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Optimization of the enzyme power source for a nano drug delivery system fuelled by glucose in blood plasma

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Abstract. A unique in vivo electrical pulse generator to improve membrane permeability for drugs and simultaneously facilitate self-powered nano devices for nano drug delivery systems (NDDS) was identified. The use of an unsupported biological catalyst component of the power supply was aimed at the NDDS instead of a conventional membrane electrode assembly (MEA). Self-powered carriers of drugs and prodrugs with improved controlled release capability to target areas using substrate available in biological matrices such as glucose in blood is envisaged. The experimental application implemented prototype designed chambers allowing the entry of premixed precursors and low ohm resistance due the absence of diffusion layers and optimised open circuit voltage (OCV). This would also minimise poisoning and rupturing of the proton exchange membrane (PEM). The model uses the isothermal experimental design (37^{o}C) parameter and the glucose is partly oxidised prior to entry and mostly oxidised at the surface of the proton exchange membrane (PEM). The experimental model used a residence time instead of the usual flow rate. The power was notably high for short periods due to the absence of carbon supported diffusion layers. The findings included low levels of glucose and glucose oxidase (GOx) are needed for OCV optimisation.

1. Introduction

Nature has over time established a chemical energy supply from biomass sources using complex metabolic pathways. Scientists are using this information to optimise in vitro power cells to power sustainable in vivo devices and other stationery device applications [1]. Where blood plasma contains approximately 90% water, drug dosage (concentrations) undergo a dilution effect coupled with an efficient blood circulatory system (systemic) without the efficacy of targeting may lead to poor therapeutic regimens and possible mutations where bacterial infections like Tuberculosis (TB and MTB) occur leading to extreme drug resistance (XDR) and deteriorating patient health where inadequate dosing is encountered. Polar drugs are more likely to be excreted via the urinary pathway further lowering the bioavailability of the intended therapeutic dose reducing efficacy. Multiple drug regimens and crushed drug (tablets) administration, to treat co–infections, are possibly affected by drug-drug interaction due to differences in the pKa (weak acid / base in pH buffered matrices)) chemistry could possibly compromise efficacy. A biologically compatible NDDS in physiological matrices is essential in maintaining the optimised pharmacokinetic (PK) levels ensuring the therapeutic drug dosage removes all symptoms and infection. The complex biological homeostasis energy balanced systems can counter the fluctuations in glucose as the precursor is consumed and the water by-product is formed. In a biological matrix the fuel source is placed in the anode chamber of the cell in the presence of a potentially biologically active catalyst (enzyme). At the anode, the enzyme (protein) oxidises the fuel source (glucose) to produce carbon dioxide and water. This chemical process does not only yield carbon dioxide and water but also yields electrons and protons.

$C_6H_{12}O_6 + H_2O \rightarrow C_6H_{12}O_7 + 2e^- + 2H^+$ ……………………………….………… (1)

The following reactions are seen to happen in a glucose fuel cell [2, 3]:

At the cathode, the electrons also flow to the positive terminal also known as the cathode through a conductive bridge for the completion of the cell. The current in this case flows from the negative terminal to the positive terminal [6].

$$\frac{1}{2} O_2 + 2e^- + 2H^+ \rightarrow H_2O \quad (2)$$

Overall cell reaction:

$$C_6 H_{12}O_6 + \frac{1}{2} O_2 \rightarrow C_6 H_{12}O_7 \quad (3)$$

Here the biological power source is defined as a conversion mechanism of chemical energy stored in bio-molecule fuel into electrical energy released by the catalytic activity of enzymes. A substrate in this process is consumed and the electrons are produced from anode glucose catalytic GOx reaction. The electrons move to the negative terminal of the cell also known as the anode to the cathode electrode depending on the open cell potential efficiency. The optimised theoretical power cell potential for glucose as a fuel source is approximately 1.256 V [2, 3]. Owing to over-potential losses this will typically show operating glucose power source cells operate at an open circuit potential (OCV) of 0.5–0.8 V.

The model assumes that the glucose and hydrogen ion transport through the cell occurs through a membrane that is temperature dependant. The glucose fuel cell domain was divided into 3 compartments namely - the anode premix chamber (APC), the proton exchange membrane and the cathode premix chamber (CPC). The schematic of the fuel cell is shown in Figure 1 with the dimensions. It is assumed that the membrane is impermeable to glucose but permeable to hydrogen ions. Thus, the concentration of the glucose is modelled only until the membrane. The reaction of the glucose to form hydrogen ions is assumed to occur in the APC, in presence of the enzyme, GOx. The unreacted glucose and the hydrogen ions diffuse across the cell to the cathode. The unreacted glucose is assumed not to react at the cathode. The model assumes that the transport of glucose is solely by diffusion and convection. The effect of the potential drop across the glucose fuel cell on glucose molecules is considered to be negligible (glucose is not a charged species). The potential drop across the membrane is considered to be a significant factor in the transport of hydrogen ions across the membrane. The effect of potential drop on the transport of hydrogen ions across the enzyme layer and the cathode catalyst layer is considered negligible given the thickness 183μm.The electrode biofouling can also contribute to the potential drop compounded by non-porous electrodes and unbound enzymes [4].

2. Experimental Methods

A scale-up power cell prototype model, Figure 1, was used as the power cell included an anode chamber contained GOx and glucose. The anode chamber contained neither facilitator nor mediator at temperature ranging from 36.9 to 37.1^{o}C. The anode chamber was degassed using a nitrogen cylinder (N_2) fitted with a regulator, the inert gas was flowed through the system for 2 hours. The buffer used was a phosphate buffer to facilitate the ion migration and pH stability. The assumed Gibbs energy was -97.5kJ.mol^{-1} [8]. The electrodes used were carbon graphite rods, 1 per chamber (4 mm carbon rod electrode diameter; 50 mm length with only flat 4mm end immersed) where the electrode reactions are controlled by the enzyme kinetics [7].

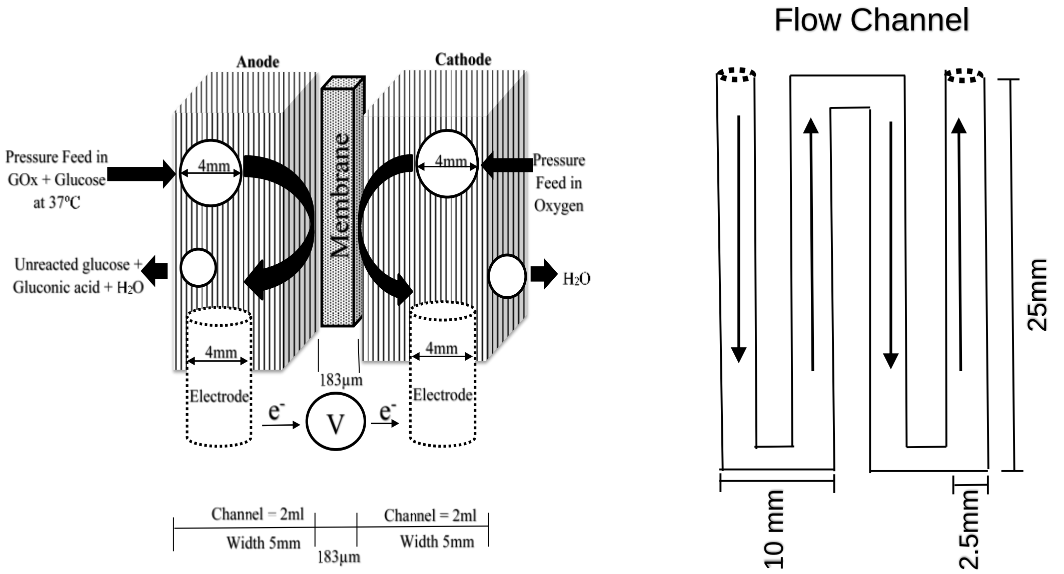


Figure 1: Power cell (EFC) with 2 electrodes and glucose / GOx feed in absence of diffusion layer

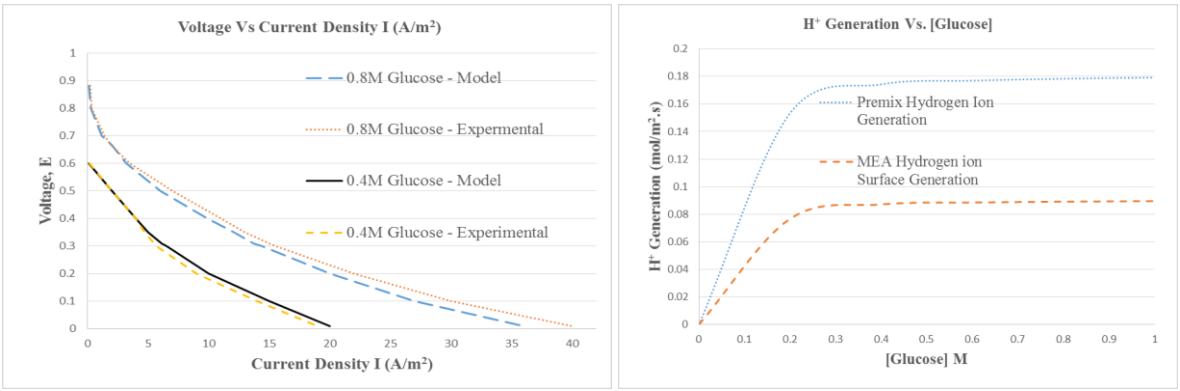
Electrical circuit was connected using insulated conductive and chrome plated electrically conductive crocodile clip connections. The clip connections were not in contact with the solutions in either chamber. The experiments were conducted under biological temperatures conditions and ranged from 229 to 331 K. The electrode area was calculated as follows.

$$A = 2\pi r^2 + 4(L_1 \times B_1) + 2(L_2 \times B_2) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

The sulfonated tetrafluoroethylene based fluoropolymer-copolymer Nafion® [6], was incorporated as it displayed sufficient stability previously [6]. The pH influence on open circuit voltage (vs. E) was measured by multi-meter (software) and Metrohm pH meter in 0.001M HCl (Kimix batch no. 264). The electrochemical activity (ECA) was studied using software attached to a sensitive multimeter. The basic mechanism of the electricity production in this cell was to allow the freely available glucose to react with glucose oxidase and co-factors in an aerobic environment. The glucose reacted with water to produce carbon dioxide, protons and electrons, Equations 1-3.

3. Results and Discussion

The influence of enzyme and substrate concentration was investigated as a redox relationship to produce hydrogen ions contributing to the OCV. The physical measurable components included the hydrogen ion concentration influencing the current density, electron and ionic charge, electrical conductivity and electrical field. The curent density is one dimensional and plotted on the x-axis, Figure 2a.



(a) **(b)**

Figures 2 (a) and (b): Modeled Polarization and comparative experimental data for varied glucose concentrations and hydrogen ion generation.

The divergence of current density depended on the hydrogen ion formation being relative to the oxidation and reduction (consumption of H^+) processes. The hydrogen ion generation occurred mainly at the anode compartment and the consumption, assuming membrane proton conductivity was optimal, was noted to occur in the cathode compartment. The hydrogen ion diffusion coefficient in water was accepted as $4.5x10^{-9}$ m^2/s.

Proton flux towards the cathode have significantly influenced the OCV. Phosphate buffer optimisation of pH dependant flux was seen to improve the potential difference at biological pH7. The flux of protons through the membrane between anode and cathode solutions is expressed as a function of the mass transfer coefficient and assuming a constant pH in the cathodic solution. The kinetic enzyme reaction rate coefficient was estimated as $1.6x10^3$ s^{-1} and the hydrogen ion surface generation (H^+_s) was calculated at 0.088mol/(s.m^2) for the MEA.

$$H^+_s = \frac{[E][G]\,2k_{cat}}{[G]+k_m} \quad \ldots\ldots (5)$$

Equation (5) where the concentrations for enzyme and glucose are estimated as $2.8x10^{-5}$ and 1.0M respectively for OCV approximately 0.45V when a membrane electrode assembly (MEA) is used, however a premix, Figure 2(b) can produce a shorter yet >2 fold power increase. There was a noticeable power surge and power spikes exceeding 42 A/m^2 recorded. The power discontinuity could be due to slow diffusion rates across the water and the PEM medium inhibiting the charged particle gradient formation affecting the OCV and depletion parameter.

4. Conclusion

The drug permeability enhancement from a NDDS with polar and non-polar drug carrying capability, in the lipophilic form and with a carrier that can enhance permeability by means of *in vivo* generated electroporisity pulses as a possible mechanism to supply pulses to the gated channels and possibly create new temporary transport channels has been supported with this research. With the ability to affect the capacitance capability of the bi-lipid membrane and the ion movement, the power source can stimulate the absorption of molecules to either support the sustained potential difference across the membranes or stimulate transport of and when necessary block potassium (K^+), calcium (Ca^{2+}) or sodium ions (Na^+) during the cells natural electrochemical operations.

Figure 2a, model fit for 2 glucose concentrations (0.4M and 0.8M) are in support of varying in vivo glucose levels to change the ECA power output. The varied concentrations support the introduction of a control mechanism that can regulate power and subsequent pulse frequency and intensity. Insulin level regulation will be monitored in future as a regulatory mechanism for ECA power outputs (influencing permeability) to adjust glucose and subsequent OCV levels.

Figure 2b, the premix power was higher than that of the MEA however short lived. Increases in potential were noted with increases in the glucose and enzyme (GOx) concentrations. OCV logarithmic dependence was evident in increased GOx concentrations that is relative to buffer concentrations. The voltage increased where the buffer concentration decreased.

NDDS design qualification criteria will influence the drug-transport efficiency in tissue once released from the carrier, where neutral drug forms are considered favoured in the pharmacological context being lipophilic. The buffer role and significance was highlighted during the proton formation and transport, as the proton formation can lower the pH however increased buffer concentrations have been noted to lower the OCV. The maintenance of the pH will become highly significant depending on the PKa of the drug in the carrier. The carrier (possibly a Micelle) will ideally be polar with the capability to carry non-polar drugs. Drugs which are considered weak acids will easily maintain their neutral configuration in a matrix pH below their PKa values. There may be a need for a compartmentalised power source and storage cell during the courier of weak bases as the pH needed is above the PKa to maintain the neutral electronic status of the molecules. The substrate oxidation and proton generation may form ionisation of the drugs considered weak base. The neutral glucose molecule can be oxidised however a passing glucose molecule in plasma can be secured within the NDDS carrier by phosphorylation ionisation, with a charge of -1.8 easily binding to the enzyme by electrostatic interaction.

The optimized model will include glucose receptors and binding sites ensuring the constant supply of glucose in a porous electrode anode chamber. The use of over expressed proteins coating the electrode and membrane. In the absence of chemical mediator's, transportation is depended on the mechanical entry and distribution effects of pressure supplied substrate in the blood matrix to facilitate the ECA. Transportation is also depended on effective OCV's and proton concentration gradients. A logarithmic dependence exists where increased enzyme levels exceed the buffer concentrations. Although molecules of similar polarities attract each other the drug permeability will depend on the matrix pH and miscibility of the medium solvents to ensure the diffusing molecule is able to permeate within lipophilic mediums. It seems with time the overall output of the power density decreases however this does not occur in a linear manner. The voltage output was within the range of 0.5 V and 0.8V. This was possible at low glucose concentrations which is promising as OCV increases is then possible at higher glucose concentrations. There was evidence of hydrogen ion consumption (C_o/C_L) ratio increases with the generation and subsequent increased consumption of the proton. This created a voltage gradient also due to the boundary condition near the electrode surface as well as the distance travelled by the proton.

Future micelles with ECA capability for drug delivery following spray drying for microencapsulation, directional flow studies and micro fluidic system design could aid in the effectiveness in prototype advancements to further develop working models. The model will be most effective where the carrier can be inserted into the blood matrix and function optimally utilising the biological conditions without having to make changes to the matrix needed for an optimised OCV.

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