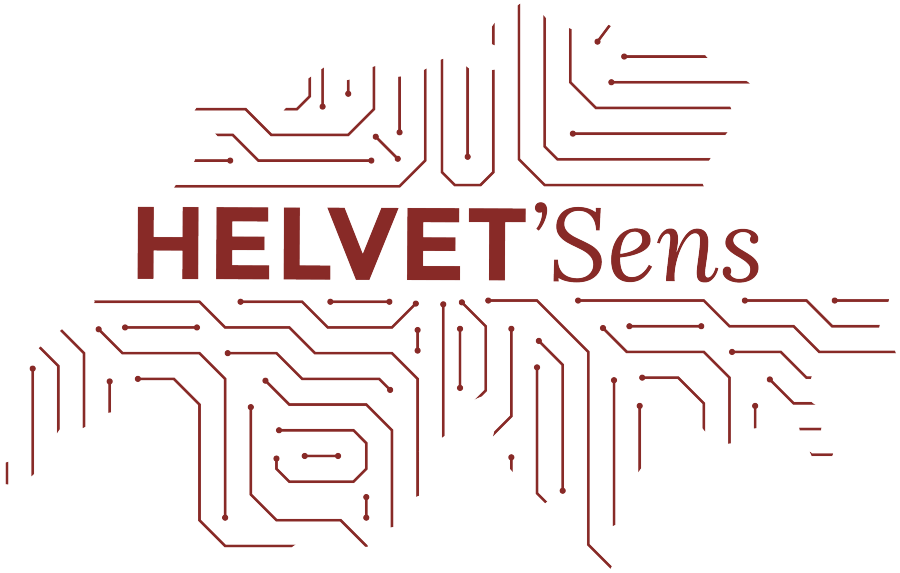
Ecole Polytechnique Fédérale de Lausanne

RENO: Rapid Electrochemical impriNted pOlymer



Team members

Emma Barbier
Ahmed Ben Romdhane
Loïc Bruchez
Roxana Buga
Alix Buu-Hoang
Aurélie Ducrot
Caroline Juliano
Syrine Mansour
Maxime Marchionno
Nicolas Minder
Laura Puyet
Margaux Roulet
Tiberiu Totu
Nina Wahler

Supervisors

Thamani Dahoun
Philippe Renaud

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Contents

1 Summary for the SensUs website

Patients suffering from epilepsy see their lives affected in drastic ways. Not only do they experience seizures and unusual behaviors but their prescribed treatment, Valproic acid, often causes important side effects. It is paramount to effectively monitor their concentration of free VPA (fVPA) to keep secondary effects at bay. To do so, we have devised **RENO**, a **R**apid **E**lectrochemical impri**N**ted p**O**lymer based sensor.

Our small, portable device is designed to help neurologists quickly optimize and monitor their patient's VPA dosage, which is presently a lengthy and costly process. Additionally, RENO performs a fast electrochemical assay, determining fVPA concentration in under 5 minutes. Such rapid output will facilitate dose management by doctors, benefiting patients and insurances. RENO is user-friendly and smartphone connected via an app. Doctors simply deposit a drop of serum on our designed electrodes and insert them in the device which then displays the result on the screen. The sensing mechanism is based on a competition between fVPA, present in blood serum, and a probe known as ferrocyanide, already present on the electrode. Our electrodes are engineered with a molecular imprinted polymer, ensuring the selectivity of the assay and the biorecognition of fVPA. Learn more in our report!

2 Biosensor system and assay

2.1 Molecular recognition and assay reagents

The detection of free VPA is performed via electrochemical sensing based on a technology known as molecular imprinting (MIP). This technique generates artificial binding sites and receptors within polymers [1]. The polymer is prepared by using the targeted drug as a template, and incorporating additional compounds known as functional monomers. These groups provide complementary interactions with the target such as hydrogen bonds and ionic interactions, thereby increasing its affinity to the binding cavity. In our case, VPA serves as the template, and the selected functional groups are α-Cyclodextrin (CD) and acrylamide (fig.1a). These chemical groups were selected after investigating the binding of VPA with its natural targets: Albumin and Histone deacetylase (HDAC). As such, molecular recognition is ensured not only by size and shape of the cavity, but also by the weak interactions between the functional monomers and VPA. The carboxylic group of VPA will form an electrostatic bond with the NH_2 provided by the acrylamide, and the CD will form a hydrophobic interaction with the aliphatic chains of VPA, providing a "guest-host" interaction, and resulting in a chemically stable complex.

The assay, devised for free VPA monitoring, is based on a competition between the anti-epileptic drug and an electroactive probe known as ferrocyanide. At the surface of the VPA-molecular imprinted polymer (V-MIP), both probe and drug will compete for the binding pockets (fig.1b). Ferrocyanide will enter the binding site solely by interacting with the CD, where it will undergo an oxydation reaction that will be recorded. However, in presence of VPA, which by design has a higher affinity for the binding pocket, the probe will be blocked by the drug, unable to enter the cavity, resulting in a measurable drop of oxydation and thus a drop in the measured current.

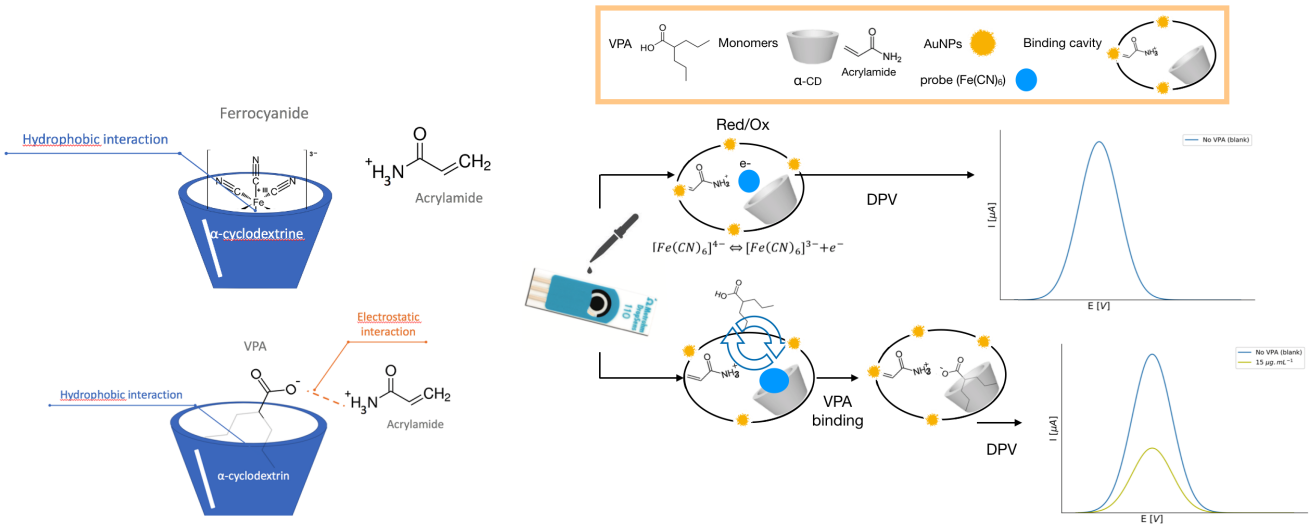


Figure 1: **a)** Schematic representation of molecular interaction at the MIP for both VPA and ferrocyanide.
b) Sensing mechanism of the competitive assay for the detection of free VPA

2.2 Physical transduction

During the assay, both molecules will be dropped on the screen printed gold electrode coated with MIP. Gold nanoparticles are embedded in the polymer to ensure a good conductivity of the electron emitted during the ferrocyanide oxydo-reduction. The device will then perform a differential pulse voltammetry with a $0.05V/s$ sweep rate. The ferrocyanide will produce a redox current measured by the potentiostat. As VPA competes for the electrode, the resulting current peak decreases. A peak detection will then be performed by the *"EmStat Pico module"* and sent to the Micro Controller Unit (MCU). The concentration is then computed using the difference between the blank measurement I_0 done by the manufacturer, and

the measurement I_1 done by the user. Finally, the device will convert the drop in current $\Delta I = I_0 - I_1$ to a VPA concentration with the calibration curve.

2.3 Cartridge technology

The device cartridge is the functionalized screen printed electrode, on which a small capped reservoir is mounted. The reservoir initially contains 30μL of ferrocyanide at $8.3mM$ in PBS at $pH7$. The user collects 20μL of serum using commercially available micro-pipettes, opens the reservoir and deposits the liquid onto the cartridge reservoir. The obtained 50μL of solution will homogenise by simple diffusion.

2.4 Reader instrument and user interaction

The user has two ways of communicating with the device, a touch-screen interface integrated on the biosensor and a phone application. The touch-screen will first show the main menu offering the three following options: start the measurement, open the information tab or turn off the device. If the user selects "Start" a reminder will ask him to make sure the electrode is plugged into the device and ask him to enter the blank value provided by the manufacturer. Once the measurement is done, the device will show the measured concentration on the screen. If the information tab is chosen the device will show the important information about the device such as its handling, VPA side effects and advice in case the user experiences side effects. Finally, the device will shutdown by choosing the off button. Figure 2 shows the main steps the user must follow to operate the biosensor.

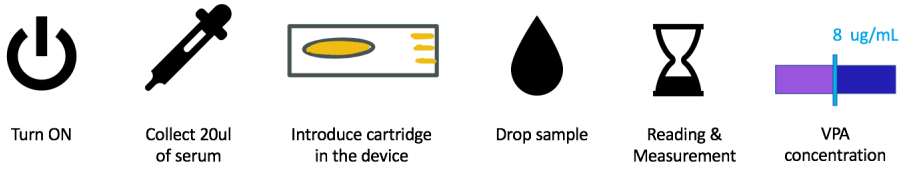


Figure 2: Main steps to operate the biosensor and perform the measurment reading

The device is separated into four parts if counting the WiFi module. The *LCD touch screen TFT adafruit 2.8 resistive* [2] is used as the interface between the sensor and the user. The screen is driven by an *Arduino mega 2560 rev 3* [3] which is the intermediate between the user interface and the potentiostat. The Arduino also performs the conversion from current measurement to VPA concentration. The potentiostat used is the *EmStat Pico Development Kit* [4] and performs the sample measurements. The circuit block scheme can be found in the annex. The MCU also sends the data through WiFi by communicating with an *ESP8266 module*. The parts are connected according to fig.7.

The software uploaded to the Arduino handles the communication with the potentiostat. In particular, sending the start signal and retrieving the measurements. It also registers the blank value to convert the measured current peak into the corresponding VPA concentration. The final dimensions of the device are $130 \times 70 \times 72$mm.

Furthermore, the user's interface goes beyond the physical device by adding a mobile application to the product set. This allows the storage of the patient's VPA concentrations, leading to a statistical overview. The communication between the Arduino board and the mobile application is done via Bluetooth, using the ESP8266 module and the programming is done using Blynk. The app prototype regroups 3 tabs. First, the "User's manual" which contains a simple using guide to allow the device handling by any individual, regardless of their background. Second the "Test's result" indicating VPA level and comparing it to a healthy value. Lastly, the "Stats" tabs offers a global view of the results in a clear chart that may be communicated to the affiliated specialist by a click of a button.

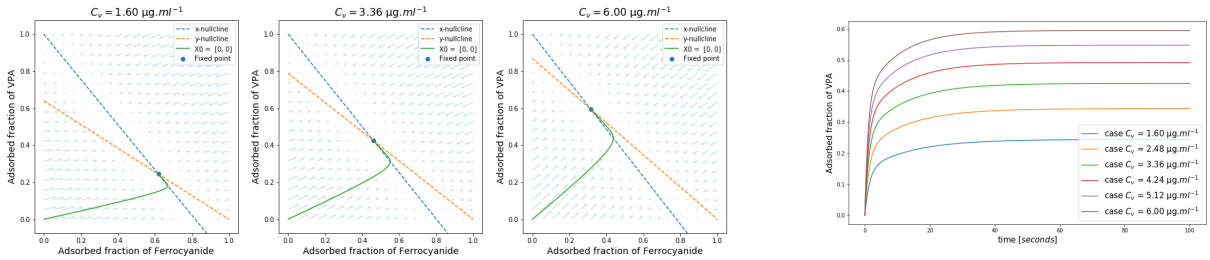
3 Technology feasibility

3.1 Simulation of the biological assay

The model used to simulate the biosensor electrochemical assay is inspired from the Lotka-Volterra model of competition between species. It can be used to describe the competition between an analog molecule, assumed to be the prey, and a target assumed to be the predator, over the same fixed ligands or capture molecules. The constraint here is that the analog must have a smaller affinity to the ligand in comparison with the target molecule, so that the targets are able to 'displace' the analog molecules off the ligands [5]. In the same way, we can assume that fVPA and ferrocyanide are competing for the occupation of the MIP cavities, as target and analog molecules, respectively. Such model can be written as Langmuir equations [5] describing the change of surface coverage in function of time for each molecule.

$$\begin{aligned} \frac{du}{dt} &= k_u C_u(1-u-v) - k_{-u}u - \beta C_v u \\ \frac{dv}{dt} &= k_v C_v(1-u-v) - k_{-v}v \end{aligned} \tag{1}$$

u and v are the adsorbed fractions of ferrocyanide and VPA respectively, with respect to the total number of cavities (ligands). C_u and C_v are the bulk concentrations of analog and target molecule close to the adsorbance sites in $[M]$. The adsorption and desorption rates are denoted as k_i and k_{-i}, with units of $[M^{-1}.s^{-1}]$ and $[s^{-1}]$. Finally, the $(1-u-v)$ term refers to the fraction of free ligands available for binding. The competition is enabled by the β term ($[M^{-1}.s^{-1}]$) corresponding to the displacement rate of analogs by the targets. We assume that the analog molecules do not affect the target [5].



(a) (b)

Figure 3: (a) Phase plane trajectory (with vector flow) and (b) Evolution of the adsorbed fraction of VPA in function of time, for different initial concentrations of fVPA in the therapeutic window : 1.6μg/mL - 6μg/mL.

Simulation results analysis

The simulation was performed for different fVPA concentrations located in the therapeutic window of $1.6-6$ µg.mL^{-1} (*i.e.* $4-15$ µg.mL^{-1} in the original non-diluted sample), starting from an initial state of zero adsorbed molecules, a fixed concentration of 5 mM ferrocyanide, with rate constants and competition term values as defined in section D.2 of the Appendix. Results are shown in Figure 3. It can be seen that an equilibrium is quickly reached between the two molecules, as they both converge rapidly (less than 1 minute) towards a fixed surface coverage marked by the stable fixed point (Figure 3(a)). This equilibrium largely promotes VPA binding (respectively ferrocyanide displacement), which increases with fVPA concentration in the sample volume, reaching approximately 60% of VPA coverage for the maximum concentration (and approximately 32% of ferrocyanide coverage). Hence, this supports the idea of measuring different peak current variations (*i.e.*$\Delta I = I_{blank} - I_c$) for different free concentrations of target. Note that if no fVPA is present in the measured sample, system (1) reduces to a single Langmuir model equation (*i.e.* first one with no competition term and $v = 0$) characterizing the adsorption/desorption of ferrocyanide, this later reaching up to 83% of surface coverage at equilibrium (after approximately 10s, not shown). Finally, from the kinetic curves presented in Figure 3(b), the time taken for the reaction to reach equilibrium at the electrode surface could be extracted (40s). Combined with the 12s needed for the Differential Pulse Voltammetry (DPV) measurements, this means that it would only take around 52s to perform the measure with this device, considering this preliminary model.

Current estimation

Since lab experiments could not be performed, DPV measurements were not obtained. These are indeed required to obtain a calibration curve over the therapeutic range defined above, that ultimately relates the decrease in peak current (ΔI) to fVPA concentration. However, a rough estimation of the maximum peak current (i_p) was computed using the Randles–Sevcik equation [6] at 25°C: $i_p = 2.69 * 10^5.n^{3/2}.A.D^{1/2}.C.v^{1/2} \approx 26$ µA, where A is the electrode surface area (taken as the area of the working electrode = 0.0314 cm^2), n the number of transferred electrons during the redox reaction ($n = 1$ for the probe used here) v is the scan rate (0.05 $V.s^{-1}$), C the electroactive species concentration (5.10^{-6} $mol.cm^{-3}$) and D its diffusion coefficient ($7.6 * 10^{-6}$ $cm^2.s^{-1}$ [7]). This maximum current is in the range of the used potentiostat but remains an approximation, since the addition of a MIP layer will certainly result in a decrease of the active area, hence a decreased current.

3.2 Electrochemical impedance spectroscopy

The a.c. Electrochemical Impedance Spectroscopy (EIS) represents one of the essential techniques [8] allowing the study of the electrical response of the system under study in terms of complex impedance, dielectric constant or conductivity, amongst others. Through EIS, it is possible to extract our functionalized electrode's electrical equivalent circuit. Such circuit represent the first requirement for designing a feasible interrogator circuit that should replace the current "EmStat Pico module", providing the interrogator electrical restrictions. Moreover, the EIS measurements have the critical advantage of an accurate assessment of the influence that the interaction between the sensor and the plasma solution has on the MIP's charge transfer ability, being the most suitable electrode characterization method. The real equivalent circuit, as well as the associated electrical elements, cannot be theoretically determined, requiring practical measurements. However, a priori knowledge about possible circuit configurations and the range of measured electrical parameters are usually necessary for extracting a reliable final model. Our expected equivalent circuit is presented in Figure 4. It consists of two parallel RC circuit that models two relaxation times, the reasons for choosing this model are available in the Appendix, section B.4. We expect that the AuNP resistance to be around a few hundreds kΩ [9], the value of the associated capacitance of AuNP is of few hundreds of nF [10], the solution resistance about 16 kΩ [11], while the charge transfer resistance could be about 128 kΩ [12]. The constant phase element (CPE) and the Warburg impedance cannot be associated with any initially imposed value, remaining with unrestricted degrees of freedom.

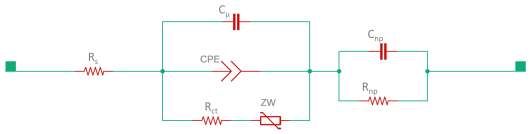


Figure 4: Electrochemical cell equivalent circuit : R_S - solution resistance, C_μ - Nagami-Havriliak general capacitor, CPE - constant phase element, R_{ct} - charge transfer resistance, ZW - Warburg impedance, C_{np} - NP capacitance, R_{np} - NP resistance

3.3 Limitations and future work

It is important to note that the provided model is only valid if certain assumptions are fulfilled (see Appendix, section D.1). Mainly, we considered here that reaction time is not delayed by diffusion, following ideal Langmuir kinetics. Furthermore, the rate constants were approximated in a semi-empirical (Appendix, section D.2) way to provide a proof of concept. The next step would be to do wet lab experiments to assess how the model performs against real data, which will enable to improve it and to measure the required constants. More importantly, experiments will allow us to calibrate the sensor for therapeutic ranges of fVPA, and to extract important parameters such as the sensitivity. Furthermore, a plasma sample pre-treatment (*e.g.* naffion membrane filtration) would be necessary to get rid of competitor molecules (Appendix, section D.3) and other unwanted proteins to promote the assay's success.

4 Originality

4.1 Team statements

The relatively low specificity and sensitivity of available electrochemical sensors were the main problems to overcome for a Therapeutic Drug Monitoring of VPA. With our device, we pushed the selectivity further thanks to molecular imprinting, and improved the biosensor's sensitivity through the gold nanoparticles [7]. The novel use of Cyclodextrine as functional monomer helps mimic the natural binding site of VPA, providing an additional tethering site to the polymer and hence promoting molecular recognition.

With the proposed sensor, the free fraction of VPA is directly measured, thus requiring no downstream computation of free VPA with regard to other molecule's concentration such as Albumin. We therefore ensure more accuracy in the measurement of free VPA levels.

We believe that the device originality also stands in the sample dispensing system.

Moreover, the sensor's digital interface as well as the application makes the biosensor system easy to operate for a user with no scientific background, while allowing an extensive data management easily made available to the medical body.

During the development of the biosensor's working principle and its prototyping, our team proved itself to be highly creative and autonomous. Indeed, molecular imprinting is not a topic of research in our university, which pushed us to educate ourselves on the subject and extrapolate the principle to VPA detection. Therefore, the presented protocol, models as well as simulations and hardware development were made entirely by our team members.

4.2 Team supervisor statements

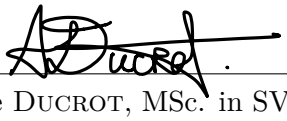
As SensUs is a student competition, leaving the choice of the technique to our students and letting them express their creativity was essential to us. After guiding them through the beginning of the competition and providing them with our experience from past editions, we supported them in choosing their detection principle. After exploring various technique, each having its advantages and down-falls, they chose to use a new technology in a promising field. Not only does this technique directly measures the free fraction of VPA in a rapid and precise way but using it to detect different molecules would be relatively easy and fast.

The EPFL Team demonstrated their autonomy while seeking guidance from various experts, gathering knowledge from different background and putting it together.

What they bring to this competition is not only a device using technologies that are rarely used in the case of bio-medical sensors, but also a prototype of an app and interface that are easy of use.

Philippe Renaud, Prof.
Full Professor, LMIS4

Thamani Dahoun, PhD.
Scientist, LMIS4



Aurélie Ducrot, MSc. in SV
Team Captain

Maxime Marchionno, MSc. in MT
Team Captain

5 Translation potential

5.1 Business Canvas

5.2 Stakeholder desirability

5.2.1 The need in the market

Epilepsy is a disease still poorly understood. There are several types of epilepsy and its causes are diverse, ranging from trauma and strokes to congenital malformations. In Europe, 1% of the population suffers from epilepsy, i.e. around 4.5 mio people are affected, including 900K children [13]. With 70% of epilepsy being treated with medication, meaning 3 mio patients are generally subjected to pharmaceutical treatment. However, finding the right drug and dosage may take time, which can be a significant burden for patient learning to live with their disease. To date, more than 20 drugs to treat epilepsy are available on the market [14]. The drugs administered in the first instance are generally new generation drugs, which do not require routine biological monitoring [14]. Older generation drugs such as valproate or phenitoin are still administered to a large number of patients who have generally been stabilized on the drug for some time.

Therapeutic drug monitoring (TDM) for epilepsy pharmaceutical treatment has never been done before. Dr Jan Novy, specialist in epilepsy pharmaceutical treatment, revealed that phenytoin (PHT) is a prototype substance for TDM. It has indeed non-linear kinetics, interactions with other drugs and a narrow therapeutic range. Conversely, valproate routine TDM is not used because the benefits could not be demonstrated. In light of this discussion, Helvet'Sens believes that it would be more advantageous to target PHT since the Swiss stakeholders, including specialists, might be more interested in the product if it is in line with their former research. This is why the sensor presented in this business plan will target PHT instead of VPA.

Both the Swiss macro environment and the industry being favourable to the development of new business in biotech put us in a great position to start a business (see Appendices E.1 and E.2). EPFL is located in the heart of the Health Valley, experts in the field of biotech. The proposed solution is very innovative and has never been seen on the market of therapeutic drug monitoring yet.

5.2.2 Value proposition

In average, an epileptic patient has quarterly follow-ups with his neurologist to monitor his PHT levels and have dosage adjustment if needed, resulting in a lengthy process. On a face-to-face consultation, the doctor takes a blood sample from the patient and send it to the laboratory for analysis. The laboratory determines the level of PHT in the blood by High Performance Liquid Chromatography (HPLC) or immunoassay. The neurologist will then receive the results and call the patients in case of dosage adjustment. This process can take up to 3 days from seeing the patient to receiving the results.

The RENO device makes the process easier by eliminating the need for a lab, making it more efficient and affordable. The neurologist can conduct the blood analysis himself by taking just one drop of blood, placing it on the cartridge and inserting it into the RENO device. The results are displayed on the LCD screen within one minute. The neurologist can give immediate feedback to the patient. The smartphone application is directly connected to the device and helps with the patient's data management on the long term.

We offer neurologists a device that they can monetize throughout their careers. Indeed, the price of the laboratory analysis for the patient is currently CHF 120 for HPLC and CHF 60 for the immunoassay. The purchase price of a consumable is fixed to CHF 50, making our prices competitive.Therefore, the healthcare for epileptic patients become more affordable and health insurances have less to reimburse.

In conclusion, the RENO device is user-friendly, portable, affordable and gives instant results. It facilitates the process of TDM and offers a better management of dosage and side-effects with minimum discomfort. There is currently no device on the market that can give doctors such timeliness and flexibility in the management of pharmaceutical treatment of epileptic patients.

5.3 Business feasibility

2021-2023: In the first year of R&D, we plan to use incubators of EPFL to have access to all the materials (chemicals, electronics, etc) and facilities necessary. Expertise from professionals for both the technical and business aspects of our biosensor would also be provided. We will take part in many start-up events to increase our visibility in the field of biosensors development and access grants [15], [16], [17] to generate enough incomes for development. From the outset, we want to hire a qualified and experienced regulatory employee who will facilitate our entry into the market. Based on the experience gained during the first year, we plan to obtain a partnership with Beckman&Coulter the next year, an industry already selling devices for monitoring of phenytoin, to take profit of their scale up potential for a future entry in the market. It would also allow us to outsource the development of the casing and the disposables. We will produce our final biosensor and begin the clinical tests to obtain the Swissmedic approval [18], allowing us to sell our product in the European union.

2023 : We will find neurologists who are interested in our device and willing to try it. We want to build a relationship of trust with them. Their feedback will be key in helping us adapt our product.

2024: This year marks the first entry trial in the Swiss market. We will target private neurologists and hospitals, using the distribution channel of Beckman&Coulter industry.

2025-2026: We will further expand our lead in Swiss market and enter the European market in 2025, targeting countries that use PHT the most as Spain and UK) [19]. After 2 years, we project to obtain the FDA approval and further expand to the American market, the biggest seller of PHT in the world. After that, we will use the versatility of our product conferred by the use of disposables to extend to other market segments such as detection of viruses, bacteria or to determine concentration of other drugs.

We already contacted some start-ups as bNovate and Caulys for partnership, to provide us useful tools to rapidly enter the market. Palm'Sens was also contacted and were of great help by providing us the potentiostat and electrodes for our prototype's development. After some emails, the possibility of having Palm'Sens as an OEM hardware supplier was determined.

5.4 Financial viability

Our product is sold to neurologists and hospitals. The neurologist makes a one-time purchase of the device and then buys the cartridges as a consumable. The selling prices are set at 400CHF for the device and 50CHF for the consumable. The application is offered free of charge. The cost structure and manufacturing procedures assure a considerable margin for selling the devices without losing competitiveness on the market. These include the fees of approval processes, patent, production, employees' salaries and marketing. Detailed cost and time to revenue estimation can be found in Appendices E.3 and E.4.

In the next two years, main charges would include salaries of the two co-founders and R&D costs. We first enter the Swiss market in 2024. We predict that we will sell our device to one third of neurologists, i.e. 200. On average, we can count 12 patients per neurologist and 3 tests per year per patient, which would make a total of 7'200 cartridge sales. We enter the European market in 2025. We estimate that we will sell our device to one sixtieth of the neurologists on the European market, i.e. 800 neurologists. The number of cartridges sold on the European and Swiss market combined now increases to 38'400. Obviously, our charges will increase. We will increase our number of employees to 15. Scale-up fees will also increase.

Our entry in the US market in 2027 is key. Indeed, we become profitable from this year on with a net profit of 3mio CHF over the year and a cumulative profit of 0 over the 6 years. We will already reach a constant revenue thanks to the consumables, which ensures a steady cash inflow every year while the market will continue to grow. In parallel, we can now expand our activities and allocate a substantial budget to the R&D department to start research on the detection of new molecules, bacteria or viruses. Our goal in the long term is to become a market leader in the therapeutic monitoring of small volatile molecules.

6 Team and support

6.1 Contributions of the Team Members

Emma worked on an alternative detection principle using fiber optics. Afterward, Emma worked on coordinating and perfecting the TRD report.
Ahmed was responsible for the simulation of the sensor's reaction at the interface and an estimate of the measurement speed. He helped designing the assay and is one of the creativity pitchers.
Loïc was in charge of the backup solution with Maxime. Loïc worked on the business feasibility and on the translation potential pitch. He made the device animations.
Roxana worked on developing an alternative technique based on optodes with metalloporphyrin enhanced membranes. She worked as well on the development of EIS equivalent circuit of the chosen technique.
Alix was the team leader for the business team and helped design the device's interface. She took part in different interviews and was in charge of social medias.
Aurélie is one of the team captains, ensuring the communication with SensUs organizers and keeping track of deadlines. She investigated the use of employing molecular imprinting for fVPA sensing, worked on designing the assay and functionalization protocol. She also helped with the business.
Caroline worked on another detection technique, which was not retained as the final choice. She joined the chemistry team and helped with the functionalization protocol. She worked on the technical pitch and is one of the pitcher. Also, she helped design the app and with social media.
Syrine is a microengineering student, who worked on the business part and on the conception and development of the device interface.
Maxime is one of the team captains, ensuring the communication between Helvet'Sens and SensUs organizers as well as keeping track of various deadlines. He was in charge with Loïc of the backup solution. He worked on prototyping the device. He also did the pitches' videos.
Nicolas He was one of the pitcher and worked on the translational pitch. For the device he helped with the communication between the different module of the device.
Laura worked with Emma on another technique that was not retained for the final competition. Laura did the device touch screen interface and worked extensively on the pitches.
Margaux worked in the business and chemistry team. She took part in different interviews. She wrote part of the protocol for the electrode functionalization. She also worked on the teaser pitch, designed the logo and managed the orders.
Tiberiu worked on selecting and modeling the EIS equivalent circuit, as well as the PCR regression model used for the sensor calibration. He also supported Roxana with one of the alternative techniques.
Nina worked on an other possible technique that wasn't retained for the final competition. She helped various sub-team. She also worked on the pitches.

6.2 People who have given support

Dr. Jan Novy is a neurologist at the CHUV who specializes in pharmaceutical treatments for epilepsy. He has provided us with valuable informations on the customers' journey and on current research in the field in Switzerland. He advised us to target PHT rather than VPA for our business plan.
Epi-Suisse is an association for epilepsy. It forms a large network of patients in Switzerland. The association has agreed to publish our patient survey in its newsletter so that we can contact patients directly and obtain their opinion on therapeutic drug monitoring for the treatment of epilepsy.
Caulys is a start-up formed by alumni students of the EPFL. We were able to discuss cost estimation and time to revenue with them. In particular, they advised us to have a short and long term business strategy.
Arnaud Bertsch is a chemist, scientific collaborator working in Prof. Renaud's laboratory. He has a strong background in microfluidics. Thanks to him, we were able to write a clear and detailed protocol to continue this project in the laboratory, once access has been restored to students.

Irene Taurino is one of the experts in Molecular Imprinting which supervised and guided us for the development of the assay. She also provided advice on pitches and shared her experience on electrochemical sensing.
Sandro Carrara is one of the professors from EPFL who initially agreed to give us access to his lab prior to the Covid-19 pandemic. Despite this, he agreed to continue to support our project by guiding us and supervising us for the elaboration of the assay, the electronics and simulations.
Maria Antonietta Casuli is an expert on electrochemical sensing with cyclodextrin who has followed us throughout this project. She has shared her knowledge and experience which was invaluable to elaborate this assay and device. She has also guided us for the selection of the electroactive probe so as to obtain the best electrochemical signal possible.

6.3 Sponsors

7 Final remarks

We envisage further development of the device, as we want to make it possible to test starting from whole blood instead of serum samples. In that way, we hope to minimize even further the time and steps required to perform the assay. Moreover, we would like to work on improving the re-usability of our device and the disposal process so as to make it more sustainable.

Our device also offers promising perspectives for future uses in clinical settings. In fact, our technology may be adapted to other targets by devising novel functionalizations which would be tailored to the detection of different molecules.

Finally, we are very thankful to the SensUs organizers who helped us navigate through these uncertain times and still manage to keep the competition going. We are also grateful to our supervisors, Prof. Renaud and Dr. Dahoun, for giving us pertinent advice. We hope next year's competition will be restored to its original format for future students, but we are happy with our challenging experience and what was accomplished.

Appendices

A Study of VPA's binding site

VPA's main target in human serum is albumin. The interaction between the drug and protein is somewhat complex. Indeed, there are 10 distinct secondary binding sites, and more importantly, there are 2 primary binding sites characterised by an association constant of $22.6x10^3$L/mol [20]. Additionally, VPA is known to interact with a number of drugs such as Warfarin, benzodiazepines and profens as they, as well, target those primary binding sites [21]. In order to identify specific binding requirement for VPA, these primary binding sites were studied. Looking at Figure 5, it can be seen that ibuprofen's carboxylic group interacts with Arginine, Lysine and Tyrosine residues via hydrogen bonds. Similarly, Warfarin's oxygen atoms forms a hydrogen interaction with the same amino acids. Keeping in mind that VPA competes with these drugs and has somewhat similar chemical composition, it can be hypothesized that it uses similar interaction when competing for these binding sites.

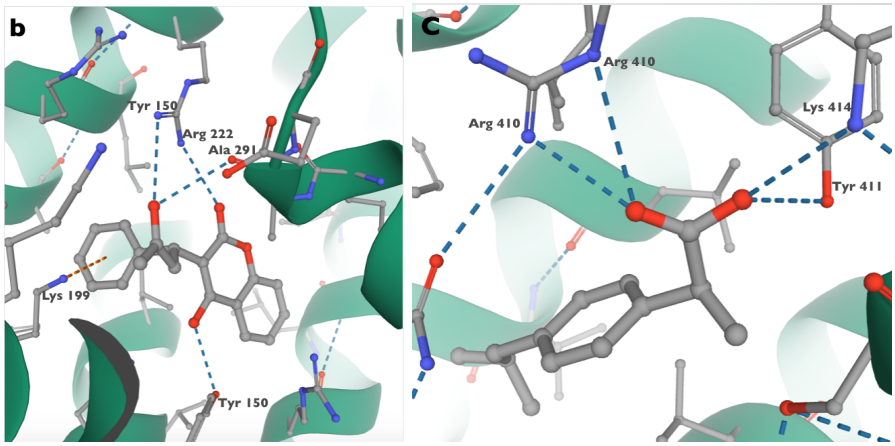


Figure 5: **b)** Interaction *Warfarin* binding site 1 of HSA in subdomain IIA. Source: PDB 2BXD. (Blue line: hydrogen interaction; orange line = cation pi interaction) **c)**Interaction *Ibuprofen* binding site 2 of HSA in subdomain IIIA. Source: PDB 2BXG.(Blue line: hydrogen interaction)

Recently, it has also been shown that VPA might be an effective cancer drug due to its ability to inhibit HDAC class I and class II proteins. It is hypothesised that VPA performs this inhibition by binding to the catalytic center of the enzyme [22, 23]. Figure 6 shows the interaction of VPA with the catalytic site of HDAC8. Looking at the binding site, weak interactions are taking place between the carboxylic group of VPA and Tyrosine and Lysine residue. Moreover, potential hydrophobic interactions forms between the aliphatic chain of the drug and hydrophobic amino acids such as phenyalanine or alanine and valine present at the Hydrophobic Active Site Channel (HASC) (fig. 6).

Taking all this into consideration, it can be hypothesized that to have adequate binding of VPA to the electrode surface, it requires: a lipophilic group for hydrophobic interaction with its aliphatic chains, a chemical compound to form hydrogen bonds with its carboxylic group, ensuring molecular stability and a NH2 group for possible electrostatic interaction to improve the binding affinity.

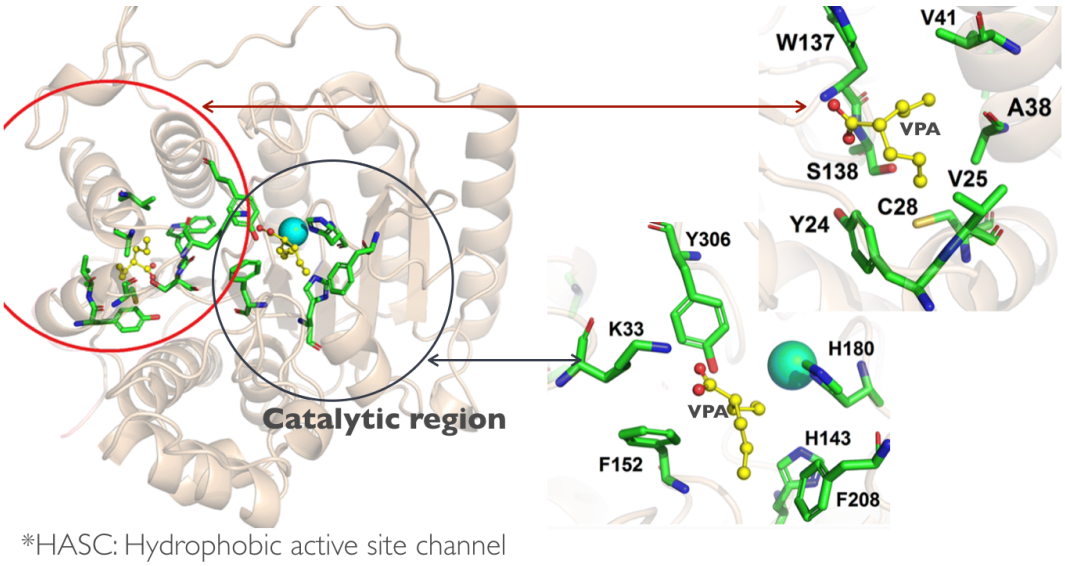


Figure 6: Interaction between VPA & the hydrophobic active site channel (HASC-red) and catalytic region (grey) of HDAC8 enzyme. (Figure adapted from original paper: "Exploring the inhibitory activity of Valproic acid against the HDAC family using an MMGBSA approach", Sixto-López et al., J Comput Aided Mol Des, 2020 [24])

B Protocol

The objective of this protocol is to functionalize the surface of a screen-printed electrode for the detection of free VPA in human serum. The protocol is heavily based and inspired by the following paper [7]

Molecular imprinting: Functionalization is performed using the technique of molecular imprinting. Molecular imprinted materials are prepared using a template, generally the analyte of interest (VPA), and functional monomers. Functional monomers assemble around the template forming additional interaction with the template. Gold nanoparticles present good optical, electrical and catalytic properties, and therefore can be used in combination with molecular imprinted polymers to amplify the output signal. For this work, it's their notable electrical properties which is of interest. AuNPs enhance the conductivity by allowing a faster electron transfer between the redox probe and electrode surface. The AuNPs solution, the chemical mixture of monomer as well as the template (VPA) are deposited onto the screen-printed electrode. A polymerization step is required to cross-link the monomers and create the polymer matrix, i.e the molecular imprinted polymer (MIP). Finally the template is removed and what is left is a cavity complementary in size and shape to the template with high affinity and specificity to the target molecule VPA.

Competition assay: Potassium ferrocyanide (K4[Fe(CN)6]) is used as the redox probe to characterize the electrochemical sensing. The molecule is small enough to fill the recognition sites left by the MIPs and compete with VPA when the latter is present in the sample. Cyclic voltammetry (CV) is first used to characterize the intensity peaks of the functionalized electrode before extraction of the template. It can also confirm the surface modification by comparison of the peak with the bare electrode. Differential pulse voltammetry (DPV) can then be used as a technique to measure the electrochemical activity of the sensor in presence of different known VPA concentrations. The obtained voltammograms show different intensity peaks of ferrocyanide; the lower the peak, the higher the VPA concentration. A calibration curve can then be fitted to determine a linear relationship between the intensity and the concentration.

Element	Concentration
VPA	pure
Ethanol	90%
gold (III) chloride trihydrate (HAuCl4-3H20, 99.99%)	99.99%
N'-methylene-bisacrylamide (NNMBA)	71 mg/ml
Acrylamide (AAM)	6 mg/ml
Beta-CD	6 mg/ml
N,N,N',N-Tetramethyl-ethylenediamine (TEMED)	5%
Ammonium persulphate(APS)	13mg/ml
Potassium ferrocyanide (K4Fe(CN)6)	5mM
PBS	0.01M, pH 7
Distilled water (DW)	-
Human serum	-
Trisodium citrate dihydrate	0.5g/50ml

Table 1: Reagents. Concentration adapted from Motia et al. [7]

PPE: Gloves, Eyewear, goggles, Lab coats, Fume hood

Materials: Listed reagents (see table 1), Screen-printed electrode (Palmsens, AC1.W1.RS Dw = 2): WE material: gold, CE material: gold, RE material: silver. Magnetic beads for stirring, Beakers, Pipettes, Condenser, Double necked flask (250 or 150 mL)

Instruments: Heater, Hood, Stir plate, Electrochemical Analyzer/Workstation : Model 600C Series Electrochemical Analyzer/Workstation (CHInstruments, Inc., Austin, TX)

Molecular imprinting chemical mixture preparation

1. AAM (6 mg/mL), Beta-CD (6mg/ml) and NNMBA (71 mg/mL) were mixed in a PBS buffer (0.01 M, pH 7.0) in 0.5:0.5:1 vol ratio.
2. 1 mL of APS solution (13 mg/mL) and 20 μL of TEMED (5%) were added to the previous mixture to initiate and accelerate polymerization, respectively.
3. A volume of 1 mL VPA (1 mg/mL in DW) was also added to the previously obtained solution.

Electropolymerization

1. Pre-treatment of Au-SPE was performed by rinsing with ethanol and distilled water (DW).
2. 50 μl of final chemical mixture is mixed to 1ml AuNPs solution
3. Au-SPE functionalized with the final chemical mixture prepared. 50 μl of the mixture is deposited onto the screen-printed electrode.
4. Electrochemical analyzer is used for electropolymerization using cyclic voltammetry:
   - Temperature: room temperature
   - Scan rate: 50mV/s
   - voltage window: -0.4 to 0.2V
   - maximum nb of polymerization cycle to optimize
   - Init P/N: to optimize

C Electronics

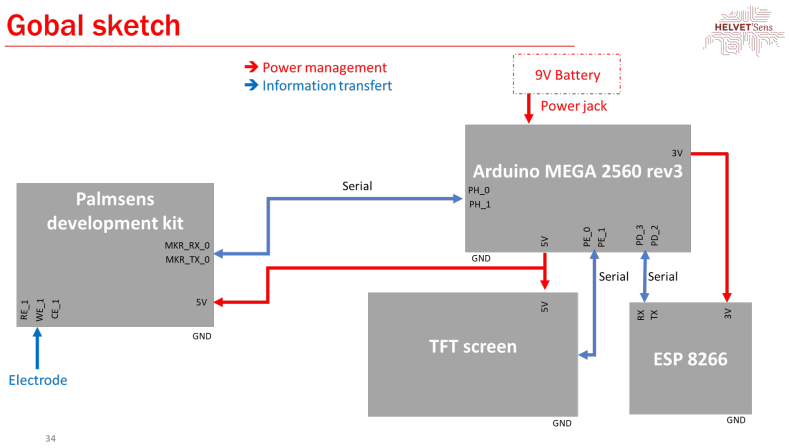


Figure 7: Box scheme of the electronics device

D Competition assay simulation

D.1 Model assumptions

The simulation done previously for the competition assay considered that the occupation of the imprinted cavities via adsorption of either the target molecule (fVPA) or the electroactive probe, can be described by a Langmuir model. The choice of this kinetics was further motivated by the fact that these molecules are retained in the MIP cavities by mean of non-covalent and weakly reversible interactions, *i.e.* target (and probe) are constantly captured and dissociated (at a smaller rate though).

Hence, it is important to keep in mind that the provided competition model is only valid if certain assumptions are fulfilled [25]. Mainly, a Langmuir kinetics assumes that the adsorption process is not limited by diffusion, and hence we considered in this work that the diffusion time to the capture sites is very small compared to the reaction time. This was particularly motivated by the nature of the assay and the small volume considered for the measurement (*i.e* dropping target and probe directly on the functionalized surface). Moreover, we considered that only one functional monomer (*i.e.* the CD) is responsible for most of the binding at the molecularly imprinted cavity, and hence we neglected the interaction between VPA and acrylamide, given that multivalent interactions (as well as interactions between free target molecules) are excluded in a Langmuir model of adsorption. Instead, the effect of this additional interaction has been translated to a higher affinity of the cavities towards VPA. Other assumptions include the need for the adsorption process to be reversible, and for the binding sites to have the same probability of adsorption, with an adsorption event assumed to be independent of the others. These latter assumptions are assumed to be valid considering our functionalization scheme (Figure 1), *i.e.* MIP cavities that bind the target with non-covalent binding; plus, the cavities are all similar. Additionally, the free concentration of adsorptive molecule is assumed to be constant in solution (*i.e.* the number of adsorbed molecule is negligible compared to that in solution). This could be accomplished by, for instance, carefully selecting and optimizing the concentrations of functional monomers used in the electropolymerization process so that the number of cavities is sufficiently small compared to the number of adsorptive in the sample volume. Finally, the adsorption is assumed to happen in monolayer formation only. Since the thickness of the MIP film generated through the electropolymerisation process can be controlled with ease [26], we can think of this value to be very low, in order to approximate a monolayer formation. A simplified scheme of the adsorption at the electrode surface (MIP cavities), the one we consider for our modeling, is shown in Figure 8.

Figure 8: Representation of the simplified adsorption process at the MIP cavities. α-cyclodextrin (CD) is shown in grey, working electrode (functionalized with AuNPs) in gold.

In conclusion, this model is only valid within the framework of the hypotheses mentioned and, even if one assumes that most of them are validated, these simulations must be compared with experimental results in order to ensure that the model is indeed adequate.

D.2 Choice of model parameters

The adsorption rates k_{on} and k_{off}, as well as the dissociation constant relating them ($K_D = \frac{k_{off}}{k_{on}}$) can be determined experimentally by kinetic measurements [25]. This being impossible since no experimentation could've been done for this investigation, an approximation of these values was made.

Considering that the selectivity of the molecularly imprinted cavities (MIP) maximizes the VPA (v) binding, it is reasonable to assume a lower affinity for the ferrocyanide (u), *i.e.*, a higher dissociation constant:

$$K_D^u = \frac{k_{-u}}{k_u} > K_D^v = \frac{k_{-v}}{k_v} \tag{2}$$

A measure of the dissociation constant between VPA and its Antibody (Ab) was reported to be of $K_D = 90 \pm 21$ µg/ml or $\approx 6.24 * 10^{-4} M$ considering a value of 90 µg/ml [27]. In the model, we supposed that the binding affinity of the MIP cavities towards VPA is of $K_D = 6.24 * 10^{-6} M$ (100 folds higher compared to Ab), with $k_v = 10^4 M^{-1}.s^{-1}$ and $k_{-v} = 6.24 * 10^{-2} s^{-1}$, considering that MIPs can yield a selectivity and a binding affinity on par with those of Abs [28] and that these cavities are highly selective and specific to VPA. $k_u = 1.10^2\ M^{-1}.s^{-1}$ and $k_{-u} = 1.10^{-1}\ s^{-1}$ were chosen in order to satisfy the condition (2). The concentration of ferrocyanide (analog) is a constant ($5mM$) chosen for the assay and the displacement factor β is set to be equal to 10 times the adsorption constant of ferrocyanide.

D.3 Some metabolites similar to VPA that are present in the blood

To identify possible candidates which could interact with the sensing, the serum metabolite database was searched using the available "ChemQuery Structure Search" [29]. As specifications for the search, VPA's structure was given to the algorithm in addition to a molecular weight range of 100-200 $g.mol^{-1}$, and a similarity ratio of 0.75. Besides, we looked among "Detected and quantified" metabolites. From the 18 molecules which appeared as a result, only 6 were present in human blood (*i.e.* serum): these latter represent example of potential competitors that could bind to the MIP cavities. We can particularly mention caprylic acid and capric acid with natural concentrations that can be higher than the therapeutic range of $4 - 15$ µ$g.mL^{-1}$ fVPA adressed in this work. This, in addition to their structure that is very similar to VPA (aliphatic chain and carboxyl group) would greatly facilitate their adsorption within the cavities. Hence, an adapted prea-treatment strategy needs to be carefully evaluated for our sample.

D.4 EIS equivalent circuit

The electrochemical method allows the study of the characteristics and behavior of the modified sensor with MIP-Au NPs. For the impedance measurements, it should be used an electrochemical system with a three-electrode cell arrangement: the working electrode (SE) being our sensor - the modified electrode, the counter electrode (CE) with the associated reference electrode (RE). At the level of the reference electrode, it is measured the potential ($V_{measured}$). On the CE is applied the input voltage (V_{input}) and here the total current is provided (I_t). At the level of the SE, it is measured the output current ($I_{measured}$).

The applied frequency range should be from 100 mHz to 1.0 MHz, and the applied a.c. amplitude may be considered to be 0.005 V [12]. The data integration will be set at 5 cycles with 20 steps per decade.

The Nyquist plot should include two semicircles observed at high and medium frequencies accompanied by a straight line at a lower frequency. The semicircle from the high-frequency domain allows the assessment of the charge transfer resistance, R_{ct}. Also, the electrochemical impedance investigations determine some of the critical electrical parameters of the modified electrode such as electrical conductivity or relative permitivity. The relative electrical permitivity could be calculated from the measured geometrical capacitance and the geometrical characteristics of the MIP, width and surface, and it depends on the MIP's homogeneity. The dielectric constant gives essential information on the charge transporting ability within MIP-AuNP. Higher would be the dielectric constant value, then a better ability of the MIP to hold a more substantial charge will be recorded. In a complex polymeric matrix as our MIP, the presence of AuNP influences the dielectric permitivity and electric conductivity with a significant influence on the charged species transport mechanism [30]. The electrical conductivity , allows us to correlate the distribution of the charges and their mobility within the MIP-AuNP modified electrode. The classical EIS experiments are based on the assumption that the potential drops homogeneously across the MIP-AuNP. However, such a hypothesis is not valid as the composite system MIP-AuNP might be inhomogeneous, having insertions that generate potential changes with different profiles across the polyacrylamide matrix. If the content of the nano-filler –AuNPs is low, then the assigned equivalent electrical circuit could be associated with a simple Randall's circuit. In such a case, a single semicircle would be expected on the Nyquist diagram. However, when the nanoparticles concentration is significant in such a way to make a difference in the MIP's behavior, then an elaborated equivalent circuit should be considered. A capacitive element, C_{np}, in parallel with another resistance (R_{np}), should be introduced. Much more, following the possible inhomogeneity of the MIP-AuNP matrix structure, a capacitive element for modeling the inhomogeneities have to be also considered, C_{μ}. This capacitance termed Nagami-Havriliak general capacitor is usually exploited during the selection process of equivalent circuits in order to model unexplained nonlinearities. The electrochemical behavior of the modified electrode based on the MIP-AuNP matrix could be modeled using the equivalent circuits presented in Figure 4 from the Section 3.3.

The proposed equivalent circuit generates on the electrochemical impedance spectra two semicircles that might be or not fully resolved. The semicircles indicate two relaxation processes. As a consequence, the MIP-AuNP system could be characterized by two different time constants. The presence of any linear part in the low-frequency range associated with the included Warburg impedance in the equivalent circuit, shows that the exchange process at the level of the modified electrode occurs with diffusional control.

After the immersion of the modified electrode in the plasma sample containing valproate ion, the MIP-AuNP should swell, facilitating the electron-transfer process between the electrode and the solubilized redox species [12]. As a consequence, the charge transfer resistance is expected to decrease. Various level in the R_{ct} decreases would show the ability of the MIP-AuNP membrane to sense the VPA.

E Translation Potential

E.1 PEST analysis

Political factors: In Switzerland, the Health2030 plan has just been implemented. The Federal Council intends to further improve the Swiss health system so that everyone will benefit in the future from a high-quality and affordable health system.
Economical factors: Helvet'Sens grows in the heart of the Health Valley and benefits of high skilled workforce. Education in bioengineering is of the highest quality and international renown. But, the Swiss healthcare system is under great pressure. Health costs are rising, the number of non-communicable diseases is also increasing. Depending on socioeconomic status, access to care is unequal. Some households have difficulty paying their health insurance.
Socio-cultural factors: The resident population enjoys a long life expectancy and good access to the health care system with the system LAMAL. Every citizen benefit from basic insurance. However, for people with epilepsy, it is very difficult to have access to complementary insurance. Increasingly, individualized care is being developed. In Switzerland, therapeutic drug monitoring is already widely used by patients suffering from diabetes or mood stabilizers, but is only beginning to be used for epilepsy.
Technological factors: Microfabrication is becoming extremely efficient in terms of production. Innovative measuring methods are constantly being discovered. Data analysis tools are also evolving; Machine Learning is starting to be used in biotechnology to identify compounds.

E.2 Porter's 5 forces

Threat of new entrants **(medium):** First class technology, infrastructure and research institutes promote start-ups and spin-off. Capital investment in biotech companies continues to grow. Switzerland has a large range of modern research laboratories and production facilities for pharmaceutical, biological and medical technology products. The presence of more than 700 life science companies, including capital-intensive and world-leading pharmaceutical companies (Novartis and Roche), opens up numerous opportunities for the commercialization of intellectual property products.
Threat of substitutes **(high):** VPA and PHT are old generation drugs. Today this substance is not given in the first instance, hence market growth is limited. New generation drugs are preferred. A second potential substitute is all non-pharmaceutical treatments for epilepsy, including inhibitory stimulation and surgery. But for now, these alternative treatments are very expensive.
Bargaining power of customers **(medium):** Hospitals, clinics and pharmacies do possess a certain bargaining power. They can ask for affordable, simple biosensor kit. Otherwise they might prefer to send directly the sample to laboratories which will manage the analysis. As well, individual customers will ask for affordable product, since such a kit could not be covered by their health insurance.
Bargaining power of suppliers **(low):** Both workforces and material resources are abundant. Our technology depends on the pharmaceutical industry in the sense that they control the production of valproate. We depend also on subcontractors who take care of functionalizing surfaces and molecules.
Competitive rivalry **(medium):** Competitive intensity is significant as there are few barriers to entry and the threat of substitutes for valproate should be considered. However, TDM monitoring for the treatment of epilepsy is not yet applied in Switzerland and very innovative. PHT is a prototype drug for TDM. We would be the first on the market offering this product and service.

E.3 Cost estimation

price of raw material of the sensor (prototyping)

Sector	What	Cost (CHF)	Cost (USD)*
Electronics	EMStat Pico	2.62	2.88
	PCB	100.00	110.00
	Small material (cables, connectors, ...)	10.75	11.83
Prototyping	Shield tactile TFT arduino	48.55	53.41
	ESP8266 WiFi, Adafruit	21.75	23.93
	9V Battery	9.35	10.29
	MCU arduino Mega2560	46.75	51.43
	3D printing	5.00	5.50
Others	**Total cost**	**250.52**	**257.57**

* The change rate is 1 CHF = 1.10 USD (current situation on 7th of August 2020)

Cartridge cost per use

Sector	What	Cost (CHF)	Cost (USD)*
Chemistry	Chemicals	0.30	0.33
Electronics	Screen-printed electrodes (Gold)	1.50	1.65
Others	External risks	3.20	3.52
	Total cost	**5.00**	**5.50**

* The change rate is 1 CHF = 1.10 USD (current situation on 7th of August 2020)

E.4 Time to revenue

(CHF)	**2021**	**2022**	**2023**	**2024**	**2025**	**2026**	**2027**
Charges	-500'000	-780'000	-757'000	-1'622'000	-4'530'000	-4'242'000	- 5'634'000
Income	200'000	500'000	5000	440'000	3'040'000	5'320'000	8'560'000
Net Loss/Profit	-300'000	-280'000	-752'000	-1'182'000	-1'490'000	1'078'000	2'926'000
Cumulative Loss/Profit	-300'000	-580'000	-1'332'000	-2'514'000	-4'004'000	-2'926'000	0

	Key activities	Market	Employees	Sales	Charges (CHF)	Entries (CHF)
2021	R&D Prototyping Fundraising	-	2 Co-founders 1 Regulatory	-	Salaries : 450'000 R&D : 50'000	Grants 200'000 (EPFL)
2022	Partners and outsourcing Clinical tests Certifications	-	1 CEO/CTO 1 Regulatory 2.5 Engineers 0.5 Admin	-	Salaries : 750'000 Swissmedic : 20'000	Grants 0.5 mio (Innosuisse)
2023	Transition year from R&D to commercialization Scaling-up	CH: first clients 20 neurologists	1 CEO/CTO 1 Regulatory 2.5 Engineers 0.5 Admin	Device: 20 Cartridge: 720	Salaries: 750'000 Production costs Device : 5'000 Cartridge: 2'160	**Sales** **Device: 5'000** **Cartridge: 0**
2024	Enter the Swiss market Scaling-up partners	CH : 1/3 markets 200 neurologists	1 CEO/CTO 2 Regulatory 1 marketing 4 Engineers (outsourcing) 1 Admin	Device: 200 Cartridge: 7200	Salaries: 1.35 mio Production costs Device : 50'000 Cartridge: 21'600 Procedural: 100'000 Scale-up costs: 100'000	**Sales** **Device: 80'000** **Cartridge: 360'000**
2025	Enter the European market Expand Swiss market Certification US	CH : 2/3 market 400 neurologists EU : 1/60 market 800 neurologists	1 CEO 1 CTO 2 Marketing 3 Regulatory 8 Engineers (outsourcing) 2 Admin	Device: 1000 Cartridge: 52'800	Salaries: 3 mio Procedural: 500'000 Scale-up costs: 500'000 FDA: 16'000 Production costs Device : 250'000 Cartridge : 264'000	**Sales** **Device : 400'000** **Cartridge : 2.64 mio**
2026	Expand swiss market and EU market	CH : entire market 600 neurologists EU : 1/30 market 1600 neurologists	1 CEO 1 CTO 2 Marketing 3 Regulatory 8 Engineers (outsourcing) 2 Admin	Device: 1000 Cartridge: 98'400	Salaries: 3 mio Procedural: 500'000 Production costs Device : 250'000 Cartridge : 492'000	**Sales** **Device : 400'000** **Cartridge: 4.92 mio**
2027	Enter US market Expand EU market R&D other moleucles	CH : 600 neurologists EU : 1/20 market 2400 neurologists	22	Device: 1800 Cartridge: 156'800	Salaries: 3.3 mio Procedural: 700'000 R&D costs: 400'000 Production costs Device : 450'000 Cartridge : 784'000	**Sales** **Device : 720'000** **Cartridge: 7.84 mio**

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