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Electrografting of isophthalic acid monolayer and covalent attachment of antibody onto carbon surfaces: Construction of capacitive biosensor for methotrexate detection

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ABSTRACT

In this study, a 5-diazonium isophthalic acid was synthesized and electrografted onto glassy carbon (GCE) and screen-printed carbon (SPCE) electrodes. SPCE was used to demonstrate fabrication of a miniature device and to compare with conventional glassy carbon electrodes. The isophthalic acid (IPA) electrografted thin film was used for the immobilization of anti-methotrexate antibody (Ab) using carbodiimide activation chemistry to form antibody modified surfaces, GCE-IPA-Ab and SPCE-IPA-Ab. The GCE -IPA-Ab and SPCE-IPA-Ab surfaces were used as capacitive biosensors for the detection of methotrexate (MTX) in phosphate buffer (pH 7.4) using capacitive electrochemical impedance spectroscopy (EIS). The EIS data was analyzed using singular value decomposition (SVD). Principal component regression analysis gave R^2 values of 0.99 for both the GCE-IPA-Ab and SPCE-IPA-Ab surfaces. The detection limit from the calibration curve of the GCE-IPA-Ab and SPCE-IPA-Ab was calculated to be 7.0 pmol.L^{-1} and 5.5 pmol.L^{-1}, respectively.

1. Introduction

Methotrexate (MTX) is an antifolate metabolite that inhibits DNA synthesis and cellular replication [1]. It has been successfully used for the treatment of rheumatoid arthritis (RA) but its most important use is as a chemotherapy agent [2–6]. However, MTX has been found to cause several side effects such as gastrointestinal disorders, hepatic dysregulations, dermatitis, hematologic disorders and nephrotoxicity pneumonitis [1,7–9]. In chemotherapy, the effective concentration range of MTX is limited to a relatively narrow therapeutic window. At low concentrations the therapeutic activity toward cancer cells is poor while an unacceptable toxicity toward healthy cells is observed at high concentrations. The dosage of MTX and its level in the blood needs to be regularly controlled and individualized to establish the dose response and side effects [9]. There is therefore high demand for rapid and highly sensitive methods for monitoring MTX in real time and online. Today, the measurement of MTX in blood samples is often conducted using techniques such as high-performance liquid chromatography (HPLC) [10], oxidative fluorimetry [11], electrospray ionization tandem mass spectroscopy (ESI-MS) [12] and capillary zone electrophoresis (CZE) [13]. These techniques offer high accuracy, sensitivity and selectivity, but they require highly trained personnel, large amount of chemicals, bulky instrumentation and tedious sample preparation. This makes these techniques not amenable for on-site or point-of-care (POC) applications.

Development of alternative methods, based on electrochemical detection, has recently received increased attention [14] and this is due to the low-cost, rapid analysis and the possibility of miniaturization for POC applications. Amongst the various electrochemical methods, electrochemical impedance spectroscopy (EIS) has emerged as a promising technique for biosensor development [15]. EIS offers the possibility for the development of relatively cheap capacitive biosensors that can be miniaturized and used for *in-situ* analysis. Impedimetric biosensors are either faradaic or non-faradaic [16]. The faradaic impedimetric biosensor comprises of a redox probe (label) while the non-faradaic impedimetric biosensors are label free and may not need a reference electrode. The biosensors that are based on non-faradaic EIS are used to mon-

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