Application Note # 161

Polymerization kinetics and morphology changes of thermo-responsive polymer

Multi-Parametric Surface Plasmon Resonance (MP-SPR) measures surface changes in real-time. High sensitivity and minute volumes make the measurement method excellent for thin film characterization providing layer thickness and refractive index.

Thermo-responsive poly(N-isopropylacrylamide) (PNIPAM) polymerization kinetics was measured during atom transfer radical polymerization (ATRP) in real-time using MP-SPR. Polymer growth was linear with a rate of ~20 nm/min up to 100 nm after which chain termination gradually reduced the growth rate. Brush height during the polymerization was determined without the need to estimate the refractive index. Additionally, brush conformation at different temperatures was studied, showing collapsed polymer at 40°C (69 nm) and extended at 22°C (243 nm).

Introduction

Responsive polymers alter their morphology upon changes in the physiochemical environment and are used for example in repellent and anti-friction coatings. Lately, their use in functional materials and smart interfaces has attracted a lot of attention (Emilsson *et al*. 2017). Applications of PNIPAM polymer range from drug delivery, cell culturing to various lab-on-chip applications.

Surface Plasmon Resonance (SPR) is a well-established method used for measuring binding affinity and kinetics of molecular interactions. However, the physical phenomenon is also applicable to material characterization. Comprehensive 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, thus making MP-SPR an outstanding tool also for material characterization.

MP-SPR measures adsorption of molecules in real-time, and the same measurement provides also layer thickness and refractive index. For layers that do not absorb light, thickness can be measured from ångströms up to micrometers in gaseous and in liquid environments.

Sensor slides can be easily coated *in situ* and *ex situ* by various methods, such as spin coating, dip coating, ALD and self-assembly. MP-SPR uses an elastomer-coated prism and thus allows oil-free operation, which in turn enables further characterization of the coatings by AFM, SEM and other surface sensitive techniques.

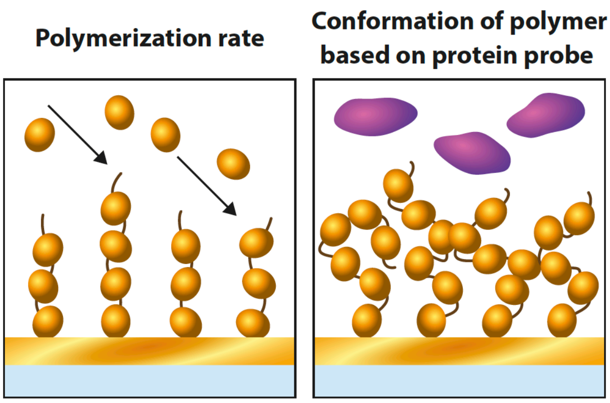

Figure 1. Rate of atom transfer radical polymerization of *in situ* growth PNIPAM (poly(N-isopropylacrylamide)) polymer measured using MP-SPR. Conformation of thermo-responsive PNIPAM polymer in various temperatures was determined by using a protein probe.

Materials and methods

Experiment was performed using MP-SPR Navi™ 220A NAALI equipped with high chemical resistant flow-cell (PEEK).

MP-SPR gold sensor slides were cleaned *ex situ* (H_2O:H_2O_2:NH_4OH 5:1:1 v/v at 75°C for 10 min). Thermo-responsive polymer poly(N-isopropylacrylamide) (PNIPAM) was coated utilizing so called surface-initiated polymerization (SIP) *in situ* and *ex situ*. The solvent for the polymerization was a 1:1 mixture of methanol and water (v/v) and the final concentrations were: NIPAM 0.48 M, $CuBr_2$ 0.6 mM, PMDETA 6.4 mM and Ascorbic acid 10 mM. The height was monitored during the polymerization process using MP-SPR.

The height of polymer was determined also in phosphate buffered saline (PBS), in air and at different temperatures. A new method for thickness evaluation was developed using BSA (20 g/L) as a probe which does not bind on the PNIPAM polymer brushes, and thus the MP-SPR response is solely obtained from solvent refractive index changes during probe injection. Utilizing total internal reflection (TIR) part of the SPR curve and curve fitting, thickness and refractive of the layers were determined. Calculations were based on the Fresnel equations.

Results and discussion

Thickness of polymer brushes is typically measured only after the reaction is stopped, using atomic force microscopy (AFM) or ellipsometry measurements. Usually, the thickness is evaluated only in air since it is considerably more difficult to accurately determine the height in liquid. MP-SPR can monitor the polymerization rate and measures layer properties in air as well as in liquid without any change in the instrument setup.

BioNavis

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

During the polymerization, no change in total internal reflection (TIR) part of the complete SPR curve was observed, confirming that the response was solely obtained from binding (and not from bulk/solvent refractive index changes) (Figure 2).

Refractive index of the PNIPAM was 1.44 ± 0.004 based on MP-SPR data, which is in good agreement with the literature. Considering the poor solubility of PNIPAM in solvent used (methanol/water), the refractive index of the polymer film is not expected to vary to a high degree. Thickness of the growing polymer increased linearly up to 100 nm after which the rate decreased (Figure 3). Thicknesses up to 300 nm were measured. In terms of size of the monomer (0.3 nm), the polymerization rate was determined as 1.4 monomer units per second.

Height (conformation) of *in situ* and *ex situ* grown polymers was quantified in water-based buffer. *Ex situ* grown films were also characterized at two temperatures and in the dry state. The height was almost five times higher at 22°C when compared to 40°C, showing the collapsed state of the polymer above the lower critical solution temperature (LCST) (Figure 4).

Additionally, polymer resistance against serum samples was measured. For brushes polymerized for 2.5 min, the film was highly protein resistant at 22°C. However, at 40°C (above LCST) the adsorbed serum protein amount increased drastically to over 100 ng/cm2, but BSA still did not bind. MP-SPR results were in good agreement with the complementary Quartz Crystal Microbalance (QCM-D) and Atomic Force Microscopy (AFM) data.

Conclusions

MP-SPR enables comprehensive characterization of thin films, providing thickness, refractive index, conformation, interactions and bound mass as shown with PNIPAM polymer. Possibility to measure in various temperatures and environments allows throughout characterization of various materials.

Instead of probe injection, the multiwavelength configuration of MP-SPR instruments allows both thickness and refractive index to be determined based on full SPR curve fitting at multiple wavelengths. For more information see Application Note #128.

See also how polymers have been characterized in vapor using MP-SPR Application Note #159.

Original publication:

Emilsson *et al*., Applied Surface Science, Vol. 396, 2017

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 200 OTSO, 210A VASA or 220A NAALI equipped with high chemical resistant flow-cell (PEEK) and additional wavelength -L

Sensor surface: Au, other metal or inorganic coating

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

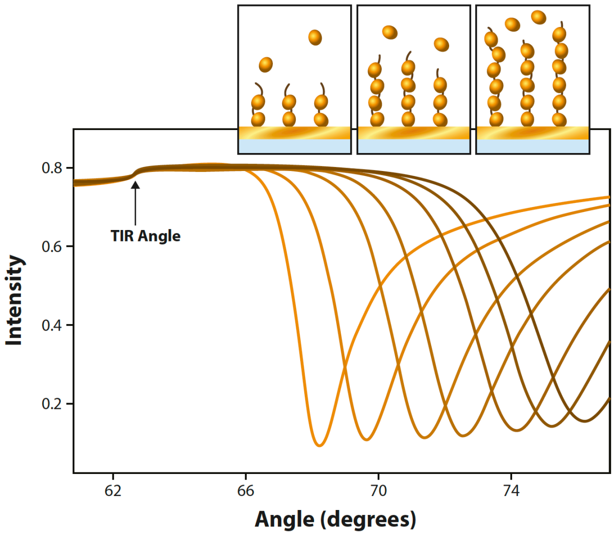

Figure 2. SPR peak minimum position shifts to the right due to growth of the polymer chain. The total internal reflection (TIR) angle does not change and thus confirms that no changes in liquid composition (bulk) are occurring during the process.

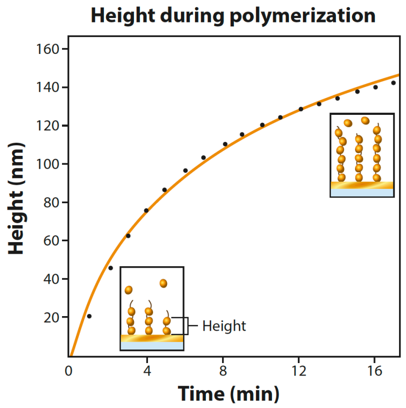

Figure 3. PNIPAM height monitored during polymerization *in situ* in methanol/water.

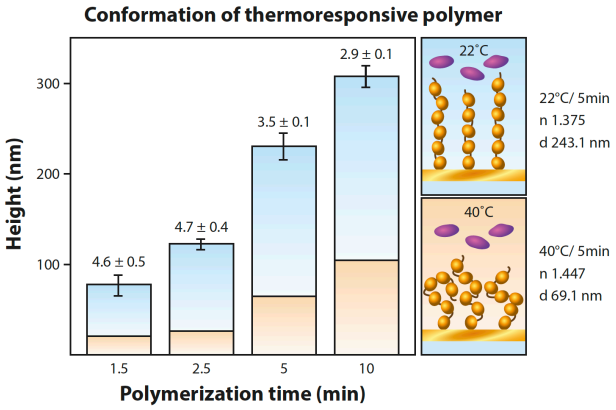

Figure 4. PNIPAM polymer is collapsed at 40°C and extended at 22°C when measured in PBS buffer. Above each bar there is a ratio between the height in extended and collapsed state.