Gold nanoparticle-based biofuel cell catalytic efficiency reliance on medium pH

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Abstract. Over the past several decades, biology, chemistry, and medicine took advantage of gold nanoparticles (AuNPs) for their fascinating characteristics. Today these nanomaterials can be synthesized in various methods from biosynthesis, when particles are made by microbial culture, to physical. Using the fast scan cyclic voltammetry, AuNPs can be electrodeposited from chloroauric acid ($HAuCl_4$) directly onto the surface of a graphite electrode. This method provides high reproducibility and allows to control the size of the nanoparticles (from 4 to 75 nm) and electrode coverage by changing $HAuCl_4$ concentration, scan speed, and a number of applied cycles. Gold nanoparticles are known to express comparable catalytic activity to natural enzymes such as glucose oxidase or horseradish peroxidase. Despite the advantages over natural enzymes, inorganic enzyme mimicking catalysts exhibit limited catalytical efficiency and selectivity. To increase efficiency, we used low size AuNPs which increase reactivity; the lack of selectivity in a biofuel cell is advantageous since AuNPs can reduce/oxidize several substrates in the medium. Biofuel cell based on AuNP's has a superior life span, as the main active part of the system is inorganic, and thus it is not affected by oxygen stress, where typical microbial biofuel cells are struggling. The observed catalytic activity had a minimal dependency on temperature change. pH was changed from 4 to 10 with the smallest synthesized AuNPs. However, pH has an impact on the reaction speed. Results showed that biofuel cell is suitable for raw wastewater as it usually deviates from pH 7, and usable current can be generated.

1. Introduction

Biofuel cells are devices that transform chemical energy into electrical energy utilizing reactions that involve biochemical pathways. Biofuel cells can be divided into two distinct groups: microbial fuel cells and enzyme-based fuel cells. Microbial fuel cells use whole living organisms to complete enzyme pathways, while enzyme-based fuel cells use isolated and purified enzymes to catalyse specific electrochemical reactions [1].

The use of biofuel cells for the generation of electrical power has several advantages. Firstly, biofuel cells are substantially less harmful to the environment than traditional batteries since they provide power from non-toxic materials that occur naturally, such as glucose. Secondly, biofuel cells

can operate under mild conditions (20-40^{o}C and around neutral pH), making them desirable for application where harsh conditions are unwanted. In addition, biofuel cells can consume a wide variety of fuel substances [2, 1].

Enzyme-based fuel cells are promising in being applied in implantable medical devices, including pacemakers, drug pumps, and blood glucose meters. Current implantable medical devices use lithium-ion batteries for power, which require replacement from time to time. This replacement process leads to high medical costs and causes pain to the patients. Enzyme-based biofuel cells are biocompatible and can have long lifetimes due to the fact that they use glucose in the blood for power and oxygen as an oxidating agent. They do not require recharging as the body supplies the fuel and have high efficiency. Integrating enzyme-based biofuel cells into implantable medical devices would reduce medical costs and alleviate pain for the patients [3].

However, several drawbacks prevent the practical usage of current enzyme-based biofuel cells. A considerable problem is an instability. Enzymes are commonly active in a solution at body temperature for only a few days. This can be overcome to increase enzyme stability for up to 45 days by immobilising the enzymes. Most enzyme-based biofuel cells also depend on cofactors such as NAD+ and NADP+, which poses another instability issue. Mediated biofuel cells also suffer from another layer of instability. Organometallic mediators tend to suffer from loss of their ligands even if they show reversibility over multiple voltammetric scans. Most organic mediators experience a loss of stability due to dimerisation and can lead to the deterioration of electrodes in the cell. Enzymatic biofuel cells also carry a risk of being harmful to the body as they operate close to living tissue, and bodily fluids passing by the cell may suffer from denaturation [4].

Another issue is mass transfer—the diffusion of the fuel limits the efficacy of biofuel cells to the active sites of the catalysts. When there is high resistance to the fuel transfer, a concentration difference tends to build up between the bulk phase and the active site of the catalyst, which slows down the rate of the reaction and can cause polarisation of electrodes. Lastly, the power density of biofuel cells is a big issue. Power density is usually measured by power output per surface area of the electrode. A necessary step to achieve high power density is high enzyme loading. For example, when glucose oxidase was randomly packed as a monolayer on a flat surface was only 1.7×1012 mol/cm^2, which was determined by the physical size of the enzyme. The theoretical highest current density for this layer was calculated at about 0.2 mA/cm^2. Glucose oxidase is known as one of the most efficient redox enzymes. Therefore other enzymes with lower specific activity should produce even less power [5].

Nanozymes are catalytic nanomaterials with enzyme-like characteristics. While natural enzymes are selective, highly efficient and active catalysts, due to some of their intrinsic drawbacks such as high cost, laborious preparation, degeneration, sensitivity of catalytic activity to environmental conditions and difficulty of recycling, natural enzyme practical application is limited. Nanozymes surpass natural enzymes in these terms (while also being biocompatible) as they are simply prepared, low in cost, highly stable and easily recycled [6,7]. Besides excelling in certain aspects compared to natural enzymes, nanozymes in addition exhibit unique features: their size, structure and composition can be adjusted, changing catalytic activity. Their high surface area can be easily modified and bio-conjugated, they have multiple uses besides catalysis and have a smart response to external stimuli [6]. Yet, nanozymes, unlike natural enzymes, lack in substrate selectivity and catalytic activity, leaving space for improvement [8]. Lack of substrate selectivity could be used as an advantage when constructing nanozyme-based fuel cell. It is discovered that AuNPs could also act as a glucose oxidase mimic catalytically reacting glucose with oxygen to form gluconic acid and hydrogen peroxide [9]. The reaction rate is slower at lower pH, and because one of the products of glucose oxidation is gluconic acid, therefore the reaction is self-limiting. Adding a base can compensate for the pH drop [10].

The AuNP catalysed oxidation of glucose reaction follows an Eley-Rideal mechanism. In summary, the hydrated glucose anion adsorbs to the AuNP leading to electron-rich AuNP. After that, oxygen is activated by forming a dioxogold intermediate, then two electrons are transferred from the adsorbed glucose to oxygen via the dioxogold intermediate forming H_2O_2 and gluconate anion [6].

The aim of this paper is to evaluate biofuel cells catalytic efficiency reliance on medium pH using gold nanoparticles as catalyst in glucose oxidation reaction.

2. Materials and Methods

2.1. Materials

The 0.05 M phosphate-acetate buffer solution (PABS) was prepared by dissolving 0.05 M CH_3COONa, 0.05 M NaH_2PO_4, 0.05 M Na_2HPO_4 in distilled water. To increase buffer solutions conductivity 0.1 M KCl was added to it. 0.1 M $HAuCl_4$ solution was prepared in 0.5 M H_2SO_4. All chemicals were purchased from Sigma-Aldrich (Steinheim, Germany). Glucose (≥98%) and potassium ferricyanide (≥99.0%) were purchased from Carl Roth (Karlsruhe, Germany). All solutions were prepared in buffer. Glucose solution was left to mutarotate overnight before use.

2.2. Graphite electrode preparation

A small piece of the graphite electrode rod (150 mm, ø3 mm, low density, 99,995% trace metal basis) was cut and sanded with 4 different grit size sandpaper, to finish polished with paper until shinny surface is achieved. Then prepared electrode is washed with 97% ethanol to remove any grease.

To deposit gold nanoparticles onto the electrode surface fast-scan cyclic voltammetry was used. Deposition was performed using three-electrode electrochemical cell. Earlier prepared graphite rod used as a working electrode, platinum wire as an auxiliary electrode and the Ag/AgCl/KCl (3M) electrode as a reference electrode. All the components (borosilicate glass titration vessel with plastic mounting ring and lid, platinum, and 12.5 cm length Ag/AgCl/KCl (3M) electrodes) were purchased from Metrohm AG (Herisau, Switzerland). The deposition of AuNPs was performed by cycling in 0.1 M $HAuCl_4$ solution at different number of cycles at 12.5 V/s scan rate, in range from 0 to +850 mV. All AuNPs were deposited at the same conditions, to form differently-sized nanoparticles number of scans changed from 10 to 500. To verify that gold was deposited onto the electrode regular cyclic voltammetry in range from -0.7 to 1.3 V, at a scan rate of 0.1 V/s was performed to observe oxidation and reduction peaks of gold and gold(III).

2.3. Electrochemical measurements

Electrochemical measurements were performed using DorpSens μ400 potenciostat (Utrecht, the Netherlands) and DropView software. All experiments were carried out in ambient temperature (at 20 ◦C) in phosphate-acetate buffer solution (pH from 4 to 10) under aerobic conditions. The cycles were measured five at a time, and the last one was plotted in the result section. If the concentration of additional materials were changed, it was done sequentially by adding it to the same measuring cell. In between, additions solution was stirred for 30 s and left to sit for 1 min. All measurements were repeated 5 times, and the average values were derived. Cyclic voltammograms were recorded at a scan rate of 0.1 V/s in the range from −0.7 V to 0.7 V.

2.4. Calculations

The Hill equation is used to determine how many ligand molecules bind to a protein macromolecule to produce a functional effect. However, the Hill coefficient gives an accurate estimation of how many ligands bind to a protein only if there is extreme cooperativity between the binding of the first and subsequent ligand molecules. Therefore, the Hill equation is sometimes used to describe cooperativity among multiple ligand binding sites, and the Hill coefficient can be thought of as an “interaction” coefficient [11].

Electrochemical measurements were evaluated by Hill's equation:

$$J = \frac{C^n}{k^n + C^n} \quad (1)$$

where: J is current density, C is the concentration of substrate (glucose), k is the half-maximum concentration constant, and n is the Hill coefficient.

3. Results and Discussion

Cyclic voltammograms were recorded using graphite electrode modified with 40 nm size gold nanoparticles, 750 µM of potassium ferricyanide and incrementally changing glucose concentration form 0 to 50 mM and then changing medium to fresh with different pH value and oxidation peaks were plotted as dependency on glucose concentration (Figure 1). pH value was changed from 4 to 10 by increments of one. In all samples, current density increased with the increase of glucose concentration. The most significant change was observed at 9 pH, from 90 to 125 µA/cm^2. The 9 pH could be contributing to neutralising gluconolactone and, in this way, pushing reaction towards the product side.

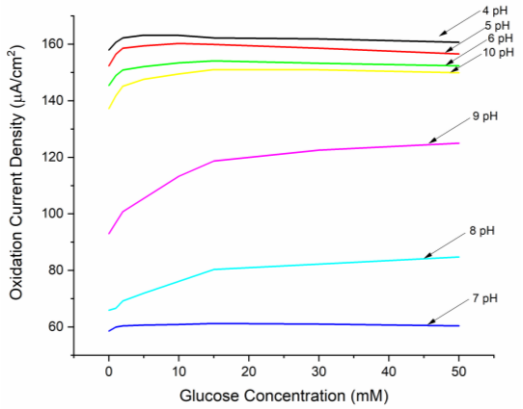

Figure 1. Current density dependency on glucose concentration when medium pH was changed from 4 to 10. The medium consists of PABS of different pH (from 4 to 10), 0.75 mM of potassium ferricyanide, and glucose.

The oxidation and reduction peaks of current density from voltammogram were plotted as glucose concentration dependence and evaluated by Hill's equation (Equation 1) (Figure 2). At the 0.15 V potential, the Hill coefficient n was higher than one (n=1.26), and at the 0.35 V potential, n was also higher than one (n=1.09), indicating positive cooperativity at both oxidation and reduction processes. If glucose concentration increases, then the efficiency of charge transfer by glucose molecules also increases. Half the maximum concentration constant k (k_{ox}=8.123) was lower at oxidation potential than at reduction potential (k_{red}=9.08), which emphasises that oxidation reaction rate was faster.

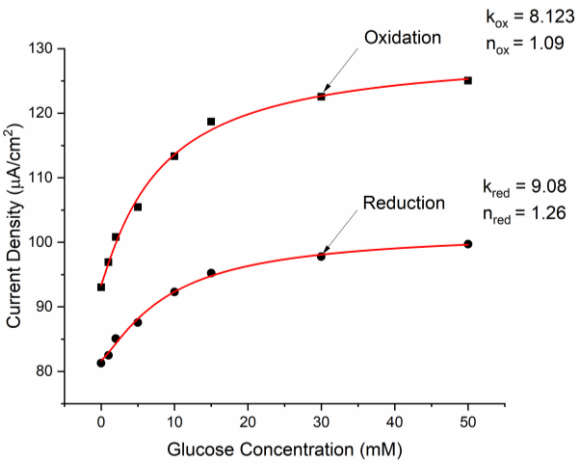

Figure 2. The peaks of current from voltammogram as a dependency on glucose concentration. Oxidation peak registered at +380 mV; Reduction peak registered at +150 mV.

The highest current density achieved at a glucose concentration of 30 mM. For that reason, other AuNP-modified graphite electrodes were tested at 9 pH and 30 mM glucose concentration.

Cyclic voltammograms were recorded at 750 µM of potassium ferricyanide, in pH 9 and 30 mM glucose concentration, using graphite electrode modified with different size gold nanoparticles, from 4 to 75 nm (Figure 3). Experimental data showed that the highest current density of 181 µA/cm^2 was achieved when particle size was 5 nm. And when particle size reaches 40 nm, current density decreases substantially and reaches plain graphite electrode.

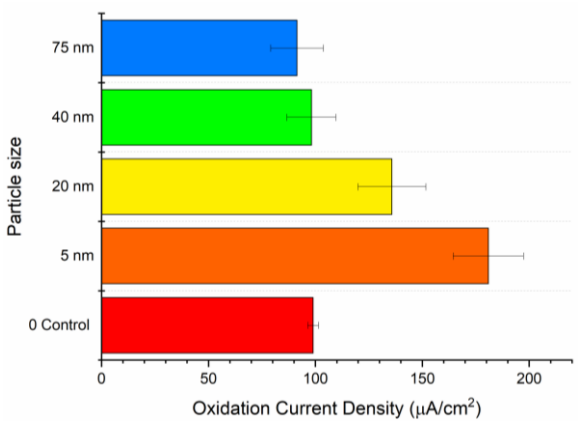

Figure 3. Oxidation peak current density dependency on particle size. Oxidation peak at +380 mV; Solution: PABS of pH 9, 0.75 mM of potassium ferricyanide, and 30 mM glucose.

The system with 5 nm AuNP's were evaluated by fitting the peak current was from voltammograms to Hill's equation (Equation 1) (Figure 4). The highest current density was achieved when AuNP size was 5 nm at a maximum peak current density of 183 µA/cm^2.

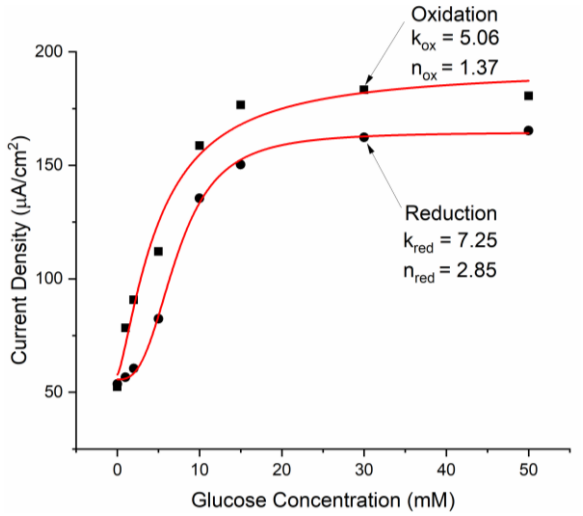

Figure 4. The peaks of current from voltammogram as a dependency on glucose concentration. When pH is 9; 0.75 mM of potassium ferricyanide. Oxidation peak at +380 mV; Reduction peak at +150 mV.

At the 0.15 V potential, the Hill coefficient n was higher than one (n=2.86), and at the 0.35 V potential, n was also higher than one (n=1.37), indicating that positive cooperativity was observed at oxidation and reduction peaks. It means if glucose concentration increases, then the efficiency of charge transfer by glucose molecules also increases. Half maximum concentration constant k (k_{ox}=5.06) was lower at oxidation potential than reduction (kred=7.25), emphasising that the reaction rate was faster at oxidation. The built system can still be improved by removing sulfur from gold particles, thus providing a larger surface area for the catalytic reaction.

Summary
A biofuel cell based on the gold nanoparticles concept was designed and evaluated. This research shows that the optimal medium pH for such biofuel cells would be 9. This could be because it can withstand a higher concentration of gluconolactone, which provides better efficiency in the system. After evaluating the optimal pH for the medium, it was used to research the impact of the gold nanoparticle size on the system performance. The highest current density (183 $\mu A/cm^2$) was observed when AuNPs size reached 5 nm, and glucose concentration was 30 mM. It increased linearly with decreasing size of the particles. Smaller size nanoparticles could be used to improve current density further. And according to Hill's equation, all reactions have positive cooperativity, however, reactions speed increased when smaller particles were used. As synthesis process uses sulfuric acid gold could have some sulfur residue on its surface, thus the reaction has less surface area to take place. This system could be further improved by the removal of sulfur from the gold surface. Our future research will be related to measuring power output and upgrading the current design of the system.

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