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Carbamazepine Biosensor Development for Epilepsy Patient Screening

Esteban J. Pino[1], *Senior Member, IEEE*, Francisca Pucheu[1], Fabián Alvarado[1], Britam Gómez[1], Marta De Diego[2], Sigrid Mennickent[2], Claudio Aguayo[3], Carlos Peña[4] and Andrés Rodríguez[5]

***Abstract*— The San Carlos population in Chile is an example of an underserved community due to lack of timely access to regular controls and laboratory results. One particular challenge is the adherence to treatment of Epilepsy patients. In this work, we present the design and proof–of–concept of a Point of Care Device (POCD) to measure carbamazepine levels in saliva to screen for correct dose prescription among epilepsy patients. We present the Screen Printed Electrode design and activating circuit and preliminary results to verify feasibility of the biosensor. Future steps include the fabrication of the device itself and validation with the target population.**

I. Introduction

Epilepsy is a disease characterized by presenting brief pictures of involuntary movements in a partial or generalized way in a person, which in some cases may be accompanied by a loss of consciousness [1].

Current epilepsy therapy is symptomatic, as there is no effective prophylaxis or cure available, thus, treatments aim to achieve a balance between preventing seizures and trying to minimize the risks of drug's side effects which must be taken daily for years, in order to maintain the best quality of life for the patient. These long-term therapies and drug side effects can lead to treatment failure or poor adherence [2].

For disease control, treatment must achieve adequate therapeutic concentrations and maintain stable levels to protect the patient all day long, so it is very important to check blood levels of anticonvulsant drugs, and optimize treatment in biological matrices, due to the great pharmacokinetic variability and their narrow therapeutic margin [3].

One of the available treatments is the administration of carbamazepine (CBZ), an oral anti–epileptic drug. The initial dosage in adults is 100 to 200 mg every 12 hours, being increased from 100 to 200 mg every 2 weeks until reaching at maintenance doses that are usually 800 mg to 1.2 g per day. This medicine, despite having good treatment efficiency, is usually associated with adverse reactions [4].

Knowing if the patient is taking the correct dose generally requires the use of invasive procedures, that is, obtaining a blood sample in place, ensuring certain conditions and medical equipment. After this, the sample needs to be sent to a laboratory, where it will be analyzed and informed. Considering the aforementioned, it is easy to conclude that it corresponds to a procedure that requires several days and specialized equipment.

In the Ñuble region, in Chile, according to the official records from 2010, 2497 patients have an epilepsy diagnosis [5]. Most of them are cared in Community Health Centers, close to their rural residencies, with low or rare access to specialist physicians. From those centers, samples for plasma monitoring are sent to laboratories located in Santiago, 400 km away, with the consequent delay in the results. The particular condition that motivates this work is from the San Carlos population, a small city in Ñuble. At present, the control of CBZ levels of patients is performed only once a year, with results taking one week, making it difficult to detect critical situations that require therapeutic intervention.

Our proposed solution is inspired by the increase in the development of Point of Care Device (POCD) that allow obtaining results immediately without the need of elaborate laboratory devices. A characteristic of POCD is the compact size and portability, and its advantages include low price, low sample volume, ease of use, speed of results, and that it can be used at or near the patient's point of care eliminating or reducing sample transport time [6].

The technology has been growing enormously in the last years. One of the greatest advances in this area are the small-scale biological and chemical sensors, which has allowed the creation of very tiny sensors based on electrodes [7]. The importance of having analytical tools that allow rapid delivery of results motivated the development of this work. In this work, we present the design and first prototype of a POCD for easy and fast CBZ therapy assessment based on screen-printed sensors (SPE) and saliva samples.

II. Materials and Methods

The instruments used for electrochemical characterization include the following:

- MT100 incubator (Universal Labortechnik).
- Laminar flow safety cabin AH-100 Telstar BioSTAR.
- Bipotenciostat CHI812B, CH Instruments, Inc., controlled by software.
- Multi Potenciostat/Galvanostat μStat 8000, Metrohm-DropSens S.L.
- Electrodes, buffers and test solutions.

The electrochemical characterization of CBZ is obtained by cyclic voltammetry and lineal sweep in a potentiostat, using a 3–electrode cell.

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[1]Electrical Engineering Department, Universidad de Concepción, Concepción, Chile epino@ieee.org

[2]Pharmacy Department, Universidad de Concepción, Concepción, Chile

[3]Clinical Biochemistry and Immunology Deptartment, Universidad de Concepción, Concepción, Chile

[4]Institute of Applied Chemical Sciences, Universidad Autónoma de Chile, Temuco, Chile

[5]Unidad de Farmacia, Hospital de San Carlos, San Carlos, Chile

The supporting electrolytes employed are KCl 0.05 mol/L and phosphate buffer 0.05 mol/L. CBZ concentrations tested are in the 1 – 8 mmol/L. The sweep conditions are: 50 mV/s, 10 – 5 V/A sensitivity. The electrochemical sweeps are preformed to obtain the anodic and cathodic curves.

To produce custom–made SPE, a LPKF Protolaser S-Series for PCB prototyping is used on flexible copper sheets is used, for low–cost, disposable electrodes

III. Proposed Solution

Considering that anticonvulsant drugs require plasma concentrations within certain ranges to obtain an adequate control of the disease [8], it is very important to have analytical tools that allow the rapid determination of these drugs.

In this project, a prototype of a portable device, a POCD, will be created to facilitate the determination of CBZ levels in saliva of patients with epilepsy. This device will allow the measurement of CBZ levels at the patient's point of care and outside a laboratory, which has several advantages compared to the conventional techniques currently used [9], [10], [11], such as HPLC or ELISA, among which the following stand out:

- Speed of the result, which reduces the time needed for therapeutic intervention and early recognition of critical situations.
- Portable, remote testing.
- Easy to use (requires less training).
- Minimal sample processing.
- Sample transport time eliminated or minimized.

It is important to mention that although the advantages are clear, there are some disadvantages to be taken into account. One of them is the possibility of measurement inaccuracy, which can lead to differences in relation to the results provided by the laboratory [6]. To minimize this effect, the results obtained with the POCD will be compared with conventional techniques and calibrated.

The portable device will be developed based on a biosensor to determine the concentration of CBZ in saliva of patients with epilepsy, by means of an antigen-antibody reaction. Saliva will be used, as it is an alternative biological fluid to blood, easier to sample and less invasive. Due to its physicochemical properties, CBZ concentration in saliva and plasma/serum is highly correlated [3], [12].

In this project, CBZ is selected considering that it is one of the most widely used anticonvulsants for the treatment of epilepsy in Chile [13]. It has a low aqueous solubility, and a metabolism induced by other drugs and also by autoinduction, which generates a poor dose-effect relationship. This added to the risk of adverse effects, and its narrow therapeutic margin, make it advisable to monitor its plasma concentrations to obtain safe and effective therapeutic regimens.

IV. Results

A. Electrochemical Characterization

The sweep results show that CBZ presents an adequate response close to 1.2 V in the anodic direction, as shown in Fig. 1. The response is proportional to the concentration between the levels of detection (LOD) 0.3 mM to 8 mM.

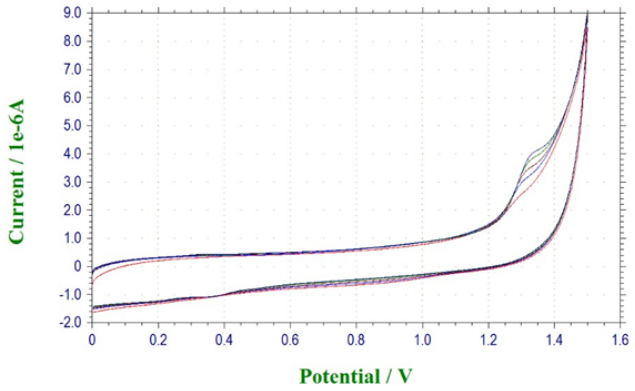

Fig. 1. Anodic sweep, cyclic voltammetry for different CBZ concentrations.

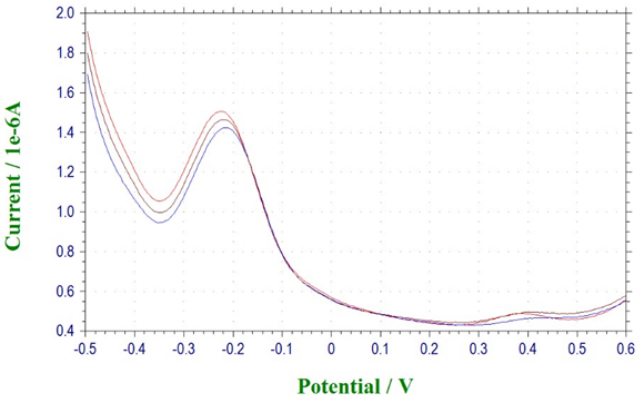

Fig. 2. Cathodic sweep, lineal sweep voltammetry for different concentrations.

After a cathodic sweep, shown in Fig. 2, the response is found near -0.25 V, proportional to the concentration between LOD 0.3 mM to 4 mM.

The difference in lineal intervals is probably due to the presence of oxygen in the solution. A nitrogen purge is necessary in case the cathodic zone needs to be quantified.

Molecularly Imprinted Polymers (MIP) were synthesized from commercial CBZ. The results of the MIP–CBZ affinity will be studied in a future work. The use of synthetic polymer for CBZ targeting facilitate detection and electrode production.

These results validate the capability of detection of CBZ in a solution using SPE and MIP in the active site.

B. Electronics design

1) Screen Printed Electrodes: In the search for low-cost electrodes that can be produced on a large scale, ½ oz. flexible copper plates with a thickness of 0.8 mm are used. The design is based on commercial screen-printed electrodes such as DS-220BT. The shape of the electrode allows use of standard equipment to fit the three terminals: work electrode (WE), reference electrode (RE), and counter electrode (CE). The sample site of the WE has to be uniform and its size

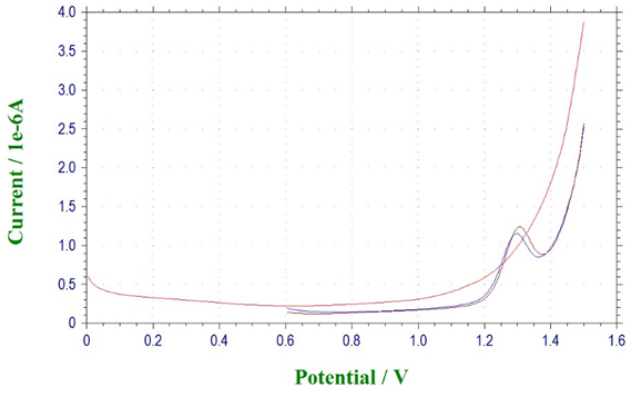

Fig. 3. Comparison, CBZ at different concentrations vs target solution, lineal sweep voltammetry.

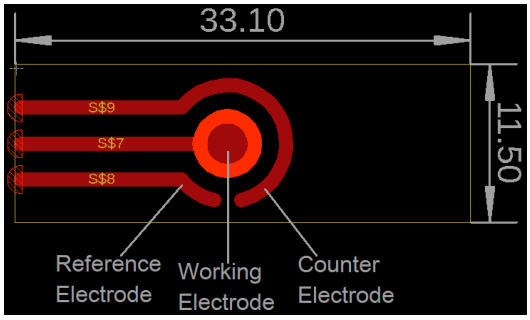

Fig. 4. SPE design in Eagle for LPKF etching in flexible copper PCB.

such that little solution is required to achieve an oxidation–reduction reaction (Redox) to indicate the concentration of the target enzyme. Also, about 10 mm at the end of the electrode have been left unused for easy handling. The electrode was designed in Autodesk Eagle electronic design software and implemented using an LPKF Protolaser S-Series LPKF printed circuit board printer. The final design can be seen in Fig. 4 and the printed electrodes are shown in Fig. 5.

2) Potentiostat Circuit: An analog circuit is required to generate the necessary potential to make the substrate react and obtain a measurement proportional to the CBZ concentration at the electrodes [14]. This circuit has three parts: isolation stage, differential control stage, and comparison

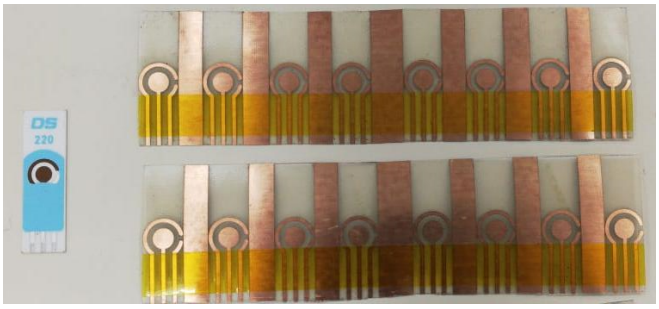

Fig. 5. Commercial SPE (left) and compatible electrodes printed at our lab (right).

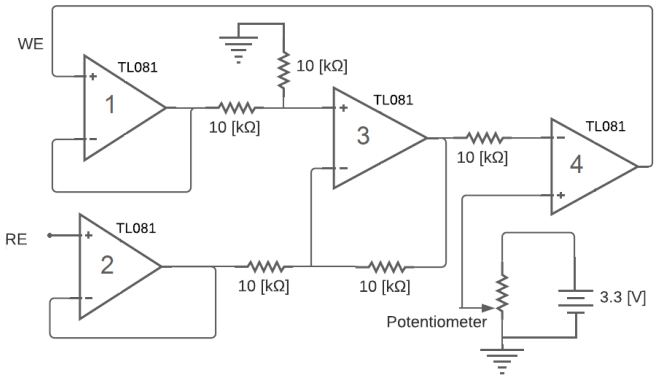

Fig. 6. Potentiometric circuit schematic, implemented with Texas Instruments TL081 OpAmp.

stage, and is shown in Fig. 6.

The isolation stage consists of buffers that are connected to the RE and WE terminals. These voltages go to the differential control stage, where a differential amplifier with unit gain evaluates the error between these electrodes, so the difference between the working and reference electrode voltages remains constant. This difference is defined by the 4^{th} operational amplifier that acts as a comparator between a voltage divider (implemented with a digital potentiometer) and the voltage obtained by the differential control amplifier, which maintains this difference through a feedback to the buffer of the WE. This results in a current from the CE that represents the reaction of the solution.

The critical parameter for a definitive design is the test voltage applied to the electrode. This voltage must be determined by the characterization tests of the electrochemical behavior of CBZ. From the results shown in Fig. 1 and Fig. 3, the voltage will be around 1.3 V, but a precision potentiometer is considered for final adjustment. Once this voltage is defined, the design will be finalized and implemented as a printed circuit for reliable operation.

3) POCD design: At this point, the actual POCD is yet to be designed and 3D printed for our first tests with patients. However, the conceptual design defined the following requirements:

- Hand–held form factor.
- Battery operated.
- SPE port for easy measurement.
- Bluetooth connection to a mobile phone.

The device will be used on the field, to assess both rural and urban patients. A Bluetooth connection using an ESP32 chip will send the converted SPE measurement to a mobile phone, where patient data will be stored. Upon presence of internet connectivity, that data will be sent securely to a repository for queries by the attending physician.

V. Future Steps

The COVID-19 pandemic forced the project to a halt in March 2020. Our plans for this year, after we are able to return to our laboratories, are:

- Validate quantitative determination of CBZ from saliva using HPLC chromatographic method.
- Calibrate CE measurement to CBZ concentration in saliva.
- Fabricate and assemble POCD prototype with Bluetooth connection.
- Program simple Android app to report measured CBZ concentration on the field and store and forward measurements to centralized repository.
- Determine and compare CBZ levels from patients' saliva samples from POCD and HPLC method.
- Test synthetic MIP affinity for CBZ.

This project has been approved by the University's ethics, bioethics and biosafety committee. For the final validation with voluntary patients we will obtain authorization from Servicio de Salud Ñuble's Institutional Review Board.

VI. Conclusion

We developed a proof–of–concept biosensor for CBZ detection. This electrochemical sensor, combined with a SPE–based POCD can facilitate screening for correct maintenance doses and avoid adverse reactions due to poor medication. The proposed saliva–based test would facilitate the screening and dose adjustment of a population that currently has limited access to timely healthcare. The selected components and design of the POCD can be easily replicated for mass production.

The described methodology to produce and test a biosensor can be applied to other medications, provided they can be detected in saliva or other easily acquirable fluid. The development of new easy–to–use POCD can provide new means for timely and objective screening of patient adherence for many chronic conditions, supporting both their own treatment and the health personnel in charge of their care.

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