Towards an Implantable Bio-Sensor Platform for Continuous Real-Time Monitoring of Anti-Epileptic Drugs

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***Abstract*— The need of continuous real-time monitoring device for in-vivo drug level detection has been widely articulated lately. Such monitoring could guide drug posology and timing of intake, detect low or high drug levels, in order to take adequate measures, and give clinicians a valuable window into patients' health and their response to therapeutics. This paper presents a novel implantable bio-sensor based on impedance measurement capable of continuously monitoring various antiepileptic drug levels. This portable point-of-care microsystem replaces large and stationary conventional macro-systems, and is a one of a kind system designed with an array of electrodes to monitor various anti-epileptic drugs rather than one drug. The micro-system consists of (i) the front-end circuit including an inductive coil to receive energy from an external base station, and to exchange data with the latter; (ii) the power management block; (iii) the readout and control block; and (iv) the biosensor array. The electrical circuitry was designed using the 0.18-um CMOS process technology intended to be miniature and consume ultra-low power.**

Keywords — Micro-system, real-time monitoring, implantable bio-sensor, in-vivo detection, antiepileptic drug (AED).

I. Introduction

Epilepsy is the most common chronic neurological disorder after stroke with a prevalence nearing 1%. Common causes include brain tumors, stroke, head traumas and genetic mutations. Antiepileptic drugs (AEDs) are the first line of treatment and most patients will need to take them daily over an extended period of time or for their whole life. After absorption, these AEDs will eventually reach a peak level (point where theoretically the patient is most protected against a seizure and when the risk of dose-related side effects is highest) and then gradually decrease to a trough level (when protection against seizures is lowest). Measuring AED levels in the bloodstream or tissue in vivo would give clinicians a valuable window into patients' health and their response to therapeutics. This device could allow for example: a) a better understanding of (intra- or inter-individual) drug level variations whether it be over 24h, days, weeks or years, at various ages, during certain concomitant physiological or pathological conditions; b) a comprehensive assessment of non-adherence by patients (which could be as high as 30-60% according to some studies); c) a thorough causality assessment between drug levels and side effects or seizure protection; d) a better assessment of the impact of switching from brand-name to generic AEDs.

There are various monitoring techniques utilized for AED detection including high performance liquid chromatography[1], gas chromatography[2], capillary chromatography[3], chemiluminescence[4], spectrometry[5], and amperometry[6], most of these techniques cannot be reproduced in portable devices, are time-consuming and rely on heavy apparatus. Thus arises the need for a miniature, portable, and point-of-care biosensor enabling the continuous monitoring of those AEDs.

Electrical Impedance Spectroscopy(EIS) is one method performed in impedance measurement systems and has been widely used for applications such as bio-molecular, DNA, metabolites and cancer cell detection. EIS provides precise and fast impedance measurements with variations in electrode's surface. Once extrapolated, measurements are then utilized in order to detect, or specify changes in fluid concentration. However, none of existent EIS based impedance systems offer continuous detection of AEDs. Therefore, we propose the utilization of EIS as a method for the detection of AEDs.

Indeed, our group has found that carbamazepine, a drug widely used in the treatment of seizure disorders [8], has a significant effect on impedance. Impedance measurements conducted using a VMP-300 potentiostat at Polystim Labs have signified that impedance decreases with the increase of carbamazepine concentration. EIS also revealed different impedance spectra with different concentrations of AEDs.

Compared to other techniques such as voltammetry and amperometry, EIS is a more promising technique due to its precision, the ability to detect a much wider variety of particles not necessarily electroactive ones and little to no variation or damage to analyte measured. Two challenges with EIS are the need to adjust the potential of operation outside the oxidation/reduction window of unwanted particles, and the need of finding functionalized electrodes for the specific detection of the drug to be measured. This ensures that unwanted particles have minimal to zero effect on our measurements.

The proposed implantable micro-system consists of four components: (i) the front-end circuit including an inductive coil to receive energy from an external base station, and to exchange data with the latter; (ii) the power management block; (iii) the readout and control block; and (iv) the biosensor array consisting of the fabricated ion-selective functionalized electrodes. The implantable bio-system will consume ultra-low power, display high sensitivity to AED

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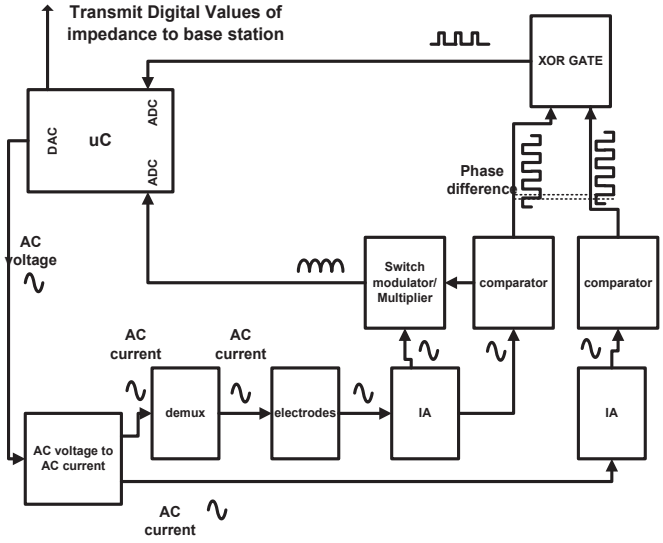

Figure 1: Proposed Impedance Measurement Biosensor Block Diagram

level variations and wirelessly transmit these AED concentrations to a user's smart device (base station).

II. Biosensor Block Diagram

The complete block diagram of the proposed biosensor is presented in figure 1. The microcontroller will be used to generate an AC voltage which will then be changed into an AC current to be sent to its respective electrode. Once excited, the voltage across the electrode with reference to the reference electrode will then be amplified and multiplied by its corresponding square form generated through a fast comparator. This results in the magnitude of impedance measured across the electrode's surface. The microcontroller offers a variety of amplitudes and frequencies to be adjusted according to the drug to be measured. As for the phase, the comparator generated square wave of the voltage across the electrode will be compared with that of the original signal. This difference generated by a xor gate will resemble the phase of our impedance.

III. Impedance Measurement Circuit

The EIS system chosen to be integrated on the implantable biosensor was proposed by P. Kassanos, I. F. Triantis, and A. Demosthenous in[7]. Figure 2 presents a CMOS magnitude/phase measurement chip. This technique extracts both the phase and the magnitude of the voltage across the particles tested. After applying a differential alternating current through a pair of surface electrodes to the body tissue, the resulting voltages across the unknown impedance and the known current-sense resistor (R_{Isense}) are measured by two instrumentation amplifiers and then converted into square waves using two comparators. An XOR gate then produces a pulse signal, with the pulse width representing the phase delay introduced by the unknown impedance at that particular frequency. The square wave generated by the comparator is then used as the clock required by a switch modulator for synchronously rectifying the measured signal which is then low-pass filtered to obtain a full-wave rectified signal dc average voltage.

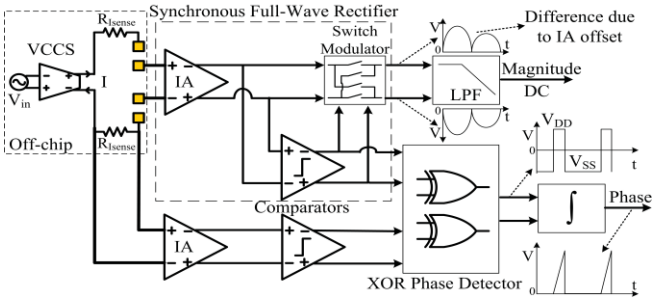

Figure 2: Adopted Impedance Measurement Circuit

The phase (ϕ) of the unknown impedance from the output of the phase detector is then calculated by:

$$\phi = 360° \cdot \Delta t \cdot f \quad (1)$$

Δt is the pulse width and f is the frequency of the applied signal.

As for the magnitude (M) of the unknown impedance, it could be calculated by:

$$M = \pi * \frac{Vdc}{2 * Av * I} \quad (2)$$

V_{dc} is the full-wave rectified signal dc average voltage, A_v is the gain of the instrumentation amplifier and I is the amplitude of the injected current.

A. Instrumentation Amplifier (IA)

The main common-mode interference in bio-impedance measurements occurs at the working frequency and is produced by the current injected into the body to make the measurements[8]. The differential signal measured between the pair of electrodes can be as small as a few tenth of a microvolt (μV) whereas the common-mode interference can be in the hundreds of millivolt (mV) range. Therefore a high gain amplifier with a high common mode rejection is needed in order to amplify such a small measured signal while rejecting as much noise as possible.

There are two basic approaches to the design of an IA: resistive feedback and current feed-back. Both approaches offer good performance, whereas the current-feedback offers higher CMRR because of both isolation and balancing techniques. The IA presented in figure 3, uses a current feed-back topology and is comprised of an input transconductance stage and two output transimpedance stages to render it fully-differential. This design has a variable gain which can be achieved by modifying the ratio of the integrated resistors, which offers us an advantage while varying the range of impedances measured. Additionally to having a high CMRR, this IA uses a capacitive neutralization transistor (M0) in order to diminish the mismatch in the drain capacitances of the input pair transistors. As for the output DC voltage of the amplifier, it can be set using a DC voltage source (usually generated using a band-gap voltage reference circuit).

The instrumentation amplifier was biased at a current of 37.5uA and an output DC voltage was set to 1.42V in order to be used in the comparator stage.

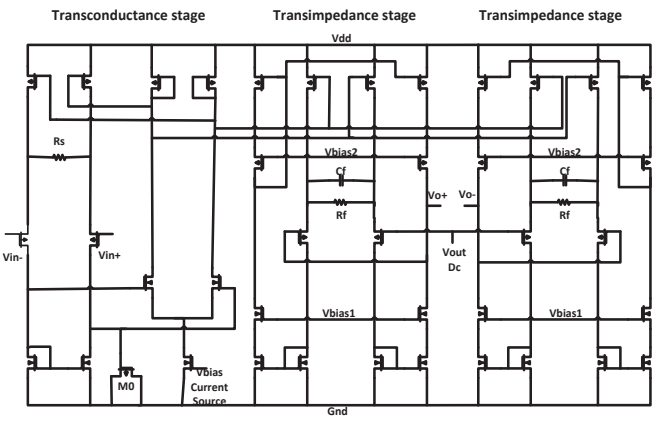

Figure 3: CMOS 3-stage Instrumentation Amplifier

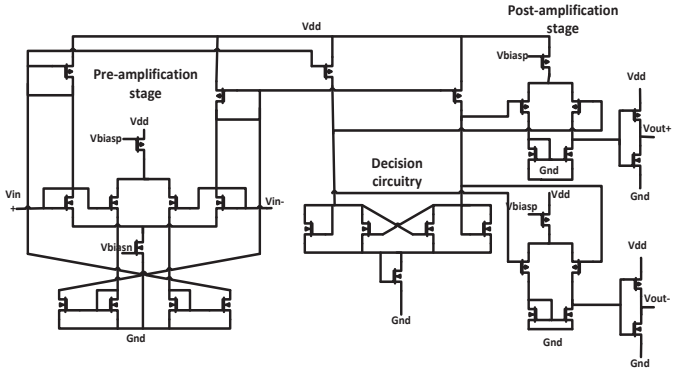

Figure 4: CMOS comparator with rail-to-rail input common-mode.

B. Multi-stage Comparator Circuit

The comparator design is a clock-less continuous time multistage comparator as provided in figure 4. It includes both a PMOS and an NMOS differential amplifier feeding a decision circuit, and the output buffer is a PMOS differential amplifier driving an inverter. In order to be used as a clock for our switch modulator, the comparator must be in-phase with the signal generated from the instrumentation amplifier. The delay of the comparator will introduce an error in the calculation of the magnitude. The equation of multiplying the sine-wave output of the IA with the square wave generated from the comparator is provided below.

$$Vout = \frac{2Vo[\cos(\emptyset) - 2\cos(2\omega t + \emptyset)]}{\pi} \qquad (3)$$

By providing a square wave directly in phase with IA sine-wave output we remove the additional gain factor of cos(Ø).Therefore the delay of our comparator needs to be minimized.

IV. Biosensor Array

The biosensor consists of an array of electrodes upon which selectivity could be provided. Various electrodes can be modified in order to offer a certain selectivity towards the drugs, which will enable us to distinctly measure the concentration of the drugs. Such electrodes exist, and are labeled as functionalized electrodes, which exhibit a high sensitivity towards our various drugs. As an example Graphene-Gold nanoparticle modified electrodes are highly

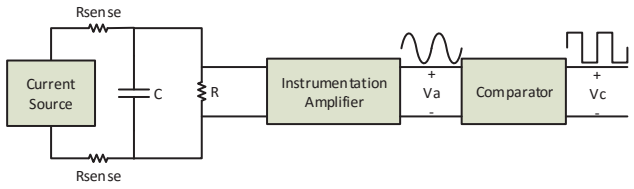

Figure 5: Combined IA and Comparator

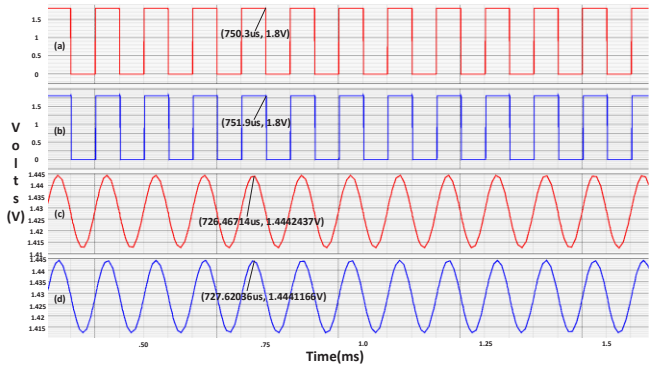

Figure 6: Varying C from 1p to 100p displaying variable amplitude and phase. (a) Square wave at C = 1 pF, (b) Square wave at C = 100 pF, (c) Sine wave at C = 1 pF, (d) Sine wave at C = 100 pF

selective to carbamazepine[9] and phenytoin selective electrodes are proposed by M. El-Tohamy[10].

In order to detect the different concentrations, there will be a switching technique which operates the electrodes separately and records the corresponding measurements. This will result in separate measurement of each drug. When necessary, these electrodes can be easily modified to render our biosensor universal able to detect not only AED levels but lactate and glucose concentrations, dissolved O2 and pH levels.

V. Simulation Results

In order to test the operation of the designed circuit, the IA was combined with the comparator as provided in figure 5. A transient analysis was performed while varying the capacitance C and keeping R constant. Both voltage signal were recorded. The first was an amplified sinusoidal voltage signal (Va) generated by the IA, while the other was the square wave signal (Vc) generated by the comparator. The capacitance C was changed from 1pF to 100pF in order to test if the circuit can detect any changes in impedance. Effectively, as provided in figure 6, a phase shift was recorded between both square waves (a) and (b), and the amplitude of the sinusoidal waves (c) and (d) varied. The phase shift is due to the increase in voltage lag caused by the capacitor, whereas the change in amplitude refers to the change in the magnitude of impedance measured.

VI. In-vitro Experimentation

Attempting to monitor the concentration of the AEDs through EIS, requires the drug to alter the impedance of the electrodes surface. This means that a change in concentration of the drugs must result in change in opposition to current flow between the working and counter electrode. In order to test the validity of this assumption we performed in-vitro tests of impedance versus concentration of drugs.

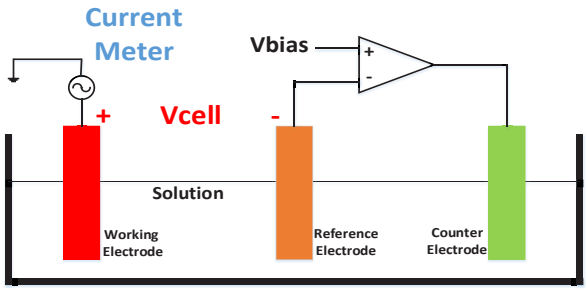

Figure 7: Three-Terminal Electrode Setup

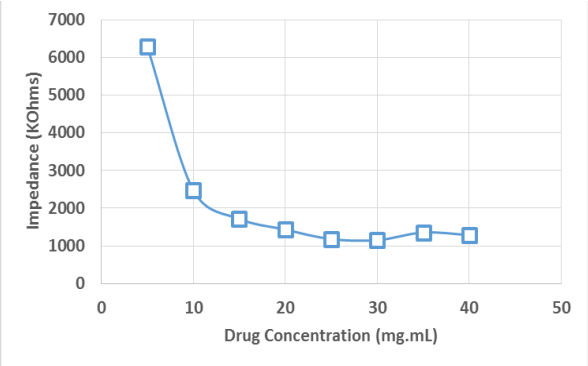

Figure 8: Impedance vs. Carbamazepine Concentration

White carbamazepine powder with a molecular weight of 236.27 was ordered from Sigma-Aldrich and dissolved in Isopropyl alcohol to form concentrations over the range of 5-40 (mg/mL). Impedance measurements were taken by a VMP-300 potentiostat at Biostim laboratories. Such is a multichannel workstation able to perform EIS measurements. A 3-electrode terminal setup was used to validate the concept with a gold working electrode, a platinum counter electrode and an Ag/AgCl reference electrode. These electrodes are used in most basic electrochemical measurement and were provided by EDAQ (ET014 EChem Electrode Kit). A 3-electrode terminal setup was chosen since it offers better measurements when compared to a normal 2-electrode setup; measurement is less affected by residual impedance and contact impedance at low frequencies.

Figure 7 represents the system operated on the potentiostat. In electrical impedance spectroscopy a sinusoidal voltage is varied according to both amplitude and current whilst measuring the current and varying drug concentration. The voltage is then divided by the current to generate a graph of impedance versus concentration.

Figure 8 provides the in-vitro graphs generated of impedance versus concentration of carbamazepine, using impedance spectroscopy. A total of 32 measurements were conducted, four measurements for each concentration, with a total of 8 drug concentrations, and the average impedance for each concentration was calculated and plotted. It is clear that the impedance decreases as carbamazepine in isopropanol concentration increases. This implies that the drug increases the current flow in the solution; as concentration increases the current opposition decreases yielding a decreased impedance value.

VII. Conclusion

Detection of AEDs using electrical impedance spectroscopy is a promising technique which offers a wide range of detection and precision. Three-electrode setup measurements performed using the potentiostat showed success when detecting the increase of concentration of drugs in-vitro, and the circuitry designed detected change in impedance through changing the value of capacitance, resistance or inductance. Analog circuit components were designed in cadence 180nm technology requiring a voltage supply of 1.8V. EIS enables various recording while varying electrodes, and operating ranges enabling the detection of multiple drugs.

Acknowledgments

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