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Drug Under Skin Meter (DUSM): a portable bio-impedance spectroscope for assessing transdermal drug delivery

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Abstract **- An instrument prototype, based on a measurement method relating bioimpedance and amount of drug present in a tissue under test, was realized. The instrument assesses the amount of drug conveyed through the skin by measuring impedance spectrum before and after administration. In compliance with safety regulations, the stimulus signal does not exceed the peak value of 200 μA well below the threshold of 424 μA provided by the IEC-60601 standard. In this paper, after illustrating the conceptual and physical design of hardware, firmware, and software interface, a preliminary metrological characterization of the prototype is described. This realization opens interesting scenarios to pursue strategies of immediate efficacy assessment for all non-invasive systems for intradermal conveying.**

1. INTRODUCTION

The topical use and the transdermal delivery of drugs allow the action of the active ingredient to be localized, by minimizing systemic effects [1]. At present, shared and recognized $in-vivo$ assessment methods are missing for topical drugs, with the sole exception of the class of corticosteroids: a colorimetric scale measures the vaso-constrictive effect of the active principle through the whitening extent of the treated tissues [2]. The absence of effective methods and recognized measuring in vivo for locally acting drugs makes problematic the scientific use of the concept itself of dosage for topical drugs [3]. Skin biopsy and suction blister allow precise measurements but invasive and not immediate. The tape-stripping and microdialysis techniques are less invasive but they are hard to standardize [4]. Other recent and promising methods such as Raman spectroscopy require very expensive equipment and still allow a direct analysis of only the most superficial layers of the skin [5].

For biomedical applications in general, and in particular also in dermatology, the measurement of impedance is extensively studied. It was used as containment factor of reproducibility problems in the context of penetration measurement for $ex-vivo$ drugs [6]; as a tool to measure variations of the hydration state of the horny layer [7], and, in this way, to predict its permeability [8]; as a method to differentiate the interaction of diverse pharmacological principles with the skin tissue [9]; as a tool to measure the blood flow caused by treatment heat transfer [10]; and, last but not least, as a method to diagnose the presence of cancer [11].

In this paper, a prototype of the Drug Under Skin Meter (DUSM), a portable bio-impedance spectroscope for assessing transdermal drug delivery, is proposed. The instrument is based on a measurement method relating bioimpedance and amount of drug present in a tissue under test. The DUSM assesses the amount of drug conveyed through the skin by measuring impedance spectrum before and after administration. After illustrating the conceptual and physical design of hardware, firmware, and software interface, a preliminary metrological characterization of the prototype is described.

2. REQUIREMENTS

In this Section, the functional and metrological requirements that drove the design of the of the DUSM are illustrated.

The IEC 60601 standard [12] for the patient/wearer safety and effectiveness of medical electrical equipment specifies the limits of patient leakage currents and patient auxiliary currents under normal conditions and single fault conditions . These current limits are important parameters in the circuit design. The maximum DC current allowed to be sourced in the body in normal conditions has to be less than or equal to 10 μA and the maximum DC current under single fault condition in the worst scenario is 50 μA. The maximum AC current allowed to be sourced in the body in normal conditions depends on the frequency. If the excitation frequency is less than or equal to 1 kHz, the maximum allowed RMS current is 10 μA. If the excitation frequency (f) is greater than 1 kHz, the maximum current is defined by the Equation 1

$$I_{AC_{MAX}} = \frac{f}{1000\,Hz} \cdot 10\,\mu A_{RMS} \qquad (1)$$

A preliminary experimental campaign for measuring the drug absorbed by a biological tissue in laboratory emulation of dermatological topical treatments was carried out [3]. The procedure for determining the percentage variation of impedance magnitude at varying the amount of injected substance in pulp eggplant was defined and validated.

3. CONCEPTUAL DESIGN

In this Section, (i) the basic ideas, (ii) the architecture, (iii) hardware design, (iv) firmware design, and (iv) user interface of the DUSM are illustrated.

A. Basic ideas

The conceptual design of the DUSM was based on the following main basic ideas:

* to measure the amount of drug injected in a biological tissue by assessing the difference in impedance spectrum measured by Electrical Impedance Spectroscopy [3];

* to carry out an initial acquisition of the tissue spectrum in the range from 200 Hz to 50 kHz to assess the impact of the type of tissue on the measurement sensitivity; as a matter of act, different tissue conditions (in particular the thickness and the state of hydration of the stratum corneum) determine variability in the measurement sensitivity. At the end of the transdermal delivery of the drug, the impedance measurement at the frequency of 1 kHz of stimulus signal allows the amount actually penetrated to be calculated. This amount is related by a linear relationship to the percentage of current impedance change with respect to the initial one [3];

* In line with the design goal of building an integrated device with existing mesoporation instrumentation, an on-chip impedance spectroscope (i) operating in the frequency range of interest, (ii) simultaneously ensuring the accuracy target, as well as (iii) respecting the abovementioned safety regulations for biomedical devices [12] was found on the market.

B. Architecture

The conceptual architecture of the DUSM is illustrated in Fig.1. Once generated a sinusoidal current at a given frequencies, the impedance spectroscope acquires the corresponding voltage drop through the electrodes and the signal conditioning stage. The spectroscope calculates both the spectrum for all the frequencies and the relative change in impedance at a fixed frequency. The impedance spectrum is then employed by the classifier block to recognize the peculiarities of the tissue under investigation. The relative change in impedance is employed by the inverse model block to calculate the amount of drug transmitted through suitable coefficients identified by the classifier block.

C. Hardware design

The main hardware components are: electrodes, signal conditioning (that ensure that no dc voltage appears across the electrodes and guarantee the maximum allowable excitation current), precision ac voltage source, high precision current meter, precision differential voltage meter, DFT block (to calculate real and imaginary components of impedance).

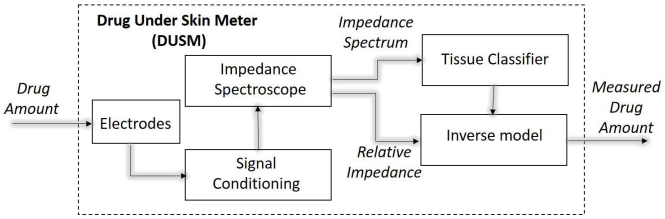


Fig. 1. Architecture of the DUSM.

D. Firmware design

The firmware design was based on the software package ADuCM350BBCZ provided by Analog Devices to write applications for ADuCM350. The boot kernel, startup, system and driver source code, driver configuration settings, driver libraries, sample, documents and the OSAL (Operating System Abstraction Layer, a wrapper around the optional RTOS) package, are provided. For the ADuCM350BBCZ on-chip peripherals configuration, the Device Drivers and System Services are adopted. The ADuCM350BBCZ is designed to work with IAR Embedded Workbench for ARM and the Micrium uC/OS-II RTOS for ARM Cortex-M3.

E. User interface

A Windows client application for the interaction with the DUSM from laptop was developed. It allows impedance measurement of a body tissue and the subsequent data processing and storage to be carried out. For its development, the environment Microsoft Visual Studio 2015 - Community Edition was chosen by using the .NET Framework 4.0 and the C# programming language. The application was developed with object-oriented, multi-threaded architecture and provides error handling and application exceptions beyond the trace of the operations performed. The data is persisted to the database report. The application implements the communication protocol of virtual serial port with the device ADuCM350 and allows its initialization and sending commands specific to the necessary measurement operations.

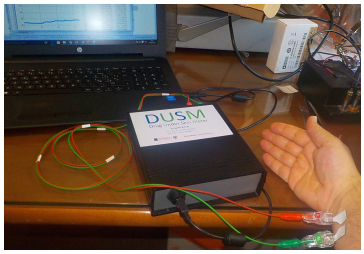
4. REALIZATION

The realized prototype of the DUSM is illustrated in Fig. 2.

A. Hardware architecture

DUSM is based on the chip of Analog Device ADuCM350 (Fig. 3) [13], with a 16-MHz processor ARM Cortex-M3, equipped with an analog front end specifically designed for high-precision data acquisition. It has an integrated system for complex impedance measurement and employs an integrated parameterizable wave generator. The ADuCM350 houses an excitation control with high precision loop that applies an alternating voltage to the electrodes. A sine wave DDS generator produces the

(a)



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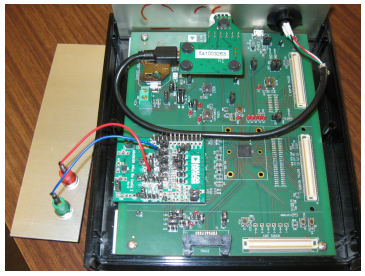


Fig. 2. The realized instrument: (a) operation view, (b) motherboard.

alternating stimulus by means of a 12- bit DAC. An ammeter with high precision using a trans-impedance amplifier (TIA) for the conversion of current into voltage and to measure the latter with a precision ADC whose gain is determined by an external resistor: RTIA. A significant both analog and digital filtering is performed on the measurement for the rejection of interfering and noise. The ADC converts the current measurement with a rate of 160 kS/s. The ADuCM350 realizes a discrete Fourier transform (DFT) to 2048 points of the ADC on the output to 16-bits by calculating the real and imaginary part of the impedance. To comply with the IEC-60601 standard, the ADuCM350 is used in combination with an amplifier to external instrumentation (AD8226), to complete high-precision absolute measurements using a 4-wire measurement technique. Considering stricter rules on the continuing tensions with respect to alternating they were placed on the line of isolation capacitor electrodes. It was also inserted in the circuit of a 3 kΩ protective resistor so that the current, with an applied voltage of 600 mV, does not exceed the peak value of 200 μA well below the threshold of 424 μA provided by the IEC standard 60601. The device is powered via USB from laptop during in vivo testing only works with its own battery.

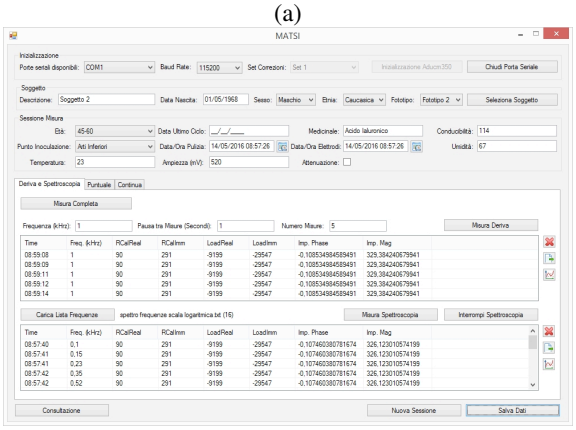
B. Firmware implementation

The firmware, implemented in C, consists of an application software running on ADuCM350. During the initialization stage, power up and calibration steps are performed by paying attention to set 125 ms of delay between each stage. Then, the 2 DFT measurements are performed, invoking the "$adi_AFE_RunSequence$", where 4-wires input was selected. From the results, the real and imaginary values for the current and the voltage are assessed. The algorithm then calculates the magnitude and phase of the DFT results by achieving the final results. All the arithmetic is performed using fixed-point types and functions defined in CMSIS DSP library. The final results are reported by adopting a custom fixed-point type, with 28 integer bits and 4 fractional bits.

5. DUSM WORKING

The DUSM is configured by means of the user interface illustrated in Fig. 4a, developed with the use of different user controls (label, textbox, lists, combo box, etc.). The user can define the information on the measurement session is performing, ask the types of necessary measures and to enforce measures. The data received by the device are properly processed and displayed Fig. 4b. The database of data is then available for further processing, research, visualization and plotting.

(a)



(b)

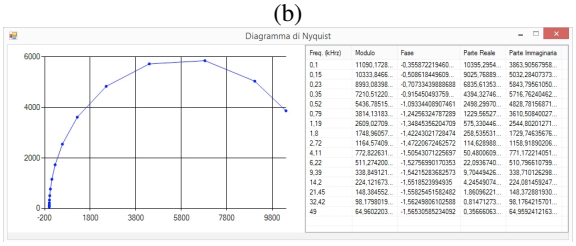


Fig. 4. User Interface of the impedance spectroscope DUSM: (a) configuration and (b) results of the measurement.

6. PRELIMINARY METROLOGICAL CHARACTERIZATION

The DUSM was characterized metrologically both in laboratory and in vivo. In the former case, the impedance spectroscope was tested, while in the latter case the DUSM as a whole.

A. Laboratory tests

The sample was constituted by a parallel impedance of a resistance (5831.5 Ω) and a capacitance (50 pF). The resistance was obtained by means of a precision resistor in

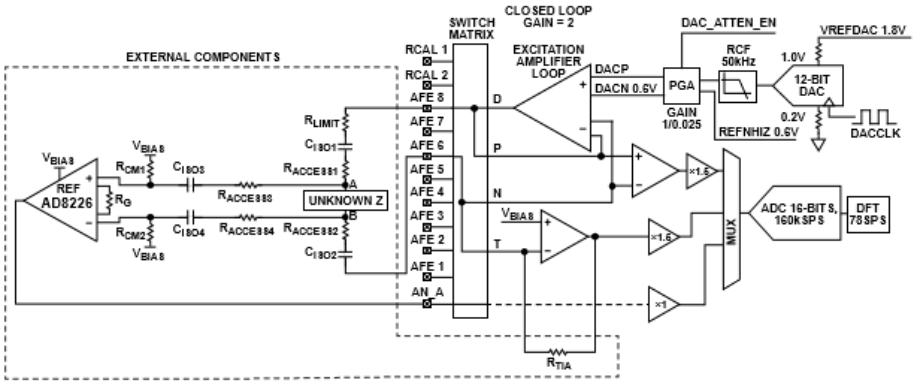


Fig. 3. Architecture of the on-chip impedance spectroscope ADuCM350 [13].

decades (Decade Resistor General Radio 1433-M) while the capacity of a sample capacitor (type R 509 General Radio Co.). A total of 500 measurements were carried out in the range 1 kHz -50 kHz by increasing by 1 kHz step for a total of 50 measurements. For each frequency, 10 impedance measurements (modulus and phase) were carried out and the average of the measured value with the percentage standard deviation were calculated. The results in the bandwidth up to 8 kHz are reported in Figs.6 (a), modulus and (b) phase. The impact of the stimulus signal on repeatability was verified experimentally: the amplitude that provides the best 1-sigma repeatability (0.06%) is 0.52 V. By increasing or decreasing such an amplitude value, the sigma grows at least by an order of magnitude.

B. In vivo tests

The experiments were made at the medical study of a cosmetic surgeon and have scheduled the realization of measurements in the course of ordinary activities of (Fig. 7).

Drug was injected 0,5 cm below the surface. The experiments were repeated for 20 times for the following levels of injected drug: 0.0, 0.05, 0.10, 0.15 , 0.20, and 0.25 ml, by measuring the percentage variation from the initial impedance magnitude and phase. This helps to soften the impact of the initial conditions and common mode noise. The measurement conditions are summarized in Tab. 1. A linear behavior was experienced as pointed out in Fig 8. Sensitivity [$ml^{-1} \cdot 10^{-1}$], percentage $1 - \sigma$ repeatability [%], percentage nonlinearity [%], and resolution [ml] were computed (Tab. 2).

The nonlinearity was assessed as the standard deviation of the linear regression error of the calibration straight [14]. The resolution arises from the indetermination of the measured amount of drug, assessed by the uncertainty of the calibration curve. Therefore, it was assigned as the standard deviation of the regression error assessed through analysis of variance (one-way ANOVA).

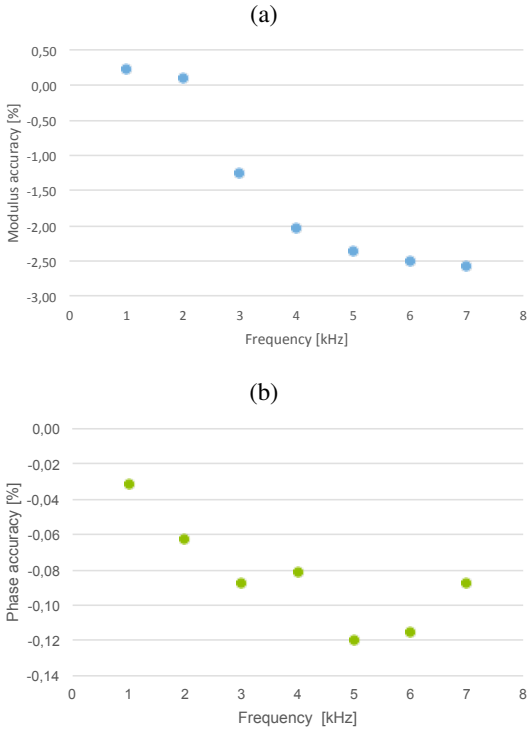


Fig. 5. Percentage accuracy of the DUSM impedance spectroscope: (a) modulus and (b) phase.

Table 1. Measurement conditions in the metrological characterization of the proposed procedure

Signal Frequency	Electrodes Area	Electrodes Gap	Signal Amplitude
1.00 kHz	1 cm^2	1 cm	520 mV

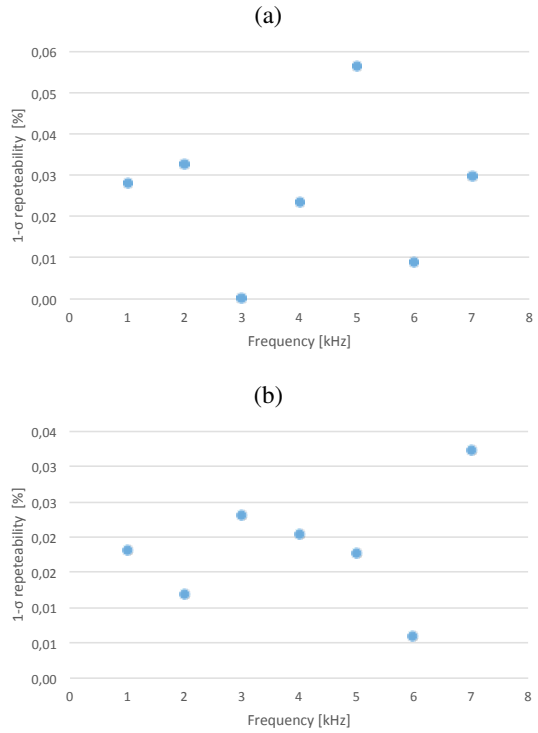


Fig. 6. 1-sigma percentage repeatability of the DUSM impedance spectroscope: (a) modulus and (b) phase.

Table 2. Metrological characteristics of the proposed procedure

Sensitivity [$ml^{-1} \cdot 10^{-1}$]	1 - σ Repeatability [%]	Non linearity [%]	Resolution [ml]
14,97	0,18	1,63	0,032

7. CONCLUSIONS

An instrument for measuring the amount of drug present in a tissue under test was prototyped. The instrument proved to be capable of assessing the drug amount by measuring impedance spectrum before and after administration. In compliance with safety regulations, the stimulus signal does not exceed the peak value of 200 μA well below the threshold of 424 μA provided by the IEC-60601 standard. The instrument exploits a 5-pole probe in order to both (i) not take into account the impedance of the amperometric electrodes, and (ii) to compensate the short-circuiting action of surface conductive fluid layer. The results of a preliminary metrological characterization of the prototype provide satisfying indications. This realization opens interesting scenarios to pursue strategies of immediate efficacy assessment for all non-invasive systems for intradermal conveying. In the future, the portable measurement instrument will be enhanced by implementing a differential approach in order to further improve the signal-to-noise ratio.

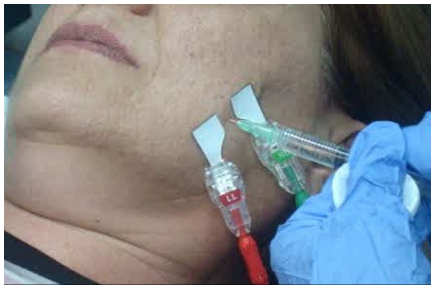


Fig. 7. In vivo experiment during activities of mesotherapy.

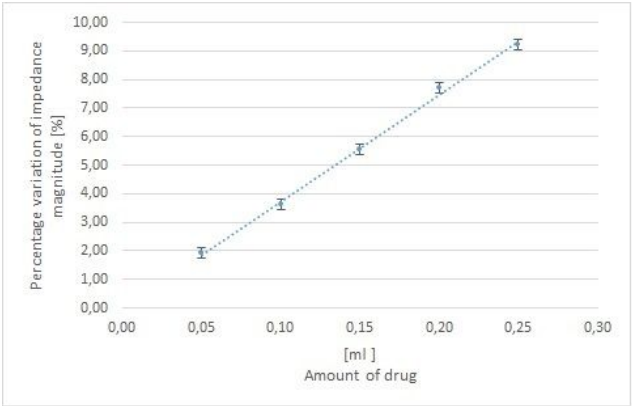


Fig. 8. Calibration curve in measurements conditions of Tab. 1.

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