Application Note # 126

# Cellulose model surfaces for studying protein interactions using MP-SPR

*New type of point of care devices and other consumer analysis devices need new cost effective and preferably renewable new materials in order to be economically and environmentally feasible. Multi-Parametric Surface Plasmon Resonance can be used to study new material coatings, such as modified cellulose model surfaces, for studying interactions of new materials and analysts.*

## Introduction

The adsorption of human immunoglobulin G (hIgG) and bovine serum albumin (BSA) on native-, carboxymethylated cellulose (CMC) -modified and chitosan-modfied cellulose surface, was studied using MP-SPR and quartz crystal microbalance with dissipation monitoring (QCM-D). The different cellulose derivatives are known to affect protein binding by different electrostatic and non-specific interactions, and can either enhance or reduce the binding of different proteins.

The prevention of nonspecific adsorption of biomolecules is a key element in affinity adsorption as it defines the detection sensitivity of the system as well as its suitability in different applications. As such, the adsorption of BSA on cellulose, CMC-, and chitosan-modified cellulose was studied by SPR.

In the diagnostic field, the ability to detect certain pathogens requires for the manufacture of an support with specific binding affinities. Therefore, the functionalization of cellulosic substrates with hIgG could render a platform for detection of these biopolymers.

The results of this study show how it is possible to tailor biomolecule binding by choice of appropriate surface modifier and pH of the medium. The findings of the present study can be used in e.g. diagnostic, medical and bioactive-paper fields.

## Materials and methods

The SPR experiments were performed using a BioNavis SPR Model Navi 200. The thickness of adsorbed layers and the surface excess concentration were calculated according to equations 1 and 2 in the original publication [1].

The supports for cellulose consisted of the polystyrene-coated gold SPR sensors. Trimethylsilyl cellulose (TMSC) layer was deposited on the supports by Langmuir-Schaeffer-deposition and then desilylated to cellulose by vaporized hydrochloric acid. Further details of the sensor preparation can be found in the original publication [1].

Both CMC and chitosan were adsorbed from aqueous solutions (0.5 mg/mL) on the cellulose surface until signals from the SPR indicated adsorption plateau. All measurements were performed at 25 °C under continuous flow rate of 100 µL/min. Thereafter, the cellulose surfaces were rinsed to wash away any unbound molecules.

BSA and hIgG (0.1 mg/mL) were allowed to adsorb on cellulose, CMC-modified cellulose, and chitosan-modified cellulose at different pH (BSA at pH 4, 5, 6.2, and 7.4; hIgG at pH 5, 6.2, 7.4, and 8). The adsorption was continued until the plateau was observed, and any unbound molecules were rinsed out.

Details of the QCM-D experimentation can be found from the original publication [1].

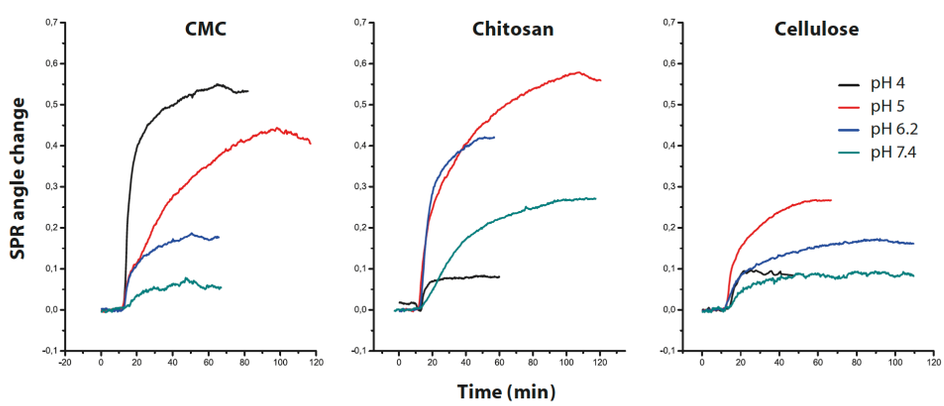


**Figure 1.** SPR sensograms for the adsorption of BSA on cellulose, CMC-modified cellulose, and chitosan-modified cellulose. The measurements were done at pH 4, 5, 6.2, and 7.4.



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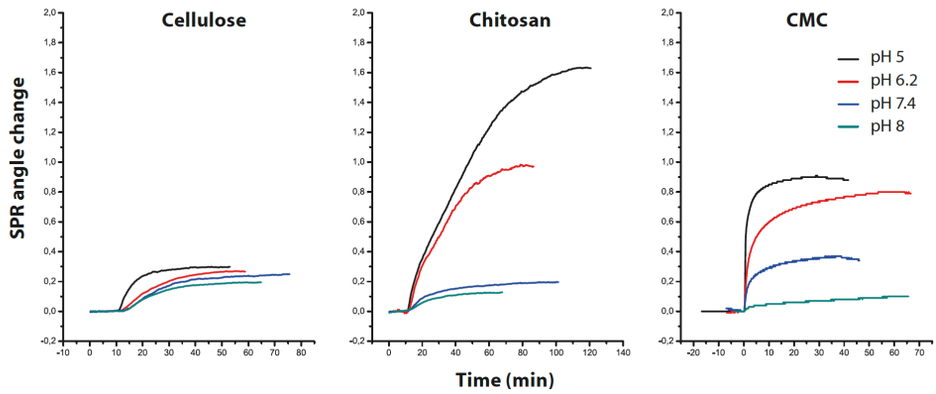


**Figure 2.** SPR sensograms for the adsorption of hIgG on cellulose, CMC-modified cellulose, and chitosan-modified cellulose at pH 5.0, 6.2, 7.4, and 8.0.

## Results and discussion

The adsorption kinetics of CMC and chitosan was Langmuir-type and revealed to have similar adsorption mechanisms. SPR data indicated that the adsorption plateaued after 30 min equilibration time and no desorption was evident after rinsing. QCM-D measurements confirmed the high hydrogel-like nature of CMC and at lower extent also that of chitosan. The prevention of nonspecific adsorption of biomolecules is a key element in affinity adsorption as it defines the detection sensitivity of the system as well as its suitability in different applications. As such, the adsorption of BSA on cellulose, CMC-, and chitosan-modified cellulose was studied by SPR.

Figure 1 illustrates the adsorption of BSA at different pH onto cellulose, CMC-modified cellulose, and chitosan-modified cellulose. Maximum areal BSA adsorbed mass was calculated from the SPR data at different pH values. Briefly, in the case of chitosan-modified cellulose, a maximum adsorption of BSA can be clearly observed to be close to its isoelectric pH due to electrostatic attraction forces between the molecules. In the case of CMC-modified cellulose the strongest affinity was observed at more acidic pH due to the increased anionic charge of BSA.

Preadsorbed CMC increases the adsorption of positively charged proteins while preventing adsorption of negatively charged and hydrophobic proteins. Vice versa, preadsorbed chitosan layer increases the adsorption of negatively charged proteins.

The presence of the CMC and chitosan significantly increased the adsorbed mass of hIgG at acidic conditions. This is likely due to the attractive electrostatic forces between the layers and hIgG. The adsorption of hIgG on cellulose, CMC-modified cellulose, and chitosan-modified cellulose was found to be irreversible.

Figure 3 summarizes the results of adsorption of BSA and hIgG at different pH.

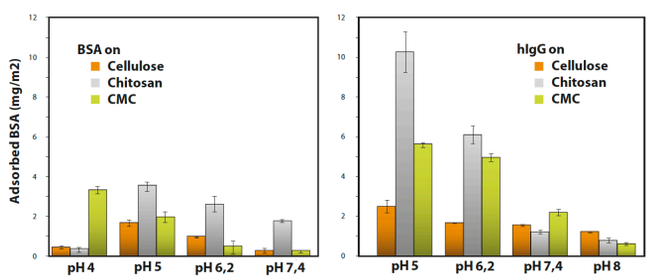


**Figure 3.** Adsorbed mass of BSA (left) and hIgG (rigth) on cellulose, chitosan-modified cellulose, and CMC-modified cellulose. The adsorbed amount were calculated from the SPR data.

## Conclusions

MP-SPR analysis enabled the investigation of the usability of CMC- and chitosan-modified cellulose in protein adsorption and detection. It was found that the type of the cellulose modification and pH strongly affected to the level of protein binding. The developed platforms based on cellulose model surfaces and physical attachment of appropriate proteins is expected to offer new applications in e.g. diagnostic, medical and bioactive-paper fields.

**References:**
[1] Orelma *et al.*, Biomacromolecules, 12(12)



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