Phase transition of pNIPAM grafted on plasma-activated PEO monitored in-situ by quartz crystal microbalance

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Abstract. Thermoresponsive poly(N-isopropylacrylamide) layers were grafted on plasma-activated poly(ethylene oxide)-like substrate. Analysis by ToF-SIMS confirmed the presence of a pNIPAM film, which shows a phase transition temperature at 28 - 32 °C with hysteresis, as determined in-situ by quartz crystal microbalance. During the transition, the frequency of the pNIPAM coated quartz sensor exhibits a pronounced and unexpected minimum while the dissipation changes monotonously. This peculiar behaviour is explained by temporary formation of water-rich regions, which may cause delayed release and uptake of water during the collapse and re-swelling of the layer.

1. Introduction

Temperature-switchable polymers, such as poly(N-isopropylacrylamide) (pNIPAM), exhibit a reversible phase transition from a swollen, hydrophilic state below the lower critical solution temperature (LCST) to a collapsed, hydrophobic state above the LCST. When deposited on a surface, thin films of pNIPAM are envisioned as “smart”, bioactive coatings for controlled protein and cell attachment [1], and could be utilized in devices such as reusable biosensors [2]. It was shown recently that thin pNIPAM films prepared by photo-grafting [3] or plasma-assisted chemical vapour deposition (PACVD) [4] on different substrates have indeed a reversible phase transition. Furthermore, the affinity for soluble proteins at room temperature is one order of magnitude lower than at 37 °C [5]. To fully understand this switching behaviour, it is, however, necessary to follow the phase transition in-situ, i. e., to monitor characteristic properties of the film as a function of temperature while passing through the LCST. Therefore, we have studied the viscoelastic properties of pNIPAM, which was grafted on plasma-activated poly-(ethylene oxide)-like film that was previously deposited on a QCM crystal by plasma-CVD. The frequency shift and the dissipation during the phase transition, while the pNIPAM layer collapses upon heating and re-swells upon cooling, reveal some unusual transitional features, which can be monitored only by in-situ measurements. These features are interpreted as being caused by the trapping of small, water-rich regions during the phase transition.

2. Experimental

Poly-(ethylene oxide)-like films (called PEO films subsequently) were deposited on SiO₂ precoated quartz crystal sensors (Q-Sense QSX 303) in a homebuilt reactor by pulsed RF (13.56 MHz, 5 W, 10%
duty cycle) plasma-assisted chemical vapour deposition using diethylene glycol dimethyl ether as precursor, as described elsewhere in more detail [6]. Deposition of PEO-films on SiO$_2$ precoated sensor crystals ensures good adhesion of the film. This is most probably due to the presence of –OH groups on the SiO$_2$ surface, which allows covalent bonding of the PEO precursor. The latter is not possible when standard Au covered QCM sensors are used instead. The films used for the experiments described below had a thickness of 20±3nm. After washing in ultrapure water and drying in N$_2$, the PEO films were activated in another reactor by applying continuous wave Ar plasma (10 W input power, working pressure 34 mTorr) for 30 s. Graft polymerization of NIPAM on the activated films was carried out in aqueous solution. The monomer was purified by recrystallization from pure hexane prior to use. The monomer solution was prepared by dissolving purified NIPAM in a mixture of ultrapure water and methanol (20% v/v), followed by degasification in the reaction flask for at least 1 h by bubbling a stream of N$_2$ through the solution. The activated PEO-coated samples were placed at the bottom of the flask and the solution was tempered for 2 h on an oil bath at 32 °C while the N$_2$-stream was maintained during the entire reaction. After the polymerization, the grafted samples were washed overnight in a 3:1 mixture of ultrapure water and methanol. The next day, they were sonicated for 1 min in ultra-pure water in order to remove any weakly bound residuals from the surface. Finally, the samples were dried in a stream of N$_2$ and stored for a maximum of 3 days at room temperature (RT) in Al-foil until further use.

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) data were obtained using a ToF-SIMS V instrument (ION-TOF GmbH). The primary source was a 25 keV bismuth liquid metal ion gun, and the total flux was kept below $10^{13}$ ions/cm$^2$. Temperature-dependent QCM measurements were performed with a temperature-variable instrument with dissipation monitoring (QCM-D E4 from Q-Sense; temperature stability 0.02 K). Shifts of the fundamental resonance frequency (nominally 5 MHz), including up to the 13th overtone, as well as the corresponding shifts in the dissipation were measured simultaneously as a function of time and temperature at a heating rate of 1.7 K/min. Water contact angle (CA) measurements were made with a GBX Digidrop MSE apparatus using a homebuilt sample holder equipped with a Peltier element for temperature control.

3. Results and discussion
ToF-SIMS analysis of the sample surface was performed after the final preparation step. Si samples with chemically polymerized, spin coated pNIPAM were also prepared as reference. Characteristic and particularly important for the identification of pNIPAM are the nitrogen and nitrogen/oxygen containing peaks, and fragments of isopropyl groups are also common for pNIPAM spectra [7]. Examples for these peaks are C$_3$H$_5^+$ (27 amu), C$_3$H$_7^+$ (43 amu), C$_3$H$_6$O$^+$ (54 amu), C$_6$H$_7$N$^+$ (58 amu), C$_6$H$_6$NO$^+$ (73 amu), and C$_6$H$_7$NO$^+$ (113 amu). All of these peaks are present in the spectra of the grafted pNIPAM surfaces (Fig. 1), and the comparison with the spin coated reference sample showed very good agreement between the two types of samples and hence confirms the presence of pNIPAM. The thickness of the pNIPAM layer on top of the PEO after the ultrasonic bath was (10±0.5) nm at
a refractive index of 1.6, as measured by ellipsometry. Corresponding AFM measurements at RT showed a smooth surface (rms roughness 0.51 nm). CA measurements at 15 °C, where pNIPAM adopts a swollen, random coil configuration, and thus has a hydrophilic character, gave a value of 55°, whereas at 45 °C, where the polymer is hydrophobic and has a compact and rigid, collapsed globular conformation, the CA is 80°. This indicates that the phase transition takes place in this temperature region, as expected for pNIPAM.

**Figure 2:** $\Delta D$ and $\Delta f$ of the pNIPAM coated PEO/quartz crystal during heating and cooling.

**Figure 3:** Dissipation and frequency change of the pNIPAM layer as a function of temperature after subtraction of the contribution of the PEO coated sensor.

The change of the frequency ($\Delta f$) and the dissipation ($\Delta D$) of a QCM sensor functionalized with PEO and coated subsequently by pNIPAM during variation of the temperature are shown in Fig. 2 as a function of the annealing time. Since $\Delta f$ and $\Delta D$ of the PEO-coated sensor itself are also temperature dependent, the signal of the sensor without pNIPAM was measured separately (dotted lines) and was subsequently subtracted from the signal of the sensor with pNIPAM attached (dashed line); the result, which represents the pNIPAM layer only, is included as solid lines. Both $\Delta D$ and $\Delta f$ change pronouncedly when the temperature is raised from 20 °C to 37 °C and again during the cooling process. For better visibility of the phase transition, the $\Delta D$ and $\Delta f$ signals of the pNIPAM layer, corrected for the contribution of the PEO-coated crystal, are also plotted as a function of the temperature in Fig. 3. In the heating process $\Delta D$ sharply decreases, and the $\Delta D$ curve has an inflection point around 34 °C. In the cooling process $\Delta D$ increases again and reaches the original value at around 20 °C, but this time the inflection point is at 28 °C, i.e., the transition has a hysteresis. Such behaviour of $\Delta D$ is expected when the layer collapses above the LCST, where the pNIPAM becomes hydrophobic and expels water while the layer becomes more rigid. Upon cooling, it swells again and adopts a softer, hydrophilic state. The behaviour of $\Delta f$ upon variation of the temperature is, however, unexpected. During the heating process $\Delta f$ decreases and has a pronounced minimum at the same temperature at which $\Delta D$ has the inflection point, after which it increases until a plateau is reached.
above 36 °C. This behaviour is reversible, i.e., the same $\Delta f$ (and $\Delta D$) values are obtained after the cycle is completed by re-cooling, which holds true for at least five cycles in sequence. $\Delta f$ has a hysteresis as well: the minimum during cooling is at a lower temperature than during the heating process. The two minima of $\Delta f$ are at the same temperatures as the inflection points of the corresponding $\Delta D$ signals.

These findings for $\Delta f$ cannot be explained by the common model, according to which water is continuously expelled during the collapse of the layer, because in that case, the layer should become more rigid (as indicated by a decrease in $D$), while the effective mass should decrease (which would be indicated by an increase in $f$). We therefore propose that small water-rich regions are formed in the pNIPAM layer during the collapse, which leads to a transition that consists of two steps. Upon heating, when the layer starts to collapse, water may be trapped in these pore-like regions, which leads to a more efficient coupling of the water mass to the shear vibration of the crystal and causes the initial decrease of $\Delta f$. This way $\Delta f$ and $\Delta D$ change into the same direction. Eventually the water is released and expelled from those regions, and $\Delta f$ increases again, while $\Delta D$ continues to decrease with increasing rigidity of the layer. When the temperature decreases again, these two processes proceed in the opposite sequence. This view is additionally supported by the presence of a hysteresis, which can be observed in adsorption-desorption processes in porous and nanoporous systems when a liquid is confined by capillary condensation. The pronounced nonlinear relation between $\Delta D$ and $\Delta f$ (Fig. 4) also indicates that the structure of the pNIPAM film changes dramatically during the transition, and that the collapse and re-swelling are not continuous under these conditions but rather involve the formation of transient structural features.

4. Conclusions
Thermoresponsive pNIPAM was grown on plasma-deposited PEO after activation of the substrate surface in Ar plasma. The phase transition from the swollen to the collapsed state and back was monitored in-situ by QCM-D as a function of the temperature. The transition is reversible and shows pronounced transitional features. This behaviour is most likely the result of trapping of water in transient water-rich regions, which are formed during the phase transition. This can lead to a more efficient coupling of the water mass to the shear vibration of the quartz crystal.

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