

Monitoring of polyelectrolyte multilayer build up and cross-linking using QCM-D

Polyelectrolyte multilayers were introduced by G. Decher and co-workers in 1992 and have since attracted extensive attention because of their extraordinary advantages in a wide range of applications. The polyelectrolyte multilayers are particularly interesting to characterize by QCM-D which is a real-time technique sensitive for mass changes in the ng/cm^2 regime. In particular, the monitoring of changes in the dissipation gives important information about the viscoelastic properties of the polyelectrolyte multilayer as well as the structural changes induced by, for example, cross-linking reactions.

Introduction

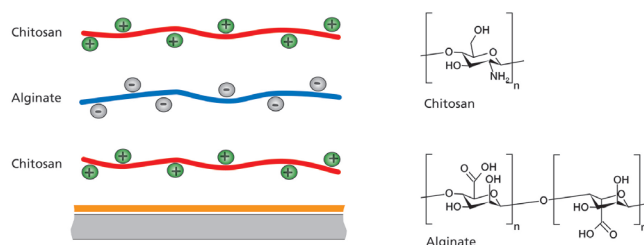
This application note describes QCM-D data obtained during the formation of a polysaccharide polyelectrolyte multilayer. It is an example of a biomedical application of the polyelectrolyte multilayer approach. These applications are attractive due to their ease of preparation and the fine control over the material structure. A high degree of versatility is also offered, including the capability of incorporating high loadings of different types of biomolecules in the films and adjusting the film robustness under ambient and physiological conditions. In particular, natural polysaccharides, such as alginate and chitosan, have attracted interest since they resemble components in the extra cellular matrix and show good biological properties while being accessible to chemical modifications.

Here, polysaccharide multilayers were created following a protocol described by Alves et al¹ based on alternating deposition of the positively charged polysaccharide chitosan and the negatively charged polysaccharide alginate onto gold coated QCM-D sensors. The resulting multilayer structure is schematically shown in figure 1 together with the chemical structures for the polysaccharides. The experiments were performed using an automated QCM-D setup (Q-Sense E4 Auto) allowing 18 h long measurements including a terminal cross-linking step of the chitosan/alginate multilayer.

Experimental

Measurements were conducted based on the procedure described by Alves et al¹. Briefly, 31 layers of chitosan and alginate were built up on a gold coated QCM-D sensor with chitosan as the first and as the terminal layer. Both polysaccharides were dissolved in pure water to a concentration of 0.5 mg/ml and adjusted to $\text{pH} \approx 4$ with 100 mM citric acid. Glutaraldehyde (0.5%) was added as a cross-linking agent after the terminal chitosan layer. 150 mM NaCl

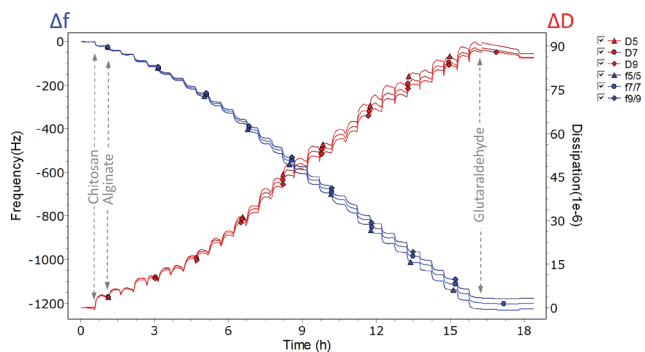
was used as rinsing buffer between each deposition. A flow rate of $50 \mu\text{l}/\text{min}$ and temperature of 22°C was used for all steps in the measurements.



[Figure 1]: a) Schematic illustration of the polyelectrolyte multilayer assembly of positively charged chitosan and negatively charged alginate on a gold coated QCM-D sensor. b) Chemical structures of chitosan and alginate.

Results and discussion

The QCM-D frequency and dissipation shifts during the build-up of 31 alternating layers of the polysaccharides chitosan and alginate are shown in figure 2. Each layer deposition can be distinguished by stepwise decrease and increase in frequency and dissipation, respectively. Mass changes are related to changes in oscillating resonance frequency, Δf , where a negative frequency shift is equivalent to mass increase. The dissipation, ΔD , is related to the energy dissipation of the crystal oscillation, and a positive shift indicates a less rigid structure. The relatively small ratio between the dissipation and the frequency shifts suggests a continuous build up of a quite rigid and compact polyelectrolyte multilayer as is expected for these highly charged polysaccharide chains. This rigidity is also reflected in the small separation of the



[Figure 2]: QCM-D sensorgram for a typical build up of 31 layers of chitosan and alginate shown for the 5th, 7th and 9th overtones. In the last step, a cross-linking agent was added to the multilayer structure (0.5% glutaraldehyde).

frequency overtones. The measurements were performed using the E4 Auto setup. The system proved great stability over the entire measurement sequence of 18 hours under constant flow conditions and the magnitudes of each of the deposited layers increased during the first 6-7 layers to become close to linear for the additional layers. The characteristics of the deposition were similar to the published data on this system including the terminal cross-linking step with glutaraldehyde 1. The action of the cross-linking agent gave only a small dissipation shift and close to no frequency shift. These small responses were rationalized in the study by Alves et al, where infrared spectroscopy showed that only the top chitosan layer was cross-linked. Cross-linking is an attractive way of controlling the stability of polyelectrolyte multilayers, enabling for example control of cell adhesion, wettability or release of incorporated biomolecules.

Conclusions

Polysaccharide multilayers of chitosan and alginate were successfully built up using the Q-Sense E4 Auto setup for 18 h measurements while allowing minimization of the hands-on time to approximately 1 hour. The obtained results proved close to linear build up of 31 layers of a quite rigid structure. Cross-linking at the end of the experiment had little effect on the QCM-D responses and probably only occurred in the top chitosan layer. This study shows the E4 Auto as a reliable and efficient tool for long-time measurements and enables experimental setups that would have been cumbersome to perform by manual sample handling.

Acknowledgements

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References:

- [1] Alves, N.M., Picart, C. & Mano, J.F. Self Assembling and Crosslinking of Polyelectrolyte Multilayer Films of Chitosan and Alginate Studied by QCM and IR Spectroscopy. *Macromolecular Bioscience* 9, 776-785 (2009).