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A compact hybrid-multiplexed potentiostat for real-time electrochemical biosensing applications

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ABSTRACT

The architecture and design of a compact, multichannel, hybrid-multiplexed potentiostat for performing electrochemical measurements on continuously-biased electrode arrays is presented. The proposed architecture utilises a combination of sequential and parallel measurements, to enable high performance whilst keeping the system low-cost and compact. The accuracy of the signal readout is maintained by following a special multiplexing approach, which ensures the continuous biasing of all the working electrodes of an array. After sampling the results, a digital calibration technique factors out errors from component inaccuracies. A prototype printed circuit board (PCB) was designed and built using off-the-shelf components for the real-time measurement of the amperometric signal of 48 electrodes. The operation and performance of the PCB was evaluated and characterised through a wide range of testing conditions, where it exhibited high linearity ($R^2 > 0.999$) and a resolution of 400 pA. The effectiveness of the proposed multiplexing scheme is demonstrated through electrochemical tests using KCl and $[Fe(CN)_6]^{3-}$ in KCl solutions. The applicability of the prototype multichannel potentiostat is also demonstrated using real biosensors, which were applied to the detection of IgA antibodies.

1. Introduction

The need for easy-to-use, rapid and accurate detection of target analytes in applications such as for food safety, environmental monitoring and clinical diagnostics has driven the development of a range of biosensors. The family of electrochemical biosensors has garnered particular attention due to their sensitivity, miniaturisability, compatibility with microsystem technology and cost effectiveness (Wang, 2006). Today's electrochemical biosensors with micrometer sized electrodes are usually designed in array forms incorporating multiple sensing sites on a single structure, in order to perform parallel measurements that improve efficiency and cross-validate the results for increased confidence.

The proposed architecture targets the growing application area of electrochemical sensor arrays, where a single bias voltage is required for all the elements of the array. An example of this application is a multiplexed array system based on a sandwich format which is modified with different capture elements (i.e. antibodies or DNA sequences). The capture elements interact with the corresponding target molecule in the sample, and detection is carried out using a mixture of enzyme-labeled detection probes. An enzymatic substrate is then added and by reaction with the enzyme label an electroactive product is generated and detected. The combination of capture and detecting probes gives the system the required selectivity in analyte detection and therefore there is no need to label the different detection probes with different enzymes. From the detection point of view, this simplifies and facilitates the operation of the system since the application of only one potential is required for detection.

From the point of view of a readout system, electrochemical sensing involves translating the progress of a chemical reaction, taking place on the surface of electrodes, to an electrical signal. For most experimental processes, this signal is produced under controlled bias conditions. In the case of a potentiostatic readout for a three-electrode electrochemical "cell" (counter, reference, working), a controlling bias voltage between the reference and working electrodes will induce an electrical current, proportional to the concentration of the electroactive species on the surface of the working electrode.

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Owing to the fact that electrode arrays can be exploited for many applications, the required signal dynamic range and resolution can vary largely, depending on the sensor electrode size, the electrochemical species, the experimental process, etc. General purpose laboratory based potentiostats are designed to cover an extensive range of input and biasing signals, but are usually bulky and relatively expensive instruments.

To address the possibility of having a portable potentiostat for *in situ* use, various small sized systems have been reported for controlling and reading output signals e.g. amperometric current, from electrochemical sensors. Many of these are designed to be application specific, tuned for their limited range of operation, offering optimised readout capabilities. This is especially the case for integrated circuit (IC) readout designs, which are integrated together with the sensing elements (Zhang et al., 2010; Levine et al., 2009; Zhang et al., 2005; Duwe and Chen, 2011), compacting the system on a single chip performing a specific task. Other examples of presented readout designs include stand-alone measuring systems, either in IC (Hwang and Sonkusale, 2010; Ng and Yusoff, 2010) or discrete forms (Pittet et al., 2008; Rowe et al., 2011), connected externally to the electrochemical elements.

This paper describes a hybrid sequential-parallel architecture to perform real-time multichannel potentiostatic measurements, that may be applied in either stand-alone discrete or IC, or single chip designs, as the implementations mentioned above. Multiplexing and circuit reusability concepts, such as those found in various IC implementations, e.g. Zhang et al. (2005) and Ayers et al. (2007) become important for large array applications with hundreds or thousands of sensing elements. These concepts are used and are extended by acting on groups of sensors, making the system compact and cost effective, while maintaining a targeted sampling performance.

Following a hybrid multiplexing technique, the readout system gains the scalability to be used with a variety of array sizes, trading off between the benefits and disadvantages of parallelisation and multiplexing. On this proposed architecture, it is ensured that the multiplexing scheme does not interfere with the correct and continuous biasing of all the electrodes of a sensor array, thus letting it virtually respond as its fully parallelised counterparts.

Additional improvement of the system readout accuracy is achieved using a simple and effective calibration process. It involves two independent variables, namely the bias voltage and the sensor load, to minimise the gain and offset errors both in electrode biasing and in signal conversion.

The feasibility of the proposed approach is demonstrated using a discrete prototype implementation of a 48-channel design, targeting multiplexed biosensing applications. Our prototype was implemented using discrete components that has interesting advantages, such as the low cost associated with its fabrication compared to the cost of an IC, when producing small quantities. Today's commercial discrete components such as operational amplifiers (op-amps), digital-to-analog converters (DAC) and analog-to-digital converters (ADC) offer excellent features, such as high gain, very low offset voltages and bias currents, low voltage and current noise, high linearity, etc. The performance is analysed using extensive characterisation and actual biosensor measurements.

2. System design

The block diagram of the potentiostat system is shown in Fig. 1(a). The configuration of the electrode array uses a common counter and a common reference electrode with each working electrode connected separately to the system. The design of a sample electrode array that was used during evaluation of the system, is organised in 3 rows of 12 elements each, where a separate microfluidic channel is placed over every row. Two rows of connection pads serve as the electrical interface to the readout.

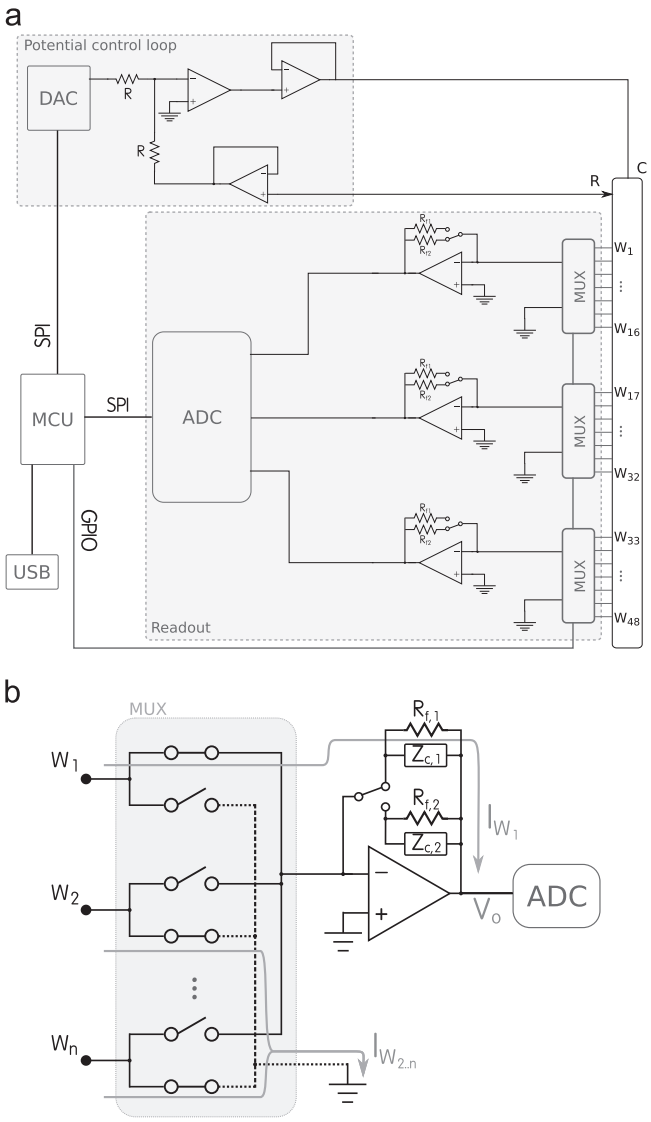

Fig. 1. Block diagram of (a) multichannel potentiostat system using hybrid sequential-parallel multiplexing and (b) current to voltage converter connected to the working electrodes through SPDT multiplexing.

2.1. Overview of subsystems

The basis of the implemented system is formed by a potential control loop and a current-to-voltage (I/V) converter, which constitutes the core of potentiostats for three-electrode cells (Bard and Faulkner, 2001). Building on that, multi-channel readout capabilities are added using analog multiplexing units with single-pole, double-throw (SPDT) switch characteristics. A programmable gain control is provided using switched feedback resistors on I/V converters. A DAC unit produces the waveform of the potential difference applied between the working electrodes and the reference electrode, and an ADC unit gives a readout of the measurement. Lastly, a microcontroller (μC) unit controls the behaviour of all subcomponents.

2.2. Digital control and communication

The μC unit is responsible for the control of the individual readout components, as well as for the communication of the