Application Note # 165

Cellulose fiber-based yarn development for capturing estrogen residues from aqueous matrices with MP-SPR

Estrogen hormones are widely used in contraceptive pills and in pharmaceutical veterinary prescriptions to increase meat production. Extended use and consumption of such preparations lead to a high release of hormone residues and metabolites into the environment. As a result, environmental risks created by estrogen-based human and veterinary hormone residues in waste waters have increased significantly. Excessive estrogen intake can cause developmental abnormalities, increase the risk for cancer and cardiovascular diseases and decrease the size of fish population (Adeel et al., 2017). Therefore it is essential to develop materials which can bind hormone residues from water and be applied in waste water purification process.

In situ preparation of chitosan and cyclodextrin-functionalized cellulose fiber yarns and capture of the synthetic estrogen hormone 17α-ethinyl estradiol (EE2) from water was investigated to address this problem. The thickness of functionalized chitosan and cyclodextrin layers were determined. The effectivity of the hormone capture was determined by determining the thickness of adsorbed EE2 layer.

Introduction

Surface Plasmon Resonance (SPR) is a well-established method for measuring binding affinity and kinetics. Innovative Multi- Parametric Surface Plasmon Resonance (MP-SPR) instruments can perform measurements in an exceptionally wide angular range (40-78 degrees) and at more than one wavelength, allowing a wider range of applications than traditional SPR. MP-SPR measures adsorption of molecules in real-time, but also provides the possibility to calculate layer thickness and refractive index.

Additionally to the acknowledged high sensitivity and label-free detection of conventional SPR, MP-SPR provides new methods and tools for biosensor development, particularly for characterization of biosensing surfaces as used in portable (point-of-care, field) devices. Functionalization for biosensing purposes can be chosen from various surfaces: metal electrodes for electrochemistry, plastics for well-plate assays, cellulose for printed biosensors, glass for traditional chemistry, nanoparticles for SERS etc. MP-SPR enables development of bioassays on various materials which avoids assay transfer steps and allows measurement of real samples (100% serum, urine, sewage water etc.), unlike many traditional SPR instruments. After MP-SPR measurements, the sensor surface can be further characterized with other methods, such as AFM. This is made easier by the oil-free operation of MP-SPR, using a prism coated with an optical elastomer.

Materials and methods

Experiments were performed with an MP-SPR Navi™ 210A VASA instrument. The measurements were performed at 23 °C with a flow rate of 10 µl/min.

Cellulose nanofibril (CNF) thin films were formed by spin-coating onto PEI coated MP-SPR gold sensor slides. First 0.5 g/l chitosan was injected onto the CNF surface for 20 min, followed by the conjugation of 0.5 g/l TEMPO-oxidized β-cyclodextrin (TO-βCD) with 0.06 g EDC and 0.106 g NHS for 20 min. The unreacted TO-βCD molecules were removed from the surface by a 30 min rinse with running buffer. The running buffer and solution for chitosan and TO-βCD was 50 mM NaOAc at pH=5. After this, the running buffer was changed to 50 mM phosphate buffer pH 7.4. EE2 binding was determined with 2 µg/ml EE2 in buffer for 20 min. The difference in binding of EE2 between the TO-βCD conjugated chitosan CNF surface and the chitosan CNF surface was determined. Sensor slide functionalization and EE2 capture is shown in Figure 1.

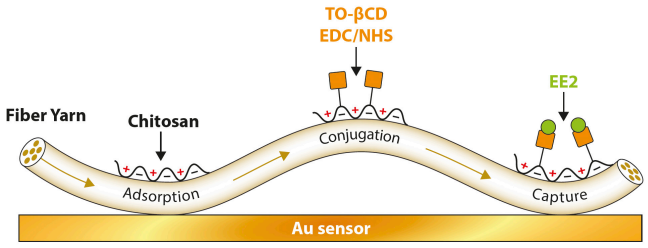

Figure 1. CNF coated with chitosan by adsorption. TO-βCD is conjugated to chitosan with EDC-NHS chemistry. TO-βCD captures EE2 from water.

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Results and discussion

MP-SPR was used to develop a biosensor for detection of steroid hormones from waste water. First the formation of chitosan and TO-βCD monolayers on cellulose fiber yarn was monitored in real-time (Fig. 2a) and followed by studies of EE2 hormone binding to the functionalized yarn (Fig. 2b).

The thickness of the absorbed chitosan layer was approximately 0.53 nm with a surface coverage of 93 ng/cm2. The average thickness of the TO-βCD layer was 1.0 nm, being close to a monolayer of cyclodextrin. MP-SPR measurements were validated by AFM.

Cyclodextrin functionalization significantly improves hormone capture from water. The adsorbed EE2 layer to TO-βCD was 0.5 nm. EE2 adsorption to reference surface, chitosan without TO-βCD, was 0.1 nm. TO-βCD functionalization increases EE2 adsorption in five fold. The low binding of EE2 to chitosan is caused by the hydrophobicity of this steroid hormone. The binding of EE2 to TO-βCD was confirmed with UV/Vis spectroscopy. The EE2 binding capacity of the prepared yarn was 2.5 mg/g. MP-SPR experiments enabled to effectively assess the estrogen hormone binding.

Conclusions

MP-SPR proved to be a valuable method to evaluate biomolecule adsorption to biomaterials with high precision. The technique allows the user to perform real-time, label free measurements for biosensor surface characterization of in situ or ex situ prepared biosensor surfaces. Thanks to oil-free operation the sensor surfaces can be used for other measurements after MP-SPR, such as SEM or AFM. MP-SPR also enables experiments with crude samples (e.g., serum). In conclusion, steroid hormones can be succesfully captured with modified cellulose fiber yarns. Binding capacity was proven with MP-SPR.

See Application Note #160 to see how a biosensor was developed to detect bacteria in milk with MP-SPR.

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 200 OTSO, 400 KONTIO, 210A VASA and 220A NAALI

Sensors surfaces: Au or other inorganic coating

Software: MP-SPR Navi™ Control, DataViewer and LayerSolver™ for MP-SPR Navi™

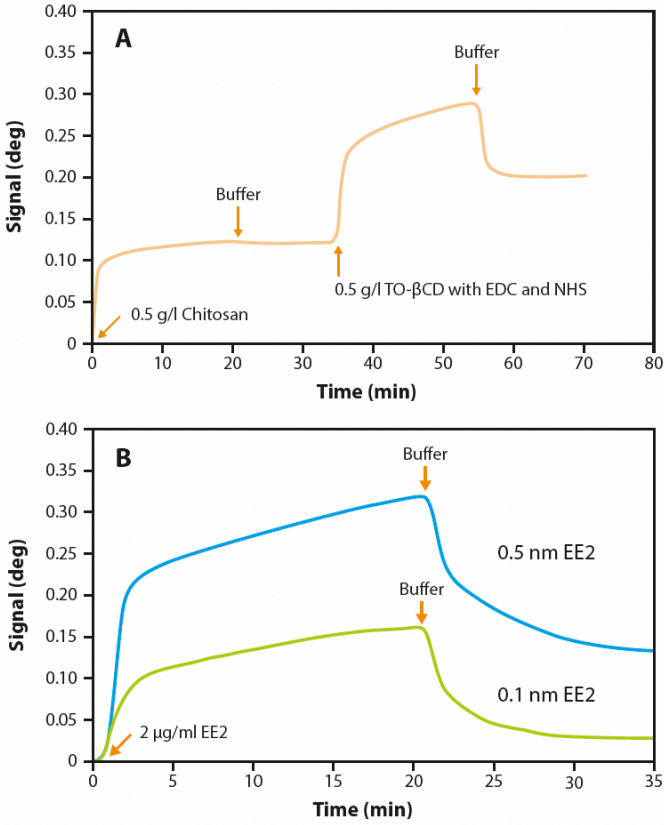

Figure 2. A) Sensogram of the chitosan adsorption to cellulose fiber yarn, followed by TO-βCD conjugation. B) Sensogram of EE2 adsorption to TO-βCD functionalized surface and to reference chitosan surface without TO-βCD.

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Orelma *et al.*, Biomacromolecules Vol. 19 (2), 2018

Reference:

Adeel *et al.*, Enviroment International Vol. 99, 2017

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