AixSense

RWTH AACHEN UNIVERSITY

Team Results Document

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SensUs 2020

Team members: Luca Fehlings, Jerome Jayakar, Anirudh Kudiramurthy, Hendrik Kupper, Stefan Leisten, Abarna Ravichandran, Anish Russel, Shilpa Suresh

Supervisor: Prof. Dr. Sven Ingebrandt **Coach:** Dr. Vivek Pachauri

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Summary for the SensUs website

We developed a sensor based on electrical impedance spectroscopy (EIS) using micro-scale inderdigitated electrodes (IDEs) coated with a metal-organic framework (MOF) for the detection of valproic acid in a time frame of 5 minutes. Here we use microsystem technology methods in order to minimize and integrate our sensor, reducing the detection limit and fabrication costs in the process. The novel MOF material allows us to achieve precise measurements, even for low concentrations, by creating a porous, crystalline functional interface with a high effective surface area. Additionally, due to the system being based on the readout of electrical properties, the system is reliable, easy to use and requires next to no maintenance or trained personnel to operate. Further, the readout data can be directly processed and the corresponding valproate concentration can be calculated and displayed to the user.

1 Biosensor system and assay

1.1 Molecular recognition and assay reagents

Gold interdigitated electrodes (IDEs) (Fig. 1.1) functionalized with a Terbium-based metal-organic framework (MOF), $[Tb(BTC)(H_2O)_6]$, are used for the recognition of valproic acid (VPA). MOFs are solid crystalline materials which have distinctive porous compounds possessing metal centres bonded to organic linkers (Molecule bridging ligand). The MOF provides a high surface area along with good mechanical and chemical stability, making it a promising candidate for reliable sensor measurements. With this surface functionalization, measurements based on luminescence, field effect, and impedance are made possible [1]. Here, the VPA molecule size agrees with the pore dimension of the MOF, allowing us to capture and recognize the VPA using our surface functionalization.

1.2 Physical transduction

Measurements are done using electrical impedance spectroscopy with a three-electrode setup. Here, one of the finger pairs, the working electrode is coated with the MOF, while the other finger pair is left uncoated. The reference electrode is a commercial miniature Ag/AgCl reference electrode supplied through an inlet.

Upon contact with the analyte, the impedance change over time of the working electrode is registered at a specific frequency. To get reliable measurements, both the impedance after a specific time frame as well as the slope of the impedance change over time are considered.

1.3 Cartridge technology

The sensor consists of 7 x 7 mm chips with a PDMS microfluidic system on top of the chip. These chips are glued on a chip carrier and electrically connected with bonding wires, which are passivated using PDMS. The cartridge consists of these chip carriers including the microfluidic layer and the chips and can be connected to the device by
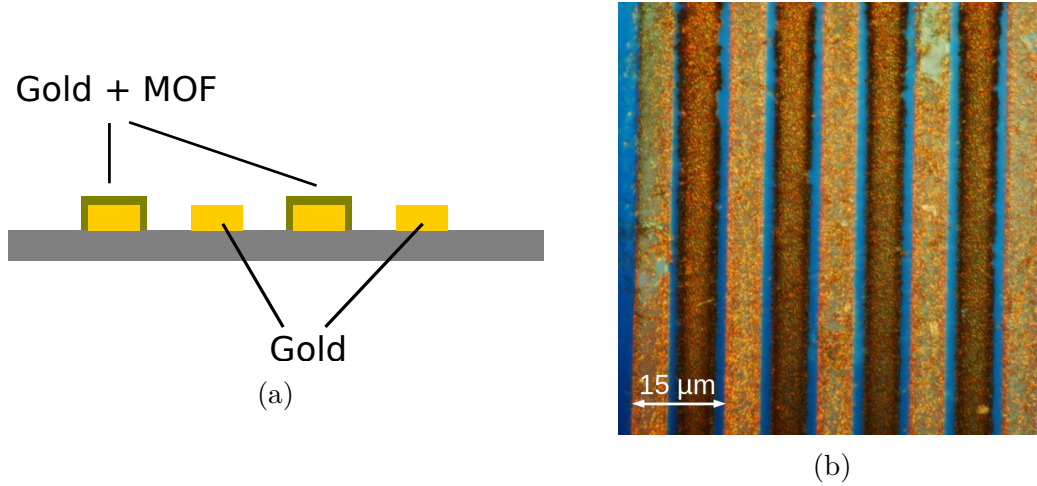
Figure 1.1: (a) Schematic of funtionalized IDE platform , (b) Microscope image of functionalized IDE platform, the dark fingers are coated with a thick MOF layer, the brighter fingers with a thinner layer.

insertion into a chip socket.

The PDMS microfluidic system provides an inlet and an outlet where the sample can be inserted and removed using a pipette. The system uses capillary channels to transport the analyte over multiple IDE sites in series or parallel, allowing for differential measurements or use of the IDEs for dielectrophoretic separation.

## 1.4 Reader instrument and user interaction

Readout of the impedance measurements is done by an EmStat Pico (PalmSens BV, Houten, Netherlands) which is connected to the 16 IDE working electrodes via multiplexers. All channels share a common counter and reference electrode which are directly connected to the EmStat Pico. The EmStat Pico communicates its readout data with a microcontroller via UART, so that the microcontroller can calculate the VPA concentration using a calibration curve. The calculated concentration is communicated to the user using a display. Additionally, raw data and a log of results are stored on the integrated memory.

Cartridge change can easily be done by the user by removing the cartridge from the spring-loaded socket and inserting the new cartridge. The whole prototype package has footprint of 110 x 80 x 70 mm

2 Technological feasibility

The sensor uses micro-scale gold interdigitated electrodes (IDEs) on a glass substrate fabricated using standard clean room lithography technology processes. Therefore, fabrication is cheap, allows mass production and very high reproducibility. These IDEs allow precise electrochemical impedance spectroscopy (EIS) as well as functionalization of every single electrode separately as done here using metal-organic frameworks to enhance the impedance readout. This allows different MOF-layer characterizations on a single chip.

The electrochemical MOF deposition process, using cyclic voltammetry in a voltage range of -1.5 - 1.5 V with a scan rate of 100 mV/s, has shown high reliability and reproducibility. Additionally, the deposited MOF films showed good mechanical stability over the time frame of the project. The MOF functionalized IDEs show a higher surface roughness and therefore a higher surface area than the gold IDEs, as indicated by the enhanced cyclic voltammetry response using a ferrycynide redox couple (Fig. 2.1 (a)). This allows a precise sensing even in small concentrations. In the presence of the VPA, the impedance of the MOF functional layer increases in the low frequency range, presumably due to the VPA accumulating in the pores of the MOF structure.
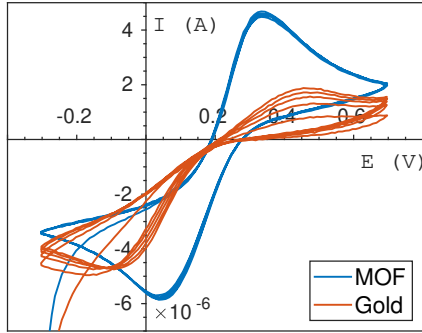
(a)

(b)

Figure 2.1: (a) CV redox couple response of MOF funtionalized IDEs. (b) Packaging of the sensor prototype, including cartridge socket.

One problem, however, is that the reliability and long term term stability of the MOF layer under test conditions (e.g. blood plasma) are not investigated properly and no

procedure is available to clean the functional layer of residue. Additionally, the noise added by varying molecule and ion concentrations across different blood plasma samples has not been considered yet.

The IDE chip with 16 electrode pairs is designed with a microfluidic system in mind and therefore allows further microfluidic integration for fluid handling and separation. Using the EmStat pico and multiplexers controlled by the Pico's GPIO ports, readout can be performed on all electrode pairs in order to take advantage of different functionalized areas, make blank measurements, and rule out false positives.

Fig. 2.2 shows the impedance of one MOF-coated IDE at different frequencies and VPA concentrations in the clinically relevant range. While these first results show a promising trend, further experiments need to be conducted and statistically evaluated to verify this behaviour and obtain reliable calibration data.
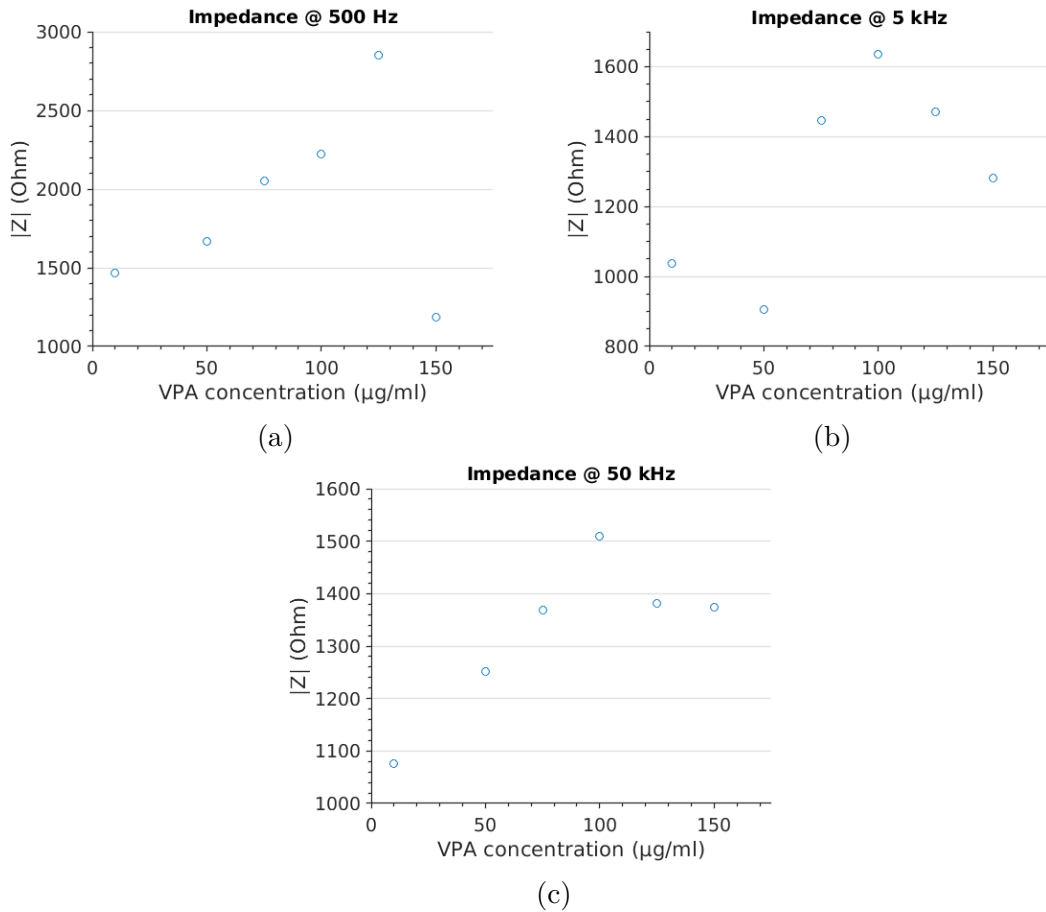

(a)

(b)

(c)

Figure 2.2: Impedance of one MOF-coated IDE at different frequencies and VPA concentrations.

3 Originality

Team part

The concept of using MOFs to sensor VPA has never been done before. MOFs however have been used in biosensors to detect e.g. Nucleic acids [2] specific ions for DNA sensing [3] and Bacteriophage [4] using different sensing strategies e.g. fluorescence or electrochemiluminescence.

The sensing strategy we are using in this work is the electrochemical impedance spectroscopy which requires a much simpler equipment than the others which also may allow portable devices. Our team developed a process to deposit MOFs on the gold electrodes with controlled parameters using cyclic voltammetry. The choosing of the kind of MOF, in our case terbium, was done outside the group, since it required deep chemical understanding. Then the team developed sensing protocols for VPA sensing, firstly starting with PBS solution and then eventually progressing to blood serum.

Supervisor part

The technology platform under development by the AIXSENSE team from IWE1, RWTH Aachen University involves taking advantage of novel metal-organic frameworks (MoF) as high performance transducer elements, integrated in a point-of-care (PoC) biosensor format. The MOFs are a group of emerging nanomaterials for development of advanced nanoscale interfaces stemming from their hierarchical organization at nano and sub-nanoscale and wide variety of chemical functionalities available as an intermix of metal centres and organic ligands. The MOFs can therefore display selectivity at molecular scale due to size-selection, as well as specific chemical interactions.

Selective interactions of MOF has therefore been explored in the chemistry and material science domains in recent years. MOFs have found leading position towards fabrication of nanoporous membranes, and realization of advanced matrices for gas storage, slow-release and gas-sensor applications. Several MOFs being optically active have been a topic of interest for studying host-guest mechanisms and development of optical sensor platforms. Systematic integration of MOF with modern electrical microsystems technology is, still largely missing and likely to be picked up in near future. Here, AISENSE makes an early entry into the use of MOFs as active transducers materials for biosensor

applications and attempts at micro/Nanosystems integration, making it highly innovative platform and getting ahead of the technology competition.

AIXSENSE's choice of Terbium based MOF material [Tb(benzene-1,3,5-tricarboxylic acid)(H2O)6] was based on theoretical findings and is expected to show size and chemical selectivity towards valproate molecules. Realization of the sensor interface and technology integration of MOFs for monitoring of valproate, is therefore a highly original concept. Despite the high-risks of working with a novel material interface, Aixsense was successful in realization of the sensor platform based on photolithography fabrication of the microscale IDEs and working out a new electrochemical approach for the realization of MOF thin-films site-specifically. The method, has the potential for scaling up and render extreme control in tuning the properties of the sensor interface at nanoscale. Biosensor measurements for Valproate screening, so far has been carried out in concentrated buffer solution and show promising results for further optimizations to achieve analytical performance for real applications i.e. valproate monitoring from biological samples. I am confident that, optimized MoF interfaces based on the current platform of Aixsense will be fully system-integrated in near-future for point-of-care deployment. Amperometric and potentiometric readout mechanisms for sensor readout will enable miniaturized analytical prototypes with industry requirements and fit within medicine 4.0 framework.

4 Translation potential

4.1 Business model canvas

PROBLEM	SOLUTION	UNIQUE VALUE PROPOSITION	UNFAIR ADVANTAGE	CUSTOMER SEGMENTS
• Epilepsy patients have to go to hospital, get their blood drawn for valproate monitoring • Daily monitoring highly inconvient • Doctor cannot immediately interact with patient based on test results (delay) • Existing devices for testing are expensive, require a skilled operator	• Affordable, in home device for rapid valproate sensing based on electrical impedance spectroscopy • Highly mobile device can be used from anywhere (results shared with doctor via mobile internet connection/app) ->lifestyle	• Save money for insurance companies (lower costs) • Eliminate time in waiting rooms (additional risk of infection) • No redundant interaction with medical staff (save time and money) • Convenience for patients • Synergy with growing telemedicine sector • Potential reduction of side-effects of valproate by superior dose adjustment • Doctors can assess patient medication adherence	–	• Women of fertile age stand to benefit most from daily monitoring • Hospitals/insurance companies (both have interest in lowering costs; provide better service to patients) • Alternative: Direct-to-consumer market (expensive studies to prove clinical benefit not mandatoy)
EXISTING ALTERNATIVES • ELISA testing kits and chromatography are alternatives but don't adress the above issues	KEY METRICS • Successful sensing of fVPA in blood serum • User/tester feedback		CHANNELS • Provide prototype to hospital for testing (gain market feedback or even support from medical professionals) • Hire advisors to establish contacts • Promote prototype/sensor concept at startup meetup/conference	EARLY ADOPTERS • University hospital that receives prototype for testing

COST STRUCTURE		REVENUE STREAMS
• Protype to market-ready	20.000€-40.000€	• Initial sale of sensor platform (Probably around 600€ per device); either to hospitals or directly to consumer • Constant revenue stream due to sales of cartridges (e.g. 2€ per cartridge per customer); daily testing: 730€ per year per customer; 40k estimated customers -> <u>28m€ potential yearly revenue</u>
• Patent/counceling	5.000€	
• CE-certification	14.800€	
•		
• Total	40.000€-60.000 €	

Figure 4.1: Canvas showing our assessment of the market

4.2 Stakeholder desirability

As it stands, routine daily monitoring of free VPA (fVPA) does not seem to be widely practiced [5]. It is only recommended if co-administration of drugs (such as antibiotics) that are known to strongly influence pharmacokinetics is essential [6].

In an Australian study, the author bemoans that a large number of tests is taken "unnecessarily", i.e. for reason other than indications relating to assessment of compliance, toxicity or overdose [7]. According to their calculation, "at a rate of \$18.45 per test, [. . .] the unnecessary expenditure amounts to about \$13 236 a year" for one hospital. The absence of daily testing therefore seems to be strongly informed by financial considerations. We, on the other hand, believe that we will be able to offer these tests with a cost reduction of almost 90 % which could be a complete game-changer. With

our at-home solution, patients would be able to conveniently take their tests at home, eliminating the need to go to the hospital for daily testing. Conducting these tests from home also eases the burden on nurses and lab technicians, which in the long run is also going to save money for clinics and insurance companies. Established ELISA tests and HPLC-MS only work with ultrafiltrated samples, therefore requiring a volume of blood to be drawn. With our device, it should be sufficient to extract a small drop of blood from a finger prick, which is far more convenient for the patient.

Since it has been reported that the teratogenic effects of valproic acid are dose related, the benefits of daily monitoring, with the goal of maintaining the lowest possible effective dose, are invaluable for women who are at risk of pregnancy [5]. Some publications advocate for the practice of frequent testing in patient groups with specific physiological conditions, such as pregnant women and children, as well as during combination therapy and in cases of treatment failure, suspected adverse effects, and therapy noncompliance [8]. It seems prudent for us to focus our efforts on this patient group for which monitoring is already advised.

4.3 Business feasibility

To protect our IP, patenting should be looked into as soon as a functioning device has been developed. Currently however, we are still in the early stages of prototyping. It will therefore be necessary to hire some external talents for further development, notably sales strategy, user interface development and cloud connectivity. In order to pass the examination required for certification by a notified body, help from a specialized consultation firm will have to be enlisted.

Once the prototype has been finished, and the sensor's ability to reliably determine fVPA concentrations in blood serum has been proven, the next step would be to approach manufacturing companies to help us manufacture the cartridges, the socket, the PCB for multiplexers and the potentiostat module, as well as the casing and periphery. Assembly, packaging and shipping of the finished products will either have to be contracted to a company, or staff will have to be hired. If our device is supposed to work as a part of telemedicine or cloud-assisted treatment, experts need to be consulted who can coach us on EU regulations on the handling and transfer of patients' medical data. An aspect that should not be overlooked is branding and marketing. For any product it is important to carefully craft an image. We think that for our product, aspects such

as independence, simplicity and flexibility should be emphasized, thus requiring us to consult marketing agencies as well.

Additionally, the readout and cartridge concept could be adapted for other sensor applications in the future.

4.4 Financial viability

About 50 million people worldwide are affected by epilepsy. In Germany an estimate of 40.000 (0.2%) fertile women are currently taking valproate medication [9], [10], which represents our core customer target group.

The total cost of our finished prototype amounts to 685€. The driving factor of this is the EmStat Pico development board. In the next prototyping stage we plan to design our own PCB with all readout components and the cartridge socket integrated, replacing all wired connections.

The potentiostat module has a single order list price of around 500€. Small custom PCBs (80mmx50mm), including soldering service for electrical components, only cost about 5-6€ each when quantities of 100+ are ordered.

We think that the best course of action would be to offer the readout module with little to no profit at a price of approximately 600€, and turn a profit through sales of cartridges. Considering most patients will take VPA for the rest of their life, profits through cartridge sales promise a high and continuous stream of income.

For the cartridge wafers of our chip design can be ordered microsystem company for around 25€ per 4-inch wafer, which can be diced into 200 individual chips. Including the price for bonding and deposition of MOF layers, the expenses should stay below 1€, so the final product can be sold for 2€, still being 10 times cheaper than commercial tests.

If tests are conducted daily, the yearly profits per patient should be around 360€ per year; with 40.000 prospective customers in Germany, that's a potential 14.4 million euros per year. We estimate the expenses of transferring the prototype into a market ready product as around 20.000 to 40.000 dollars in our case. Counselling fees for patenting and certification are estimated with 5000€. CE certification, using the formulas provided by SGS, a swiss certification company, the total cost for an initial certification will amount to around 14.800€ for a class b device, in addition to recurring annual costs for audits. In sum, estimated initial costs therefore amount to approximately 40.000-60.000 euros.

5 Team and support

5.1 Contributions of the Team Members

- **Luca Fehlings:** Team captain, microfluidics

- **Jerome Jayakar:** Molecular recognition

- **Anirudh Kudiramurthy:** Translational part

- **Hendrik Kupper:** Team captain, fabrication of IDE platform

- **Stefan Leisten:** Readout system, Translational part

- **Abarna Ravichandran:** Molecular recognition

- **Anish Russel:** Electronic readout system

- **Shilpa Suresh:** Communications, Translational part

5.2 People who have given support

Institute of Materials in Electrical Engineering 1 (IWE1), RWTH Aachen University, Aachen, Germany:

- **Andrea Haack**: Carried out the Parylene coating process

- **Dorothee Breuer**: Carried out the Lithography

- **Jochen Heiss**: Carried out the metallization process

Central Scientific Instruments Organisation (CSIO), Chandigarh, India:

- **Dr. Akash Deep**: Provided the MOF

5.3 Sponsors

AMO GmbH, Aachen

List of Figures

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