MINIATURIZED ELECTROCHEMICAL SENSING SYSTEMS FOR IN VITRO AND IN VIVO BIOMEDICAL APPLICATIONS

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Abstract: Development of miniaturized electrochemical sensing systems for *in vitro* and *in vivo* biomedical applications is discussed. The systems are based on high sensitivity potentiostatic instrumentation, which is suitable for chemical and biochemical sensors. The *in vitro* application is an 8 channel hand-held PC-controlled system with user-friendly interface. This is capable of implementing different electrochemical potentiodynamic techniques. The *in vivo* applications are realized using two approaches: a small sized PCB with commercially available ICs, and a specially developed on-chip system. The performance of the systems is validated through electrochemical characterization of a microarray sensor.

1 INTRODUCTION

Electrochemical sensors play an important role in biomedical applications, including clinical disease diagnostics (Peng et al., 2008), (Seo et al., 2008), (Ndamanisha and Guo, 2008), (Wang and Ha, 2007), (McLaughlin et al., 2002), drug testing (Abbaspour and Mirzajani, 2007), microbiological pollutant determination (Morales et al., 2007), and detecting and quantifying DNA and proteins (Shiddiky et al., 2008). These sensors give rapid and sensitive measurements, and can detect solid, liquid or gaseous analytes. If we consider the disease diagnostic applications, this diversity can be illustrated with the following examples. Nitric oxide sensors (Peng et al., 2008) have found application in the monitoring of neural conditions such as stroke, heart attack and epilepsy, through the link between elevated nitric oxide levels and these diseases. Various glucose sensors are available for *in vitro* or continuous *in vivo* monitoring of blood, urine and saliva to diagnose diabetes (Seo et al., 2008). There are oxygen sensors (McLaughlin et al., 2002) for monitoring blood oxygen levels in pathways to different organs. Recently sensors for uric acid have been developed which indicate the presence of gout and Lesch-Nyhan diseases (Ndamanisha and Guo, 2008). Sensors, which measure pH levels within the gastrointestinal tract, can detect the presence of intestinal diseases like gastroesophageal reflux disease (GERD) (Wang and Ha, 2007). Also, electrochemical sensors for *in vivo* drug monitoring are being explored to monitor drugs administered to treat different diseases e.g. anti-inflammatory drugs for the treatment of intestinal diseases (Abbaspour and Mirzajani, 2007).

In the area of clinical diagnosis, there is a growing emphasis on early–disease detection. Through this capability, serious diseases such as heart disease and cancer can be more successfully treated. To achieve this challenging goal, developments in three key areas are essential. Firstly more sensitive and more diverse sensors should be created. Sensors with a lower limit of detection, for example, would be crucial for early diagnostics (before full-scale disease symptoms develop), and an expansion of the number of available sensors for analytes or biomarkers linked to the disease is important to improve accuracy of disease diagnosis. Secondly these sensors need to move from their current use in highly specialized clinical laboratories to areas of point-of-care (POC) such as at the patient's bedside. To enable this move, developmental work in the area of instrumentation is necessary, which will provide easy access to these methodologies for the patient and physician, e.g. user-friendly portable systems or *in vivo* systems such as implantable devices that will allow continuous supervision of the patient with access close to the source of disease. This ability would allow for more accurate monitoring of the disease and provide early disease diagnosis.

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There have been notable advances in suitable sensors for early stage disease detection (Peng et al., 2008), (Seo et al., 2008), (Ndamanisha and Guo, 2008), (Wang and Ha, 2007), (McLaughlin et al., 2002), (Abbaspour and Mirzajani, 2007), (Morales et al., 2007), (Shiddiky et al., 2008), However, there remains a need to address the developments in appropriate systems to allow progression of these sensors outside of the lab to POC and to *in vivo* applications. Currently, sensing instrumentation is mostly bench-top based equipment, which is large and not adapted to work outside of the lab environment. Therefore, the development of miniaturized sensing instrumentation is essential.

In this study, we discuss approaches to develop miniaturized sensing systems for *in vitro* and *in vivo* applications based on a potentiostatic solution, which is commonly used with different electrochemical sensors, and different sensing methods (amperometry, voltammetry, impedometry). Although, fundamentally the systems for *in vitro* and *in vivo* applications are built on similar circuitry, they vary significantly due to different requirements in size, power, sensitivity, biocompatibility and functionality. For the *in vitro* applications, we have developed a portable, hand-held, miniaturized, multichannel potentiostatic system, which is optimized for operation with a sensor array on chip. The system has the appropriate accuracy and sensitivity required by biomedical applications, and would be suited for use in a hospital or at a GP's office. For the *in vivo* applications, we have developed two variants of the system that are both miniaturized and can operate over extended periods on low power, again with the required accuracy and sensitivity. The first solution is based on commercially available low-power, low-noise, micro-sized small outline integrated circuit (SOIC) components and chips. The second device presents a specialized on-chip system that is developed in-house. The *in vivo* systems have similar performance specification but the PCB-based system costs less. Both of these systems are optimized for low-power operation, making them applicable for implantable devices, where an appliance is implanted and remains long term in the body, operating on a continued basis.

2 SENSING SYSTEM STRUCTURE

In general, for both system solutions, the electrochemical potentiostatic sensing system consists of an electrochemical cell incorporating the sensor or sensor array, and two main analog electronic units, the potentiostat and a transimpedance amplifier, and a microcontroller unit. These analog units connect to the microcontroller unit that controls the measurement process, and provides data acquisition and connectivity to the personal computer (PC) as shown in figure 1.

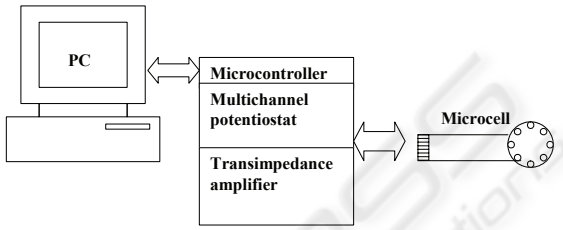

Figure 1: Block diagram of electrochemical sensing system for *in vitro* and *in vivo* applications.

The electrochemical cell is usually a three electrode structure comprising a counter electrode (C), reference electrode (R) and working electrode (W), which is immersed into or covered by sample solutions to be analyzed. Electrochemical or biochemical reactions in the cell are detected with electronics, in particular with a transimpedance amplifier. The reactions are dependent on the W potential, therefore, a stable potential at the W should be provided. The W potential stability is secured by the R, which supplies a reference potential independent of the environment, and the potentiostatic unit, which maintains this W potential, with respect to the R, to be equal to a stimulation signal generated by the microcontroller unit. The shape of the stimulation signal depends on the sensing methodologies (i.e. DC for amperometry, staircase for voltametry and so on). A simplified schematic of the electrochemical potentiostatic sensing system (without microcontroller unit) is shown in figure 2

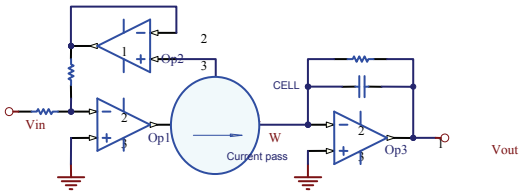

Figure 2: Simplified schematic of the potentiostatic sensing system.

Implementation of the electrochemical sensing systems for *in vitro* and *in vivo* biomedical applications is each governed by a different set of requirements. For *in vitro* real- time systems, these

requirements are high sensitivity, rapid rate of measurement and corresponding signal processing, accurate data interpretation, a multi-functional instrument and simplicity of its operation by the end user. For *in vivo* systems the main challenging requirement besides sensitivity is the size of device, which must adhere in some cases to millimeter size scale, and continuous operation over extended periods of time (i.e. up to one year, or longer for some implantable devices). Both of these restrictions impose a serious limitation on the power and size of the electronic components which can be used for the system design.

2.1 In vitro System

In keeping with these requirements for the *in vitro* applications, a multi-channel potentiostatic system, optimized for operation with an 8 working electrode on-chip sensing system, has been developed. The instrument is based on low-noise, high precision, pico-ampere input current operational amplifiers AD8602 (leakage current of 200 fA) and Analog Devices microconverter, ADuC812, which enables generation of the stimulation signal (via the in-built DAC), feeds sensing data into the system (via the in-built 8 channel ADC), and provides preliminary signal processing and communication with the PC (via an RS232 communications port). Running on the PC is dedicated software with user-friendly graphical interface, which provides final signal processing and data interpretation. The prototype of the system, with overall size 170x110x40 mm and weight 250 g, is shown in figure 3. The associated user-friendly interface software (developed in LabVIEW, figure 4) allows the user to specify the type of voltammetric technique and its setting (start and finish potential, scan rate for each channel), and to visualize obtained experimental data.

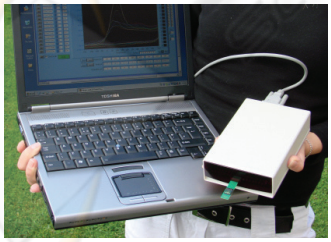

Figure 3: The multi-channel potentiostatic system suitable for in vitro applications.

In order to validate and characterize the system, a microelectrode array was fabricated using CMOS techniques. It consisted of an array of gold working electrodes surrounded by a counter electrode. The final packaged die connects to a PCB dipstick which allows for easy connection and testing of the sensor chip. The solutions with analyte can be placed directly on the sensor, or the dipstick can be integrated into an associated fluidic system, or it can be simply immersed into a container with the sample to be analysed. The performance of the developed system can be estimated by example of cyclic voltammetry applied to the 8 Ws in ferrocene carboxylic acid solution in simultaneous mode. The measurements that were carried out over different scan rates are shown in figure 5a, and the measurements that were carried out over different start and finish potentials are shown in figure 5b. These experiments demonstrate that the system's current sensitivity is greater than 1nA, with a noise level less than 50pA p-p.

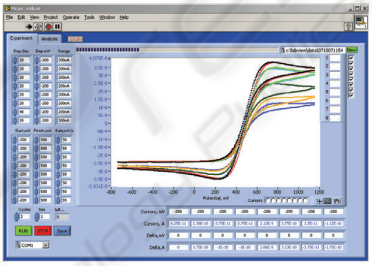

Figure 4: PC Interface for the multi-channel potentiostatic system.

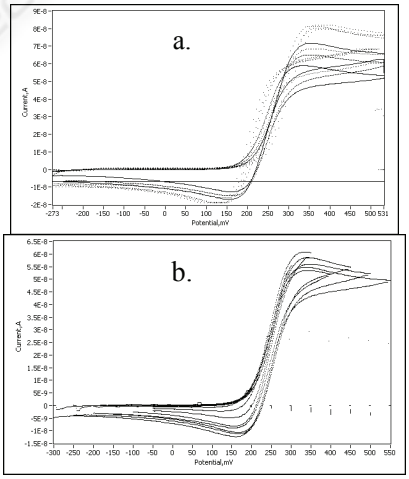

Figure 5: Cyclic voltammograms recorded simultaneously with the instrument for oxidation of Ferrocene carboxylic acid at an eight microelectrode array: (a) – with different scan rates set for each channel (from 50mV/s to 400mV/s), (b) – with different starting and finishing potentials set for each channel.

2.2 In vivo System

In keeping with requirements for the *in vivo* applications, a potentiostatic system based on a PIC microcontroller, has been developed. This microcontroller family contains a number of microcontroller models including those which are available in a smaller size, consumes less power and has more RAM memory than the ADuC, making them more suitable for this application. The PIC18F2520 has been chosen which features 6x6mm, 1256 bytes RAM and current consumption of 2.6mA during normal operation. This is in contrast to the ADuC812 features of 8x8mm, 256 bytes RAM and 6.2mA during normal operation. In the programming of the microcontroller, close attention has been given to reduce the power consumed during device operation by using appropriate electronic components with power switch on/off mode and inbuilt microcontroller possibilities. There are two realizations of this system. The first one is a PCB-based device with separate commercially-available low power and small size ICs forming a one-channel potentiostatic system in accordance with figure 1. The size of the system prototype is 12x24 mm, which is suitable for a variety of *in vivo* applications. To validate the performance of the system, cyclic voltammetry was carried out in 0.5M sulfuric acid solution at a gold macroelectrode, figure 6.

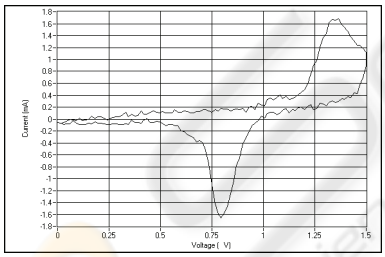

Figure 6: Cyclic voltammogram for *in vivo* PCB-based system at the macro gold electrode in 0.5M sulphuric acid solution

The second *in vivo* system is a specially developed on-chip system, which is a realization of the PCB *in vivo* device in an integrated single die. The implementation of the developed system on a silicon substrate was made by using a C5P0 CMOS 5 micron silicon breadboard. There is a digital core of 1248 gates and an analog oriented section containing 32 operational amplifier blocks, 1500 units of poly-poly capacitor, approximately 2MΩ of resistance in 40 sticks of 6 segments each, and a range of more specialized components. The schematic of the breadboard operational amplifier unit and final chip appearance, are shown in figures 7 and 8 correspondingly. The performance of the on-chip system can be seen from the example of cyclic voltammetry of a macro gold W in 0.5M sulphuric acid solution, figure 9, which substantiates its operability. At present both of the *in vivo* systems have similar performance specification but due to the initial small batch size, the PCB-based system costs less. The potential advantages of the on-chip system over PCB based device (a smaller size, a reduction in noise and power, a decrease of leakage current, which leads to an improvement of the system performance) will be realized when complete integration of sensor and electronics on one chip is developed.

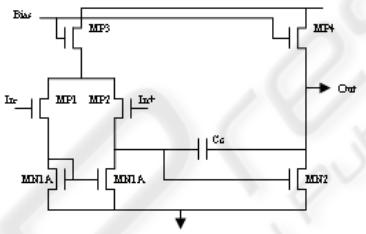

Figure 7: P-input CMOS OPAMP.

Figure 8: Fabricated on-chip potentiostat die, 8x10mm.

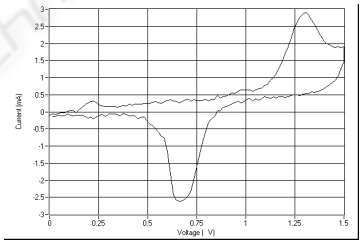

Figure 9: Cyclic voltammogram for *in vivo* on-chip system at the macro gold electrode in 0.5M sulphuric acid solution

3 CONCLUSIONS

Miniaturized sensing systems for *in vitro* and *in vivo* biomedical applications suitable for point-of-care applications have been presented that will facilitate early-stage disease diagnosis. The systems are based on potentiostatic instrumentation, which is commonly employed with different electrochemical sensors. For *in vitro* requirements, an 8 channel PC-controlled system has been developed, capable of carrying out a number of sensing methods. For the *in vivo* applications, two systems have been realized, both based on the PIC microntroller. The first is a PCB with separate commercially-available low

power and low size ICs forming a one-channel potentiostatic system. The second is a specially developed integrated on-chip system. Future work will include optimization of the developed systems for specific biomedical application according to end-user requirements. Additionally for the on-chip system the chip will be redesigned to improve its characteristics and reduce the fabrication cost.

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REFERENCES

Peng, Y., Hu, C., Zheng, D., & Hu, S. (2008). A sensitive nitric oxide microsensor based on PBPB composite film-modified carbon fiber microelectrode. *Sensors and Actuators B-Chemical, in press.*

Seo, H., Park, D., Park, J. (2008). Fabrication and characterization of platinum black and mesoporous platinum electrodes for *in-vivo* and continuously monitoring electrochemical sensor applications. *Thin Solid Films, 516(16),* 5227-5230.

Ndamanisha, J. C., & Guo, L. P. (2008). Electrochemical determination of uric acid at ordered mesoporous carbon functionalized with ferrocenecarboxylic acid-modified electrode. *Biosensors & Bioelectronics, 23*(11), 1680-1685.

Wang, M., & Ha, Y. (2007). An electrochemical approach to monitor pH change in agar media during plant tissue culture. *Biosensors & Bioelectronics, 22*(11), 2718-2723.

McLaughlin, G. W., Braden, K., Franc, B., & Kovacs, G. T. A. (2002). Microfabricated solid-state dissolved oxygen sensor. *Sensors and Actuators B-Chemical, 83*(1-3), 138-148.

Abbaspour, A., & Mirzajani, R. (2007). Electrochemical monitoring of piroxicam in different pharmaceutical forms with multi-walled carbon nanotubes paste electrode. *Journal of Pharmaceutical and Biomedical Analysis, 44*(1), 41-48.

Morales, M. D., Serra, B., de Prada, A. G. V., Reviejo, A. J., & Pingarron, J. M. (2007). An electrochemical method for simultaneous detection and identification of Escherichia coli, Staphylococcus aureus and Salmonella choleraesuis using a glucose oxidase-peroxidase composite biosensor. *Analyst, 132*(6), 572-578.

Shiddiky, M.J.A., Rahman, M.A., Choel, C.S., Shim, Y. (2008). Fabrication of disposable sensors for biomolecule detection using hydrazine electrocatalyst. *Analytical Biochemistry*, *379(2)*, 170-175.