PORTABLE INSTRUMENTATION PLATFORM FOR ECL-BASED SENSORS AND BIOSENSORS

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Abstract: A new portable instrumentation platform for electrochemiluminescence (ECL)-based disposable sensors and biosensors is described. The reader unit consists of a potentiostat and a photodiode as light-to-current converter integrated in the same instrument. To check the performance of the instrument as sensors platform, two transduction chemistries (luminol and tris(2,2'bipyridyl)ruthenium(II)) and two widely used analytes (hydrogen peroxide and triethylamine) were selected. Additionally, different working modes have been implemented in the instrument: chronoamperometry and cyclic voltammetry. The calibration functions obtained show linear dependences with dynamic ranges from 0.01 to 0.07 $mg \cdot l^{-1}$ for H_2O_2, 0.05 – 10.0 $mg \cdot l^{-1}$ for triethylamine with detection limits of 0.01 $mg \cdot l^{-1}$ for H_2O_2 and 0.03 $mg \cdot l^{-1}$ for triethylamine and a sensor-to-sensor reproducibility (relative standard deviation RSD) around 8.2 % and 3.1 %, respectively at the medium level of the range.

1 INTRODUCTION

Different techniques have been developed in order to measure the light resulting from electroluminescence reactions. Those processes are commonly controlled by a potentiostat or an amperometric unit (Gautron et al., 1980; Zheng et al., 2001) which establish potential differences or electric current flow between the electrodes of the cell, respectively. When there are no requirements regarding the size or the power supply needed for the design, a good solution to collect the weak photons generated by the ECL reaction is the use of a photomultiplier tube (PMT) (Zhou et al., 2004). These photodetectors need a very high voltage supply, which can reach thousands of volts. This fact, together with its usually bulky dimensions, makes the PMT a non convenient device for the development of portable instrumentation. Another technique for the detection of the luminescence that can be found in the literature is the use of CCD devices, such as CCD cameras and detectors (Momeni et al., 1999). These devices have the main disadvantage that for achieving good resolution they need working temperature of tens of Celsius degrees below 0º, as well as, the complexity of its use and processing.

The use of organic and solid-state photodiodes as photodetectors for the registration of the ECL radiation is also well stated in the literature (Hemmi et al., 1995; Hofmann et al., 2005). Photodiodes are devices of small dimensions with constant improvements of their features as optical detectors, thus being easily integrated in a measurement system. The sensitivity of these photodetectors depends on the inverse polarization applied to them, which can vary from few volts to hundreds of volts, in the case of avalanche photodiodes. Even in the shot-circuit configuration, this optoelectronic devices presents very good performace without requering support polarization circuitry. Therefore, photodiodes are an appropriate solution for the development of portable instrumentation to measure luminescence from ECL reactions, which is the goal of this work.

The portable instrumentation, here developed, tries to be a generic electronic platform for the

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bionanalytes determination using biosensors. This will be achieved with the inclusion, in a unique instrument, of the photodiode, the potentiostat and the reconfigurable electronics which adapts the analog processing to the ECL signal. Main advantages of our design lie on portability, low cost because of the use of a photodiode instead of a costly or bulky photomultiplier, and the use of a few microliters of sample analysis.

2 MATERIALS

The disposable cell consists of a screen printed three electrodes cell where we can found a round-shaped graphite working electrode, a graphite counter electrode and a silver pseudo-reference electrode. The screen-printed electrode was covered by a thick overlapping plastic layer with a 50-µl volume hole in the electrode area to place the sample. The sensing layer was cover by two types of solutions, one formed of luminol dissolved in 0.25 M NaCl and pH 9.0 phosphate buffer 0.2 M with H_2O_2 as analyte and another for luminophore $Ru(bpy)_3^{2+}$ with 0.25 M NaCl, pH 8.5 Tris buffer 0.2 M and triethylamine (TEA) as analyte.

3 PORTABLE INSTRUMENT DESCRIPTION

The system is composed by a photodiode with an operational amplifier integrated in the same chip, an electronic amplification stage, a potentiostat and a microcontroller that controls all elements (Figure 1). In this instrument, the PIC18F2553 of Michochip Technologies (USA) has been chosen.

3.1 Instrument Overview

In order to avoid the external illumination interferences, the electrode is placed into a dark little drawer attached to the instrument housing. The output current of the photodiode needs to be converted into voltage by an I/V amplifier. This converter can be included into the photodiode case, in the same chip, or can be external. Before amplification, the output voltage of the I/V converter was filtered in order to reduce the electrical grid interference (Figure 1). The filtered output is amplified by a Programmable Gain Amplifier (PGA). The gain of this amplifier can be configured by the microcontroller, and as consequence, the dynamic range can be adapted depending on the emission intensity of the ECL reaction. We used the PGA103 of Texas Instruments with 1, 10 and 100 gain factors that can be selected easily. Finally the results are storage in an EEPROM memory.

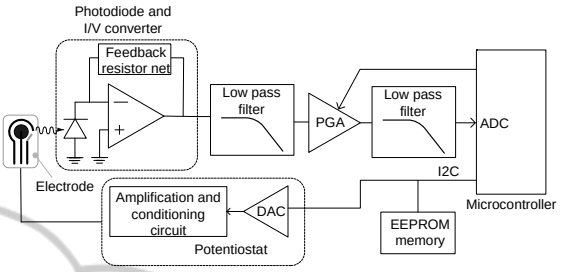

Figure 1: Block diagram of the luminometer.

The potentiostat is the electronics in charge of starting the ECL reaction by applying voltage pulses. Basically, it consists of a Digital-to-Analog-Converter (DAC) and analog circuitry to amplify and to shift the voltage output. In section 3.2 the pulse configuration will be described in detail. Finally, the presented luminometer in this work can be controlled via USB by a computer. An *ad hoc* software application has been developed for allowing the remote configuration and downloading of results.

3.2 Selection of the Photodiode

We studied two photodiodes without an integrated I/V converter: S1227-66BR and S1227-1010R (Hamamatsu, Japan), with an active area of 33 and 100 mm^2 respectively. To achieve a gain factor high enough, a T net resistor was used as the feedback converter resulting in an effective resistance of 5.2 GΩ. Others three photodiodes with a built-in I/V amplifier were tested: the S9269 and S9270 (Hamamatsu, Japan), with an active area of 33 and 100 mm^2 respectively; and the OPT301 (Texas Instruments, USA), with an area of 5.2 mm^2. The S9269 and the S9270 have an internal resistance of 1GΩ and the OPT301 internal resistance is 1MΩ. In the last one, an additional external T net resistance was added to reach a value of 5.2 GΩ. The output currents of the different photodiodes are plotted in the Figure 2.

As expected, the most sensitive photodiodes were the S9270 and the S1228-1010BR due to their bigger sensitive area. We selected the most sensitive photodiode, the S9270. In addition, this device includes a built-in I/V converter, showing better interference immunity.

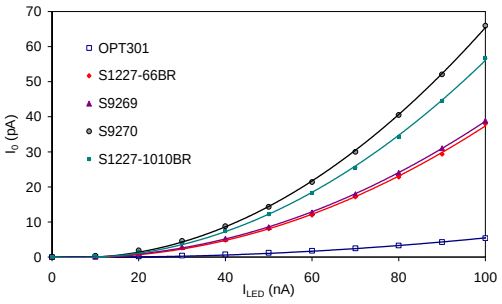

Figure 2: Output current of the studied photodiodes.

3.3 Potentiostat

Potentiostat of this instrument is based on a previous design (Martinez-Olmos et al., 2009) with some modifications. A reference voltage and bipolar power supply in order to produce negative and bipolar pulses were added. Moreover, an additional resistor has been included between the complementary electrode (CE) and reference electrode (RE) to avoid saturation of CE operational amplifier when the solution is not deposited on the screen-printed electrode. In this work, we have used mono-polar pulses in chronoamperometric form for $Ru(bpy)_3^{2+}$ and TEA ECL determination, and a cyclic voltammetry mode for luminol and H_2O_2. In the Figure 3, some voltage waveforms of the Working-Electrode (WE), Reference-Electrode and the Complementary-Electrode are plotted.

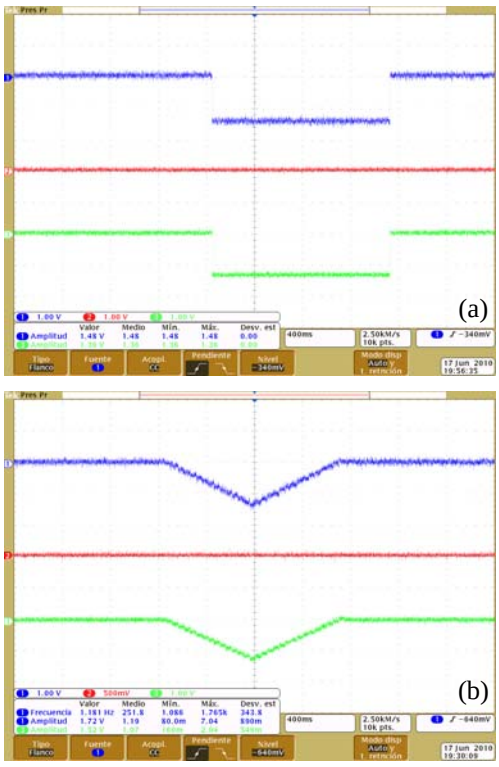

Figure 3: Output voltages of the potentiostat: RE in blue, WE in red, and CE in green. (a) Monopolar pulse. (b) Cyclic voltammetry.

The instrument can configure the applied pulses by varying the maximum voltage value (positive and negative), the rise and fall times.

4 EXPERIMENTAL RESULTS

4.1 Measurement Conditions

The instrumentation is equipped with the electrochemical tools for the different types of ECL analytical signals. The intensity of the pulse of the collected light shows a direct relationship with peroxide or triethylamine concentration. However, it is necessary to study several pulses in time of the same concentration to see the profile of the obtained peaks to discriminate and eliminate the intensity of the blank signals or by the necessity to obtain reproducible emission peaks. In the case of ruthenium complex system, the signal is obtained working at chronoamperometric mode generating 10 pulses. The measurement conditions were: a) applied potential (1.3 V); b) time between pulses, being 30 s for better sensitivity; c) pulse time with 1.5 s. The analytical signal was the average of the last four pulses due to blank signal generates overlapping intensity peaks in the first pulses.

For the luminol system, the ECL signal is more reproducible if we use a fast cyclic voltammetry. For that purpose, we generate 4 pulses configured as follows: a) voltage range: from 0 to 1 V; b) time between pulses: 30 s; c) rise time: 3s; d) fall time: 3s. The analytical signal was the different pulse maxima for each peroxide concentration. The best analytical parameter corresponds to the maximum of the third pulse. The sample volume in the screen-printed electrode was studied, selecting 50 µl as optimum volume.

4.2 Analytical Parameters

For the evaluation of the presented luminometer, two calibrations were performed with the two chemistries involved in this work. For H_2O_2 determination we obtain a linear response from 0.01 to 0.07 $mg \cdot l^{-1}$ using 178 $mg \cdot l^{-1}$ of luminol solution in all cases (Figure 4). In the case of TEA determination, we obtain a linear calibration from 0.05 to 10 $mg \cdot l^{-1}$ using a 374 $mg \cdot l^{-1}$ concentration of the luminophore $Ru(bpy)_3^{2+}$(Figure 5).

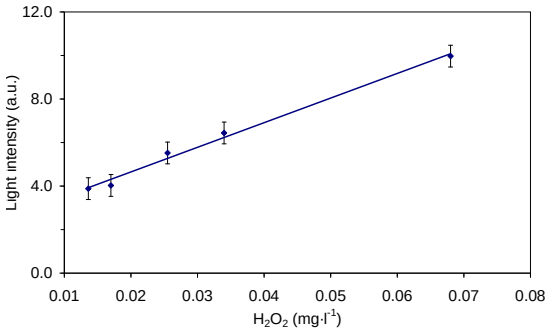

Figure 4: H_2O_2 calibration curve.

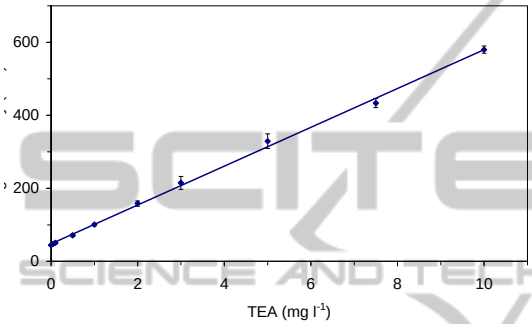

Figure 5: TEA calibration curve.

As we can see in Table 1, the analytical parameters show a good linearity in both cases

Table 1: Analytical characteristics.

Parameter	TEA	H_2O_2
Linear range ($mg \cdot l^{-1}$)	0.05 – 10.0	0.01 – 0.07
Intercept (a.u.)	47.2	2.4
Slope ($l \cdot a.u. \cdot mg^{-1}$)	57.3	113.1
r^2	0.999	0.992
Detection limit ($mg \cdot l^{-1}$)	0.03	0.01
RSD blank (%)	1.4 %	4.2 %
RSD sample* (%)	3.1 %	8.2 %

*In the middle of the linear range

5 CONCLUSIONS

A novel portable instrumentation based on a photodiode and a simple potentiostat is presented, suitable for ECL measurements. In the same instrument, is possible to work in chronoamperometric and cyclic voltammetry modes. This paper represents a first step in this research and further work will be done to test it for biological fluids such as uric acid and cholesterol.

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