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Direct Electrochemistry with Nitrate Reductase in Chitosan Films

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Abstract: Stable films made from chitosan (CS) on pyrolytic graphite electrode (PGE) gave direct electrochemistry for incorporated enzyme nitrate reductase (NR). Cyclic voltammetry of CS / NR films showed a pair of well-defined and nearly reversible redox peaks at about -0.430 V *vs.* SCE at pH 7.0 phosphate buffers.

Keywords: Nitrate reductase, chitosan, cyclic voltammetry, chemical modified electrode.

Nitrate reductase (NR) is a homodimeric enzyme with each subunit containing a ~100kD polypeptide, and it contains three internally electroactive sites such as flavin adenine dinucleotide (FAD), heme-ion and molybdenum-molybdopterin (Mo-MPT)[1]. In serves of plants, algae, and fungi it as a central point for integration of metabolism by governing flux of reduced nitrogen. NR catalyzes the first step of nitrate assimilation in all these organisms, which appears to be a rate-limiting process in acquisition of nitrogen in most case[2]. In order to limit the strong adsorption of NR at the bare electrode, we prepared a chemical modified electrode, which may provide a unique microenvironment for electrode reaction and improve the electrochemical properties of NR. NR incorporated into CS films demonstrated to enhance electron transfer with underlying PGE. Direct electrochemical properties about CS / NR films cast on PGE surface was observed by cyclic voltammetry (CV).

NR and CS were from Sigma Co.. PGE was from Beijing Normal University, geometric area 0.159 cm^2. The three-electrode cell featured SCE as a reference, a platinum flake as a counter and the modified electrode as the working electrode.

CS films were prepared by casting 10 μL of 1 mg/mL CS solution onto the PGE surface. After water was evaporated, 10 μL phosphate buffer in the presence of NR solution was cast onto CS / PG modified electrode[3]. In 0.1mol/L pH 7.0 buffer solutions, the CS / NR / PGE system showed a pair of well-defined, nearly reversible peaks at about -0.430 V (**Figure 1b**), but no voltammetric responses were observed at CS films electrode under the same condition (**Figure 1a**). There was a good linearity in peak current with scan rate from 0.01 to 0.5 V/s (**Figure 2**). The results are characteristics of thin-layer electrochemical behavior[4]. The pH of the solution strongly

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affected the direct electron transfer of NR-CS films electrode. An increase of pH of solution led to a negative shift in potential for both reduction and oxidation peaks for CS / NR films (**Figure 3**). The formal potential had a linear relationship with pH between 2.0 to 10.0 with a slope of -56.6 mV/pH. The value is close to the theoretical value of -57.6 mV/pH for a reversible, one-proton coupled single electron transfer[5].

Figure 1 CVs of the CS / PGE (a) and CS / NR / PGE (b) at scan rate 0.1V/s.

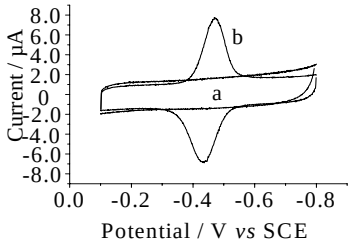


Figure 2 CVs of the CS / NR / PGE at different scan rates (V/s).

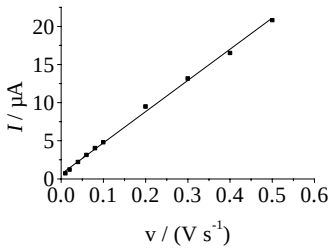
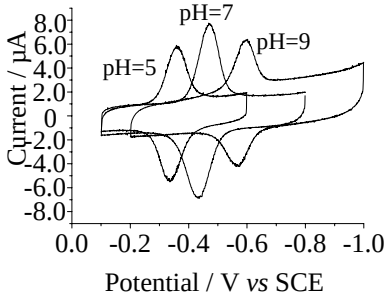


Figure 3 CVs of the CS / NR / PGE at scan rate 0.1 V/s in 0.1mol/L different pH (5.0, 7.0, 9.0) phosphate buffers.



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