AUSENSE 2020

AUC THE AMERICAN UNIVERSITY IN CAIRO

AFRICA & MIDDLE EAST REPRESENTATIVE

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SensUs

THE AMERICAN UNIVERSITY IN CAIRO 100 YEARS

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Table *of* Contents

Summary

AUSense team has developed an innovative biosensor with a great potential to measure the free valproic acid in blood plasma. We focused on providing a quick, easy-to-use and efficient biosensor at the lowest possible cost.

Our conceptual biosensor is based on the ability of valproic acid to inhibit a certain enzyme (SSDH). The enzyme reaction usually involves oxidation-reduction reaction or simply electron transfer which could be measured in terms of current. The inhibition by valproic acid will cause the measured current to decrease, and the amount of decreased current can then be correlated to the concentration of valproic acid. The biosensor is fairly simple, it consists of a multiwalled carbon-nanotube modified electrode, a potentiostat, and a reader instrument. We modified the surface of the screen-printed electrode with carbon nano-tubes to provide a better current measurement. Also, we used a protective Nafion membrane to improve the stability of the enzyme. The use of enzymes will provide better specificity towards the free valproic acid, which is the clinically-relevant concentration, rather than the total (bound and unbound) valproic acid concentration. We hope that our biosensor will help epileptic patients to lead happier and better lives.

2. Biosensor System & Assay

The biosensor is based on enzyme inhibition. The enzyme used is succinic semialdehyde dehydrogenase (SSDH) which is involved in the degradation of gamma aminobutyric acid (GABA), as well as catalyzing the conversion of succinate semialdehyde (SSA) to succinate, as well as catalyzing the conversion of succinate semialdehyde (SSA) to succinate, as shown in the following equation: **Succinate Semialdehyde + NAD^+ + H2O ⇌ Succinate + NADH + 2 H^+**

Since valproic acid is a potent inhibitor of succinic semialdehyde dehydrogenase, and the reaction involves electron transfer, an amperometric biosensor could be developed based on enzyme inhibition. In other words, the decrease in enzyme activity caused by valproic acid could be measured in terms of decreased current, and correlated to the concentration of valproic acid. In addition, the involvement of enzymes provides better selectivity and specificity towards the free or unbound valproic acid, which is the clinically-relevant concentration.

2.1. Molecular Recognition & Assay Reagents

The molecular recognition part consists of succinic semialdehyde dehydrogenase, succinate semialdehyde, and NAD^+. The enzyme molecules are cross-linked with each other to increase their stability using homobifunctional cross-linker such as Suberic acid-bis-(3-sulfo-N-hydroxysuccinimide ester) (BS3) which has an amino-reactive Sulfo-NHS ester on both ends of the cross-linker. fVPA inhibits SSDH resulting in decreased enzyme activity. Molecular interaction is illustrated in

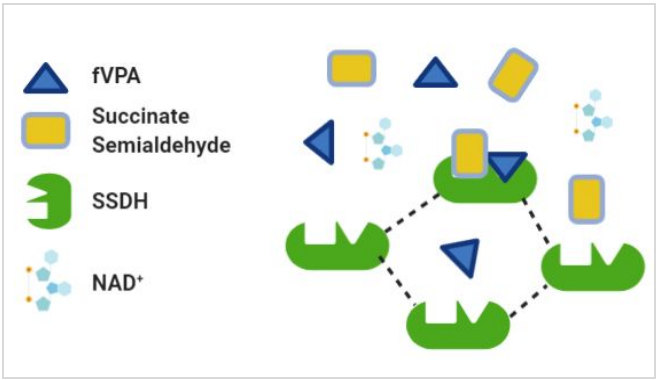

Figure 1: SSDH inhibition by fVPA.

A reaction mixture will contain the substrate, enzyme and cofactor. The concentration of the substrate to be used at Vmax as well as the Km value can be determined experimentally using the Lineweaver-Burk plot (plotting 1/v against 1/[s]). One unit of enzyme will catalyze the conversion of 1 μmol of succinate semialdehyde to succinate per minute at pH 8 and 25°C. Since the inhibition is irreversible, the most suitable measurement protocol to provide increased sensitivity will be based on residual activity. Firstly, 5 μL of sodium phosphate buffer will be added on the surface of the working electrode, followed by 10 μL of substrate and NAD^+ solution with concentration (as determined by the Lineweaver-Burk plot) and the signal is registered after incubating for 1 minute. Next, the liquid portion is removed and 15 μL of plasma sample containing valproic acid is added and left to incubate for 3 minutes. Finally, the electrode is rinsed several times with distilled water and then 5 μL of buffer is added, followed by 10 μL of substrate and NAD^+ solution, and the residual enzyme activity is measured. The residual activity is according to the equation $\%I = [(i_0 - i_i)/ i_0] - 100$, where i_0 is the initial activity and i_i is the activity in the presence of the inhibitor. To determine the linearity range, a standard curve will be made using different inhibitor concentrations.

2.2. Physical transduction

The enzyme will be cross-linked using BS3, then immobilized by physical adsorption on the working electrode of a multi-walled carbon nanotube-modified screen-printed electrode MWCNTs-SPE; Metrohm, Switzerland. The outline of the biosensor is shown in Figure 2. The cross-linker will be prepared by dissolving 10 mg of BS3 into 350 µL of 25 mM sodium phosphate buffer, then will be added to the enzyme solution with ratio of 20:1 (cross-linker: protein) so that the final concentration is between 0.5 to 5 mM, and left for 45 minutes. Finally, excess cross-linker will be quenched and reacted with 25 mM Tris, then removed using dialysis.

To immobilize the cross-linked enzyme, 2 µL of the enzyme with known units of activity will be placed on the surface of the working electrode and allowed to dry, and the excess can be washed with sodium phosphate buffer, thus, the enzyme activity will be 1 unit/mm^2 of the working electrode surface. After drying, an additional membrane such as sulfonated tetrafluoroethylene-based fluoropolymer (Nafion) will be added to act as a protective membrane to prevent enzyme leakage and anionic interferences. The membrane created by adding 4 µL of 0.5% Nafion solution to the surface of the electrode and left to dry.

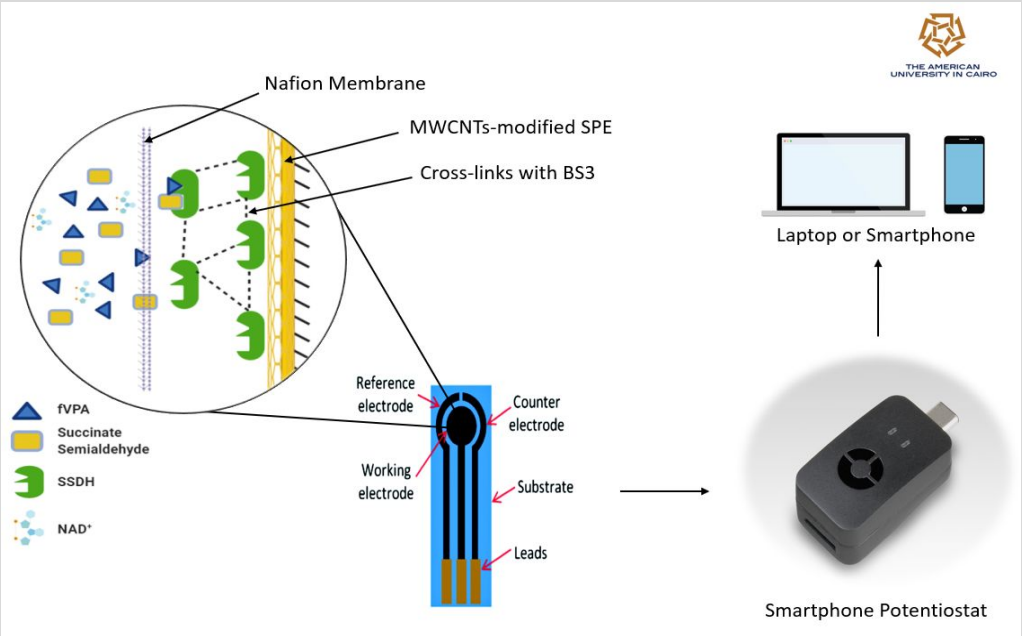

Figure 2: Biosensor Design

2.3. Cartridge Technology

The cartridge design is simple, it consists of the MWCNT-modified SPE (33 x 10 x 0.5 mm) and the portable potentiostat (43 x 25 x 11 mm); PalmSens, Netherlands. The sample is placed on the surface of the working electrode, which is then inserted into the potentiostat for measurement (current ranges 100 nA to 5 mA).

2.4. Reader Instrument & User Interaction

The reader instrument will consist of the portable biosensor (potentiostat and SPE) connected to a smartphone or laptop. Our own easy-to-use application built using PalmSens SDKs for Xamarin and WinForms for android and PC will feature cloud storage so that patients and physicians can have access to medical history. Patients will measure fVPA concentration according to the

recommended protocol using the electrode connected to the potentiostat and PC/ smartphone, then the concentration of fVPA will be displayed using the application.

3. Technological Feasibility

SSDH (E.C 1.2.1.24) belongs to the oxidoreductase enzyme group. We have performed molecular docking experiments using *AutoDock*1.5.6rc3® suite which have shown that free valproic acid is a potent inhibitor of SSDH. Enzyme kinetics parameters were also modelled, such as Km (6.3 mM for SSA, and 125 mM for NAD^+), pI (7.66), optimum pH range (8-9), and temperature (25°C). The average binding energy of fVPA and SSA to SSDH was -5.33 Kcal/mol and -4.51 kcal/mol. Moreover, in-silico studies revealed that free valproic acid is an irreversible and competitive inhibitor of SSDH, with Ki of 229.03 µM. The interaction between valproic acid and SSDH is shown in Figure 3. The three-dimensional interaction is also shown in Figure 4.

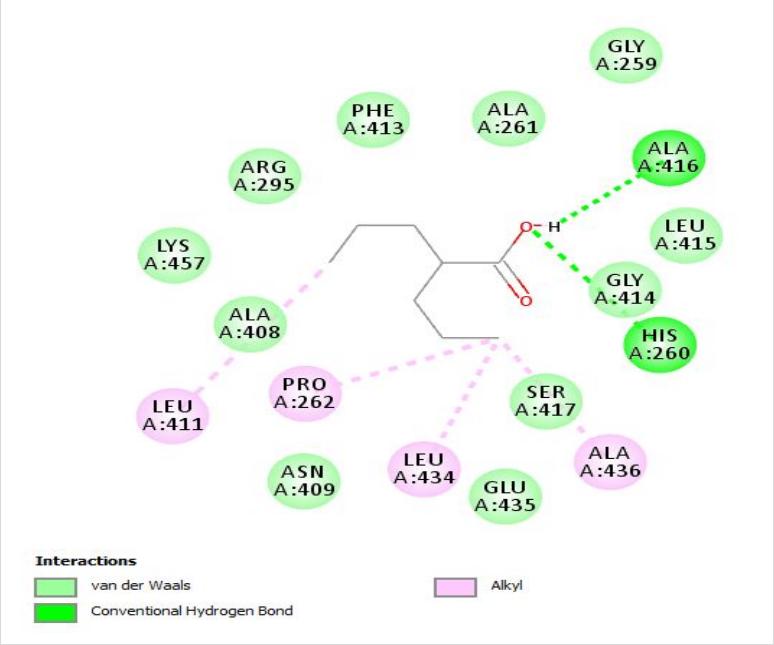

Figure 3: Two-dimensional binding sites scheme, showing the interaction between the ligand S1 (valproic acid) and the amino acid HIS A:260 of succinate semialdehyde dehydrogenase (SSDH) via H bond formation between O3 of valproic acid (refer to the numbering system in Table 1) and the Nitrogen atom of the HIS 260A with a hydrogen bond length of 3.21A^0. There is another interaction betweenO3 H4 (refer to the numbering system in Fig.1) of the OH of valproic acid and the O atom of the amino acid ALA A:416 of SSDH with a hydrogen bond length of 3.0A^0. The docking simulation of the binding energy of such hydrogen bond interaction is computed to be -4.97 *Kcal/mol*. Refer to Table 1 for these data.

Ligand	Run no.	Interaction residue in succinate semialdehyde dehydrogenase (SSDH)	Interaction atoms (amino acid ...ligand) HB length (A°)	H bonds formed	Binding Energy *Kcal/mol*	Inhibition constant K_i, µ*M*
valproic acid, S1	17	HIS260.A ALA416.A	N.....O3 (3.21) O.....H4O3 (3.0)	2	-4.97	229.03 µ*M*

Table 1: Docking molecular interactions of VALPROIC ACID docked succinate semialdehyde dehydrogenase (SSDH)

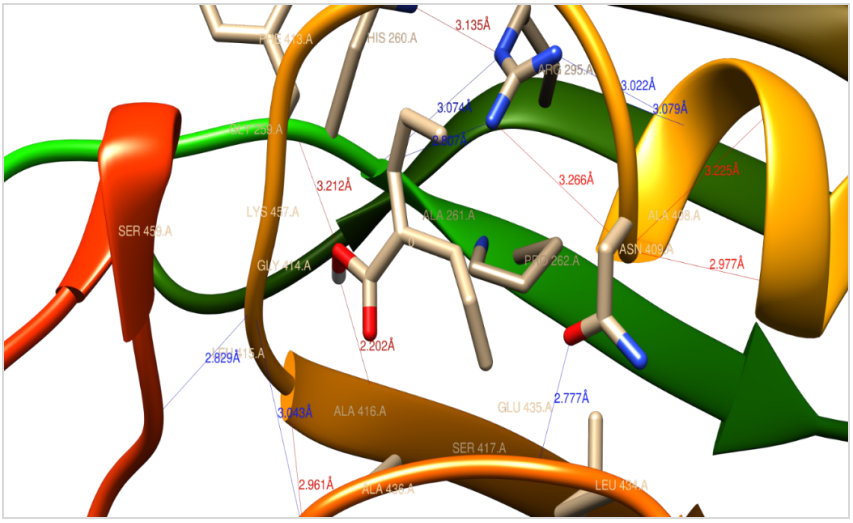

Figure 4: Three-dimensional view of the docking binding sites scheme, showing the interaction between the ligand S1 (valproic acid) and the amino acid HIS A:260 of succinate semialdehyde dehydrogenase (SSDH)

We analyzed the van der Waal and hydrogen bonds interactions between Valproic acid and succinate semialdehyde dehydrogenase using Discovery studio, as shown in Figure 5. It clearly indicates that the interaction mainly takes place between the hydroxyl acidic side chain donor and the amino acid Ala 416 and Ala 436 and the oxygen atom as a polar side chain acceptor.

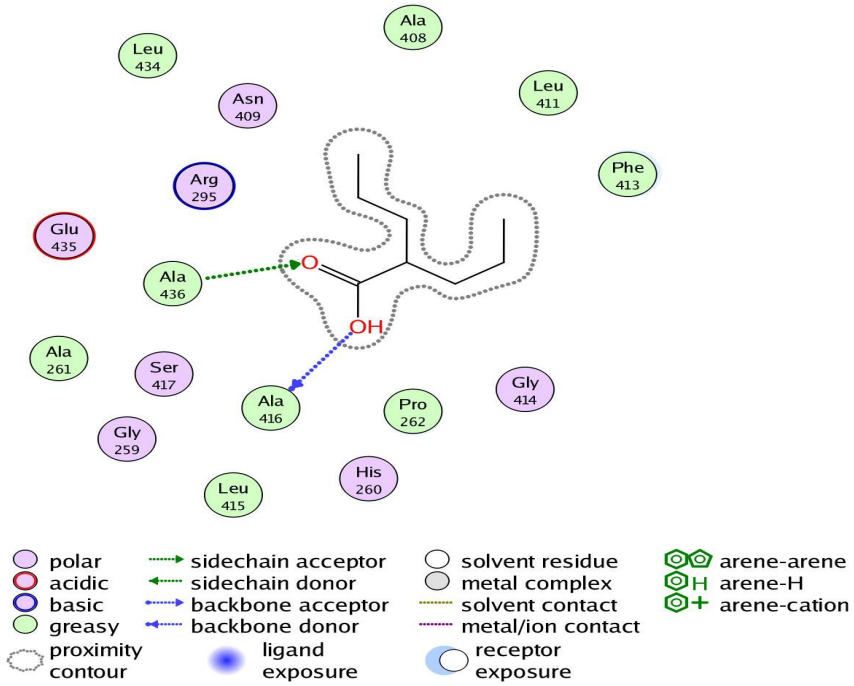

Figure 5: Various types of interactions as a result of the docking of valproic acid in SSDH

Finally, to confirm the specificity of SSDH inhibition to the fVPA, a control experiment can be done in which the enzyme assay will be carried out using samples containing only free valproate, another containing free valproate, and control sample containing no valproate.

4. Originality

Biosensors based on enzyme inhibition are already available on the market. The most popular is the acetylcholinesterase biosensor used to detect sarin and pesticides such as carbamates and organophosphorus compounds. Our main goal was to develop a cheap, user-friendly biosensor that can measure the concentration of free valproic acid, rather than the total valproic acid. Moreover, we wanted to reduce or eliminate any requirement for plasma pretreatment or the use of hazardous chemicals by epileptic patients.

Although we faced a lot of challenges, given the COVID-19 pandemic that resulted in laboratory-access restrictions, we managed to develop the best conceptual approach for biosensor development through extensive literature review, chemical modeling and consultation with experts. The proposed biosensor concept herein requires little sample preparation and minimal use of safe chemicals. Furthermore, the easy-to-use mobile application allows for enhanced user experience for patients and better follow-up procedures for physicians.

We independently reviewed and revisited many biosensors from published research, and selected the best approach for the development of the biosensor, we then fine-tuned the technique to suit our biosensor application. The new developments include the use of multi-walled carbon nanotubes-modified screen-printed electrodes (MWCNTs-SPE), which provides enhanced electron transfer and current measurements, as well as the use of chemically-inert Nafion membrane that protects the enzyme layer, in addition to its high permeability to cations and prevention of anionic interferences. The disposable screen-printed electrode provides a better and cheap alternative to measure the free valproic acid. Moreover, the chemical modeling shows good inhibition kinetics and specificity of free valproic acid towards succinate semialdehyde dehydrogenase, which offers a promising potential for the measurement of free valproic acid.

Supervisor Name: Dr. Hassan Azzazy **Signature:**	**Team Captain Name:** Bishoy Abib **Signature:**	**Team Captain Name:** Abdelrahman Nabih **Signature:**
		

5. Transitional Potential

5.1. Business Model Canvas

<table>
<tr>
<td rowspan="2">Partners

Hospitals and Medical Centers (Providing them with market data and analysis to get marketing benefits in return)
Metrohm
Palmsens</td>
<td>Key Activities

Programming
Production
Maintenance
Training
R&D</td>
<td rowspan="2">Value Proposition

1. Innovative Chemistry
2. Affordable assays
3. Higher sensitivity and fast testing
4. Specific for free valproic acid
5. Minimum sample handling
6. Patients' progress, and genetic history record and analysis.
7. Patients' Guardians virtual community</td>
<td>Customer Relationships

1. Patients & Guardians community
2. After sales service</td>
<td rowspan="2">Customer Segment

1. Egyptians with epilepsy symptoms whose guardians have technology literacy and stable internet connection.

2. Epilepsy Doctors and specialists who have work permissions and licenced clinics/medical centers.</td>
</tr>
<tr>
<td>Key Resources

Assets and raw material.
Patients' database and history.
Staff.</td>
<td>Channels

1. Hospitals
2. Medical Centers
3. Schools
4. Pharmacies</td>
</tr>
<tr>
<td colspan="3">Cost Structure

Electrode + Assembly costs
Logistics.
Fixed costs (staff, R&D and taxes).</td>
<td colspan="2">Revenue Streams

Sales & Ads.
Notify & other premium services subscription.
Doctors reservations transactions.
Device maintenance expenses.</td>
</tr>
</table>

5.2. Stakeholders Desirability

1 out of 4 newly diagnosed epileptic patients worldwide is a child who faces daily challenges on the medical, economic and social levels. Up to 70% of seizures are stoppable with early diagnosis and proper treatment that are not affordable in middle to low-income countries. Furthermore, epileptic children commonly struggle with the traditional education system due to the countless restrictions and limitations placed upon their movement and social engagement.

AUsense offers an integrated solution to ease, afford diagnosis procedures, and enhance children's social experience. Our triple solution consists of a modern biosensor/potentiostat, wearable device and web-mobile platform. The first component, the biosensor/potentiostat, enables instant blood plasma testing. This component is composed of a potentiostat, in partnership with PalmSens, our key service provider, and multi-walled carbon nanotubes-modified screen-printed electrodes, innovated by our technical team. Our unique biosensor has been developed using innovative chemistry to provide affordable assays, higher sensitivity and fast testing. Another competitive advantage is that our biosensor is specific for free valproic acid.

Second, our wearable device, in partnership with Empatica, monitors children's temperature and heartbeats, enabling them to live the standard social life with no restrictions on their social engagement as it regularly alerts parents with their child's case and of any possible sudden seizures. Patients' data are recorded and shared, through our platform, with physicians, specialists and parents. The whole solution is designed to give parents and guardians, our major stakeholders, full access on their kids' medical progress and medication efficiency. It also makes our holistic and integrated solution more attractive to other main stakeholders such as medical insurance companies, hospitals, clinics and ministry of health.

5.3. Financial Viability

AUsense financial viability will be obtained through our solid strategic management plan and diverse revenue streams. Our major revenue stream is from direct sales of our kit, biosensor, potentiostat and the wearable device, to both, epileptic patients and doctors. Device maintenance expenses will also contribute to AUsense financial stability. Second, AUsense online platform will offer premium services to epileptic patients and their guardians, allowing them to approach doctors and each other virtually. Within two years, these databases will provide an overall view of the epileptic community that could be sold to/interchanged with our partners from medical centers, hospitals and research institutes. Using Machine Learning Modelling, the platform will serve, the other way, as a medical advertising channel, our fourth revenue stream.

An estimation of 50 million patients, of all genders and age groups, compose the epileptic market, according to the World Health Organization. Egypt, our kickoff market, recorded 60,000/5,000,000 active cases are children suffering from epilepsy. In our very first year, we will target around 10% of the Egyptian market which represents 6,000 epileptic patients that use 12 cartridges per year per patient. Therefore, we estimate to sell 6000 potentiostats and 72000 cartridges. Furthermore, we assume 3% of the Egyptian market will subscribe to our premium digital services. The estimated cost per assay is $11.6, which includes the price of the electrode, enzyme and chemicals. Thus, the price is affordable for the market segment we are targeting

5.4. Business Feasibility

Three productive departments work collaboratively to evolve AUsense ultimate solution for epileptic children. Our technical team – bioassay developers and R&D leads – develops the biochemical technique and modelling. User experience and interaction are enhanced by the efforts of our software engineer and product designers, while a business developer maintains our strategic management and commercialization plans.

The international PalmSens is our key potentiostat partner and software provider, being integrated within our solution exclusively for two consecutive years. Launched in the Egyptian market, AUsense will receive direct support by an advisory board from AUC The American University in Cairo, Egypt.

6. Team & Support

6.1. Contributions of the Team Members

AUSense is a multidisciplinary team that comprises 5 undergraduate and 2 graduate students.

Bishoy Abib (Chemistry), Aya Allam (Chemistry), Rofida Zaghlol (Biology), Sohila Rabie (Biology); Worked mainly on the molecular recognition element by selecting the best technique or approach, checking the availability of the components and chemicals, molecular docking and modeling of valproic acid and SSDH, enzyme reaction kinetics, enzyme immobilization and cross-linking, and conducting surveys with patients and physicians.

Abdelrahman Nabih (Mechanical Engineering), Antonios Farouk (Physics), Zahraa Gamal (Computer Science); Worked mainly on the business model and plan, translational potential, biosensor design, selecting a suitable potentiostat, designing the user-interface and reader instrument parts.

6.2. People Who Have Given Support

Dr. Hassan Azzazy, Professor of Chemistry, AUC
As our supervisor, Dr. Hassan provided support and consultation regarding the biosensor technique, business plan and modeling, as well as ensuring the team was on track.

Dr. Mohamed El-Shakre, Professor of Chemistry, Cairo University
Provided consultation with molecular docking and modeling.

7. Final Remarks

We would like to thank everyone who has given us support. Also, we wanted to thank the SensUs organization for giving us the opportunity to participate in the competition this year. Although we faced many challenges due to COVID-19 crisis, we were able to find a good conceptual approach and provide promising data for the success of the biosensor. We look forward to continuing to work on this biosensor concept and developing the prototype.

8. References

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