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Electrochemical Detection of Phenolic Compounds by Immobilized DeniLiteTM Laccase

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Laccase from DeniLite™ was covalently assembled on a silane-modified platinum surface and the electrode was characterized with respect to response time, sensitivity, linear range, detection limit, pH dependence, and long-term stability. 2,2□-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), diammonium salt (ABTS), *p*-phenylenediamine (PPD), and *p*-aminophenol (PAP) were selected as the test substrates and detected based on the principle of enzymatic oxidation of substrate and following electrochemical regeneration. The sensitivities of the electrode are 75, 330, and 385 nA/㎛M with linear ranges of 0.6 ~ 15, 0.14 ~ 29, and 0.12 ~ 22 ㎛M for ABTS, PPD, and PAP, respectively. The detection limits (S/N = 3) are 45 and 40 nM for PPD and PAP, respectively, with around 5 nA background noise. The response time ($t_{90\%}$) is less than 2 seconds for PPD and PAP. The immobilized laccase shows high affinity to PPD and PAP with $K_{m,app}$ values of 55 and 85 ㎛M, respectively. The pH, temperature, and inhibition effects on the activity of the immobilized laccase show that characteristics of the immobilized enzyme are similar to those of free one. The long-term stability of the electrode is over two months (retaining 80% of initial activity). The very stable and fast response, and the remarkable long-term stability of the sensor are the principal advantages of currently developed sensor. The laccase electrode could be applied successfully to detect neurotransmitters and quinone containing compounds. In addition, tyrosinase could be co-immobilized and both mono- and di-phenolic compunds were determined simultaneouly.