DeTectUs

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List of abbreviations

Name	Abbreviation
3-Aminopropyltriethoxysilane	APTES
Adverse Drug Reactions	ADRs
Alternating Current	AC
Bovine Serum Albumin	BSA
Carbodiimide	EDC
Comma Separated Value	CSV
Computer Aided Design	CAD
Direct Current	DC
Enzyme-Linked Immunosorbent Assay	ELISA
Giant Magnetoresistance Resistance	GMR
Immunoglobulin	Igs
Lithium-Polymer	Li-Po
Microprocessor	microP
Monolconal Antibody conjugated to Valproic Acid	mAb-VPA
N-hydroxysuccinimide	NHS
Point of Care	POC
Secure Digital Card	SD card
Serial Peripheral Interface	SPI
Signal-to-Noise Ratio	SNR
Thin Film Transistor Liquid Crystal Display	TFT-LCD
Valproic Acid	VPA

1   Summary for SensUs

Valproic acid is a drug, used for treating epilepsy. However, it has a very small therapeutic window, while the wrong dosage can lead to ineffectiveness of the drug or side effects. Biosensors are receiving a lot of attention these days, due to their huge potential within the field of disease monitoring. We investigate how to design a giant magnetoresistance (GMR) point-of-care biosensor to monitor the concentration of valproic acid (VPA) in blood plasma.

The initial goal was to fabricate this device using GMR. Due to COVID-19 and restrictions to access the laboratories, no analytical results were achieved. However, the electronics as well as the interface was prototyped and tested.

The GMR biosensor should be fuctionalized by antibodies, which can bind to VPA. When VPA is bound the surface of the GMR sensor, the concentration of VPA is detected by the binding of magnetically labeled antibodies that bind to another binding site of VPA. This interaction can then be measured as a change in electrical resistance propotional to the concentration of VPA. The change in the resistance happens due to the change in the relative orientation of the magnetic moments in alternate ferromagnetic layers created by the addition of the magnetic nanoparticles to the sensor surface.

Compared to traditional optical detection, GMR sensors have the advantages of being low-cost, sensitive, portable and be able to give a full electronic readout.

2 Introduction

Great progress has been achieved within the field of drug discovery against epilepsy over the last decades. Valproic acid (VPA) is one of the drugs that are primarily recommended for the administration of seizures by therapeutic guidelines. VPA can prevent unexpected seizures, which is why it is used as preventative medicine, that is taken on a daily basis [1]. However, the therapeutic window of total valproate is 50-120 µg/mL, which is a very small range. A too low dosage can lead to ineffectiveness of the drug leading to breakthrough seizures, while a too high dosage can cause side effects. Life-threatening adverse drug reactions (ADRs) may occur at high dosage exposure [2, 3]. Therefore, a strategy is needed for rapid and sensitive measurement of the valproic acid concentration in blood plasma.

Biosensors are receiving a lot of attention these days, due to their huge potential within the field of disease monitoring. In 1988, Albert Fert and his coworkers discovered the giant magnetoresistance (GMR) effect. The GMR effect reveals that the resistance of a GMR sensor changes with the magnetic field applied to the sensor. Furthermore, it is well known that micro- and nanoparticles can be used for surface based biochemical assays. When an antibody is magnetically labelled, it is therefore possible to induce a signal from that particular magnetically labelled biomolecule [4].

Here, we investigate how a cheap and portable GMR biosensor that provides rapid results, can be developed for monitoring the concentration of VPA in blood plasma.

3 Biosensor System and Assay

3.1 Molecular recognition

We want to create a biological recognition site on the surface of the GMR sensor. As seen in fig. 1 this should work by: 1) Immobilizing the primary antibodies on the GMR sensor surface. 2) Adding a sample containing a concentration of VPA, the measurement of which is desired. The VPA binds to the primary antibodies on the sensor surface. After this step a washing step will be required to remove unbound molecules from the sample. 3) Secondary antibodies with magnetic nanoparticles are added to the sensor. A washing step will be required here as well to remove excess secondary antibodies. The bound amount of magnetic nanoparticle on the sensor interface will be measured by the GMR transducer, outputting a signal propotional to the concentration of VPA from the sample.

3. Secondary antibodies with magnetic nanoparticles binds to VPA creating a measurable signal in the GMR transducer.

2. VPA binds to the primary antibodies.

**1.** Primary antibodies are immobilized on the sensor surface.
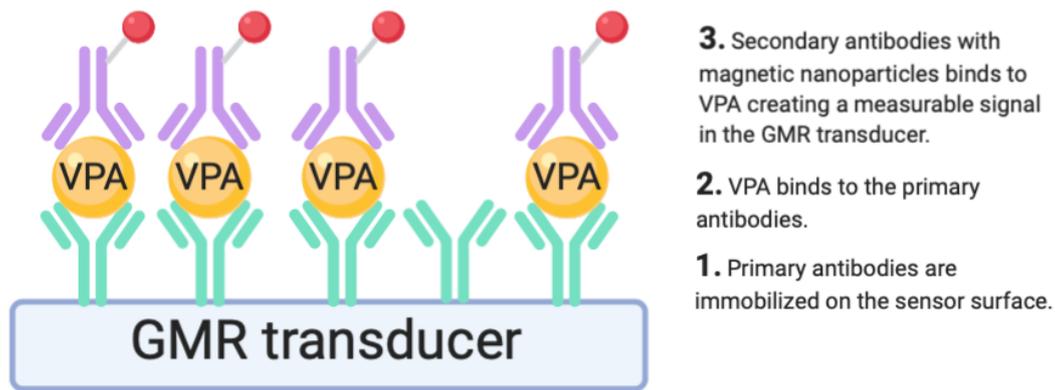
Figure 1: Biological recognition site of the sensor.

To make the biological recognition site work there are following requirements:

- VPA has to have two binding sites, that we can bind to with our antibodies.

- We have to be able to fuctionalize the surface of the sensor by immobilization of the primary antiodies.

- We have be able to bind the bind the magnetic nanoparticles to the secondary antibodies.

3.2 Testing VPA binding sites

3.2.1 Sandwich ELISA

In order to successfully design the detection part of the device, the number of binding sites of the analyzed substance needs to be determined. It has been discovered that VPA has 2 binding sites [5]. However, due to significant differences in size between the acid molecule and antibodies, it may not be possible to bind 2 antibodies to 1 molecule of the VPA. If less than 2 binding sites are present, the system won't be able to detect the analyte properly. To determine whether the presented approach can be successfully translated to the proposed measuring system, ELISA testing will be carried out. Here, a specific type of VPA-specific immunoglobulins (Igs) will be immobilized on the surface of the testing well. Then, molecules to be investigated are introduced. Finally, a second, different type of VPA-specific Igs that is the secondary antibodies are added to the well. After a rinsing, if the experimental plate shows a yellow colour reaction from the well, it will indicate that the analyte is successfully bound by 2 different antibodies simultaneously and further quantification from the absorbance value will we done, thus proving meaningfulness of the designed measurement system.

In case this sandwich structure works, it lets us measure free VPA, since there will not be room for the antibodies if the molecule is bound to albumin.

A protocol for the sandwich ELISA test of the VPA binding sites can be found in appendix A and a flowchart can be found in appendix B.

3.2.2 Indirect competitive ELISA using BSA conjugated VPA

If the primary approach fails, an alternative test will be carried out, based on the competetive assay [6]. VPA molecules will be conjugated to bovine serum albumin (BSA) and immobilized to the surface of the testing well. Then, in a separate vessel, VPA-specific Igs will be mixed with analyzed sample containing free VPA. Once the equilibrium state within the sample is established, the content will be injected into the well. The more antibodies are bound to the free VPA the less of them will be able to bind to immobilized BSA:VPA complex, resulting in decreased signal. Once the equilibrium state is achieved, the well will be rinsed from antibodies bound to free VPA and antibodies that would not bind to the immobilized complexes. Afterwards, the results are quantified by measuring the intensity of the color reaction using a plate reader, which measure's absorbance. A caliberation curve would be prepared based on different concentrations of VPA and the corresponding absorbance, that will determine the linearity of a given correlation. A stronger intensity of signal will be achieved if larger amount of free antibodies are present in the rinsed sample, indicating lower concentration of free VPA in the tested batch. Similarly, the larger concentration of the free VPA that is introduced, the smaller amount of free antibodies will remain potent to bind to the BSA:VPA complexes resulting in attenuated signal.
In order to successfully determine correlation between concentration of VPA and signal emitted by bound antibodies, a calibration curve preparation will be necessary. To do so, for known several concentrations of free VPA a constant amount of antigen-specific antibodies will be introduced, the experiment will be performed and the signals will be measured with absorbance. Overall, the obtained correlation between the concentration of free VPA and its signal should be propotional. If both of the presented methods fail to succeed, the detection mechanisms cannot be considered as reliable methods for the device design.

A protocol for the competitive indirect ELISA test of the VPA binding sites can be found in appendix C.

3.3 Functionalization strategies

To achieve the immobilization of the antibodies it is necessary to activate the surface with a way that the antibodies will be attracted to the surface. The optimal method of immobilization depends on the characteristics of the biomolecule and the solid material. Due to unknown surface material of the commercially available GMR sensor dies and lack of testing facilities to determine this, different strategies for this functionalization are planned. The chemicals that are used for the functionalization are cross-linkers that contain reactive ends to specific functional groups of mAb-VPA [7].
The structure of VPA can be seen on fig. 2.
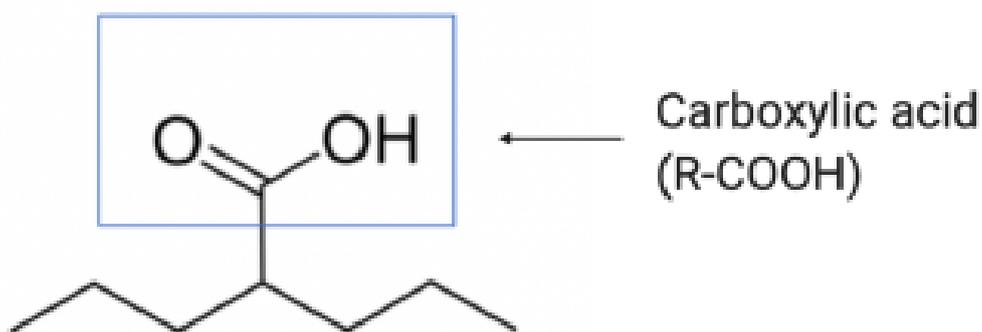
Figure 2: Structure of VPA.

As it can be seen on figure 2, the molecule contains a functional group, which is a carboxylic acid (R-COOH). After figuring out the characteristics of the biomolecule, it will first be investigated how the magnetic nanoparticles can be bound to mAb-VPA.

3.3.1 Antibody - Conjugated Nanoparticles

By using covalent bonds, it is possible to attach magnetic nanoparticles to antibodies. Especially, amide bond formation is an appropriate way, since antibody surfaces contain primary amine functional groups. Furthermore, it is also well known that primary amines are highly reactive to carboxylic acids. However, it is necessary to modify the nanoparticle, which can be done by using a chemical linker. As it can be seen on fig. 3, Carbodiimide (EDC) / N-hydroxysuccinimide (NHS) can be used in this case [7]
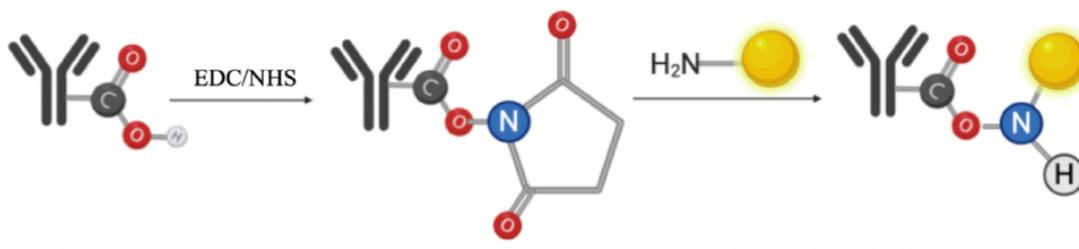
Figure 3: Amide bond formation between antibodies and nanoparticles.

A protocol for the functionalization of the magnetic nanoparticles can be found in appendix D.

After attaching magnetic nanoparticles to the antibody, it is necessary to have a strategy for functionalizing the sensor surface. A strategy for this functionalization will be considered in the following.

3.3.2 Antibody – Silica/glass/quartz surfaces

The most commonly used material for biosensor surfaces is Silica, and it can be modified by using silanization chemistry. However, the sensor surface can also be made of materials such as glass and quartz. Notably, 3-Aminopropyltriethoxysilane (APTES) is the most commonly used agent for attaching antibodies on these surfaces [8]. As it can be seen on figure 4, the sensor surface is covered with primary amines (-NH2) using the aminosilane reagent. These amines are reacted to the crosslinker Sulfo-SMCCC, resulting in a maleimide-activated surface, which is able to react with sulfhydryl groups on antibodies [9].
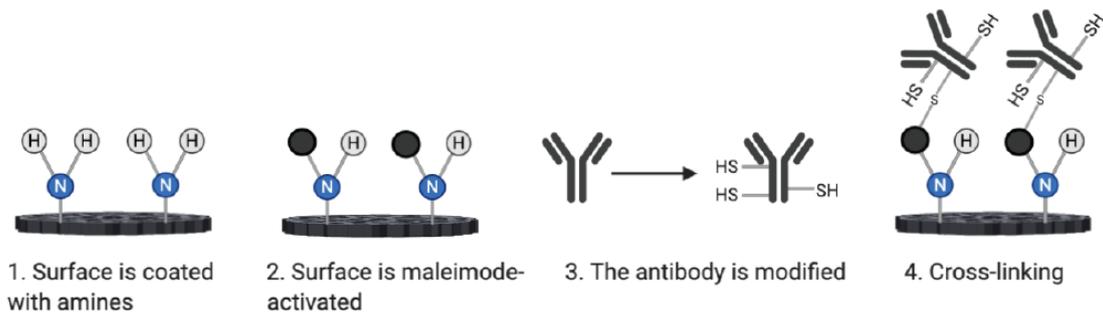
Figure 4: Attachment of antibodies to silica surface.

A protocol for functionalizing a silica sensor surface can be found in appendix E.

3.4 Physical transduction: Giant Magnetoresistance

The detection of the binding of VPA on the sensor surface is done using a GMR sensor chip and magnetic gold-shelled nanoparticles, functionalized in order to bind to the antibodies.

3.4.1 GMR sensor

(a) GMR is a multilayered configuration of either parallel or antiparallel magnitised ferromagnetic materials and a non-magnetic material between them.

(b) GMR Magnetoresistance curve for FE/CR multilayers in a perpendicular orientation with various levels of impurities in the lattice structures.
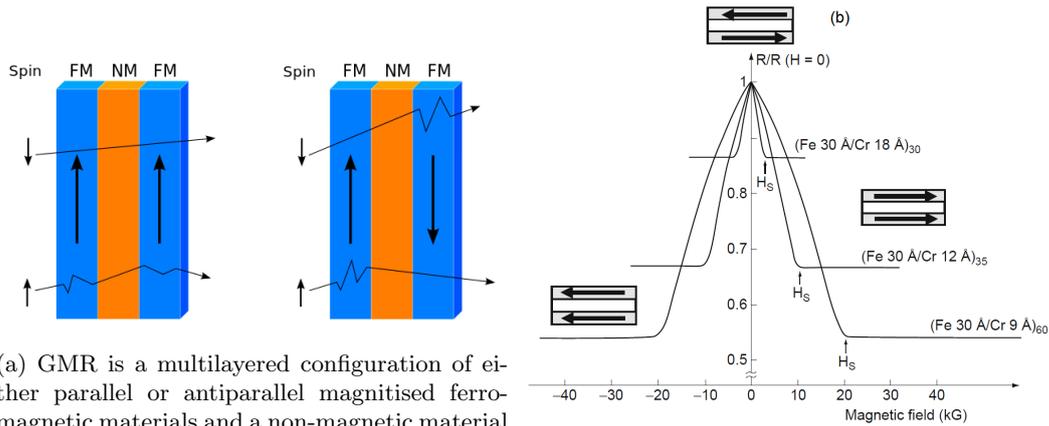
Figure 5: Working principles of a GMR sensor

GMR is a quantum mechanical magnetoresistance effect observed in multilayers composed of alternating ferromagnetic and non-magnetic conductive layers. This effect relies on the quantum property inherited by electrons called spin. Spin describes the way that an electron moves in the presence of a magnetic field. In the case of GMR, this is used in ferromagnetic conductive materials to effectuate a sort of drag to some electrons, thus changing the effective resistivity of the material, this effect is conceptually illustrated in fig. 5a. The layered structure of the GMR enhances this effect to the point where a GMR sensor on the scale of $10^{-5}m^2$ can detect variations in the Earth's magnetic field, which is on the scale of a few micro tesla. A theoretical plot of the resistivity ratio of the GMR sensors can be seen in fig. 5b. Compared to traditional optical detection, GMR sensors are cheaper, more sensitive, portable and give a full electronic readout. The fabrication of GMR biosensors is also compatible with the current very large scale integration technology. This is why GMR biosensors can be easily integrated with electronics and microfluidics to detect several analytes on a single chip [10].

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3.5 Cartridge technology

A GMR sensor works on the basis of a antibody functionalized surface and a solution with functionalized magnetic nano-particles. The most common way to reuse these functionalized surfaces is to wash the surface with a buffer solution. Applying this to our GMR sensor should work great, however, washing is not seen as simple and user friendly. When adding another step for the final user, the risk of error is increased as well.

To simplify our GMR biosensor removing this washing step is detrimental for having a simple Point-of-care (POC) device. The initial concept for achieving this was to have the functionalized surface on a super-thin, non-magnetic film instead of on the GMR sensor itself. There is a risk that this might cripple the GMR sensor performance and thus make it unsuitable. Further testing will have to be done to find out if it is possible to have a film thin enough where the increased distance between the ferromagnetic GMR layer and the magnetic nano-particle will not attenuate the magnetic field lower than needed for detection.
If this film is possible, it will help with the usability of the device. Otherwise, a different approach has to be tried. A fail-proof cartridge could be a cartridge containing the GMR sensor itself, and thus replacing the sensor part after every measurement. This will introduce a lot of GMR sensors being used, however can go hand in hand with a "return and get-new" delivery style for cartridges.

3.6 Reader instrument and user interaction

Besides the sensor technology, a lot of peripherals are needed to have an actual point-of-care testing device. There has to be power, data reading, user interfaces, control signals. That is, electronics. Most of this is controlled through an Arduino Mega 2560, connected to a TFT-LCD shield with touch capabilities. Due to the small voltage output of the sensor, it needs amplification, created with a differential amplifier.

The whole device is powered by a 12V Li-Po battery at first. At a later point, the possibility of using a power supply to charge the battery and run the device will be added as a secondary option. The GMR measurement circuit that is seen in figure 8a, have to be connected to an analog port of the Arduino, as the TFT-LCD shield takes up the first five pins, this is connected to pin A6. The Arduino only reads this pin, and doesn't interact with this circuit itself. The shield is by default required by its Arduino library to use pins 0 to 9 of the digital pins, and A0 to A5 of the analog ports alongside power outputs. The shield was built in a way that the SD read/write pins are connected to digital pin 10 to 13, on an Arduino Mega 2560. These pins don't have the required functionality for writing and reading the SD card, and so it needs to be rewired to the digital pins 50 to 53. This is due to these having the SPI functionality, a communcation protocol used for displays.

What is not present on the diagram of figure 6, is the external field that will be needed to align the ferromagnetic nanoparticles. This part is currently left out as the orientation and requirements for this external field is yet to be determined experimentally by the physics subteam. It is expected that this setup would be a simple constant current drive which is adjustable from the Arduino, and a solenoid like the one designed in section 3.6.2
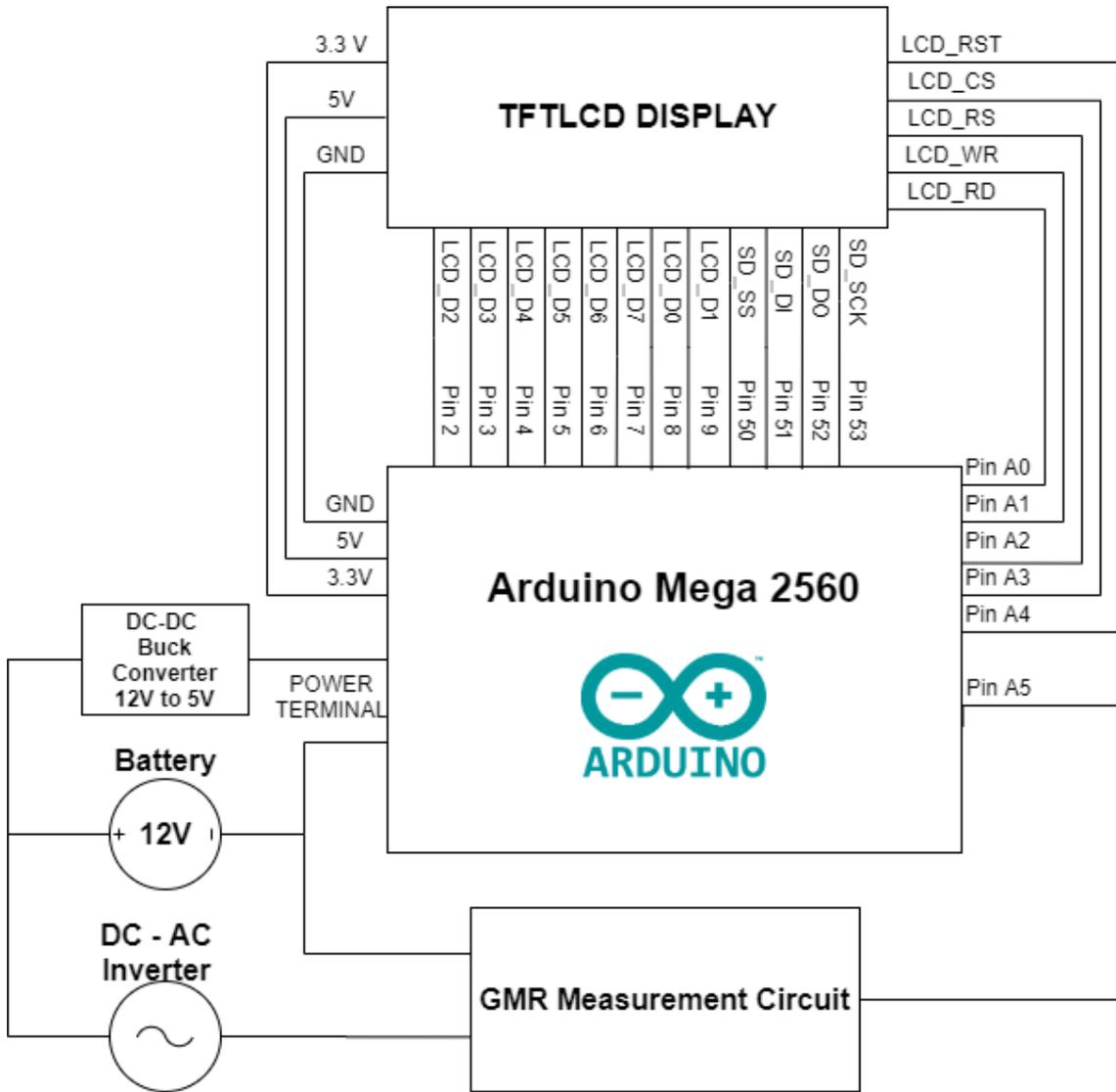
Figure 6: Block diagram showing the connections of the device

3.6.1 Interface

A point-of-care device has to be user friendly, making the interface of utmost importance. The interface, which is coded in Arduino C, was tested on the Arduino Mega 2560. It is a menu, displayed on a TFT-LCD touch screen, with menu points Measure and Calibrate. The touch screen functionality works as expected. In the measurement phase, a function for saving and writing data to an SD card has been written.
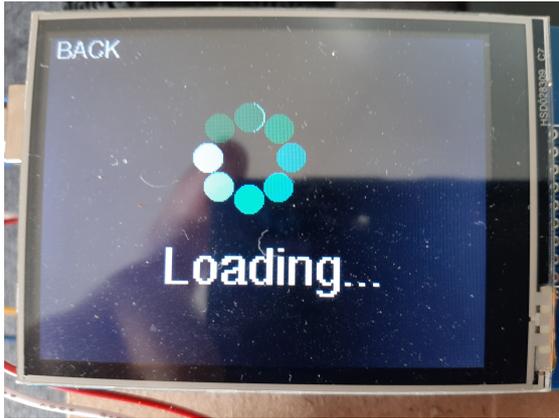
(a) Picture of interface loading screen, this runs while measurements are being taken
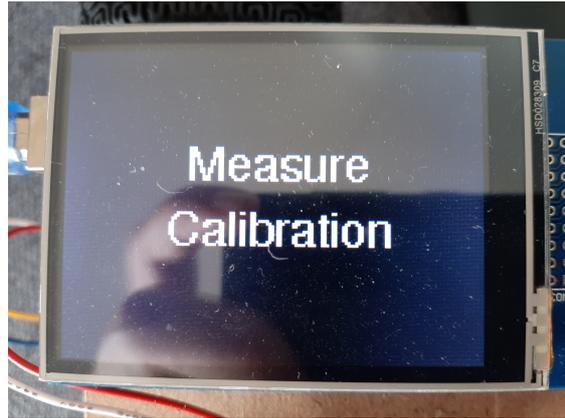
(b) Picture of interface menu screen, background will be added later

3.6.2 Experimental test setup - Electronics

The preliminary test phase consists mainly of developing a hardware configuration on test grade GMR sensors from NVE Corporation. These are commercially used in spacecraft instrumentation and geophysical instruments hardware. This is a suitable basis for experimenting with the peripherals. The sensors used has an recommended input voltage of 12-24V and can be driven by both DC and AC since the sensor in principle works as a simple resistor.

This versatility of driving frequency and the operating principle of the sensor creates possibilities for optimization of the signal to noise ratio (SNR). The sensor is designed to be sensitive to small changes of the magnetic field. However, the change in resistance is similarly small, and thus an amplifier is needed. This can be tuned in order to mitigate some amount of noise. Another way to mitigate noise is by sweeping through different frequencies of AC to test with a known magnetic field strength which configuration gives us the best signal-to-noise ratio.

To do this, a test setup is designed in CAD. This is basically a large solenoid, with room for the GMR sensor in the center. This can then generate an exact, known magnetic field, that can be measured and compared by the sensor. A signal generator sweeps through frequencies and the SNR is calculated for each frequency step. This calibration is only done once for a sensor, since it is rather invasive. Another, more frequent calibration is described in section 3.7.

The test circuitry is shown in fig. 8a. Here, 4 GMR-sensors are arranged in a Wheatstone bridge in "GMR Sensor" box, connected to a differential amplifier. Then, the GMR sensor is placed in the test solenoid, and the output is displayed and recorded on an oscilloscope.

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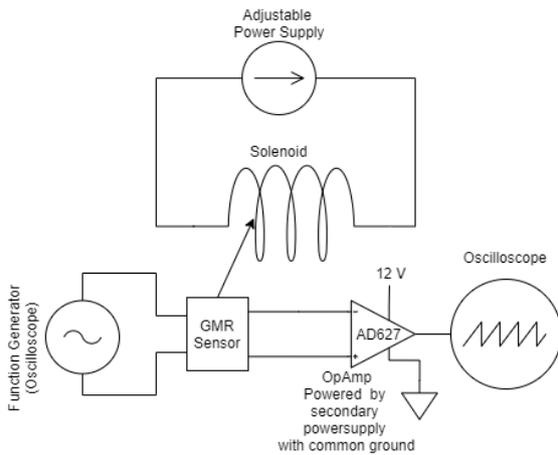
(a) Circuitry for the calibration setup.
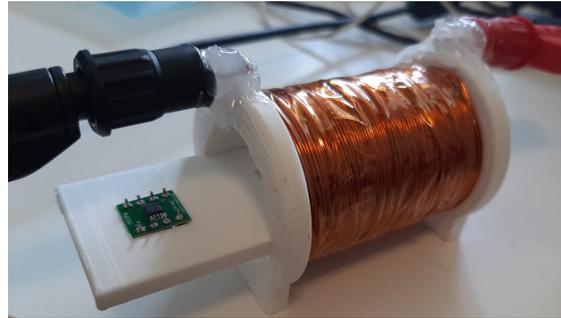
(b) Picture of GMR sensor calibration setup

Figure 8: Schematic and physical prototype of the calibration setup

Calibration results are shown in section 5

3.7   Data processing

When data enters the microprocessor (µP), it does so as amplified voltage output values of the Wheatstone bridge. In theory, it enters in discrete steps directly correlated to the number of biomolecule bindings to the surface. Additionally, an offset voltage might arise from magnetic noise in the surroundings; that is, fields from power lines, computers nearby and even the Earth's own magnetic field. It all varies with your location and how plane your test table is.

Thus, a variation of the calibration sequence mentioned in section 3.6 is needed to eliminate these factors. We can apply a known magnetic field to the sensor and use the output signal for normalization.

After normalization, the voltages are correlated to the discrete number of particles in solution bound to the sensor surface. Since the input fluid volume should be known to the microprocessor at this point, the VPA concentration can easily be calculated real time. This reliance of as exact measurements of blood volume and as much noise reduction as possible then emerges as a final limiting factor in getting precise results. In a final product, this might be controlled by microfluidics, as to reduce the risk of user error.

This concentration is then saved to an SD-card as a CSV file with the voltages and timestamps related to these.

Flow for the operation of the theoretical device can be found in app. F.

4 Novelty and Creativity

4.1 Already available

In previous studies GMR biosensors has been used for monitoring several biomolecules, such as the influenza A virus. This assay uses monoclonal antibodies to the viral nucleoprotein in combination with magnetic nanoparticles. When influenza virus is present, the magnetic nanoparticles will bind to the GMR sensor. In this project it is presented, how the GMR biosensor technology can be used for monitoring valproic acid in blood plasma. [10]. Commercially, Lab-on-A-Chip devices exist, using GMR technology to detect larger biomolecules such as pathogenic fungus and even the novel COVID-19 virus [1].

4.2 New developments

In the last decades, many trials have been done for the determination of valproic acid in human blood plasma. The patient needs to be aware and concurrently able to regulate the concentration of the valproate, to avoid any overdosing issues and experience fewer side effects. This project is aimed at the development of a novel device for monitoring valproate. By using magnetic nanoparticles, the functionalisation with antibodies became eligible to capture the free valproate in human plasma. What is novel and striking in this project, is not only the use of a POC GMR biosensor for detection of VPA in blood plasma but also that the GMR method is used with such a small molecule as VPA. The challenge of using this method with such a small molecule is approached with the competitive indirect ELISA assay and the assay is not novel in itself, but using the setup from this assay to fuctionalize the surface of the sensor is brand new.

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[1]http://zeptolife.com/our-technology/

5 Analytical Performance

Due to COVID-19, no analytical results were achieved. This is due to lab access for the biochemical team being restricted, and thus not being able to test the concept. However, the calibration setup, testing electronics as well as the interface was prototyped, letting us characterize a specific GMR sensor at a specific time, and calibrate the sensitivity.

By examining the frequency response of the commercially available GMR sensors, it was quickly discovered that they behave most linearly near DC. However, that does not mean it is linear at all. Figure 9 clearly shows a nonlinearity to be accounted for in a given sensor, making these gain curves the basis for calibration. A given measurement would then be mapped to this non-linear curve, outputting the actual magnetic field.
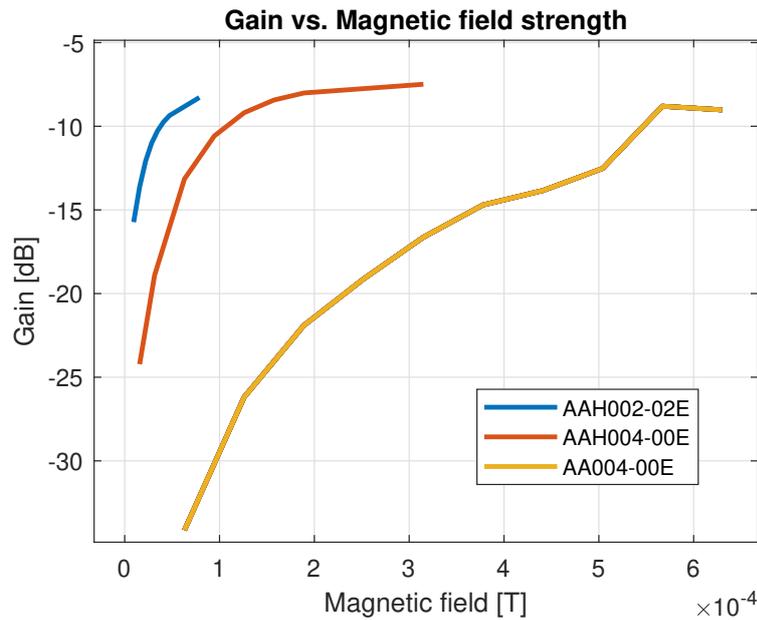
Figure 9: Gain plot at varying magnetic field strengths at 100 Hz

The SD write functionality for data acquisition has also been tested successfully with a 5 V peak-peak 0.1 Hz sinusoidal test input. For a demonstration of the Touch Functionality, a paint program is coded that works on the TFT-LCD, a video of this can be seen on youtube (https://youtu.be/B8UkAifGylA).

The therapeutic range of total VPA is 50-120µg/mL and the aim for the GMR sensor is to be able to measure concentrations of total VPA from 10-50 µg/mL.

6 Translational Potential

6.1 Business model canvas

PROBLEM 🔒

1. Medication side-effects can affect the life of the epilepsy patients negatively. In fact some have more problems with the side effects, than with the disease itself.
2. There is a lot of uncertainty involved in the treatment of epilepsy patients, especially in the initial period after the diagnosis, or in case of politherapy.
3. Going to the doctor is a lot of hassle for a child and their parents.

EXISTING ALTERNATIVES 🏷️

Adjusting the doses at home without consulting to a medical professional
Updating the doctor via phone or e-mail instead of visiting them all the time

SOLUTION 🔑

Accurate and reliable data, presented in a format that is understandable for the user
Measurement of multiple AEDs with a single device
Automated communication with medical professionals

KEY METRICS 📊

Minimum success criteria: Get acquired by a major medical company after developing a prototype.

UNIQUE VALUE PROPOSITION 🎁

Defeat the side effects of your medication, don't let them define your life!
Get your child's medication adjusted anytime, without visiting the doctor!
Ditch the uncertainty related to your treatment. Check on your condition whenever and wherever you want!

HIGH LEVEL CONCEPT 🚀

The blood-glucose meter but for epilepsy medication

UNFAIR ADVANTAGE 🚩

The device is approved by the relevant regulatory agency
European Patent

CHANNELS 🚚

Doctors' referrals
Health Insurance packages

CUSTOMER SEGMENTS 🧍

Parents of epilepsy patients
Insurance companies / Governmental entities

EARLY ADOPTERS 🥧

Parents of children that have recently been diagnosed with epilepsy and have to take medication as part of their treatment, and experience significant adverse effects of their medication.

COST STRUCTURE 🧾

Production of a single device: 120 EUR
Production of enough devices to reach 15% coverage of the target market in the initial period: 900.000(children and adolescents with epilepsy) x 0,5(experiencing adverse effects of the medication) x 0,15(market coverage) x 120(cost of a single device) = **8.100.000 EUR**
Production of enough devices to cover 15% of the new cases in the following years: 130.000(new cases yearly among children and adolescents) x 0,5(experiencing adverse effects of the medication) x 0,15(market coverage) x 120(cost of a single device) = **1.170.000 EUR/year**
Regulatory approvals - Europe: **30.000.000 EUR**
Filing for a European patent: **70.000 EUR**

REVENUE STREAMS 💰

Sales price of a single device: 200 EUR
Revenue from End-Users in the initial period: 900.000 x 0,5 x 0,15 x 200 = 13.500.000 EUR
Revenue from the End-Users in the following years: 130.000 x 0,5 x 0,15 x 200 = 1.950.000 EUR/year
Revenue adjusted by the support from insurance entities in the initial period (approximately 43% of the sales price supported): 900.000 x 0.5 x 0.15 x 350 = **23.625.000 EUR**
Revenue adjusted by the support from insurance entities in the following years (approximately 43% of the sales price supported): 130.000 x 0.5 x 0.15 x 350 = **3.412.500 EUR/year**

Figure 10: The Business Model Canvas of DeTectUs

6.2 Stakeholder desirability

In order to evaluate the translational potential of a biosensor for epilepsy patients, DeTectUs had to identify the main problems that the device could solve in order to create value to the target customer and end-user. When researching the literature related to the epilepsy, three main problem areas became apparent; patients with this condition may experience a decreased quality of life due to a.) Uncontrollable seizures, b.) Impaired social interactions or c.) Adverse effects and difficulties related to their medical treatment. Monitoring VPA in the blood of the patients has the strongest implications to the third category, and therefore this is the main problem area that was further investigated as part of the translational research.

In 70% of all the cases it is possible to control seizures with a single Anti-epileptic drug (AED) or a combination of several AEDs. A common trait of these drugs however is that they do not cure the root problem of the disease, they only serve to eliminate the seizures, meaning that a great deal of patients is bound to take these medications for life. A bit less than 50% of the patients can never leave their medication behind without experiencing breakthrough seizures . Although most of these patients are seizure-free thanks to AEDs, many of them experience adverse effect. Groups at high risk of experiencing side effects of the medication are patients who freshly started the treatment and people on polytherapy, that account for 30-40% of the total cases. All together around 50% of people with epilepsy report bothersome effects of seizure medications. In some of the cases, patients claim that these side effects are in fact as troublesome as seizures themselves. In a set of interviews published on Healthtalk.org, young epilepsy patients (between the age of 18-26) report about experiencing severe side effects such as memory loss, frequent nausea, chronic tiredness, aggression etc., negatively impacting their work, studies, social life and their mood in general. Some of the interviewees also reported physical side effects such as acne, boils and rashes

on the skin and losing some hair. Reducing side effects through better dose adjustment is therefore a potential point of intervention and serves as a basis for the value proposition of the solution.

Another potential point of intervention lies within the dose adjustment and monitoring process itself; it is assumed, that optimizing the doses and keeping track of their effects is a cumbersome, time consuming activity, due to the amount and length of visits at the doctor's office. This problem might be especially relevant for young children, who account for about 15% of the total cases, based on US statistics. In these cases, the parents are also involved in the issue as they are responsible for taking their child to the visits, update the doctor about their condition, and track whether they take the medication as prescribed. This means that both the parents and the children will need to assign time for the treatment, which might interfere with their normal schedules at school and at the workplace.

To validate the problems stated in this section interviews with epilepsy patients were carried out. The interview data confirmed the assumption, that adverse effects of the medication could indeed affect the life of a patient as hard as the seizures themselves in some cases. 33% of the interviewees experienced strong, and 33% experienced mild adverse effects of the medication over an extended period of time.

"...overdosing the medication made her dull. Sabril on the other hand made her aggressive... Every time we shift to a new treatment plan she feel better for a short period and then everything just turns upside down and the side effects become very strong and unbearable..." – Interviewee 5.

Furthermore, it was found, that generally the first months or years after the diagnosis were perceived to be hard due to the difficulty of getting acclimatized to the medication, the new restrictions, the hospital visits and the occasional seizures. Patients in this period might be more susceptible to any solutions that help them to adapt faster to the new lifestyle inherent to epilepsy. Hospital visits are perceived to be a bigger challenge for those where the patient is young, and their parents are bound to take part in these visits. These user groups would benefit greatly from a solution that can reduce the frequency or the length of the visits. The existing solutions to the problems discovered are communication via e-mail and phone with the doctor, or self-adjustment of the medication.

The problem areas identified above define the target users of the solution. It is assumed, that the device would mainly be used epilepsy patients in the first 6-12 months after diagnosis, or patients on polytherapy. The first user group implies that the device would be used by children and adolescents, as epilepsy is mostly diagnosed at a young age, and therefore this initial period after a diagnosis is also more relevant for these age groups. Children and adolescents however have no buying power, and therefore cannot be regarded as target customers of the product. Instead it is their parents, that are more likely to buy the device in order to better control the condition of their children. To understand the needs and decision journeys of these users and potential customers, the data of the user interviews were used. From these interviews the team got an overview on the costs related to epilepsy treatment as well as the monetary support they get from the government or insurance companies. Based on the findings of the interviews, when all the costs and support are summarized, and average patient in a European country does not spend more than 150 EUR a month for costs related for epilepsy. It is recognized that this data is not fully representative, but it gives an estimate on the order of magnitude of the related costs. Furthermore, there are some special medical equipment available for epilepsy treatment such as a smart glove to predict epilepsy, which are also eligible for governmental support. Based on the answer of the interviewee, the support for these equipment varies from 35-70% of the total price of the equipment. The main implication of these to customer segmentation is that governmental and insurance entities must be regarded as customers of the biosensor, as they take part in financing the solution.

When discussing the created value and the solution it is important to highlight a key discrepancy between the proposed solution and the needs of the users. The data from the interviews suggest, that screening only one type of AED is not enough to build a strong value proposition, as a large variety of medications are used by the different patients, and many of them take two or more AEDs

as part of their treatment. It is also very common, that people with epilepsy try multiple types of AEDs before selecting a specific one for extended use. Although technically it is challenging to implement a system that can be calibrated for multiple different analytes, measuring only VPA might not provide enough value to the average epilepsy patient.

To understand which features would have created the most value to the potential end-users, DeTectUs defined user needs based on the interviews, and asked the interviewees, to assign importance factors to them. The main idea was that by transforming these needs to product features, the solution provides a better response to the problems of the patients and thereby the value proposition improves. The links between user needs and biosensor features were evaluated through Quality Function Deployment (QFD), a technique that uses matrices to show the correlations between the two abovementioned domains. By using QFD it was possible to identify those biosensor features that are creating the most value to the user. These features are the accuracy and reliability of the measurement data as well as its representation on the device, the detection of multiple types of AEDs and the automated communication of the device with an external software that is used by their doctor to monitor the patients' condition remotely.

As for the unique value proposition, the main value promoted would be the capability of the device to eliminate some uncertainty in the treatment process and reduce side effects, as well as the time spent at the doctors.

6.3 Financial viability

Based on the estimation of DeTectUs, the production cost of a finished biosensor should not be more than 120 EUR, however many other costs are related to the product, such as distribution, customer service, marketing etc. One of the biggest challenge to overcome in terms of finances is the cost of regulatory approvals that are required both in Europe and in the US if a business wants to put a medical device to the market. The costs of achieving such approvals originate from the cost of regulatory consultation, document management and administration and from clinical trials and performance studies. Costs related to approvals can amount to 20-76.000.000 EUR in case of complex analytical devices.

Compared to these costs the potential market is rather small: in Europe, where DeTectUs would launch the product about 2.500.000 adults and 900.000 children live with epilepsy. As the target customers are mainly children and their parents, it is the number of young patients that serves as a basis for the potential market. On average 50% of the patients report bothersome side-effects of the medication throughout their treatment, which narrows the target market further to 450.000 customer. The estimated number of new cases among children and adolescents per year is about 130.000, which means about 65.000 potential new customers yearly. It must be highlighted however that it is impossible to realise a 100% market coverage, therefore the expected revenues are quite low, if the business model only relies on revenues from the end-users. As mentioned in the previous section an average epilepsy patient does not spend more than 150 EUR a month on their treatment, therefore the price of the product should more or less align with this amount, and cannot be sold for more than 200 EUR. Calculating with a 15% market coverage this would mean a revenue of 13.500.000 EUR in the initial years after launching the product, and 1.950.000 EUR/year in the following years. The costs of production when serving 15% of the target market, are 8.100.000 EUR in the initial years and 1.170.000 EUR/year in the following years. This way the revenue model is not sustainable, as it would require more than 25 years for the business to become profitable, assuming that the amount of new cases per year and the market coverage does not change.

In the previous section it was mentioned that many of the medication and equipment needed for the treatment of epilepsy is partially financed by the health insurance system. These systems can either be governmental or private, and they vary in the different countries of Europe, but in this business model they are key actors, as they have the power to make the model sustainable. Due to their diversity they were not investigated in detail in this project but it is suggested to map the potential of these entities in order to understand how to collaborate with them. The main goal

would be to increase the sales price of the product by 50-100% without requiring the end-users to pay more for it.

6.4 Business feasibility

Although the support of insurance entities would allow the revenue model to become sustainable, the project still needs an immense amount of resources upfront, which is why generally speaking it is difficult to enter the biosensor market. The most common phenomena for how a research project can actually become a business is through acquisition. Major companies dominate the sector of medical devices, and they have nearly unlimited resources both in terms of money and know-how. It would therefore be a main aim for DeTectUs to get acquired by one of these key players after creating a proof of concept. Besides getting access to resources that are needed for the manufacturing of the product, acquisition would also help the regulatory pathway, as the support of a big company makes the project more creditable.

A key activity would therefore be to start to investigate which companies' portfolio would support such a device and identify what values DeTectUs can offer to them.

7 Team and Support

7.1 Contribution of the team members

Subteam	Responsibilities	Team members
Biotechnical team	Surface functionalization, Epitope design	Bettina Hierzberger Efstathios Deskoulidis Jumana Merchant Karoline Valentin Jensen
Physics team	Research on and design of GMR sensor and magnetic nanoparticles	Luca Giannini
Entrepreneurship team	Business development and stakeholder management	Youssouf Traore
Electrical team	Circuit design, Interface programming, Data collection, Calibration	Aleksander Sørup Lund Carl Emil Elling

7.2 People who have given support

- Annette Holek: Administrative supervisor, anhole@nanotech.dtu.dk

- Bettina Hierzberger: Team coordinator, julfra@nanotech.dtu.dk

- Maria Dimaki: Academic supervisor, maria.dimaki@nanotech.dtu.dk

- Winnie Svendsen: Supervisor and contact person for the SensUs organization, winnie.svendsen@nanotech.dtu.dk

7.3 Sponsors

7.3.1 Blue Dot Projects DTU

The DetectUs group belongs to the prestigious Blue Dot project portfolio of DTU. The Blue Dot Projects are projects that seek to increase awareness of sutainability, and launch real life research projects.

8 Final Remarks

VPA is a successful drug for preventing seizures from occurring. However, serious problems are associated with VPA at high dosage exposure. Therefore, it is important to have a rapid and sensitive monitoring device for measuring the concentration of VPA in blood plasma. When developing a GMR biosensor for VPA detection, it is necessary to find an antibody that binds to VPA. After the antibody binding test, it is also necessary to investigate the magnetic nanoparticles- and sensor surface functionalization. The antibodies have to be magnetically labelled for inducing signals. The gold magnetic nanoparticles could be functionalized by using NHS/EDC. The magnetically labelled antibodies had to be bound to the biosensor surface. For sensor surfaces made of glass, silica, and quartz, it was found that APTES could be used.

Although the biosensor instrument itself was not finished, the project managed to characterize and test commercially available GMR sensors. A calibrating setup was created, where a series of known magnetic fields could be applied. This gave a gain curve as a function of magnetic fields, to be used in normalizing the measurement values to the actual magnetic field recieved. Measurement electronics, data writing and some processing was also implemented.

To evaluate the translational potential of a biosensor that can be used in the treatment of epilepsy desktop research and interviews with potential users were carried out. Based on the desktop research several assumptions were created and many of them were validated through the interviews.

References

[1] Epilepsy Society. *Anti-Epileptic Drugs*. `https://www.epilepsysociety.org.uk/antiepilepticdrugs#.Xu9LAJMzbep`.

[2] "Giant Magnetoresistance-based Biosensor for Detection of Influenza A Virus". In: *Frontiers in Microbiology* 7 (2016).

[3] Nikhil Bhalla, Pawan Jolly, Nello Formisano, and Pedro Estrela. "Introduction to biosensors". In: *Essays in Biochemistry*. Vol. 60. Portland Press Ltd, 2016. Chap. 1, pp. 1–8.

[4] "High sensitivity biomolecular recognition device based on giant magneto resistance effect". In: *Rare Metal Materials and Engineering* 35 (2006).

[5] R.H. Levy I.H. Patel. "Valproic Acid Binding to Human Serum Albumin and Determination of Free Fraction in the Presence of Anticonvulsants and Free Fatty Acids". In: *Epilepsia* 20 (1979), pp. 85–90.

[6] Aldo Rodaa et al. "A simple smartphone-based thermochemiluminescent immunosensor forvalproic acid detection using 1,2-dioxetane analogue-doped nanoparticles asa label". In: *Sensors and Actuators B: Chemical* 279 (2019), pp. 327–333.

[7] "Recent advances in the generation of antibody–nanomaterial conjugates". In: *Advanced healthcare materials* 7.1 (2018).

[8] CD Creativity Diagnostics. *Immobilization of Antibody on Silicon Surface*. `https://www.cdbioparticles.com/s/Immobilization-of-Antibody-on-Silicon-Surface_42.html`.

[9] Thermo Fischer Scientific. *Attach an antibody onto glass, silica or quartz surface*. `https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2FApplication-Notes%2FTR0005-Attach-Ab-glass.pdf&title=VGVjaCBUaXA6IEF0dGFjaCBhbiBhbnRpYm9keSBvbnRvIGdsYXNzLCBzaWxpY2Egb3I`

[10] Venkatramana D Krishna, Kai Wu, Andres M Perez, and Jian-Ping Wang. "Giant magnetoresistance-based biosensor for detection of influenza A virus". In: *Frontiers in microbiology* 7 (2016), p. 400.

[11] Thermo Fischer Scientific. *NHS and Sulfo-NHS*. `http://tools.thermofisher.com/content/sfs/manuals/MAN0011309_NHS_SulfoNHS_UG.pdf`.

Appendices

A Suggested protocol for sandwich ELISA to test binding sites of VPA

Materials:

- PBS
- NaHCO3
- Tween-20
- Blocking reagents
- Valproic acid
- TMB
- TMB stop solution
- Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, HRP
- Valproate antibody (Mouse, monoclonal, IgG1)

Protocol

1. Conjugate primary antibodies to the surface of wells:

   (a) Prepare a solution of used complex in PBS and introduce a constant volume into 12 wells.
   (b) Incubate this solution.
   (c) Remove the solution from the wells.
   (d) Flush wells 3 times with PBS water.

2. Pour the prepared blocking reagent into the reservoir. Block the wells with blocking reagent for 30 mins at room temperature.

3. Prepare a series of 5 different VPA concentrations in PBS.

4. Prepare a solution of VPA specific antibodies in PBS.

5. Add the solution with VPA specific antibodies to the 5 different VPA concentrations and incubate.

6. Add the mixtures to well 3-12 and the VPA specific antibodies to well 1 and 2 (negative control) and incubate.

7. Remove the mixture from the wells and wash 3 times with PBS.

8. Prepare a solution of secondary antibodies with HRP attached to their heavy chains.

9. Introduce the solution of secondary antibodies to wells 1-12 and incubate them.

10. Remove the solution from wells and wash them 3 times with PBS.

11. Add TMB substrate to the wells a 1-12, incubate them for 10 minutes in a dark place.

12. Add STOP solution to all wells to brake the color reaction.

13. Quantify the results by measuring intensity of the color reaction using a plate reader, which measures absorbance.

14. Prepare a calibration curve based on different concentrations of VPA and the corresponding absorbance, determine the linearity of a given correlation.

B   Suggested flowchart for sandwich ELISA to test binding sites of VPA

Coat the 6 wells with primary ab. and incubate.

Wash away excess ab. with PBS buffer repeat 3 times.

Make a solution row with 5 diff solution of VPA. Add these to well 2-6. Well 1 will be a neagtive control and incubate.

Washing step x3

Add secondary ab. The conc. should be double the conc. of VPA and incubate.

Washing step x3

Add tertiary ab, which has HRP attached. Make the conc. x4 VPA conc. and incubate.

Washing step x3

Add TMB substrate. Make the conc. x8 VPA conc. --> color reaction Incubate in a drak place for 10 min

Add stop solution to stop the color reaction

Quantify results in plate reader

C Suggested protocol for indirect competitive ELISA using BSA conjugated VPA to test binding sites of VPA

Materials:

- PBS
- NaHCO3
- Tween-20
- BSA
- Valproic acid
- TMB
- TMB stop solution
- Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, HRP
- Valproate antibody (Mouse, monoclonal, IgG1)
- BSA Conjugated Valproic Acid

Protocol

1. Conjugate VPA/BSA serum complex to the surface of wells:

    (a) Prepare a solution of used complex in PBS and introduce a constant volume into 12 wells.
    (b) Incubate this solution.
    (c) Remove the solution from the wells.
    (d) Flush wells 3 times with PBS water.

2. Prepare a series of 5 different VPA concentrations in PBS.

3. Prepare a solution of VPA specific antibodies in PBS.

4. Add the solution with VPA specific antibodies to the 5 different VPA concentrations and incubate.

5. Add the mixtures to well 3-12 and the VPA specific antibodies to well 1 and 2 (negative control) and incubate.

6. Remove the mixture from the wells and wash 3 times with PBS.

7. Prepare a solution of secondary antibodies with HRP attached to their heavy chains.

8. Introduce the solution of secondary antibodies to wells 1-12 and incubate them.

9. Remove the solution from wells and wash them 3 times with PBS.

10. Add TMB substrate to the wells a 1-12, incubate them for 10 minutes in a dark place.

11. Add STOP solution to all wells to brake the color reaction.

12. Quantify the results by measuring intensity of the color reaction using a plate reader, which measures absorbance.

13. Prepare a calibration curve based on different concentrations of VPA and the corresponding absorbance, determine the linearity of a given correlation.

D   Suggested protocol for attachment of magnetic nanoparticles to antibodies

The protocol is inspired by a protocol from Thermo Fisher Scientific [11].

Materials:

- Activation buffer

- Gold nanoparticles – NH2

- Valproic Acid Monoclonal Antibody (MA1-10765) from Invitrogen

- EDC

- Phosphate-buffered Saline (PBS)

NHS-ester activation:

1. EDC is added to VPA monoclonal antibody.

2. NHS is added to the reaction.

3. The reaction components are mixed and set to react for 15 min. at room temperature.

Amine reaction:

1. The buffer pH should be above 7.0. Concentrated sulfanylidenelead (PBS) can be used for pH adjustment.

2. Gold nanoparticles – NH2 is added to the solution containing activated VPA monoclonal antibody.

3. The solution is mixed well, and the reaction is set to react for 2 hours at room temperature [11].

E   Suggested protocol for attachment of antibodies to silica surface

The protocol is inspired by a protocol from Thermo Fisher Scientific [9].

Materials:

- 3-Aminopropyltriethoxysilane (APTES)

- Crosslinker: Sulfo-SMCC.

- Acetone.

- Coupling Buffer: PBS-EDTA.

- Desalting Column: D-SaltTM Dextran Desalting Columns.

- Reagent to add sulfhydryl groups: Traut's Reagent (2-Iminothiolane · HCl).

- Silica Surface.

- Valproic Acid Monoclonal Antibody (MA1-10765) from Invitrogen.

Aminosilylation of the silica surface

1. The silica surface is washed, after which it is dried.

2. A 2% solution of 3-Aminopropyltriethoxysilane in acetone is prepared.

3. The solution is covered over the surface.

4. The surface is immersed by using dry acetone.

5. The surface is allowed to air dry.

Maleimodi-activatition of the amino-modified surface

1. Sulfo-SMCC is added to Coupling Buffer.

2. The silylated surface is covered with the Crosslinker solution.

3. It is set to incubation for 1 hour at room temperature.

4. The modified surface is rinsed by using Coupling Buffer.

Adding sulfhydryl groups to antibody for Coupling

1. Coupling Buffer (PBS-EDTA, pH 7.2) is adjusted to pH 8.0 by using concentrated NaOH.

2. VPA monoclonal antibody is dissolved in the pH-adjusted Coupling Buffer.

3. Traut's Reagent is dissolved in the pH-adjusted Coupling Buffer.

4. The Traut's Reagent solution is added to the antibody solution.

5. It is incubated for 45 minutes at room temperature.

6. The modified antibody from excess Traut's Reagent is purified by using a Desalting Column equilibrated with Coupling Buffer (pH 7.2).

7. The fractions containing antibodies are identified by measuring for those having weak absorbance at 280 nm.

8. The fractions containing antibody are pooled.

Cross-linking sulfhydryl-containing antibody to activated Surface.

1. The maleimide-activated surface material is covered with the antibody solution.

2. It is incubated for 2-4 hours at room temperature.

3. The reaction solution, which contains any antibody that did not attach to the surface is removed.

4. The surface is rinsed with Coupling Buffer [9].

# F   Measurement and calibration flowchart
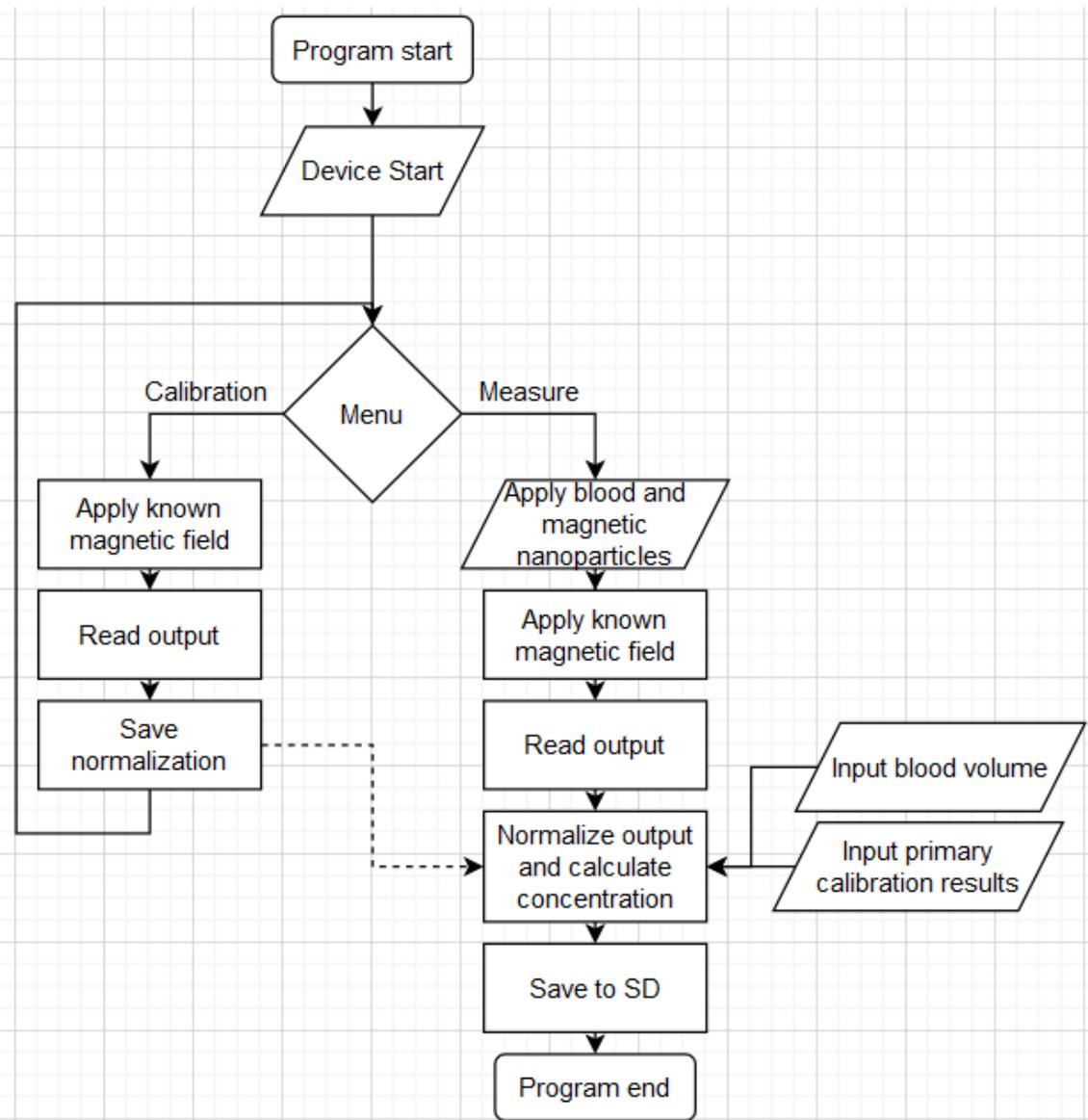
Figure 11: Flowchart showing the operation of the theoretical biosensor device.

The calibration done here is not the invasive calibration shown in section 3.6.2, but rather a mild version of this. This is intended to remove sources of noise at the given test area. Measurement then uses both this and the primary calibration from section 3.6.2 to determine the actual concentration. It also recieves blood volume as an input, in order to calculate the concentration.