obtained using both methods were compared using the least squares linear regression approach: $Y = 1.01 (\pm 0.07) \cdot X - 3E^{-09} (\pm 5E^{-09})$; ($n=5$, $r^2 > 0.99$, 95% confidence). This expression demonstrates that there is not any significant different between the standard and the proposed method.

Experimental Set-up

To evaluate the microanalyzer performance and demonstrate its versatility, experimental tests using the amperometric technique for the determination of $K_4Fe(CN)_6$ and free chlorine were carried out. The optimum applied potential for the

analyte oxidation was fixed at 250mV and 350mV for each analyte, respectively. The experimental set-up 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 base line 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 0.1M) was propelled through inlet 4 to flow constantly through the auxiliary channel where the reference electrode channel was placed (marked as 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 closing the electrical pathway between the electrodes through the ionic conductivity of the solutions.

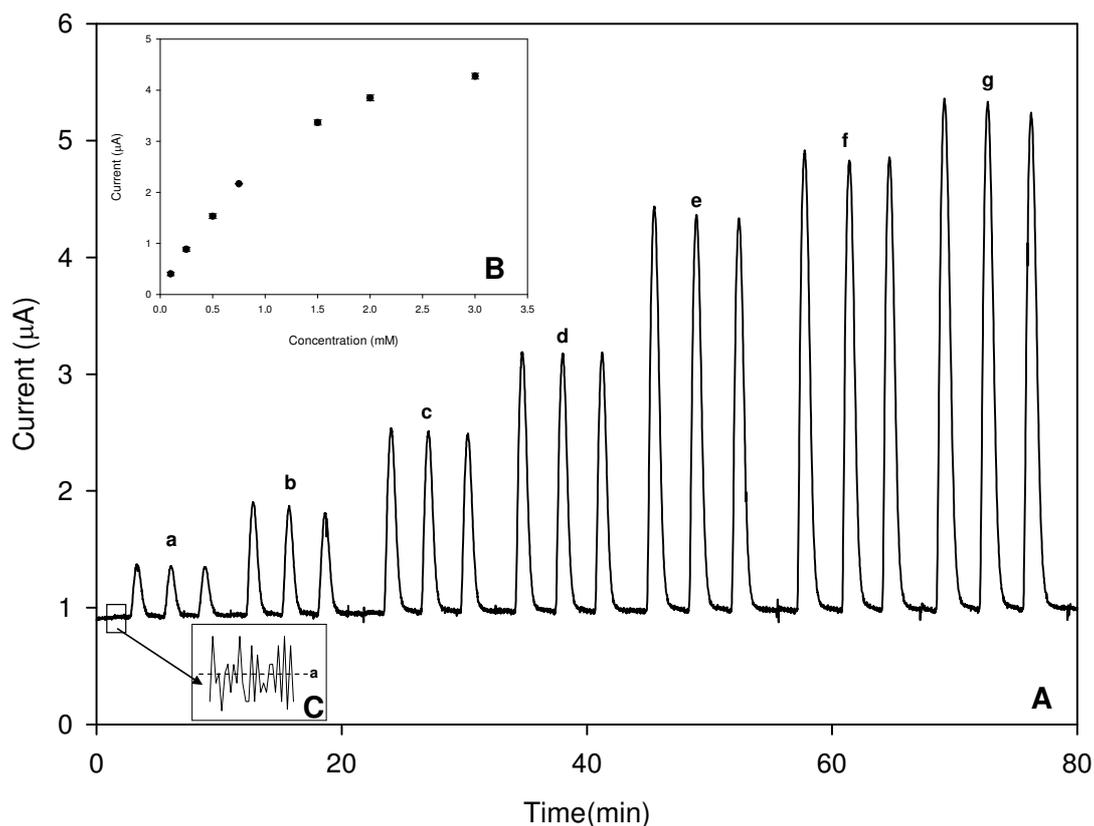
Considering the design of the microfluidic platform, this analyzer could be applied in a continuous flow mode, without the need of sample injection, by mixing constantly the carrier solution and the sample through inlets 1 and 2 respectively. This configuration would provide continuous information of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

The $\text{K}_4\text{Fe}(\text{CN})_6$ determination was performed using hydrodynamic parameters selected on the basis of a previous optimization procedure. In this way, flow rate of the carrier solution and the sample injection volume were fixed at 0.7mL/min and 500 μL , respectively, using KNO_3 (0.1M) as both, supporting electrolyte and carrier solution. The flow rate of the reference electrolyte solution (KCl 0.1M) was also fixed at 0.7mL/min. $\text{K}_4\text{Fe}(\text{CN})_6$ standards between 0.1mM and 3mM were prepared in KNO_3 (0.1M) and injected to the microsystem. The applied potential was fixed in 250mV.

As shown in figure 3, the noise reduction techniques applying both digital and analogue filtering, provided a practically noise free base line signal ($\pm 2\text{nA}$).

The calibration plot obtained allowed the observation of both the linear and the non-linear response zones of the system. Least squares linear regression applied to the system response at the linear zone (0.1mM – 0.75mM) of the calibration curve provided the following equation: $I = 1.7\text{E}^{-07}(\pm 0.5\text{E}^{-07}) + 2.7\text{E}^{-06}(\pm 0.6\text{E}^{-06}) \cdot [\text{C}]$ (n=3;

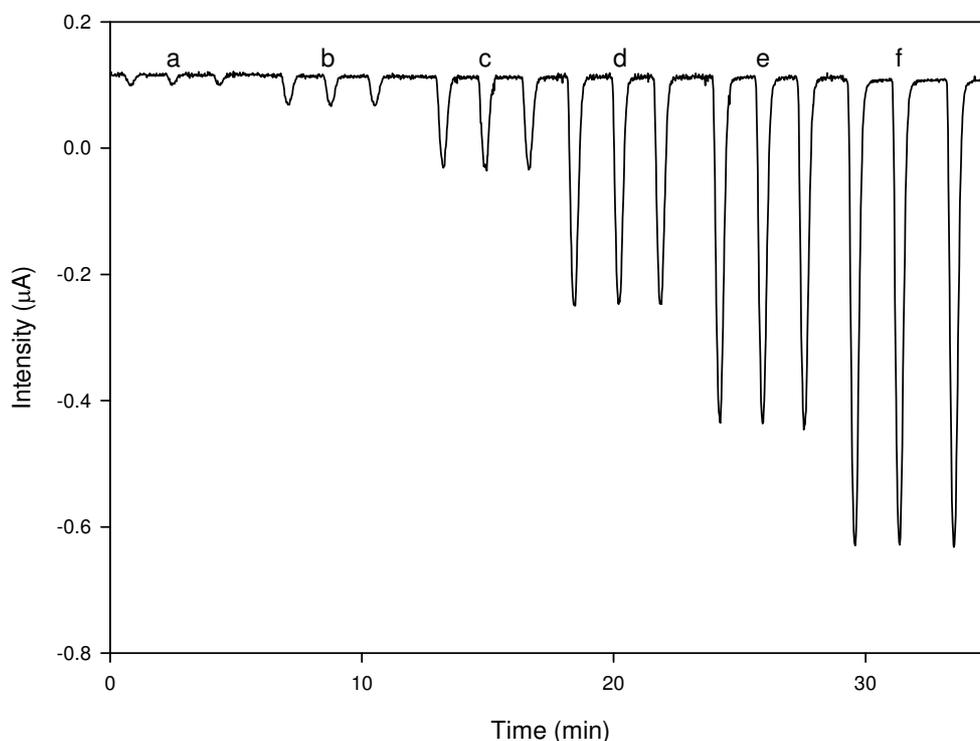
95% confidence; $r^2 > 0.997$). Where: I is the measured current in nA and $[C]$ the analyte concentration. The repeatability of the system was calculated as the relative standard deviation (RSD %) of replicate injections. For an intermediate concentration (0.75mM), the RSD obtained was 1.91% ($n = 13$, 95% confidence).



Given the device configuration and the hydrodynamic parameters used, it was possible to obtain a sampling throughput of 38 samples hour^{-1} .

As presented in figure 3, a stable base line signal with a drift barely visible 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 on the basis of an exhaustive optimization procedure previously reported.²⁹ Flow rate and sample injection volume were fixed at 1.5mL/min and 500 μ L, respectively, using KH₂PO₄ (0.1M) and KNO₃ (0.1M), mixed in a one to one ratio (pH =5.5), as carrier solution. Figure 4 shows the system response during the experiment. The flow rate of the reference electrolyte solution (KCl 0.1M) was also fixed at 1.5 mL/min. The applied potential was in this case 350mV.



The linear regression at its linear zone was founded to be $I = 3.7E^{-08}(\pm 0.1E^{-08}) - 1.1E^{-07}(\pm 0.004 E^{-07}) \cdot [C]$ ($n=3$; 95% confidence; $r^2 > 0.998$). Where: I is the current flowing in the cell and $[C]$ the analyte concentration. For an intermediate concentration (3.5mM), 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, a way to obtain a complete microanalyzer with an integration level enough to be called μ TAS is by the hybrid integration of some commercially available external microactuators (micropumps and microvalves).

CONCLUSIONS

The multilayer approach and electrical characteristics of the LTCC technology permit 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 thus, closer to a Micro Total Analysis System for real applications. The amperometric detection system included an exchangeable working electrode, increasing the microanalyzer reliability and avoiding its dependence on the life time of the working electrode. Results showed a high signal to noise ratio comparable to those obtained with conventional instrumentation. In addition, since 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 μ TAS 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.

ACKNOWLEDGMENTS

The authors would like to thank the Spanish MEC for its financial support through: Consolider-Ingenio 2010 (CSD2006-00012), TEC2006-13907-C04-04/MIC and CIT-310200-2007-29.

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FIGURE CAPTIONS

Figure 1: Amperometric microanalyzer (5.3x10.4x0.3cm). **A:** top view with the fluidics and detection system; **a:** reagent/sample inlets; **b:** reference solution inlet; **c:** outlet; **d:** exchangeable working electrode; **e:** electrical connector. **B:** bottom view with the electronics (potentiostat). **C:** fluidic distribution; **f:** reference electrode; **g:** counter/auxiliary electrode; **h:** working electrode track. **D:** working electrode set-up; **1:** metallic screw; **2:** metallic nut; **3:** flow channel; **4:** Teflon ring; **5:** working electrode (platinum sheet); **6:** channel cavity.

Figure 2: Experimental set-up for the microanalyzer evaluation. **A:** KCl (0.1M); **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; **5:** waste outlet.

Figure 3: **A:** System response to seven different $K_4Fe(CN)_6$ concentrations: **[a]**=0.1mM, **[b]**=0.25mM, **[c]**=0.5mM, **[d]**=0.75mM, **[e]**=1.5mM, **[f]**=2mM and **[g]**=3mM. **B:** calibration plot. **C:** base line amplification; **a:** $0.9\mu A \pm 0.002$.

Figure 4: System response to six different chlorine concentrations: **[a]**=0.35mM, **[b]**=0.9mM, **[c]**=1.7mM, **[d]**=3.5mM, **[e]**=5.3mM and **[f]**=7.1mM.