Bioelectrochemistry 106 (2015) 41–46

Contents lists available at ScienceDirect

Bioelectrochemistry

journal homepage: www.elsevier.com/locate/bioelechem

A glucose anode for enzymatic fuel cells optimized for current production under physiological conditions using a design of experiment approach

Rakesh Kumar, Dónal Leech *

School of Chemistry & Ryan Institute, National University of Ireland Galway, University Road, Galway, Ireland

ARTICLE INFO

Article history:
Received 22 December 2014
Received in revised form 13 May 2015
Accepted 1 June 2015
Available online 20 June 2015

Keywords:
Biofuel cell
Osmium mediator
Glucose oxidation
Design of experiment

ABSTRACT

This study reports a design of experiment methodology to investigate and improve the performance of glucose oxidizing enzyme electrodes. Enzyme electrodes were constructed by co-immobilization of amine-containing osmium redox complexes, multiwalled carbon nanotubes and glucose oxidase in a carboxymethyldextran matrix at graphite electrode surfaces to provide a 3-dimensional matrix for electrocatalytic oxidation of glucose. Optimization of the amount of the enzyme electrode components to produce the highest current density under pseudo-physiological conditions of 5 mM glucose in saline buffer at 37 °C was performed using response surface methodology. A statistical analysis showed that the proposed model had a good fit with the experimental results. From the validated model, the addition of multiwalled carbon nanotubes and carboxymethyldextran components was identified as major contributing factors to the improved performance. Based on the optimized amount of components, enzyme electrodes display current densities of 1.2 ± 0.1 mA cm^{-2} and 5.2 ± 0.2 mA cm^{-2} at 0.2 V vs. Ag/AgCl in buffer containing 5 mM and 100 mM glucose, respectively, largely consistent with the predicted values. This demonstrates that use of a design of experiment approach can be applied effectively and efficiently to improve the performance of enzyme electrodes as anodes for biofuel cell device development.

1. Introduction

Enzymatic biofuel cells (EFCs) are bioelectrochemical devices capable of converting chemical energy into electrical energy using enzymes as catalysts [1,2]. Use of an enzyme as biocatalyst rather than conventional noble metal catalysts can render the catalytic reaction more specific towards fuel or oxidant [3,4]. Enzymes immobilized at electrodes provide therefore the possibility for a membrane-less configuration of fuel cell, opening up opportunities for development of miniaturized systems for powering electronic devices [2,5]. Much interest in recent years has focused on the development of potentially implantable, miniaturized membrane-less EFCs that can deliver power using oxidation of fuel such as glucose present in the bloodstream at an anode and oxygen reduction at a cathode [6,7].

Various anode compositions, utilizing glucose oxidizing enzymes as a catalyst, have been investigated for EFC applications [8,9]. For example, a series of studies focused on improving the current signal and stability for glucose oxidation by co-immobilization of enzymes inside redox-conducting hydrogels at electrode surfaces [10]. Hydrogels based on osmium polypyridyl complexes co-ordinatively bound to polymers are widely used as mediators in EFCs, as they can "wire" enzymes by electron-hopping self-exchange within the hydrogels for connection between redox active sites of enzymes and electrode surface [5]. Osmium based systems possess advantages over iron and ruthenium based systems due to the low redox potential of Os(II/III) redox transition and relative stability of complexes in both oxidation states [11,12]. The inclusion of multiwalled carbon nanotubes (MWCNTs) in enzyme electrode films results in improved catalytic current and operational stability of the enzyme electrodes [13]. These nanostructures provide a conductive support which acts as scaffold for retention of enzyme activity, as a function of the amount deposited [3,14,15].

As an alternate approach to immobilizing redox complexes, Danilowicz et al. reported on coupling of aldehyde functional groups, distal to the osmium metal center of complexes, to amine-based polymer and enzymes to form films on electrode surfaces [12]. Boland et al. reported on coupling of osmium complexes, that contain an amine functional group distal to the metal centre, and glucose oxidase (GOx) to a carboxymethylated dextran (CMD) polymer previously anchored to functionalized electrode surfaces [16]. Similarly amine functionalized osmium complexes and enzymes have been coupled to a polyallylamine support using a di-epoxide reagent, to produce enzyme electrodes [17]. More recently, co-immobilization of amine functionalized osmium complexes and enzymes to a CMD polymer and MWCNTs on graphite electrodes using a carbodiimide coupling approach has been used to prepare enzyme electrodes [18]. Given the number of different components, and the range of amounts and methods used to prepare these enzyme electrodes, a comprehensive study is required on optimization of each component used in enzyme electrode preparation to identify the interaction or dependency of

* Corresponding author.
E-mail address: donal.leech@nuigalway.ie (D. Leech).

http://dx.doi.org/10.1016/j.bioelechem.2015.06.005
1567-5394/

these components on the performance of enzyme electrodes. Rather than adopt an optimization approach based on alteration of one factor at a time, a design-of-experiment (DoE) approach can be used to determine optimal enzyme electrode performance. For example, Babanova et al. recently reported on a DoE approach for optimization of performance of an air-breathing bilirubin oxidase-based EFC cathode [19].

Here we report on use of a response surface methodology (RSM) technique for optimization of EFC component amounts to produce glucose-oxidizing enzyme electrodes. This DoE model is developed and validated for enzyme electrode performance in pseudo-physiological conditions (5 mM glucose, 50 mM phosphate buffered saline, PBS, pH 7.4, 37 °C). The enzyme electrodes are prepared by co-immobilizing [Os(4,4′-dimethoxy-2,2′-bipyridine)$_2$(4-aminomethylpyridine)Cl].PF_6, GOx, MWCNTs and CMD using carbodiimide coupling, as described previously [17]. The DoE-optimized enzyme electrodes display a 32% improved glucose oxidation current density compared to previously reported values for the system optimized by variation of one factor at a time [18].

2. Experimental

2.1. Materials

The mediator redox complex [Os(4,4′-dimethoxy-2,2′-bipyridine)$_2$(4-aminomethylpyridine)Cl].PF_6 (Os(dmobpy)$_2$4AMP) was synthesized by ligand substitution of Os(4,4′-dimethoxy-2,2′-bipyridine)$_2Cl_2$ in ethylene glycol at reflux, upon addition of 1.1 mol equivalents of 4-aminomethylpyridine, as reported previously [17,20,21]. The Os(4,4′-dimethoxy-2,2′-bipyridine)$_2Cl_2$ was prepared from $(NH_4)_2OsCl_6$ according to literature methods [20,22]. All other chemicals were purchased from Sigma-Aldrich (Dublin, Ireland) and used as received unless otherwise stated. The CMD has an average molecular mass of 15,000 Da, glucose oxidase (EC 1.1.3.4) average activity (240 units mg^{-1}) was determined using the o-dianisidine and horseradish peroxidase-coupled spectrophotometric assay. The reaction was monitored on an Agilent 6453 UV/Vis spectrophotometer at 460 nm [23] and the MWCNTs were acid-treated by heating 20 mg ml^{-1} in concentrated HNO_3 at reflux for 6 h at ~150 °C [13]. All solutions were prepared from Milli-Q (18.2 MΩ cm) water.

2.2. Methods

A CH Instruments 1030 multichannel potentiostat (IJ Cambria) coupled to a thermostated electrochemical cell was used to perform all electrochemical measurements. Custom built Ag/AgCl reference electrode (3 M KCl) and platinum foil counter electrode (Goodfellow) were used in the cell. Graphite disk electrodes (3 mm diameter) were prepared by shrouding graphite rods (Graphite store, part # NC001295) in heat-shrinkable tubing and polishing the exposed disk on 1200 grit silicon carbide paper (Buehler) followed by thorough rinsing with Milli-Q water. Working electrodes were sonicated in Milli-Q water for 10 min and dried under nitrogen gas prior to use. All electrochemical measurements were performed in phosphate buffered saline (PBS, 0.05 M phosphate, pH 7.4, 0.15 M NaCl) at 37 °C. Currents are normalized to the two-dimensional projected area of the graphite disk electrodes to provide current density data.

The procedure for the enzyme electrode preparation involves activation of carboxylic acid functional groups of solutions of CMD (5 mg ml^{-1}) and acid treated MWCNTs (46 mg ml^{-1}), by addition of 4 μl aqueous solution of 40 mM *N*-[3-dimethylaminopropyl]-*N*′-ethylcarbodiimide (EDC) and 10 mM N-hydroxysuccinimide (NHS) in an Eppendorf for 12 min, for coupling to amine functional groups of GOx (10 mg ml^{-1}) and redox complex (4.5 mM aqueous solution). Enzyme electrodes are prepared by drop coating a volume of the resulting solutions onto graphite disks and drying the electrodes for 18 h at room temperature. The amount of different components of CMD, MWCNTs, Os(dmobpy)$_2$4AMP and GOx added in the enzyme electrode preparation step is determined by the Design Expert software (version 9, STAT-EASE Inc., Minneapolis, USA), as described in the Results and discussion section.

3. Results and discussion

3.1. Enzyme electrode electrochemistry

Cyclic voltammetry (CV) is initially used to evaluate the redox potential of the Os(II/III) transition for the Os(dmobpy)$_2$4AMP complex in the enzyme electrodes. All prepared enzyme electrodes, in the absence of glucose substrate, exhibit oxidation and reduction peaks at 0.07 ± 0.01 V (vs. Ag/AgCl), Fig. 1, which is similar to the redox potential observed for the osmium complexes in solution and also close to that previously reported for the complex immobilized within CMD films or directly coupled to a carbon electrode [16,18]. Redox peak currents vary linearly with scan rate for scan rates less than 20 mV s^{-1}, indicative of a surface-controlled response [22]. At higher scan rates, the peak currents scale linearly with the square root of scan rate, indicative of semi-infinite diffusion control of the response, as expected for multi-layered films on electrodes [24,25]. Osmium complex surface coverage (Γ_{Os}), calculated by integration of the charged passed under the redox complex oxidation peak using slow scan rate voltammetry in PBS solution [25], is comparable to values obtained previously [18] and approximately one thousand-fold that expected for monolayer coverage of osmium polypyridyl complexes [26,27]. Upon addition of glucose substrate to the PBS electrolyte, a sigmoidal-shaped slow-scan rate cyclic voltammogram characteristic of electrocatalytic oxidation of glucose is obtained for all enzyme electrodes, Fig. 1. However, a difference in potential of ~80 mV is observed between the formal potential of the immobilized redox complex in the absence of glucose and the half-wave potential, $E_{½}$, of the catalytic sigmoidal-shaped CV in the presence of glucose. It has been reported that a shift in $E_{½}$ occurs in the catalytic sigmoidal-shaped CV of mediated enzyme electrode reactions when the boundary between a mediator-limited case and a substrate-limited case is crossed [28,29], indicating that glucose substrate transport may limit the current flow under these conditions for the enzyme electrodes.

A comparison of glucose oxidation current for enzyme electrodes as a function of glucose concentration is extracted from amperometric measurements at 0.2 V vs. Ag/AgCl applied potential, selected to be 150 mV more positive of the redox potential of the osmium complex to ensure sufficient mediated glucose oxidation. Amperometric response of enzyme electrodes displays slightly higher glucose oxidation current density over

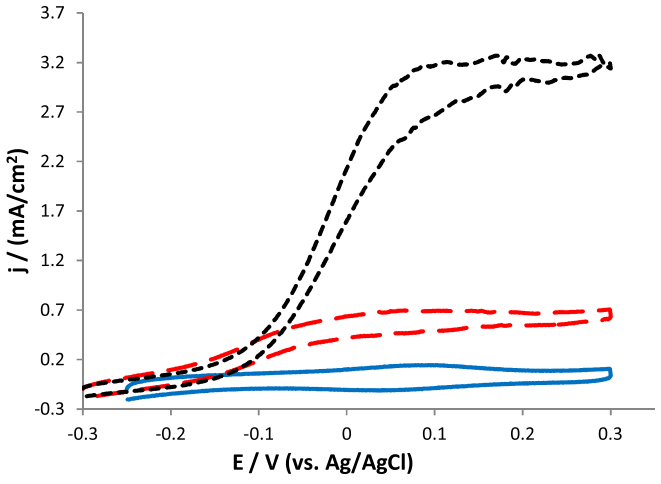

Fig. 1. CVs recorded at 1 mV s^{-1} in the presence of 100 mM (black dot), 5 mM (red, dashed) and 0 mM (blue solid) glucose in PBS (37 °C) for enzyme electrodes prepared by co-immobilizing Os(dmobpy)$_2$4AMP (30 μg), MWCNTs (400 μg), GOx (50 μg) and CMD (50 μg) on graphite electrodes.