

Gold nanoparticles with self-assembled cysteine monolayer coupled to nitrate reductase in polypyrrole matrix enhanced nitrate biosensor

Thangamuthu Madasamy, Manickam Pandiaraj, Seenivasan Rajesh, Kotamraju Srigiridhar, Kalpana Bhargava, Niroj Kumar Sethy, Govindaswamy Ilavazhagan and Chandran Karunakaran

Nitrate the end oxidative metabolite of NO is involved in the regulation of blood pressure and also as biomarker of NO generation by eNOS and nNOS. In this report we have developed a novel highly sensitive and selective biosensor for the direct determination of nitrate by covalent immobilization of nitrate reductase (NaR) in self-assembled monolayer (SAM) of cysteine on gold nanoparticles (GNP) - polypyrrole modified platinum electrode. This fabricated biosensor was characterized by scanning electron microscope (SEM) and cyclic voltammetry. The electrochemical behavior of the biosensor studied by using cyclic voltammetry revealed that the NaR immobilized electrode exhibited characteristic reversible redox peaks with the formal potential of -0.76 V vs. Ag/AgCl. Since the active site of the nitrate reductase is deeply embedded in the protein structure, redox mediator hydroquinone was used to shuttle the electrons between the active site of the NaR and the electrode surface. Argon atmosphere was maintained throughout the experiments. The biosensor exhibited a linear range of response over the concentration of nitrate from 10 μ M to 5 mM, with a detection limit of 5 μ M. The sensitivity of the biosensor

was 84.5 nA/ μ M nearly ten fold greater than the reported studies. It is thus concluded that the self-assembled monolayer of cysteine on GNP in PPy enhanced the NaR activity and shows good reproducibility. Ascorbate and urate, the common interference in biological samples were effectively eliminated perhaps due to the polypyrrole film. Using this biosensor, the levels of nitrate present in beetroot juice and serum were estimated.

