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Electroimmobilization of nitrate reductase into polypyrrole films on screen printed carbon electrode (SPCE) for amperometric detection of nitrate

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Abstract

An amperometric nitrate biosensor was successfully constructed by the electroimmobilization of the enzyme nitrate reductase from *Aspergillus niger* with and without β-NADH, a co-factor, into conducting polypyrrole films on screen printed carbon electrode (SPCE). The best performance was registered by nitrate biosensor developed without the β-NADH electroimmobolize with nitrate reducatse on the SPCE. The formation of the optimum NaR-PPy composite on the SPCE was achieved with an applied potential of 0.9 Vs^{-1} for 900s. The optimum amperometric response for nitrate was obtained in 0.1M potassium phosphate buffer, pH 7.5 with an applied potential of -0.1 Vs^{-1} and 2 mM β-NADH. Sensitivity of the nitrate biosensor was found to be in a linear range of 0.01-0.25mM with a regression coefficient of 0.97 and a relative standard deviation (RSD) of 6.6% (n=5).

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Keywords: Nitrate sensor; Biosensor; Amperometry; Polymerization; Conducting Polymer

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1. Introduction

Nitrate is a well-known pollutant to both environment and human health. Excess nitrate in environmental water systems, which is mostly due to intensive use of N-fertilizers in farming activities, causes algal blooms, a depletion of dissolved oxygen, possible eutrophication of aquatic ecosystems and contamination of fish and shellfish cultures[1]. Moreover, nitrate salts are routinely added to processed and cured meats as preservative against pathogenic microorganisms such as clostridium botulinum [2]. Epidemiologic studies have associated nitrate ion exposure through food or water with high level of nitrate to a number of medical issues such as the blue baby syndrome where oxygen uptake and transportation are compromised, spontaneous abortions, birth defects and cancer with long time exposure [2-6]. These effects have increased the demand for sufficiently sensitive, accurate and uncomplicated analytical measurements for nitrate.

Conventional bench-top techniques (e.g., UV/vis spectrometry, chromatography, and capillary electrophoresis, FTIR etc.) methods for measurement of nitrate in diverse environmental and biological materials usually require expensive and large instruments, complex procedures, and multiple reagents. Screen-printed electrochemical sensors (SPESs) offer the possibility of achieving decentralized testing as SPESs are potentially portable, simple to operate, reliable, and inexpensive to manufacture. Considering the advantage of enzymes which are strongly substrate-selective, enzyme nitrate reductase (NaR) has been found to be most useful in the development of a nitrate screen printed electrochemical biosensor. The enzyme catalyzes the reduction of nitrate to nitrite in presence of B-nicotinamide dinucleotide (B-NADH) or other suitable co-factor or mediator. As the changes caused by NaR catalyzing the conversion of nitrate to nitrite and NADH to NAD+ will result in a net change in charge at the electrode surface, a corresponding change current will result [7]. The use of a conducting polymer film such as polypyrrole (PPy) is particularly useful for achieving better detection of the net change in charge at the electrode surface. The PPy matrix will provide a porous structure with large effective surface area and high electrocatalytic activity toward the reduction of nitrate [8]. This research investigates the synthesis of a PPy/NaR matrix through the electopolymerization of a mixture of pyrrole and enzyme nitrate reductase on screen printed carbon electrodes (SPCE).

2. Material and Methods

2.1. Reagents

Nitrate reductase (E.C.1.7.1.2) from *Aspergillus niger*, potassium di-hydrogen phosphate, pyrrole and other chemicals were purchased from Sigma (St. Louis, USA). All other chemicals were of analytical grade or better.

2.2. Enzyme immobolization and characterization

Optimized conditions, which include enzyme concentration, NADH concentration, the type, concentration and pH of buffer, were determined in 96 well plates before being applied onto the screen printed electrode. The enzyme nitrate reductase and pyrrole were dropped on the working electrode of the SPCE and was allowed to react with an applied potential of 0.9 1.0 Vs^{-1} for 900s for the electroimmobilization of the enzyme. The set potential for the amperometric measurement was then determined by scanning the potential from -0.4V s^{-1} to 0.4V s^{-1} at different nitrate concentration with the

addition of 2mM NADH. The current recorded for each concentration at every potential was then divided with the current background (0 mM nitrate) to obtain signal-to-background ratio.

3. Result and Discussion

As seen in Figure 1, there are peaks at an applied potential of -0.1V s^{-1} and -0.2V s^{-1} which indicate that a reaction has occurred at both potential. It can also be observed that the peak has a larger distant from the background as the concentration of nitrate increase. The set potential -0.1V s^{-1} was chosen to be applied in further measurement due to its larger signal-to-background ratio compare to at -0.2V s^{-1}. Next, the calibration curve for nitrate detection was constructed using the amperometric technique at -0.1V s^{-1} for 300s with the nitrate concentration being from 0.001mM to 0.3mM. A linear graph of current against the concentration of nitrate was able to obtain from the concentration of 0.001mM to 0.25mM nitrate as seen in Figure 2 with a regression value of 0.97.

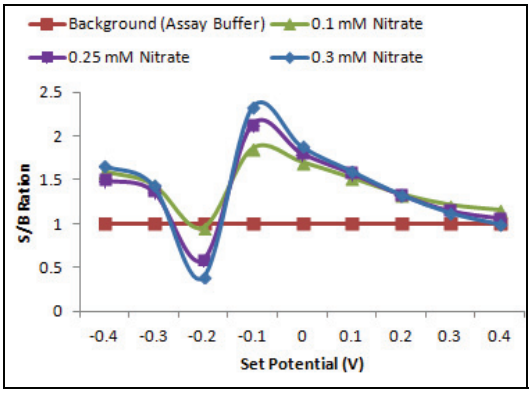

Figure 1: Set potential scanning of the redox reaction of the immobilized enzyme nitrate reductase by plotting the signal-to-background (S/B) ratio for 0.1 mM, 0.25 mM and 0.3 mM nitrate.

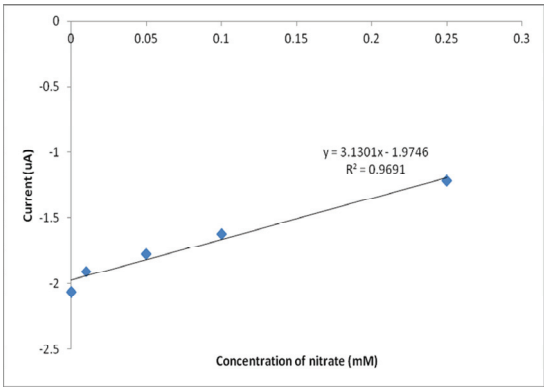

Figure 2: Current output (uA) of amperometric measurement to increasing nitrate concentration ranging from 0.00 mM to 0.25 mM.

Besides giving the required selective and sensitive response, the SPCEs turn a long and elaborated laboratory protocol into a simple task and quickly executed as the result can be obtain in 5 minutes.

The possibility to miniaturize nitrate biosensor using a transducer element with small size electrodes such as the Screen Printed Electrodes (SPE) represents an important goal in nitrate biosensors development, targeting key applications in the environmental and industrial markets. The ability to discriminate the analyte against potentially interfering species due to the integration of selective biocatalysts represents an added value for nitrate screening in food and waters samples. Owing to the redox nature of the underlying biorecognition event, electrochemical transducers in general, and amperometric/voltammetric ones in particular, are well suited for converting the catalytic reaction into a quantifiable signal. Moreover, the insensitivity of electrochemical methods to medium color and turbidity is a competitive advantage over the much less used optical approaches, whereas potentiometric and conductimetric signal transducers are more prone to interference from ionic species.

4. Conclusion

Results suggest that the electropolymerization of the enzyme nitrate reductase into polypyrrole on disposable SPCE can detect nitrate with the presence of the co-factor NADH by amperometric measurement.

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