Vortex Configuration Flow Cell Based on Low-Temperature Cofired Ceramics As a Compact Chemiluminescence Microsystem

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The integration of optical detection methods in continuous flow microsystems can highly extend their range of application, as long as some negative effects derived from their scaling down can be minimized. Downsizing affects to a greater extent the sensitivity of systems based on absorbance measurements than the sensitivity of those based on emission ones. However, a careful design of the instrumental setup is needed to maintain the analytical features in both cases. In this work, we present the construction and evaluation of a simple miniaturized optical system, which integrates a novel flow cell configuration to carry out chemiluminescence (CL) measurements using a simple photodiode. It consists of a micromixer based on a vortex structure, which has been constructed by means of the low-temperature cofired ceramics (LTCC) technology. This mixer not only efficiently promotes the CL reaction due to the generated high turbulence but also allows the detection to be carried out in the same area, avoiding intensity signal losses. As a demonstration, a flow injection system has been designed and optimized for the detection of cobalt(II) in water samples. It shows a linear response between 2 and 20 μM with a correlation of $r > 0.993$, a limit of detection of 1.1 μM, a repeatability of RSD = 12.4 %, and an analysis time of 17 s. These results demonstrate the suitability of the proposal to the determination of compounds involved in CL reactions by means of an easily constructed versatile device based on low-cost instrumentation.

Innovation in optochemical sensing is focused on the development of sophisticated transduction phenomena and their associated technologies. Additionally, direct sensing schemes based on well-known optical methods have been adapted to provide more robust, reliable, and portable instruments. Nevertheless, the required characteristics to measure real samples with complex matrixes have hindered their direct application. To solve this drawback, sensing systems including additional stages of sample treatment have been designed. The final aim is to maintain or improve the required analytical features (selectivity, sensitivity, accuracy, etc.) in a direct sensing operation by the incorporation of sample conditioning steps. In this way, miniaturization provides the tools to obtain compact and robust analytical systems, capable of rapidly and continuously supplying analytical information.

Microtechnology provides a great opportunity to satisfy the requirements derived from the need to integrate and miniaturize analytical systems, and it is currently being one of the most active research fields in this scope. Conversely, limitations of the employed technologies and materials exist. For instance, due to the restrictions of some typical fabrication techniques and materials (silicon, glass, polymers) on the construction of three-dimensional structures, it is a challenge to integrate monolithically the electronic control of devices and actuators in the same fluidic platform. In the last years, green tape or low-temperature cofired ceramics (LTCC) have been used as novel substrates to fabricate micro total analysis systems (μ-TAS), due to the combination of some of their main advantages. The most remarkable benefit is that they permit the easy and extremely rapid construction of multilayered miniaturized analytical systems without showing common handicaps, such as the need of clean room facilities or the sealing between layers to avoid liquid leakage. Moreover, these materials have been commonly used for electronic applications and are perfectly compatible with the screen-printing technology. Hence, electronics and fluidics can be easily integrated within the same substrate, allowing the procurement of a real micro total analysis system.[1] These miniaturized devices are of great interest as in some aspects they potentially improve the efficiency of conventional laboratory techniques. First, they allow for minimization of the volume of reagents, which is particularly important for those of high cost or in cases where only a little amount of sample is feasible. This also permits the use of higher reagent concentrations, and then better reaction kinetics and detection limits are attained. Furthermore, as the volume of byproducts and leftovers are reduced, this collaborates for the development of sustainable systems.

Among the different optical detection techniques, chemiluminescence (CL) shows some interesting advantages, including high sensitivity, low detection limits, and fastness (the signal is generated in a few seconds and, in some cases, is stable for some hours), and the procedures for the detection are simple enough to be easily implemented in microanalyzers. For example, Fletcher et al.[2] reported more than 200 species detectable by means of CL reactions (pharmaceutical compounds, environmental pollutants, biomedical analytes, etc.). Zhang et al.[3] reviewed interesting applications of CL reactions, as well as the latest advances in this topic. Some microanalyzers using this optical detection system have already been reported. Some of them integrate passive micromixers to enhance the mixing between reagents. On the whole, sample-reagent mixing has been achieved through a T-piece followed by an integrated spiral or compact serpentine flow cell with different 180° U-turn channels. In this sense, Nakamura et al.[4] presented an integrated spiral flow cell made of silicon, Danielson et al.[5] showed the same configuration but made of Teflon. Zhang et al.[6] proposed a compact serpentine cell located over the detector.

However, a compromise situation between a simple and fast fabrication technology and an adequate sample throughput has not been achieved yet. In order to enhance fast mixing, a three-dimensional vortex configuration can be used. Lin et al.[7] employed the self-rotation of fluids from multiple injection channels to produce three-dimensional vortexes in a circular mixing chamber fabricated on a multilayered glass substrate. In this work, we propose the use of this mixing concept to design a CL microflow cell, which can be easily integrated in a micro total analysis system. Furthermore, the use of the LTCC technology is proposed to take advantage of its multilayer fabrication capabilities.[8,9]

The efficient mixing promoted by this mixer is reached once the vortex has been generated. In this conditions, which are achieved sufficiently increasing the flow rate, a multilamination effect that favors the mixing is created.

Taking into account that one of the main characteristics of the CL reactions is the fast signal decrease once the maximum intensity has been reached, it is a great advantage to place the detection system in front of the area where the reaction is developed. The proposed fluidic platform design enables this configuration, so that light collection can be optimized and signal intensity losses can be avoided.

This paper presents the fabrication and evaluation of a versatile microanalyzer, which integrates a vortex efficient mixer acting as a flow cell allowing use of a simple, inexpensive, and miniaturized PIN photodiode detector for CL determinations.

EXPERIMENTAL SECTION

LTCC Devices Construction. Ceramic green tapes 951 (254 μm thickness) purchased from DuPont were used as described elsewhere.[1] The characteristic that makes this material so interesting for our purposes is the fact that the tapes can be easily mechanized by means of a standard milling machine or a laser in the green stage (before they are sintered), when they are still soft and pliable. In this case, the final device was the result of overlapping a total amount of 15 layers with 8 different mechanized structures. The design of each individual layer by means of a CAD software was done taking into account the shrinkage suffered by the ceramic tapes during the sintering step ($\sim$15% in each axis).[10] The mechanization was performed by a LPKF Protomat C100/HF milling machine. Once the layers were overlapped, they were placed between two stainless steel sheets with four aligning marks and laminated following a thermocompression process, which consisted of applying $\sim$3000 psi for 3−4 min at 100 °C. After that, the sintering was carried out in a furnace with programmable software (Carbolite CBCWF11/23P16, Afora, Spain), so that a concrete temperature profile could be applied.

Figure 1A shows the CAD designs of the overlapped layers for the four inlet vortexes and the three-dimensional inner structure of one of the micromixers. Figure 1B shows a mechanized layer that contains the distribution channels of the vortex chamber. Finally, parts C and D of Figure 1 show the front face of one of the vortex cells and its coupling to the photodiode detection system. Differences between the six fabricated devices are found on the diameter of the vortex flow cell (0.45, 0.9, and 1.2 cm, after the sintering step) and the number of flow inlets in the reaction chamber (4 or 8). As a final step, to seal the microflow cell, a thin glass sheet was glued to the upper face of the reaction chamber.

Apparatus and Reagents. Apart from the LTCC microflow cell (analyzer), the rest of the hybrid flow system setup consisted of a peristaltic pump (Gilson, Wisconsin), a six-port distribution valve (Hamilton MVP, Reno), 1.02 mm i.d. silicone tubing (Ismatec, Zurich, Switzerland) and 0.8 mm i.d. Teflon tubing (Scharlab, S.L., Cambridge, England).

A PIN silicon photodiode (Hamamatsu, S1337-66BQ) was used as a detector (see Figure 1). The software used (based on Labview) was specially designed for our applications.

All the reagents used were of analytical grade. Hydrochloric acid, sodium carbonate, and cobalt nitrate hexahydrated were purchased from Panreac. Luminol was purchased from Fluka, and hydrogen peroxide was purchased from Sigma. The concentrations of the reagents were modified in the course of the experiments in order to adapt them to each of the new experimental conditions. The first experimental conditions were adapted from the literature,[11] where a mixed reagent (MR) was used in order to simplify the experimental setup. The MR solution contained a luminol concentration of 650 μM and a Co^{2+} concentration of 60 μM, adjusted to 1 L with Na_2CO_3 0.1 M and at pH = 10.15 (adjusted with diluted HCl). The solution was left in a refrigerator for 48 h and kept in a dark bottle. The H_2O_2 solutions were prepared daily and kept in dark bottles, as well.
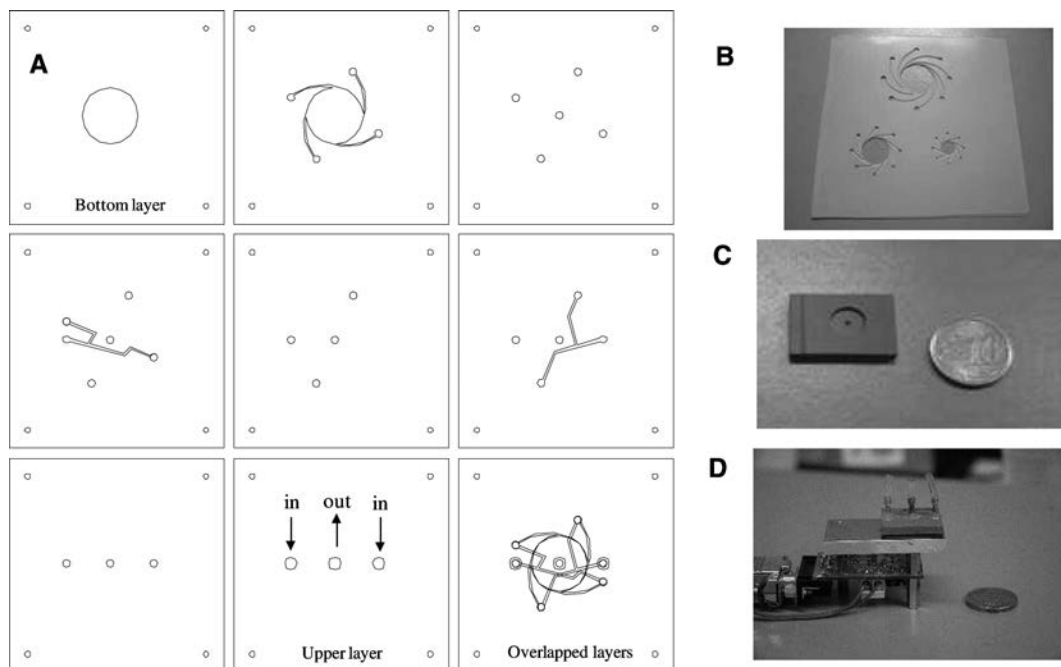
Figure 1. (A) CAD designs of one of the constructed devices with four flo inlets in the main reaction chamber and drawing of the inner three-dimensional structure; (B) ceramic layer containing the three engraved reaction chambers; (C) front side of one of the constructed device; (D) device directly coupled to the photodiode (photodetector Hamamatsu S1337-66BQ).

In order to clarify the presentation of the obtained results, each of the employed experimental setups will be shown in the results section.

RESULTS AND DISCUSSION

Device Geometry, Flow Rate, and Injection Volume Optimization. Initially, different experiments were performed to select the best flow cell geometry (diameter of mixing chamber and number of flow inlets) and to optimize hydrodynamic parameters such as sample injection volume and flow rate.

The influence of flow rate on the mixing efficiency for all the microflow cells constructed was evaluated qualitatively using phenol red and distilled water. Results obtained showed us that the higher the flow rate, the more efficient the mixing between both solutions. In Figure 2 some examples are presented showing the different mixing patterns obtained as a function of the flow rate. Low flow rates provide definite patterns with no mixing between solutions (parts A and B of Figure 2). On the contrary, high flow rates provide an adequate mixing of both solutions throughout all the chamber volume (parts C and D of Figure 2). According to these results, a high flow rate (6.67 mL/min) was chosen to obtain the most efficient mixing, which provides well-defined transitory peaks and, therefore, the best repeatability of the signals.

Given this fixed flow rate, the rest of the flow cells were evaluated using the setup depicted in Figure 3A.

For the diameter and number of flow inlets of the chamber optimization, 100 μL of H_2O_2 was injected as the sample, in a carrier solution (distilled water), which was mixed in the microflow cell with the reagent solution (MR), previously described. Results obtained are represented in Figure 4. As it can be seen, the best vortex flow cell configuration was the one with a 0.9 cm chamber diameter and four flow inlets. Since it provided the highest
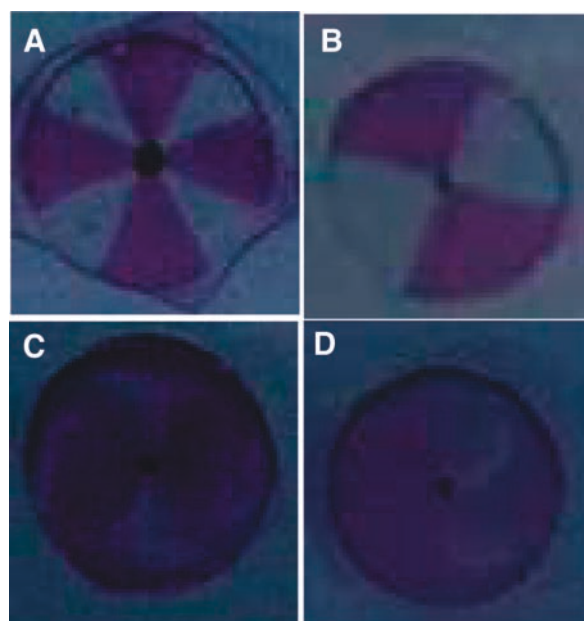

Figure 2. Mixing patterns with phenol red as the coloring solution. (A) Eight inlets and low flo rate; (B) four inlets and low flo rate; (C) eight inlets and high flo rate; (D) four inlets and high flo rate.

intensities and the best signal to noise ratios, it was chosen to perform the injection volume optimization. It is clear that a high flow rate, while generating the vortex, leads to a better mixing and that this fact gives a higher signal, but when the flow cell diameter increases, a stronger resistance to the vortex flow formation is observed. This effect is more marked for a higher number of inlets because the individual effective push of each inlet flow decreases. The total flow rate is maintained but it is distributed between more inlets. This could justify the observed difference for the 1.2 cm inner diameter cell. Another factor, which
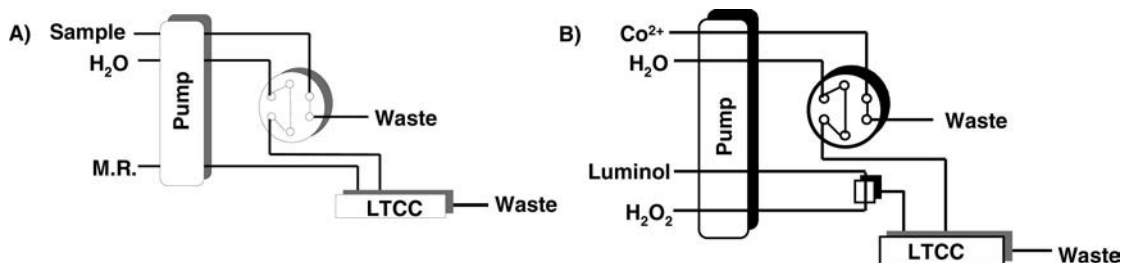
Figure 3. (A) Flow manifold used to optimize the device geometry, the injection volume, and the flo rate. (B) Flow manifold used to optimize the luminol solution concentration and pH and the H_2O_2 solution concentration.
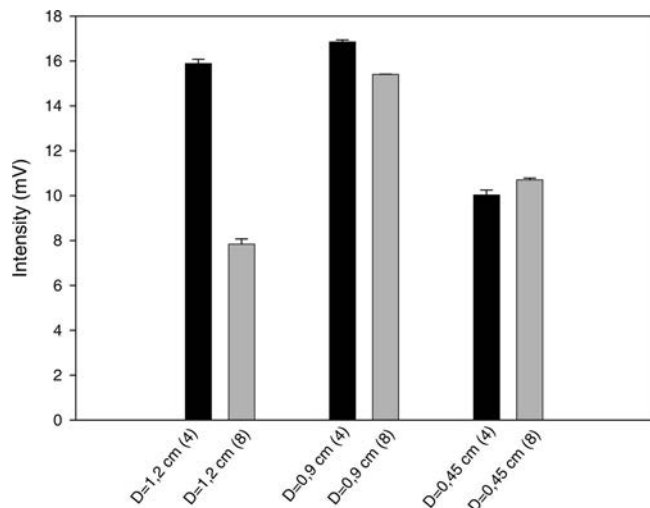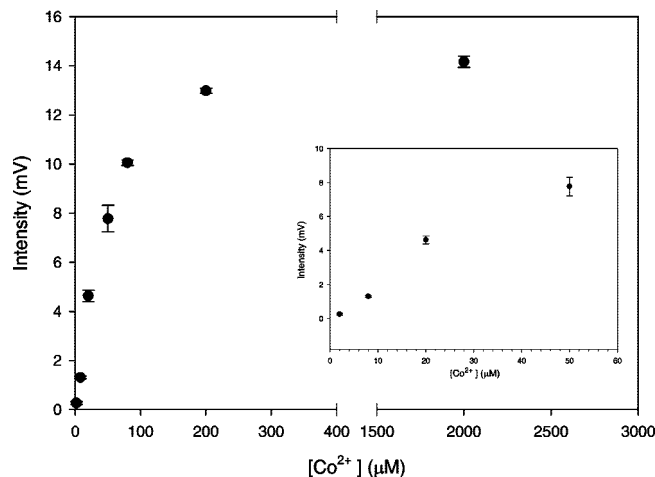

Figure 4. Optimization of the LTCC vortex configuration In parentheses are the number of flo inlets to the flo chamber. Injection volume, 100 μL; H_2O_2 concentration, 97.9 mM; flo rate, 6.67 mL/min. D, cell diameters of 1.2, 0.9, and 0.45 cm, which, respectively, correspond to 271, 152, and 38 μL cell volumes.

Figure 5. Cobalt calibration curve. Experimental conditions: 50 μL of Co^{2+}; luminol 1.5 mM at pH = 10; H_2O_2 concentration, 97.9 mM; flo rate, 6.67 mL/min. Injections are in triplicate.

would explain the signal decrease for the highest diameter cell would be the dilution of the sample as the injected volume is divided in four fractions before reaching the cell. These negative effects are not so important for the 1.2 cm diameter cell with four inlets (where two inlets are for reagents and two inlets are for the sample). Therefore, for 1.2 and 0.9 cm and four inlets, the signals are similar, whereas for 0.45 cm the signal significantly decreases for four and eight inlets. This is related to the smaller measurement area and the shorter sample residence time inside the cell for a certain injection volume.

The effect of increasing the injection volume on the signal was evaluated using a H_2O_2 concentration of 97.9 mM and working with the previously optimized conditions. It was observed that working with volumes higher than 50 μL led to auto-quenching effects on the CL reaction, diminishing the signal intensity. For this reason, the following experiments regarding the cobalt determination were performed with this sample volume.

Microsystem for the Determination of Co^{2+}. Once the hydrodynamic parameters and the microflow cell configuration were selected, a procedure for the cobalt determination was designed as a demonstration of the microsystem functionality. The manifold proposed is depicted in Figure 3B. Cobalt standard solutions were injected in a distilled water carrier solution. A new reagent solution was prepared online by mixing luminol and H_2O_2 solutions in a methacrylate T-junction previous to the LTCC vortex

entrance. Throughout all the experiments, an injection volume of 50 μL of a 80 μM Co^{2+} solution was injected as a sample solution.

In order to determine the optimal concentration of luminol, it was initially varied from 0.06 to 2.5 mM, while maintaining the concentration of the H_2O_2 solution at 97.9 mM. The signal intensity increases with the concentration of luminol; therefore, a value of 1.5 mM was chosen as the optimum as a compromise situation between the maximum intensity signal and the high consumption of reagent.

Another parameter affecting the signal intensity is the pH of reagent solution. Four pH values of the luminol solution (between 9.5−11.0) were tested, and the highest signal was obtained when the pH was adjusted to 10 at the previously optimized experimental conditions.

Finally, the effect of the H_2O_2 solution concentration on the signal was tested. As it is commonly found in the literature, the highest signals were obtained when a 97.9 mM concentration was employed.

Once all the experimental conditions were optimized, the analytical features of the proposed microsystem to determine Co^{2+} in aqueous solutions were established. Standard solutions of this metal between 2 μM and 200 mM were injected for triplicate analysis in the system to obtain the calibration parameters. The experimental results, depicted in Figure 5, showed a linear response between 2 and 20 μM. I(mV) $= -0.4(\pm0.1) + 0.24(\pm0.02)[Co^{2+}]$ $(r > 0.993)$.

The limit of detection, calculated as 3 times the standard deviation of the blank signal, was 1.1(±0.3) μM $(n = 10)$. Results

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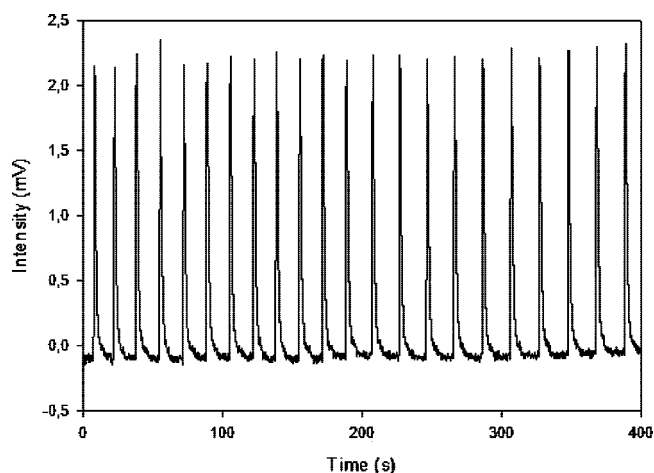
Figure 6. Repeatability study. Injection volume, 50 μL; Co^{2+} concentration, 20 mM; flo rate, 6.67 mL/min.

show that the proposed CL microanalyzer is suitable to carry out cobalt determinations in water despite the slightly high detection limit and compared to other reported systems in the literature.[12–14] It is important to point out that a PIN photodiode has been used in this demonstration instead of a photomultiplier, of higher sensitivity, which is the commonly employed detection system for CL devices. Its use entails the development of compact, portable, and low-energy consuming devices, which can be carried out for in situ and real-time analysis.

Repeatability studies were also performed using a 20 μM Co^{2+} standard solution (see Figure 6). For $n = 23$, an intensity mean of 2.29 ± 0.02 mV and a RSD of 12.4 % were obtained. The analysis time of 17 s was measured, showing a fast recovery of the baseline signal without carry-over problems, which indicates the inexistence of reagents accumulation within the vortex chamber.

CONCLUSIONS

The versatility offered by the LTCC technology has been shown in the fabrication of a three-dimensional miniaturized vortex analyzer. Because of its configuration, a low-cost and miniaturized PIN photodiode can be directly attached to the mixing chamber as a detector, thus avoiding intensity signal losses, which are a common drawback in this type of systems. Moreover and as it has already been reported, this technology would facilitate the simple and rapid construction of even more complex devices that could integrate previous mixing stages given a certain application.

After the optimization of the hydrodynamic parameters of the flow chamber, and as a proof of the general applicability of the constructed system, cobalt determination by means of a CL reaction has been proposed. Although, the detection limit was slightly higher than those obtained with conventional systems, we gave a high priority to characteristics such as portability and simplicity, which a PIN photodiode can offer, instead of the higher sensitivity of photomultipliers. However, depending on the application, a photomultiplier could be easily adapted to the CL chamber as well.