Smartphone Controlled Platform for Point-Of-Care Diagnosis of Infectious Diseases

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***Abstract*—In this work, we present the development of a point-of-care platform for the serologic diagnosis of infectious diseases. The complete system consists of magnetic particles with immobilized antigens, disposable electrochemical cells, hardware and software. The main purpose of this paper is to present the last two components. The platform is powered by a rechargeable battery and can be controlled using mobile devices, allowing point-of-care diagnosis of diseases. The platform was successfully tested for the diagnosis of foot-and-mouth disease, human and bovine brucellosis, and Chagas disease.**

***Keywords*—Point-Of-Care Diagnosis; Electrochemical Biosensor; Portable platform; Android app**

I. Introduction

Biosensors are compact analytical devices which employ a biological element in order to detect a specific substance, i.e. the analyte. After the analyte has been detected by the biological recognition element, a signal of some sort is produced which is then converted into an electrical signal with a transducer. In the case of electrochemical biosensors, the chemical signal generated by the interaction between the biological recognition element and the analyte is then converted into an electrical current via an electrochemical reaction at an electrode surface [1]. A particular case is the amperometric biosensors, where the electrochemical measurement is carried out by a potentiostat, which is an instrument that applies a voltage to a working electrode with respect to a reference electrode and measures the current flowing through that working electrode. Voltage is controlled by using an auxiliary or counter electrode.

Biosensors aimed for the detection of the presence of specific antibodies or antigens are particularly important for the diagnosis of diseases in remote environments, where carrying out immunoassays such as ELISA (Enzyme-Linked Immunosorbent Assay) is not an option. In addition to the possibility to carry out diagnosis with minimum training and equipment, point-of-care devices have additional advantages. For instance, fast, non-expensive and multiple assays can be performed with point-of-care portable devices, which can be of help in the occurrence of an epidemic spread.

This article presents the development of a compact and wireless amperometric biosensors platform, with support for Android and other operating systems that allows to diagnose infectious diseases as foot-and-mouth disease, human and bovine brucellosis, and Chagas disease. Emphasis is placed on the development of the hardware and its associated software, mentioning also the detection principle and other system components. Results obtained for those diagnosis are presented.

II. Development

A. Detection Principle and Electrochemical Cells

1) Detection Principle: Glicoconjugates and recombinant proteins were developed and covalently immobilized onto magnetic particles. Antigen-coated magnetic particles were incubated with sera and then with horseradish peroxidase (HRP) conjugated secondary antibodies [2]. The particles were magnetically collected and placed onto the surface of an electrode, where the enzymatic activity of HRP was amperometrically detected. An schematic representation of this process appears in Fig. 1. The immobilization of antigens onto magnetic microparticles allowed to reduce the incubation times and to use higher loads of antigens. Details are presented in previous articles [3] [4] [5].

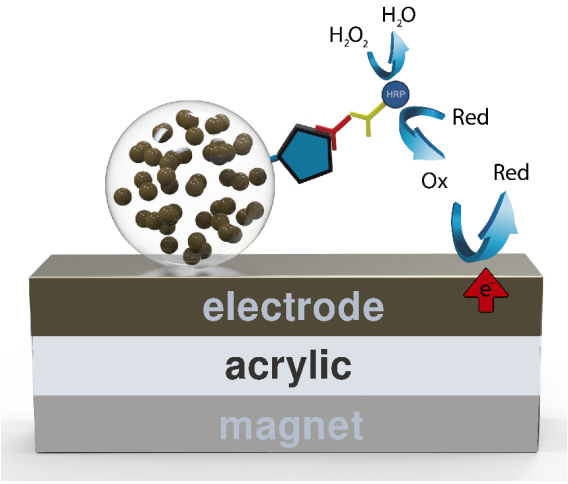

Fig. 1: Schematic representation of the detection principle.

2) Electrochemical Cells: Disposable acrylic cartridges with eight electrochemical cells were designed and manufactured with dimensions fitting an 8-channel micropipette.

Fig. 2 shows one of these cartridges. Each cell contains two carbon electrodes, the working and counter electrodes, and one Ag|AgCl reference electrode. The electrodes were screen printed onto 0.5 mm acrylic substrates and the central working electrodes were designed to be aligned with neodymium magnets so as to concentrate the magnetic particles. Each electrochemical cell has a volume of 40 μl. Commercial carbon ink (Dupont BQ242), Ag ink (Dupont 5025) and Ag/AgCl ink (Dupont 5870) were employed. Others details of materials and manufacturing process are presented in [4].

Fig. 2: Electrochemical cells cartridge.

B. Hardware Development

An 8-channel portable potentiostat was developed to allow point-of-care potentiostatic measurements based on [6]. The device can be controlled via Bluetooth using a set of basic commands, that allows to implement several types of cyclic voltametry and others electrochemical techniques. The instrument can control the working electrode voltage in the -2.5 V to +2.5 V range, allowing its use for biosensors here presented and others. According to project requirements, the valid working electrode current is in the -10 μA and 10 μA range, but the design is easy to adapt for others ranges. The device is powered by a rechargeable battery and allows to carry out measurements during charge. Also a version controlled by USB was developed, but is not presented here. For electronic protection and error-free cartridges insertion, different case models were designed and manufactured. Fig. 3 shows a prototype printed in ABS. A system block diagram is shown in Fig. 4 and its components and selection criteria are described below.

1) Power: A single-cell Li-ion battery (3.7 V) with 2600 mAh was used, obtaining an autonomy of more than 9 hours of continuous measurement. Battery charging control and voltage stepping up to 5 V, necessary for other circuit components, was implemented using the integrated standalone switching charger ACT2801, which includes an internal boost, protection circuits for high and low voltage, protection against over temperature and short circuit. Due to the bipolar voltage range required, a dual output DC/DC converter module NMA0509SC was used to power analog circuitry. This module provides -9 V and +9 V outputs from 5 V provided by the ACT2801.

2) Analog Circuitry: Electrodes control and signal conditioning for a single electrochemical cell were carried out using two different types of precision operational amplifier (OPAM). A low offset OPAM OP07 was used to control the counter electrode. To avoid loading reference electrode an LT1056 JFET input OPAM in buffer configuration was selected, providing an input impedance of (10^{12} Ohms). The current to voltage conversion, carried out in the working electrode circuitry, was also implemented using an LT1056 OPAM in transimpedance amplifier (TIA) configuration. This OPAM introduces a very low current error, typically in the range of 10 pA, and guarantied to be under 0.34 nA for the full operation range. External offset correction was not necessary in any of the cases.

3) Multiplexing: The eight channels were multiplexed by using two CMOS analog multiplexers DG408 for counter electrode voltage and working electrode current. Reference electrode voltage was not multiplexed due to its floating condition in unused channels and its very high input impedance. Also a circuit with two cheaper analog multiplexer CD4051 was successfully tested.

4) Control and Analog to Digital Conversion: The potentiostat was controlled by a microcontroller connected to a PC or mobile device via Bluetooth using an HC-06 module. This wireless module provides a serial port profile over Bluetooth V2.0 and was directly connected to the microcontroller to receive serial commands and send acknowledges and data. Checksum error checking was added by firmware. These commands, described in a complete firmware user manual, allows to turn on analog circuitry, channel selection, to configure potential step or voltammetry parameters and limits, start and interrupt assays and others. Command format is as follow:

```
#F          <- Turn on analog circuitry
E00,65,46   <- Device acknowledge
#A1005      <- Set reference electrode voltage
E00,66,45   <- Device acknowledge
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Device acknowledge includes error information and received and transmitted checksum. The microcontroller has a 10 bits A/D converter, used to measure the working electrode current and the reference voltage. Counter electrode voltage was controlled with a 12 bits PWM output.

Fig. 3: Device prototype printed in ABS.

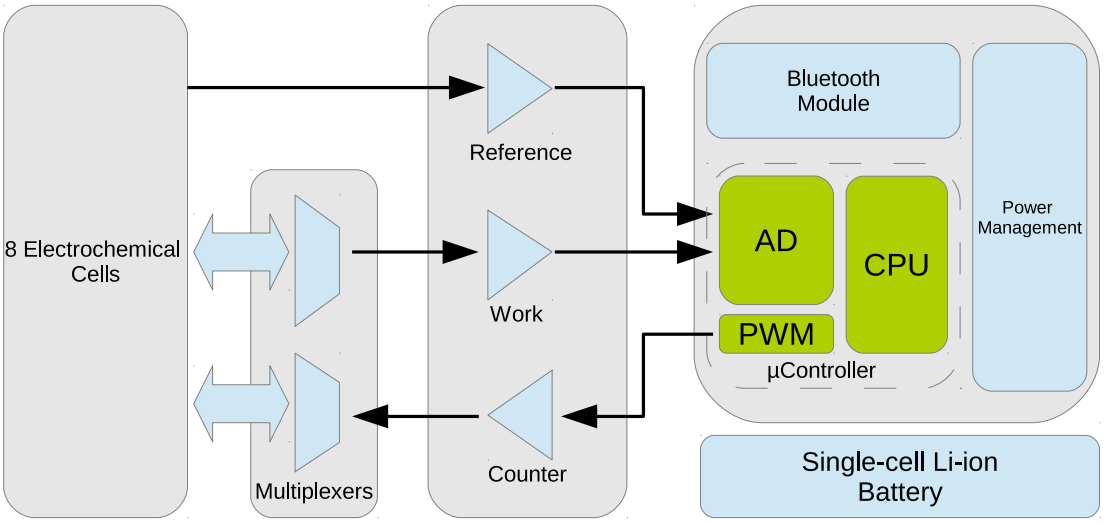

Fig. 4: Device block diagram.

C. Software Development

1) Test Software: A test software for GNU/Linux distributions was developed. It allowed to carry out amperometric records for potential step and cyclic voltammetry. In both cases filtering and an optional and configurable preconditioning step was added. User can select measurement parameters and visualize its evolution in real time. Also, the software can display simultaneously up to eight recorded assays.

Back end was developed in C++ language, and a full set of classes was written for an object-oriented device management. It manages the equipment connection, sends low level commands, process acknowledges and data, reads calibration constants and others. Perl scripting language and Gtk2 were used for front end development and communicates with the back end through pipes. This software was useful for general testing, electrodes characterization and particles validation.

2) Android App: An Android application was also developed, in Java language, with complete classes and associated documentation, compatible with API 10 (Android 2.3.3) or greater. The app allowed to carry out potential step measurements, setting assay options and detection threshold presets for several diseases. Furthermore, an Android library was written to provide specific methods related to the device, including a specific class for device management that implements the singleton design pattern, allowing to ensure only one instance of the class across the application. The class also allows to register handler objects to receive and process its status connection messages and others. This implementation simplified the software maintenance and future updates, and it lets making different applications that manage this device.

When the user starts the application, it searches and connects to a nearby device through an RFCOMM socket, by using a secure connection. Once connected, the user triggers an assay and the app sends commands to the device so as to start a potential step measurement, applying configured voltage in the electrochemical cell and reading work current. When the measurement has finished, the app calculates the average value of the work current and indicates if the detection is negative or positive, depending on the selected disease. Additionally, it saves a record for the entire measurement. For the average calculation only a certain percentage of last samples is considered, i.e. in stationary state. Fig 5 shows a screen with the progress of a measurement.

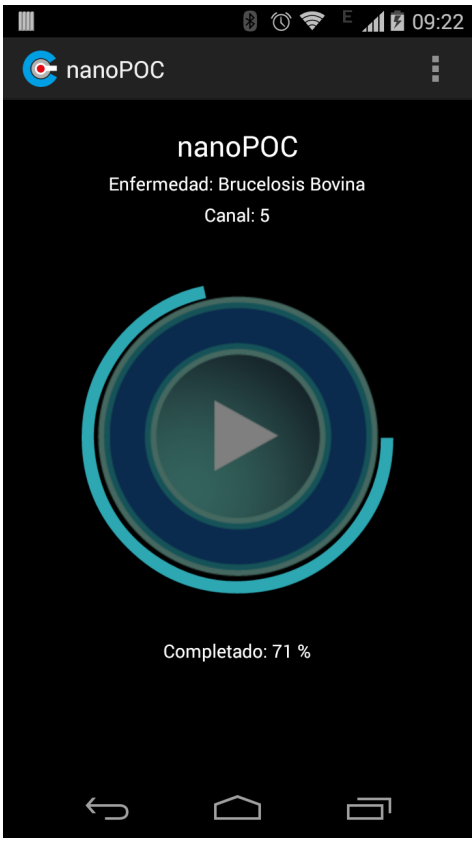

Fig. 5: Potential step measurement and diagnose with the Android application

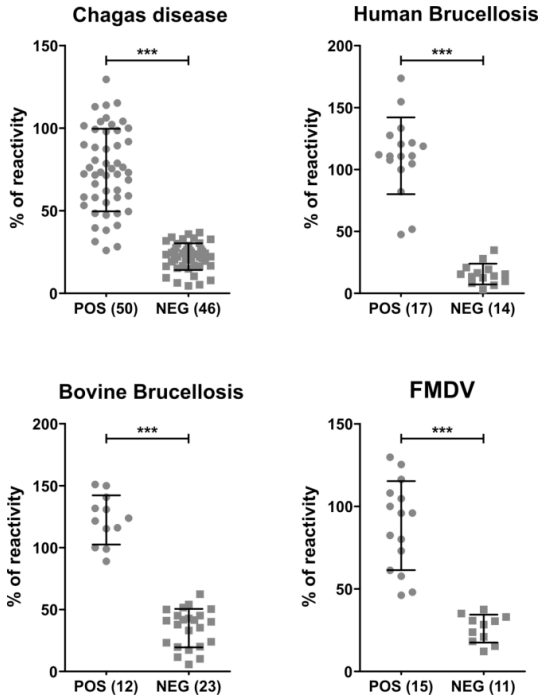

Fig. 6: Dotplots of serum samples obtained from healthy individuals and patients of different clinical groups. The mean and standard deviation for each group are indicated.

III. Results

Antigenic proteins were immobilized onto magnetic particles. A set of different antigens were used to diagnose four different diseases. These antigens were validated by other methods as ELISA and particle-based immunoassay coupled to fluorimetric detection [5]. For the serologic tests, coated particles were incubated with different sera. Then the magnetic particles were incubated with anti-Ig HRP conjugate. Finally, 5 μl of the particles dispersion was transferred to the electrochemical cell. The reference electrode potential was set at -0.23 V and the resulting current was recorded for 20 s. The average value of the limit current was used as measure of the reactivity [5]. The measured current was contrasted with a commercial potensiostat PAR 273.

The average value observed can be used to differentiate positive sera from negative sera. After following the procedure described above, in positive sera (i.e. one containing antibodies to a certain infectious disease) the peroxidase enzymatic activity from the antigen-antibody-(HRP-labeled anti-antibody) complexes is detected as a current significantly higher than those measured with negative sera. This is shown in Figure 6.

The current values obtained for negative sera were typically in the order of 0.2-0.4 μA, while a higher dispersion of current values was observed with positive sera, typically in the range of 0.8-2 μA. The difference in current values is high enough as to permit the discrimination of infected and non-infected specimens from this electrochemical enzyme-linked immunoassay.

The entire measurement process and discrimination was carried out in a compact and portable device in conjunction with an easy to use Android application using detection threshold presets.

Four different infectious diseases were tested: foot-and-mouth disease, Chagas disease, human and bovine Brucellosis.

IV. Conclusions

As the final result of the work of a multidisciplinary group, we present the point-of-care platform development for the serologic diagnosis of several diseases. The development of the platform has involved the convergence of biotechnology, electrochemistry, electronics and computer programming. The principle of the diagnostic test is an electrochemical enzyme-linked immunoassay.

The detection system is small and portable and may control assays and view results through popular devices as smartphones, tablets or similar, allowing the diagnosis in areas with poor infrastructure. The entire platform is versatile and easily adaptable for the development of other amperometric biosensors.

The diagnostic platform was successfully tested for four different infectious diseases: foot-and-mouth disease, human and bovine Brucellosis, and Chagas disease.

V. Acknowledgments

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