Detecting Solution pH Changes Using Poly (*N*-Isopropylacrylamide)-*co*-Acrylic Acid Microgel-Based Etalon Modified Quartz Crystal Microbalances

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Abstract: Poly (*N*-isopropylacrylamide)-*co*-acrylic acid (pNIPAm-*co*-AAc) microgel-based etalons have been shown to have visible color and unique spectral properties, which both depend on solution temperature and pH. In this investigation, pNIPAm-*co*-AAc microgel-based etalons were fabricated on the Au electrode of a quartz crystal microbalance (QCM), and the resonant frequency of the QCM monitored as a function of temperature, at pH 3.0. Furthermore, the resonant frequency at either pH 3.0 or 7.0 was monitored while keeping the solution temperature constant at various temperatures. In all cases, when the solution temperature was below the collapse transition for the microgels (~32 °C), the resonant frequency at pH 3.0 was lower than at pH 7.0, which we attribute to the film transitioning from a deswollen to swollen state , respectively. It was observed that the magnitude of the resonant frequency change increased as the solution temperature approached the collapse temperature for the microgels. The overall sensitivity to pH was determined to be 1.3 x 10^{-8} M [H^+] Hz^{-1} and a theoretical detection limit of

390 nM was obtained. This sensitivity will be exploited further for future biosensing applications.

Keywords: Poly (N-isopropylacrylamide) microgels, color tunable materials, quartz crystal microbalance, stimuli responsive polymers, sensing

1. Introduction

Poly (*N*-isopropylacrylamide) (pNIPAm) is one of the most well-known and extensively studied thermoresponsive polymers. At T < 32 °C, pNIPAm is fully water soluble, existing as a random coil. At T > 32 °C, pNIPAm transitions from a hydrated random coil to a desolvated, compact globule.[1-3] The temperature at which this occurs is called the lower critical solution temperature (LCST), or the volume phase transition temperature.[4] The thermoresponsive nature of linear pNIPAm can be used to synthesize pNIPAm-based particles (microgels) via free radical precipitation polymerization.[5-11] Microgels are extremely porous, and like their linear polymer counterparts, are fully water soluble (and water swollen, large diameter) at T < LCST, and expel their solvating water and collapse (small diameter) at T > LCST. When microgels expel their solvating water their viscosity increases.[12] PNIPAm microgels are also quite easily modified with other functional groups, typically via copolymerization of comonomers into the microgel at the time of synthesis. The most commonly used comonomer is acrylic acid (AAc). AAc is a weak acid, which has a pK_a of ~ 4.25, therefore, at pH < pK_a the pNIPAm based microgels are fully responsive, while at pH > pK_a the pNIPAm responsivity is hindered.[13-16] That is, at pH > pK_a the Coulombic repulsion between the deprotonated AAc groups in the microgels prevents their collapse at T > 32 °C, requiring significantly higher T to achieve a microgel response. AAc not only results in pH responsive pNIPAm microgels, but the AAc

groups can also be used as reactive "handles" to add further functionality to the microgels.[17, 18]

We have recently demonstrated that pNIPAm-*co*-AAc microgels can be used to fabricate color tunable materials by "painting" a very uniform microgel layer onto metal coated supports.[19-24] The resulting microgel layer can subsequently be coated with another thin metal layer to yield a microgel layer "sandwiched" between two semi-transparent (yet reflective) metal layers. Figure 1 shows a schematic for the assembly. We have shown that when these devices (etalons) are immersed in water, they exhibit visible color due to light interference in the cavity formed between the two Au layers. The specific color depends on the distance between the mirrors. Since the microgel's diameter depends on temperature, the distance between the mirrors can be changed, and hence the etalon's color dynamically tuned.[19-25] In this submission we exploit the solvation state changes that take place when an etalon changes color, as opposed to exploiting the actual color change, for sensing purposes. This is done by immobilizing the etalon on the Au electrode of an AT-cut quartz crystal microbalance (QCM).

An AT-cut QCM crystal can be excited electrically to undergo an oscillating shearing motion, at a characteristic resonant frequency (most commonly 5 MHz). The resonant frequency is dependent on the properties (e.g., mass, viscosity) of the material in contact with the oscillating device and is described by equation (1): [26]

$$\Delta f = -f o^{\frac{3}{2}}(n_l p_l/\pi p_q \mu_q)^{1/2} \qquad (1)$$

Where f_0 is the initial resonant frequency of the QCM crystal, Δf is the frequency shift measured by the quartz crystal microbalance, ρ_l is the liquid density, η_l is the liquid viscosity, μ_q is the elastic modulus of quartz, and ρ_q is the density of quartz.[26]

As shown by Kanazawa and others,[27,28] and is apparent in equation (1), the resonant frequency of the QCM decreases as the viscosity of the substance contacting the QCM crystal increases. Furthermore, increased viscosity leads to an increase in the "resistance" of QCM oscillation.

Here, we demonstrate that pNIPAm microgel-based etalons can be fabricated on the Au electrode of a quartz crystal microbalance (QCM). While the etalons still function as an optical device, see Figure 2 for a sample reflectance spectrum, we show that the resonant frequency of the QCM crystal likewise depends on solution T and pH. We hypothesize that the frequency shifts in response to viscosity changes that occur when the microgels transition from a swollen (low viscosity) to a deswollen (high viscosity) state.[12] As we show here, this phenomenon can be exploited for pH sensing, although this phenomenon can be exploited to detect other species that interact with the microgels of the etalons that result in a microgel solvation change.

2. Experimental

2.1. Materials

N-Isopropylacrylamide was purchased from TCI (Portland, Oregon) and purified by recrystallization from hexanes (ACS reagent grade, EMD, Gibbstown, NJ) prior to use. N,N'-methylenebisacrylamide (BIS) (99%), acrylic acid (AAc) (99%), and ammonium persulfate (APS) (98+%) were obtained from Sigma-Aldrich (Oakville, ON) and were used as received. Sodium chloride and sodium hydroxide were obtained from Fisher (Ottawa, ON). All deionized (DI) water was filtered to have a resistivity of 18.2 MΩ · cm and was obtained from a Milli-Q

Plus system from Millipore (Billerica, MA). Cr and Au were deposited using a Model THEUPG Thermal Evaporation System from Torr International Inc. (New Windsor, NY). Anhydrous ethanol was obtained from Commercial Alcohols (Brampton, ON). Hydrochloric acid was purchased from Caledon Chemicals (Georgetown, ON). Cr was 99.999% and obtained from ESPI (Ashland, OR), while Au was 99.99% obtained from MRCS Canada (Edmonton, AB).

2.2. Instrumentation

Microgel coated QCM crystals, as well as the etalon coated devices were analyzed using a QCM-200 obtained from Stanford Research Systems (Sunnyvale, CA). The crystal was placed into a specially designed holder, which allowed for water of a given pH and temperature to constantly flow over the crystal at a rate of 0.062 mL/s. This flow was maintained by a FMI lab pump model RP-G150 (Oyster Bay, NY). The temperature was controlled by placing a beaker containing water onto a Corning model PC-420D hotplate (Lowell, MA), and the water temperature measured via a thermocouple. To change the pH of the solution, aliquots of either 1M NaOH or concentrated HCl were added to the water, and the water pH measured with a Jenco model 6173 pH meter (San Diego, CA). Ionic strength was not controlled, but we confirmed that changes in ionic strength had little effect on the resonant frequency of the QCM crystal (data not shown).

2.3. Procedures

2.3.1. Microgel synthesis

The microgels used in this study were synthesized using surfactant-free, free radical precipitation procedure as described previously.[12, 19-25] Briefly, 8.5 mmol of NIPAM and 0.51 mmol of N,N'-methylenebisacrylamide (BIS) was added to a beaker, and dissolved in 50

mL of deionized water. Once dissolved, the solution was filtered via a syringe through a 0.2 µm syringe filter into a 100 mL 3-necked round bottom flask containing a stir bar. 12.5 mL of deionized water was used to rinse the beaker, which was also filtered and added to the round bottom flask. Next, a gas inlet (needle), a reflux condenser, and a temperature probe were fitted onto the round bottom flask. The solution was stirred at 450 RPM, and purged free of O_2 by bubbling N_2 gas through the solution while heating to 45 °C for 1 h. Immediately prior to initiation, 1 mmol of AAc was added to the solution along with 2.5 mL of 0.078 M ammonium persulfate solution. The solution temperature was then increased to 65 °C at a rate of 30 °C/hour immediately following initiation and was allowed to react overnight. Following the overnight reaction, the solution was allowed to cool and was filtered through glass wool to remove large aggregates from the solution. The filtrate was then diluted to 120 mL with deionized water and 12 mL aliquots were added to centrifuge tubes, and centrifuged at ~8500 relative centrifugal force for 30 minutes each at 20 °C. The microgels were packed at the bottom of the centrifuge tube, and the supernatant solution subsequently removed, and replaced with fresh DI water. Centrifugation and resuspension was repeated five more times to remove any unreacted reagents present.

2.3.2. Formation of etalons on QCM crystals

Etalons were fabricated as previously described, with some slight modification.[19-24] The Au electrodes of the QCM quartz crystal were rinsed copiously with anhydrous ethanol and dried with N_2 gas. 12.5 µL of a concentrated, viscous microgel solution was "painted" only on the "large" Au electrode of the QCM crystal and allowed to dry for 30 minutes at 35°C. Following drying, the microgels not directly bound to the Au electrode were rinsed off with DI water, and the QCM crystal was immersed in DI water overnight at 35°C. Following soaking, the

crystal was further rinsed with DI water and dried with N_2 gas. A paper template was adhered to the QCM crystal, such that only the Au electrode, which was coated with microgels was exposed. The QCM crystal was then inserted into a Torr International Inc.(New Windsor, NY) thermal evaporation system Model THEUPG. 2 nm of Cr and 15 nm of Au were deposited only onto the Au bound microgel layer at a rate of ~0.2 Å s^{-1} and ~0.1 Å s^{-1}, for Cr and Au, respectively. After application of this overlayer, the crystal was subsequently removed from the vacuum chamber and immersed in DI water overnight at 35°C. The QCM bound etalon was subsequently used.

3. Results and discussion

3.1. Temperature responsivity

PNIPAm-*co*-AAc microgels with an average diameter of 1.5 ± 0.2 µm (measured via optical microscopy) were painted on the Au electrode of a QCM crystal. A differential interference contrast microscope image of the microgels is shown in Figure 3. The behavior of the QCM bound microgels was investigated by monitoring the resonant frequency of the QCM crystal, as the temperature of the pH 3.0 solution was raised, which affects the solvation state of the microgels. As can be seen in Figure 4(a), the crystal's resonant frequency decreased as the temperature of the pH 3.0 solution was ramped from 22.6 °C to 35.8 °C at an average rate of 0.001 °C/s. The frequency reached a minimum close to 32 °C, which is the accepted LCST for pNIPAm-*co*-AAc microgels at pH 3.0. The total observed frequency change from the initial temperature to the transition temperature was 4260 Hz. The QCM frequency surprisingly increased as the solution temperature exceeded the collapse temperature. We hypothsize that the observed frequency shift with increasing temperature, up to the microgel's LCST, is a result of

the increased viscosity of the collapsed microgels.[12] Figure 4(b) shows the behavior of the same microgels after deposition of a 2 nm Cr/15 nm Au layer on top of the microgels. Similar to the uncoated microgels, the etalon caused the QCM crystal frequency to decrease as the temperature of the pH 3.0 solution was increased from 24.2 °C to 35.3 °C at an average rate of 0.001 °C/s. As can be seen from the data, the 6903 Hz frequency change of the etalon is much more dramatic than what was observed for the uncoated microgels. At this point, the reason for the enhanced response of the etalon coated QCM crystal to temperature is unknown. Although, it is known that at high temperature the Au layer on top of the microgels will be close to the QCM crystal surface, as a result of the microgels collapsing.[19-25] As a result, the QCM crystal will able to detect the increased viscosity from the collapsed microgels as well as the mass of the Au overlayer. This combined will lead to a greater QCM response. To confirm that this observed behavior was due to the microgels, and not the crystal's sensitivity to temperature, an uncoated QCM crystal was heated, in the same way as above, which shows a relatively small frequency response to temperature. Therefore we attribute the frequency changes to the presence of the microgels and etalon on the QCM crystal's Au electrode.

We wanted to further confirm that the observed behavior was truly due to the microgels, and the microgel's influence on the QCM crystal, and not due to heating the microgels at non-equilibrium conditions, i.e., a continuous temperature ramp as was done above. To do this, the samples used to generate the data in Figures 4(a) and 4(b) were heated to discrete temperatures, and the frequency allowed to stabilize. Figures 5(a) and 5(b) show the frequency data as a function of time, at various temperatures, for the uncoated and Cr/Au coated microgels (etalons). As can be seen, the trends for the QCM resonant frequency were similar to the continuous heating experiments. Most important for the study here is the ability to stabilize the QCM's

frequency at each temperature, especially at the minimum found at the LCST. Figure 6 shows the resonant frequency values at each stabilization temperature for the uncoated microgels and the etalon. As can be seen from the data, if the microgel layer can be made to reswell, while maintaining the solution temperature at the LCST, a large QCM frequency shift, back toward its frequency at low temperature would be expected.

3.2. pH responsivity

To investigate this further, we took advantage of the pH responsivity of the pNIPAm-*co*-AAc microgels. It is well known that at a given temperature, the diameter of pNIPAm-co-AAc microgels is larger at pH 7.0 than at pH 3.0. In other words, the viscosity of the microgels at pH 7.0 will be lower than at pH 3.0. Therefore, it stands to reason that at all temperatures, the QCM resonant frequency at pH 7.0 should be higher than at pH 3.0. Figures 7(a) and 7(b) show that for both the uncoated microgel layer, and the etalons, that this is the case. From the plots, it appears that a pH change from 3.0 to 7.0 is enough to reswell the collapsed microgels, and affords a frequency increase. In fact, because the change in the QCM crystal frequency, relative to its initial value, is much greater compared to the uncoated microgels, the frequency change upon reswelling at high pH is likewise enhanced. In addition, in both cases it can be seen that the QCM frequency at the LCST in pH 7.0 solution was greater than its frequency at low temperature in pH 3.0 solution. We hypothesize that this is a result of Coulombic repulsion between the deprotonated AAc groups in the microgels at pH 7.0 causing the microgels to be more swollen (less viscous) than they are at low temperature in pH 3.0 solution. This is a well-known phenomenon for pNIPAm-co-AAc microgels in solution. [12] The resulting changes in frequency in response to the solution pH change for the uncoated microgel layer and after Cr/Au addition were 5401 Hz and 8217 Hz, respectively.

We further investigated whether the pH response could be enhanced at any other temperature. To accomplish this, pH transitions from 3.0 to 7.0 were performed at multiple temperatures. As seen in Figures 8(a) and 8(b), the QCM crystal frequency plateaus at similar values after transitioning from pH 3.0 to 7.0. This means that the film's reswelling is limited; therefore the maximum pH response is achieved when the film is at the LCST. In other words, the maximum response is achieved when the microgels transition from their fully collapsed to fully swollen form. The fact that the QCM crystal's frequency returns to the same maximum resonant frequency in pH 7.0 solution at each temperature further supports the hypothesis that the microgels reswell in pH 7.0 solution due to Coulombic repulsion.

Finally, we set out to find the pH range that the device would be most sensitive. To determine this, the etalon was brought to its transition temperature at pH 3.0. At this temperature, the solution's pH was increased stepwise, while monitoring the QCM resonant frequency. The resonant frequency was allowed to stabilize at each pH value investigated, and the solution pH was continuously monitored. Figures 9(a) and 9(b) show the results of this experiment for both an uncoated microgel layer and an Au coated microgel layer. Each pH value stated was stabilized for at least 15 minutes before further addition of NaOH. As can be seen, the maximum response occurs over a two (2) pH unit range, centered around a pH of ~4.10, which is close to the accepted value for the pK_a for AAc. From the data, we estimated the device's detection limit to be 390 nM H+, with a sensitivity was calculated to be 1.3×10^{-8} $[H^+]$ Hz^{-1}. From the data, it also stands to reason that in order to make an etalon that is sensitive in other pH ranges, acidic monomers with varying pK_a's can be polymerized into the microgels.

4. Conclusions

In this submission, we showed that the resonant frequency of a microgel coated QCM crystal (and etalon) was affected dramatically by the temperature and pH of the solution it was exposed to. We hypothesize that this response was a result of the different solvation states (and hence viscosity) of the microgels as a function of temperature and pH. This sensitivity was exploited for pH sensing, by operating the materials at the microgels LCST. The response to pH was shown to be more significant for the Cr/Au coated microgel layers (etalons). The most sensitive region for the samples is at the LCST over 1 pH unit range. We can detect pH shifts as little as 390 nM [H+] within this pH range. This system will be further developed for biosensing applications as well as for detecting pH changes in different ranges.

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Figures:

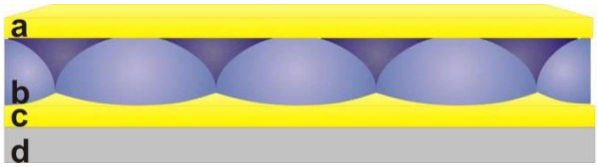


Figure 1. Structure of a poly (N-isopropylacrylamide) microgel-based etalon. (a and c) two 15 nm Au mirrors (with 2 nm Cr adhesion layer) sandwiching (b) the microgel layer. Typically, (d) is a glass substrate. For these experiments, (d) is an AT-cut quartz crystal.

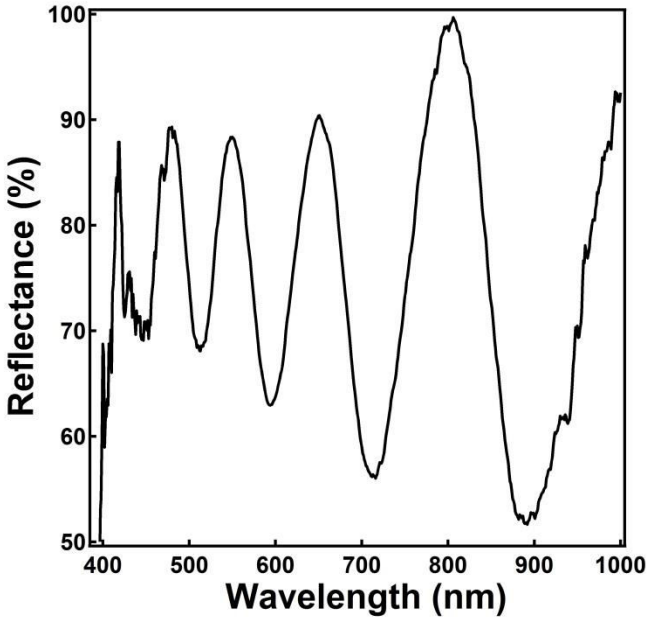


Figure 2. Reflectance spectrum of a representative etalon immobilized on a QCM crystal. The various peaks correspond to the different orders of reflection, which is typical of pNIPAm microgel-based etalons.

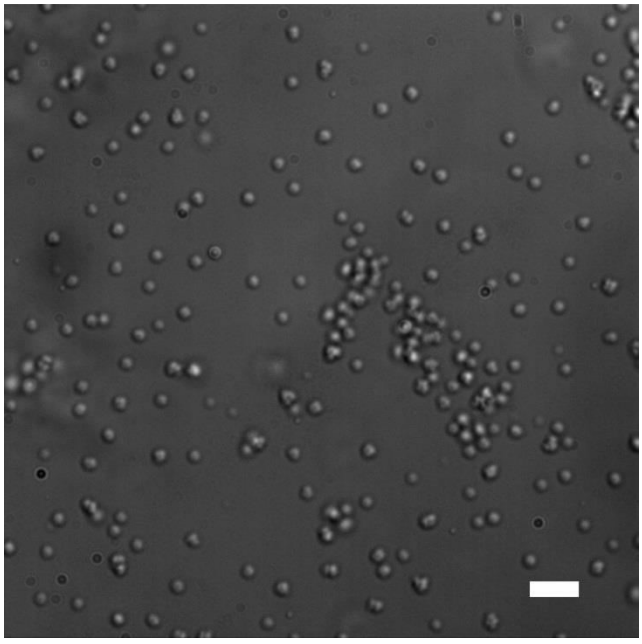


Figure 3. Differential interference contrast microscope image of the pNIPAm-based microgels used in this study. The microgels were in water, on a glass substrate. The scale bar is 6 µm.

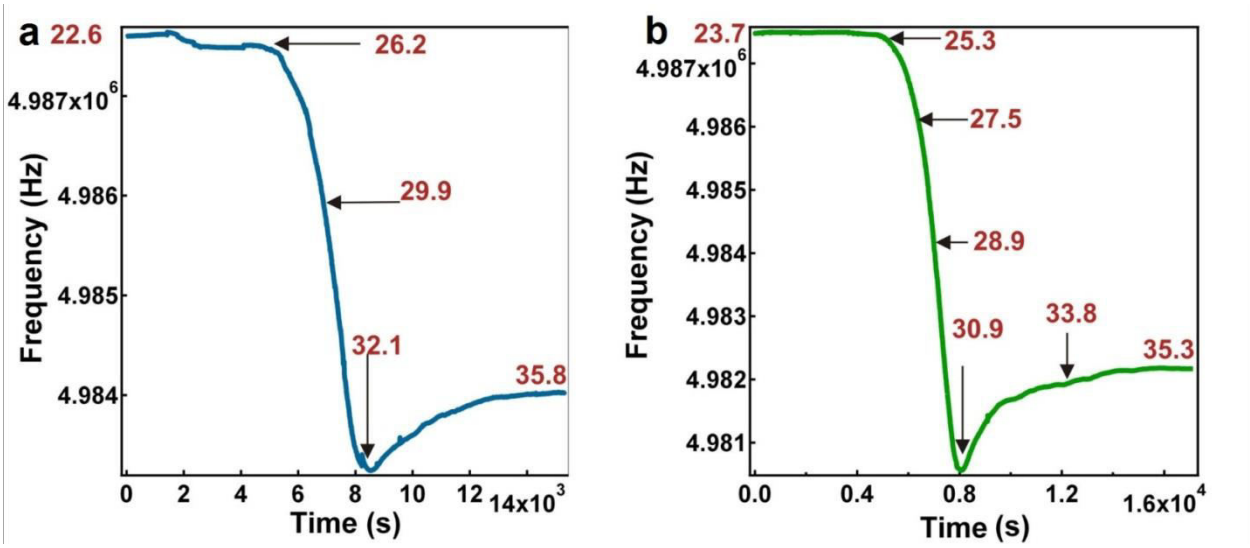


Figure 4. QCM resonant frequency as a function of time for (a) pNIPAm-*co*-AAc microgel coated QCM crystal and (b) after deposition of a 2 nm Cr/15 nm Au overlayer in pH 3.0 water. The heating rate was on average 0.001 °C/s, and the values on the individual plots correspond to the temperature (°C) at a given time.

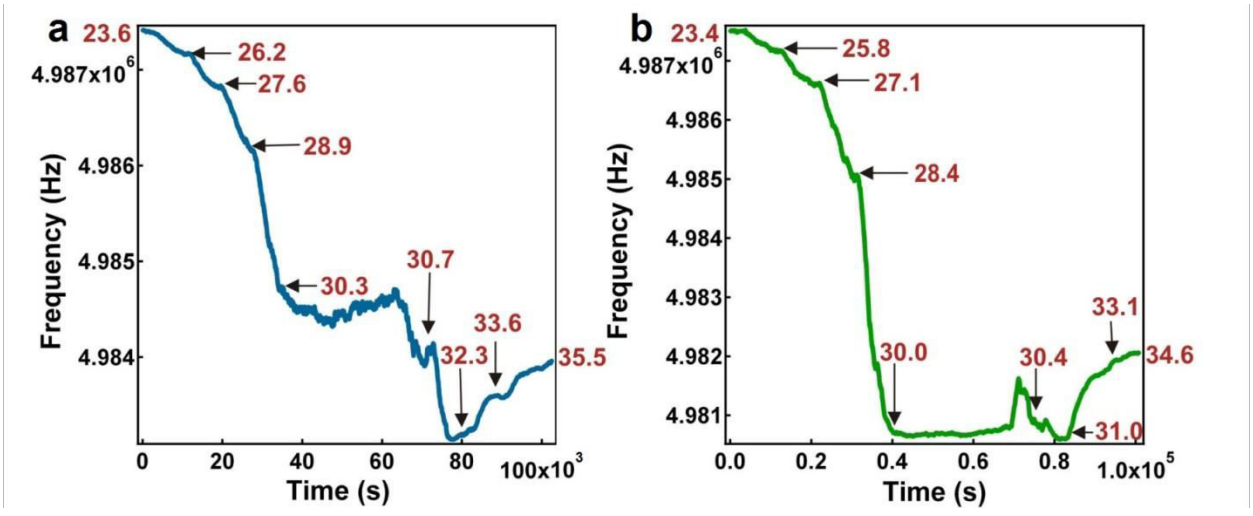


Figure 5. QCM resonant frequency as a function of time for (a) pNIPAm-*co*-AAc microgel coated QCM crystal and (b) after deposition of a 2 nm Cr/15 nm Au overlayer in pH 3.0 water. After reaching a given temperature, the QCM resonant frequency was allowed to stabilize before proceeding to the next temperature. The values on the individual plots indicate the temperature (°C) at the individual stabilization points.

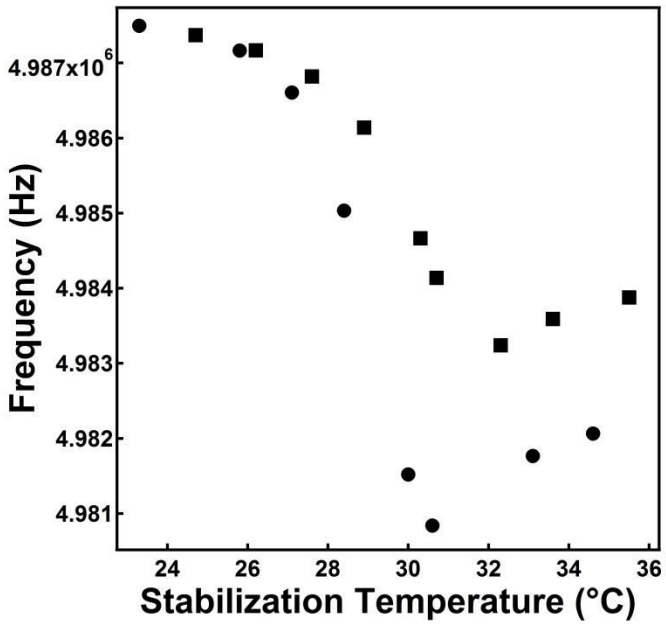
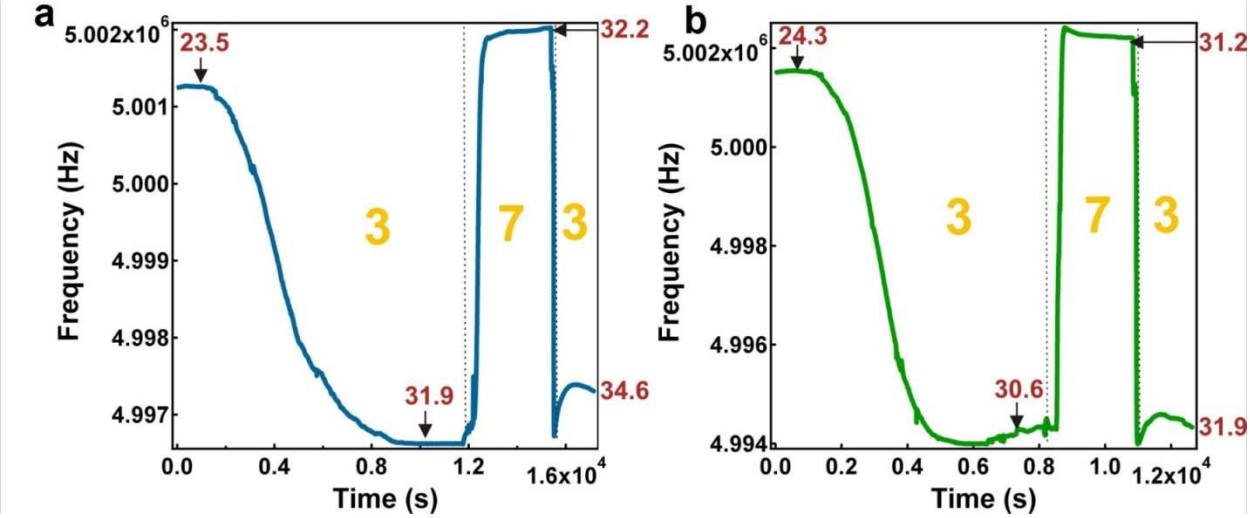
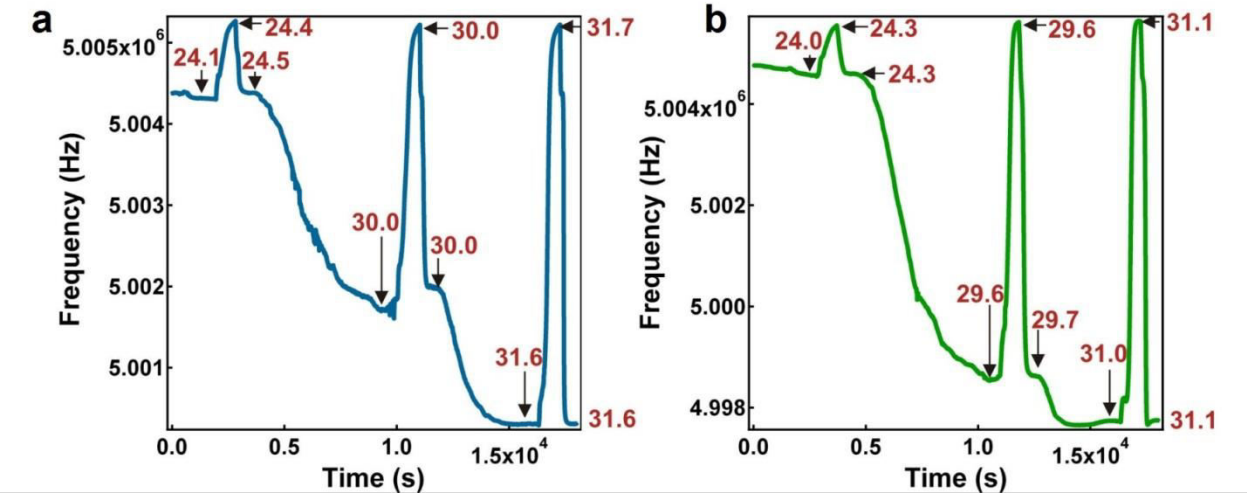


Figure 6. QCM resonant frequency at the individual stabilization temperatures in Figure 4. (■) pNIPAm-*co*-AAc microgel coated QCM crystal and (●) after deposition of a 2 nm Cr/15 nm Au overlayer in pH 3.0 water.



309

310 **Figure 7.** The pH response of a (a) pNIPAm-*co*-AAc microgel coated QCM crystal and (b) the
311 same substrate after deposition of a 2 nm Cr/15 nm Au overlayer. Initially, each was heated to
312 the film's lower critical solution temperature in pH 3.0 water. Following stabilization, the pH of
313 the water was increased to 7.0 by addition of 1M NaOH. Again, the signal was allowed to
314 stabilize, and the pH returned to 3.0 by addition of concentrated HCl. The pH and temperature
315 (°C) values are indicated on the individual plots.



316

317 **Figure 8.** The pH response of a (a) pNIPAm-*co*-AAc microgel coated QCM crystal and (b) the
318 same substrate after deposition of a 2 nm Cr/15 nm Au overlayer at different temperatures. In

each case, the QCM resonant frequency was allowed to stabilize at ~ 24 °C in pH 3.0 water. While maintain a similar temperature, the pH of the water was adjusted to 7.0 by addition of 1 M NaOH. The addition of NaOH is observed as an increase in QCM resonant frequency. Following QCM resonant frequency stabilization, the pH was again adjusted to 3.0 by addition of concentrated HCl. Following this step, in each case the temperature was adjusted to ~ 30 °C and ~ 31 °C, and the pH modulation repeated at each temperature range.

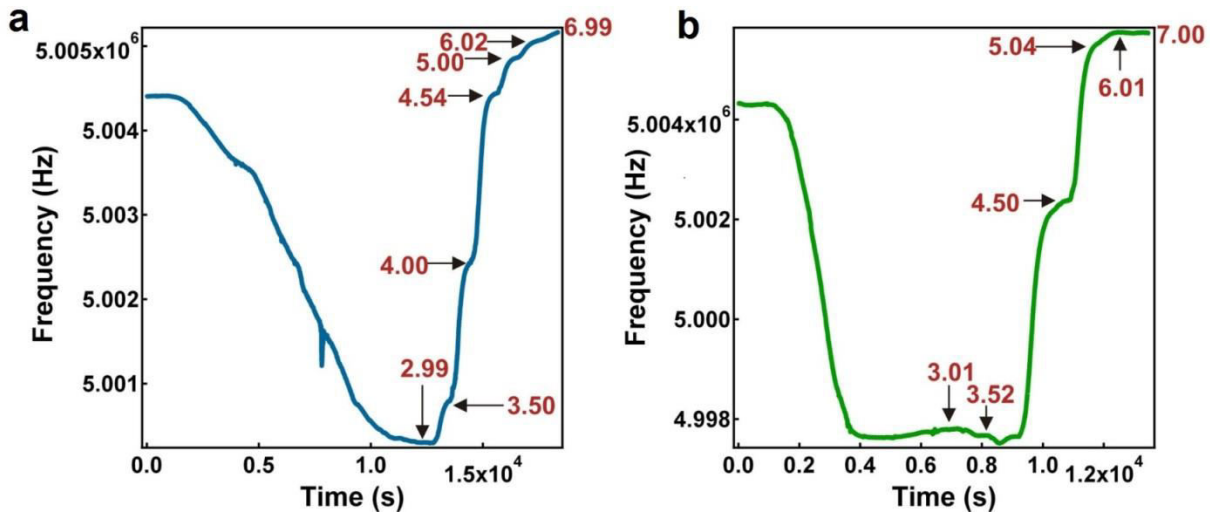


Figure 9. The pH response of a (a) pNIPAm-*co*-AAc microgel coated QCM crystal and (b) the same substrate after deposition of a 2 nm Cr/15 nm Au overlayer at the film's lower critical solution temperature. In each case, the films were exposed to pH 3.0 water and the temperature of the solution adjusted to the film's lower critical solution temperature of ~31-32 °C. Following stabilization of the QCM resonant frequency, the pH of the solution was adjusted stepwise by addition of 1 M NaOH. The actual pH values are indicated on each plot.