Electrochemical Oxidation of Immobilized Methylene Blue Monitored by MP-SPR

This Application Note explores the electrochemical oxidation of biotinylated methylene blue immobilized on the sensor surface via biotin-avidin interaction. Multi-Parametric Surface Plasmon Resonance (MP-SPR) monitors the binding of reactants in real-time as to track surface changes when Square Wave Voltammetry (SWV) is applied on the coated sensor surface at different frequencies.

Introduction

BioNavis Multi-Parametric Surface Plasmon Resonance (MP-SPR) is a highly sensitive optical sensing technique that enables real-time, label-free analysis of molecular interactions at surfaces. Advantageously, MP-SPR measures the complete SPR curve and captures multiple wavelength-dependent responses at the same measurement spot. This allows comprehensive characterization of thin films, biomolecular interactions, and material properties. When combined with electrochemical experiments, MP-SPR provides valuable insights into electrochemical reactions, surface modifications, and charge transfer processes. This integration can allow researchers to correlate optical and electrochemical signals, enhancing the understanding of complex interfacial phenomena in biosensing, corrosion studies, electropolymerization and energy storage applications.

Materials and methods

Methylene blue was immobilized onto the BioNavis Regenerable Avidin kit sensor slide using the standard procedure of the kit. Real-time MP-SPR measurements confirmed the sequential immobilization of regenerable avidin and biotinylated methylene blue (b-MB). The monitoring of the binding was performed with 670 nm and 785 nm laser wavelengths simultaneously in automated BioNavis MP-SPR Navi™ 210A VASA instrument. The BioNavis 2-channel electrochemistry flow cell (Figure 1) enables simultaneous measurements: Channel 1 is used for both electrochemistry and MP-SPR, while Channel 2 is used for MP-SPR without electrodes. Small volume of the flow cell allows rapid exchange of the liquids and is designed for kinetic electrochemistry measurements. The electrochemistry flow cell Channel 1 contains gold sensor surface as working electrode, leak-free Ag/AgCl reference electrode, and Pt counter electrode. The running buffer was PBS-T (pH = 7.4). Concentrations of avidin and biotinylated methylene blue were 25 µg/mL (with flow rate of 25 µL/min) and 500 µM (with flow rate of 15 µL/min), respectively Before the regenerable avidin molecules were introduced into the flow cell, regeneration solution 1 (included in the kit) was injected at 100 µL/min in order to remove any non-specifically bound molecules from the surface.

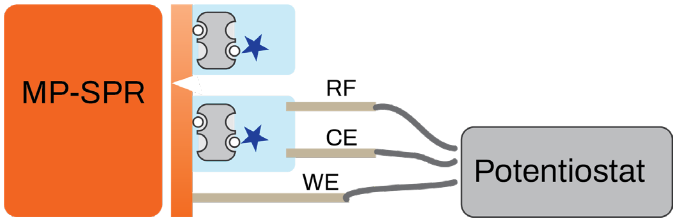

Figure 1. The MP-SPR instrument can be equipped with various user-exchangeable flow cells. The electrochemistry flow cell features two channels for simultaneous MP-SPR signal measurement. Additionally, one of the channels contains working electrode (WE), reference electrode (RF), and counter electrode (CE) connections.

Potentiostat (here PalmSense3) was connected to MP-SPR electrochemistry flow-cell and Square Wave Voltammetry (SWV) sequences were conducted at different frequencies (10, 15, 20, 30 and 50 Hz in series) from -0.4 V to 0 V (0.005 V amplitude) in 0.001 V steps to evaluate the oxidation peak of methylene blue. Equilibration time before applying the voltage was set to 5 seconds. All plotted methylene blue oxidation peaks at different frequencies had a baseline of regenerable avidin coated surface signal subtracted (non-linear).

Results and discussion

MP-SPR provides real-time data on the immobilization efficiency of biotinylated methylene blue on regenerable avidin-coated sensor slide, showing a clear shift in the SPR angle after the injection (Figure 2). The interaction between biotinylated methylene blue and the regenerable avidin layer was stable, confirming successful surface modification as seen in both channels.

BioNavis

email: info@bionavis.com
www.bionavis.com

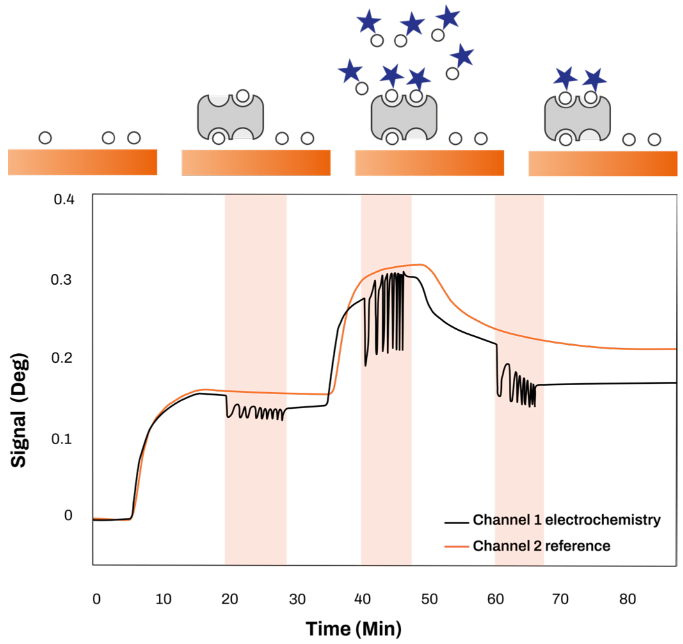

Figure 2. Square wave voltammetry (SWV) application on biotinylated methylene blue (b-MB) coated sensor slide measured. Channel 1: SWV (highlighted with orange) was applied on a biotin coated sensor slide, after Regenerable Avidin (AVD) injection, during and after methylene blue injection. Channel 2: All the injections were performed as in Channel 1, except applied voltage. Each injection end initiates washing with the running buffer (PBS-T).

Square wave voltammetry at different frequencies were performed after binding biotinylated methylene blue to the sensor surface and revealed a distinct oxidation peak corresponding to roughly between -0.22 to -0.24 V (Figure 3). These values coincide well to the previously published literature reports (1, 2). Functionalized sensor slide is used with two channel electrochemistry flow cell. When the slide is scored, the unwanted influence from the applied potential is avoided in Channel 2. The electrochemical signal intensity (current) varied with frequency, providing insights into the electron transfer kinetics of immobilized methylene blue. Faster scan rates (30 and 50 Hz) resulted in increased peak currents, indicative of surface-confined oxidation behavior. After the application of SWV on the methylene blue bound to the surface a significant reduction in MP-SPR response was seen. This can be related to the loosely bound biotinylated methylene blue molecules on the sensor surface (60-70 minutes) in Figure 2.

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 210A VASA with electrochemistry flow cell (SPR328-EC2)
Sensor surface: SPR102-AU scored sensor for electrochemistry
Software: MP-SPR Navi™ Control, DataViewer

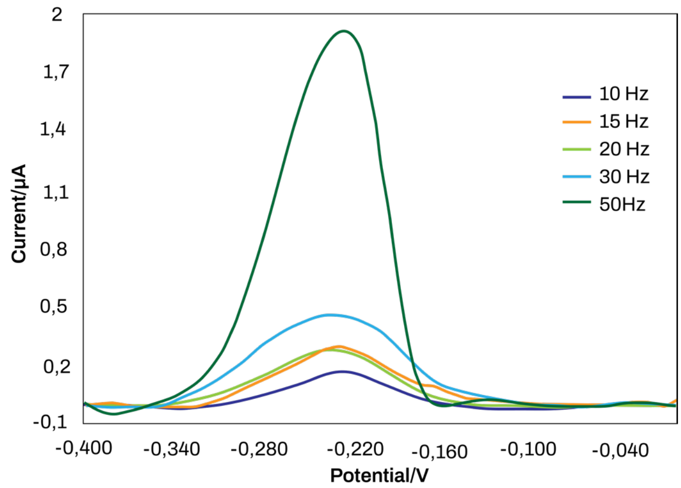

Figure 3. Square wave voltammetry (SWV) graph showing current responses at different applied frequencies coming from oxidation of immobilized biotinylated methylene blue.

The combination of MP-SPR with SWV allows simultaneous monitoring of binding interactions and oxidation of biotinylated methylene blue, offering a comprehensive view of the immobilized methylene blue behavior. The ability to correlate real-time molecular binding with electrochemical properties enhances the study of surface-confined redox species and broadens applications in biosensing, electrocatalysis, and bioelectronic interfaces.

Conclusions

The integration of MP-SPR with electrochemical techniques enables comprehensive analysis of surface-bound redox-active molecules. MP-SPR's ability to measure layer properties—such as thickness, refractive index, bound mass, and binding kinetics—during electric potential changes makes it a unique tool for comprehensive analysis. This combined approach advances the study of electroactive biomolecules and supports the development of electrochemical biosensors and bioelectronic devices.

Are you interested in static electrochemistry measurements instead? See BioNavis Application Note #142, where metal stripping and deposition were monitored in real-time using MP-SPR equipped with a static electrochemical cuvette.

References:

1. Mahlum *et al.*, Journal of Physical Chemistry C, Vol 125 (17) 2021

2. Xiao *et al.*, Nature Protocols, Vol 2, 2007

BioNavis

email: info@bionavis.com

www.bionavis.com