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Covalently electrografted carboxyphenyl layers onto gold surface serving as a platform for the construction of an immunosensor for detection of methotrexate.

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Abstract

The classical way to modify a gold electrode surface for immunosensor development is through self assembly of functionalised thiols for subsequent attachment of antibodies. Here is described a new pathway for surface modification using a diazonium salt-based immunosensor for detection of methotrexate (MTX) with electrochemical immittance spectroscopy (EIS). The diazonium salt, 4-carboxybenzenediazonium tetrafluoroborate was synthesized using a diazotization reaction and characterized by IR and cyclic voltammetry (CV). The Au electrode was electrografted with diazonium and used for anti-MTX antibody immobilization. The EIS detection of MTX was studied with and without application of redox probe; $\text{Fe}(\text{CN})_6^{3-/4-}$. MTX could not be detected using $\text{Fe}(\text{CN})_6^{3-/4-}$ as redox probe, whereas EIS measurements without redox probe and analysis of the data with singular decomposition (SVD) gave good results. A multivariate calibration model showed good linear behavior on a logarithmic scale with a detection limit for MTX of $7 \times 10^{-12} \text{ mol L}^{-1}$.

Keywords: 4-carboxybenzenediazonium tetrafluoroborate, gold electrode, antibody, Immunosensor, singular value decomposition, cyclic voltammetry, Chronoamperometry, Electrochemical Impedance spectroscopy

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