Heavy Metal Ion Detection Using Peptide-Modified Hydrogel Layers on a Quartz Crystal Microbalance

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Abstract

We investigated the use of thermo-responsive hydrogel layers in conjunction with a quartz crystal microbalance as a sensing platform for detecting metallic species in aqueous media to create a portable sensor used for potable water testing. The hydrogel poly(N-isopropylacrylamide) (PNIPAAm) synthesis was reproducible as monomer concentrations of 0.1 to 0.4 g/mL yielded dry thicknesses of 0.06 to 1.0 microns, respectively. FTIR was utilized on the bulk PNIPAAm to determine if tetra-glycine peptides were effectively embedded—results are still pending. 2.5% crosslinked PNIPAAm with no peptide polymerized *in situ* on a 5 MHz quartz crystal microbalance (QCM) exhibited frequency shifts of roughly 2 kHz upon heating at 33.2, 33.1, 32.5, and 29.7 °C, as pure water, 0.005 M, 0.05 M, and 0.38 M copper chloride concentrations, respectively, were sent to the flow cell. Hysteresis was experienced upon cooling as re-swelling occurred at 27.5, 27.0, 26.1, and 23.9 °C, respectively. Dissipation measurements were taken using the QCM-D technique that resulted in a 200-300 Hz shift upon heating the coated QCM and 250 Hz shift due to cooling. Based on our preliminary studies, we believe that viable sensors with sensitivity to much lower heavy metal ion concentrations can be constructed using these peptide-modified hydrogels and higher frequency acoustic wave sensors.

1. Introduction

Trace soluble ions, such as copper and arsenic, are toxic to the human body even at low concentrations. The development of a technique to detect such target species in aqueous systems using polymer networks that display recognition capabilities would be useful in evaluating toxicity and pollutant levels. Ion-specific polymer membranes supported on a quartz crystal microbalance (QCM) would offer portability and ultra-low sensitivity. The QCM serves as a versatile sensing platform that is commonly used in vapor, liquid, and biological monitoring applications. Using “intelligent” polymer layers specific to target analytes in conjunction with the QCM to amplify the reversible viscoelastic change of the network will allow for multiplexed detection.

Our goal is to effectively synthesize peptide-embedded thin films on a QCM to determine the LCST behavior of the modified film as well as conformational changes of the peptide moieties to trace soluble ions based on frequency shifts and dissipation energy. We have investigated the effects of different copper (II) chloride concentrations on the LCST of a cross-linked PNIPAAm thin film using the QCM as a sensing platform.

1.1 Peptide-Embedded Thermo-Responsive Thin Films

Thermo-responsive thin films such as N-isopropylacrylamide (NIPAAm) undergo a reversible viscoelastic change at a well-defined temperature called the lower critical solution temperature

(LCST). NIPAAm, more specifically, is in the swollen state, or hydrophilic, at temperatures below 32°C and collapses into the globular hydrophobic state at temperatures above 32°C (Heskins, 1968). At the nanoscale level, it is believed that the transition temperature of the hydrogel is determined by the balance of the competition between the hydrogen bonding of the carbonyl oxygen to water in NIPAAm, region 1 of Figure 1, and the hydrophobic region of NIPAAm, region 2 of Figure 1 (Schild, 1990; Ilmain, 1991; Yagi, 1992). Flory-Tanaka theory models the volume phase transition as a balance between the mixing free energy, rubber elasticity free energy, and osmotic pressure (Tanaka, 1979). Furthermore, the phase transition of PNIPAAm systems in aqueous media is affected by salts, surfactants, organic solutes and solvents (Dhara, 2000). This allows the phase transition to be employed as an effective gauge of the binding events within the network (Bulmus, 2001). To allow for selectivity, metallopeptide motifs are embedded into the hydrogel network. Metallopeptides will conjugate to target analytes such as Cu^{2+} and Ni^{2+}, to form a chelate cage. Figure 2 shows the complexation that occurs when tetraalanine conjugates with copper (II) ions.

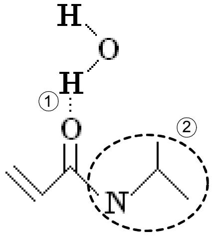

Figure 1. The balance of the competition between region 1 and region 2 of NIPAAm.

Tetraalanine is a flexible chain in the free form, and this 4-member sequence becomes a chelate cage when introduced to Cu^{2+}. There exists a high number of potential donor atoms due to the peptide backbone and amino acid side chains making the peptide-metallic interactions very effective (Kozlowski, 1999).

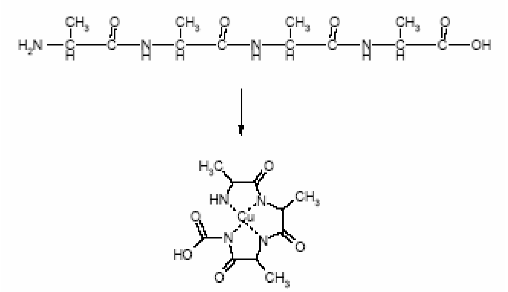

Figure 2. Complexation between soluble Cu (II) and tetraalanine.

It can be anticipated on the basis of Dhara's findings that the chelate cage within the PNIPAAm network inhibits the hydrogen bonding of the NIPAAm structure with water—shifting the balance of competition between the hydrogen bonding and hydrophobic regions within the network, and transition temperature will be a function of the binding state of the embedded peptides. When binding occurs, four nitrogen donors from the NIPAAm network are removed. Different complexes within the network can be attained through the complexing species present and the pH of the environment. With at least 20 amino acid combinations available, metallopeptide systems can be structurally very specific. Shift in resonant frequency due to metallopeptide conformation on a QCM serves as the basis of detection of specific soluble species.

1.2 Quartz Crystal Microbalance (QCM) Principles

The quartz crystal microbalance consists of an electrode deposited on a quartz wafer. Mechanical energy converts into electrical energy and *vice versa* due to the piezoelectricity of the crystal. Thus, the quartz oscillates at the crystal's fundamental resonant frequency when a radio frequency voltage is supplied to the electrodes. This allows the QCM to serve as an ultra-sensitive weighing device (<1 ng/cm^2) with the principle that mass added to, or removed from, the surface of the electrode induces a frequency shift (Czanderna, 1984).

Energy losses associated with the QCM can be monitored through the dissipation energy. This is important for applications in which a viscous thin film is supported by the QCM since energy is dissipated due to the oscillatory motion relayed within the film (Barnes, 1991). The dissipation can be measured by intermittently switching off the driving power of the oscillator driving the crystal to its resonant frequency, and measuring the exponential decay of the oscillation amplitude (Rodahl, 1995). The dissipation factor is defined by Equation 1.1.

$$\Delta D = \frac{E_{dissipated}}{2\pi E_{stored}} \qquad \textbf{(1.1)}$$

where $E_{dissipated}$ is the energy dissipated during one oscillation and E_{stored} is the energy stored in the oscillating system.

Frequency response of the crystal can be described by the Kanazawa-Gordon equation:

$$\Delta f = -n^{1/2} f_o^{\,3/2} \left(\frac{\eta\rho}{\pi\mu_q\rho_q}\right)^{1/2} \qquad \textbf{(1.2)}$$

Where ρ and η are the density and viscosity, respectively, of the aqueous media and ρ_q and μ_q are the density and shear modulus of the quartz crystal, respectively, which are constant.

The dissipation response is given by the following equation (Stockbridge, 1966; Rodahl, 1996):

$$\Delta D = 2\left(\frac{f_o}{n}\right)^{1/2} \left(\frac{\eta\rho}{\pi\mu_q\rho_q}\right)^{1/2} \qquad \textbf{(1.3)}$$

As frequency shifts and dissipation can reveal viscoelastic changes in the thin film, detection of target species can be accomplished by monitoring these viscoelastic changes in conjunction with shifts in the LCST.

2. Experimental

2.1 Materials

N-isopropylacrylamide (NIPAAm) (90%), N,N-methylenebisacrylamide (99%), 2,2 Dimethoxy-2-phenylacetaphenone (DMPA), N,N-Dimethylformamide (DMF), tetraglycine, and copper (II) chloride (99.999%) were purchased from Sigma-Aldrich Chemical Co. N-acryloxysuccinimide was purchased from PolySciences, Inc. Silicon dioxide (SiO_2) coated QSX 303 polished 5 MHz AT-cut quartz crystals were supplied by Q-Sense AB.

2.2 Instrumentation

Measurements were taken using the Q-Sense E4 instrument to obtain frequency and dissipation information on the hydrogel thin film. Solution flow cells containing a reference crystal and coated crystal, respectively, exposed one side of the QCM to constant flows using a peristaltic pump. The cells were heated simultaneously via a controllable peltier device, and a thermocouple measured the temperature. The beta-phase software of the E4 instrument was incapable of recording the temperature, so temperature was logged manually to obtain a temperature profile. The E4 offers a range of up to 65 MHz. The QCM is an AT-cut quartz crystal with SiO_2 coated on the gold electrode that serves as an electrode with a fundamental resonance frequency of 5 MHz.

2.3 Synthesis of NIPAAm cross-linked Hydrogel Thin Films

Polymerization of the ultra-thin layers of poly-N-isopropylacrylamide hydrogels was done through free radical polymerization in an inert gas atmosphere using ultraviolet light with DMPA as a photo-initiator *in vitro* on a quartz substrate. Prior to polymerization, the quartz substrate was coated with a layer of silane to anchor the hydrogel onto the SiO_2 surface through the introduction of vinyl groups. 3 μL of a solution containing NIPAAm, N,N-methylenebisacrylamide, N-acryloxysuccinimide, and DMPA in DMF is placed on the silane coated QCM, covered with a glass substrate, purged with nitrogen gas, and subsequently exposed to UV light (365 nm) for 5 minutes in a nitrogen gas atmosphere. After polymerization, the glass substrate is removed and a thin PNIPAAm film remains on the QCM.

2.4 Immobilization of Peptides in NIPAAm Hydrogels

After polymerization, the crosslinked bulk PNIPAAm layers containing N-acryloxysuccinimide were immersed in sodium phosphate buffer and contacted with a solution of tetra-glycine for 24 hours at 4°C. The reaction scheme for embedding the peptides within the network is shown in Figure 3.

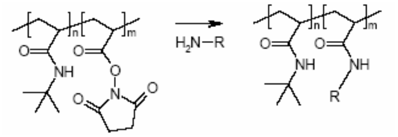

Figure 3. Reaction scheme for linking peptides to the NIPAAm/N-acryloxysuccinimide network.

Fourier Transform Infrared Spectroscopy (FTIR) was performed on the bulk hydrogels to determine if the reaction of the active ester with the peptide was a success. Results are still pending.

3. Results and Discussion

3.1 Hydrogel Thickness Information

The level of control over dry film thickness of the PNIPAAm layers as a function of the concentration of the monomer solution prior to polymerization is shown in Figure 4.

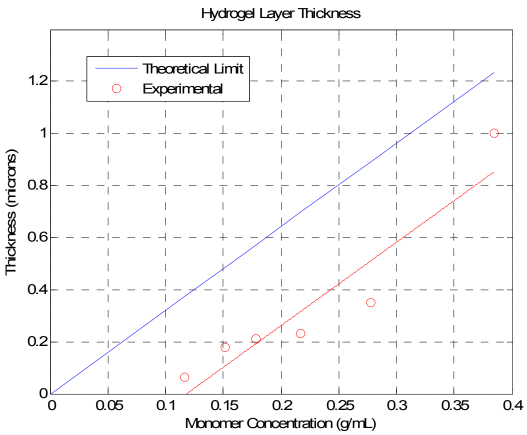

Figure 4. Variation of dry film thickness of PNIPAAm cross-linked layers with monomer concentration.

The theoretical thickness was calculated by Equation 3.1.

$$Thickness = \frac{concentration \times amount\ deposit}{density \times area\ of\ deposition} \qquad \textbf{(3.1)}$$

Film thickness is roughly proportional to the monomer concentration or the total amount of monomer. Dry film thicknesses between 100 nm up to 1 micron can be readily achieved.

3.2 Frequency Response of QCM to Hydrogel Thin Films

Frequency response of a lower critical solution hydrogel thin film coated on a QCM can amplify the nanoscale environment of the network into a macroscopic response. A 2.5% cross-linked PNIPAAm layer was polymerized onto a silane coated, 5 MHz SiO_2 coated QCM. The QCM was placed inside the flow cell and simultaneous frequency and dissipation data were obtained with a sampling time of 0.3 seconds. Heating and cooling of the cell was controlled to find the temperatures of collapse and re-swelling for the hydrogel based on frequency response, as different concentrations of copper (II) chloride in Milli-Q water were passed through the cell. A curve fit was applied to the temperature profile to relate the frequency shift to temperature. The effects of copper (II) chloride concentration on LCST, upon heating and cooling showing hysteresis effects, are shown in Figures 5 and 6.

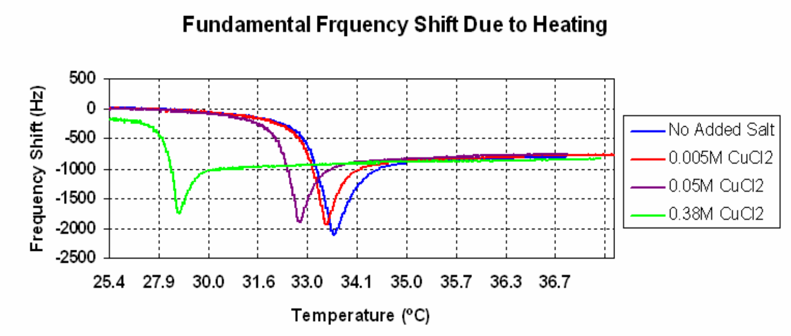

Figure 5. Copper (II) chloride concentration effects on fundamental resonant frequency due PNIPAAm hydrogel layer upon heating.

The minimum peaks represent the collapse and re-swelling of the hydrogel upon heating and cooling, respectively. Upon heating, the frequency shift due to collapse was around 2 kHz, while the frequency shift due to re-swelling was around 1.0 kHz upon cooling. The relationship of the transition temperature and the copper (II) chloride concentration is shown in Figure 7.

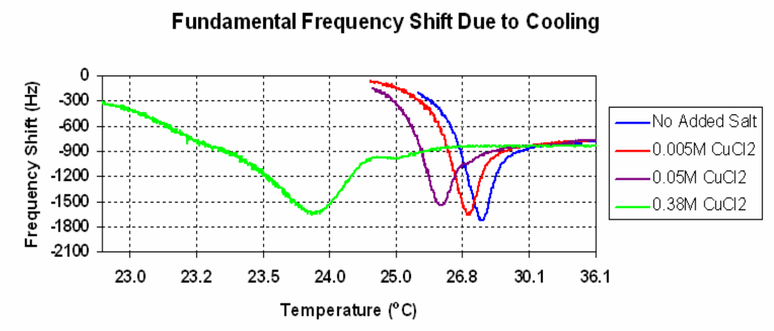

Figure 5. Copper (II) chloride concentration effects on fundamental resonant frequency due PNIPAAm hydrogel layer upon cooling.

Upon heating, the relationship between the transition temperature and copper (II) chloride concentration is linear. While allowing the coated QCM in the cell to cool, a hysteresis in the transition temperature versus copper (II) chloride concentration occurred.

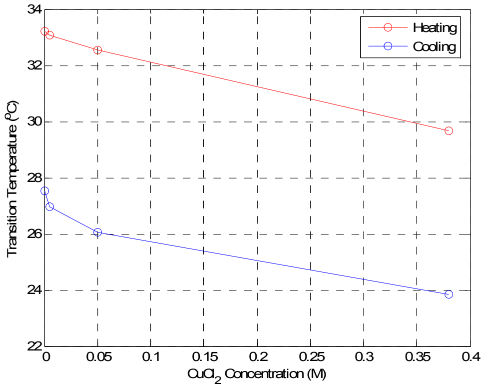

Figure 7. Effect of copper (II) chloride concentration on transition temperature of PNIPAAm hydrogel.

The dissipation shift due to copper (II) chloride concentration and temperature of the flow cell is represented by Figures 8 and 9.

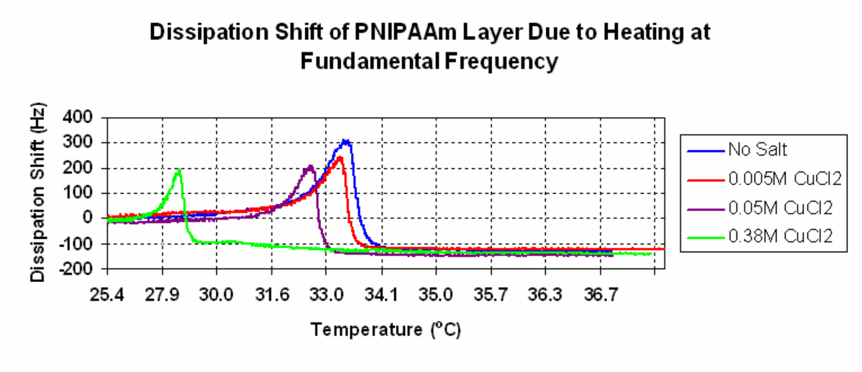

Figure 8. Copper (II) chloride concentration effects on energy dissipation of PNIPAAm hydrogel layer at the first harmonic upon heating.

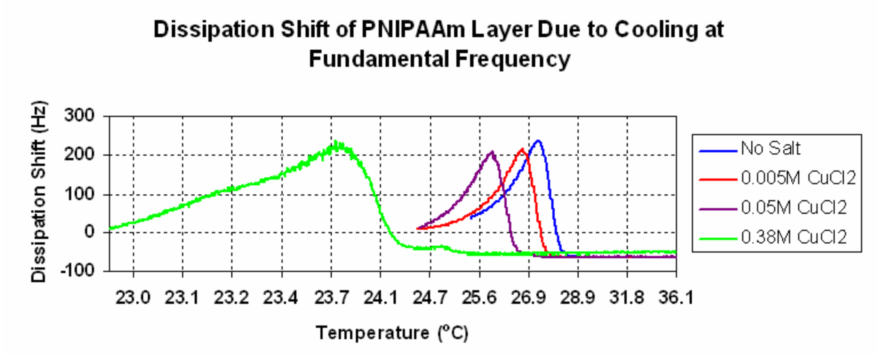

Figure 9. Copper (II) chloride concentration effects on energy dissipation of PNIPAAm hydrogel layer at the first harmonic upon cooling.

The sharp decrease in dissipation upon heating indicates the collapse of the PNIPAAm film. Likewise, on cooling, the sharp increase in the dissipation energy indicates the re-swelling of the PNIPPAm film. This reaffirms that the phase transition is attained as the dissipation energy indicates the viscoelasticity of the film. The shift in the dissipation energy at the hydrogel phase transition is affected by copper (II) chloride concentration and whether the cell was heated or cooled. This relationship is portrayed in Figure 10.

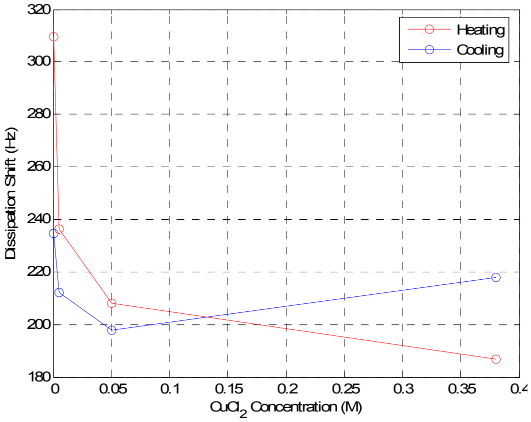

Figure 10. Effect of copper (II) chloride concentration on dissipation shift at collapse/re-swelling of PNIPAAm.

4. Conclusions

Thin peptide-modified NIPAAm networks supported on a QCM hold promise as “intelligent” sensors for detecting small target analytes. Conformational changes of peptides embedded within the hydrogel structure induce changes in the LCST of the network, allowing a nanoscale binding event to be amplified into a macroscopic signal. The fabrication of ultra-thin PNIPAAm networks has been demonstrated in this work. Effort thus far has focused on construction of the NIPAAm films on a QCM substrate and testing the frequency response and dissipation energy upon temperature changes and exposures to different copper (II) chloride concentrations. It has been shown by the frequency response and dissipation energy recovery upon heating to the hydrogel LCST that detection would offer reproducibility and reusability. The shift in LCST due to copper (II) chloride concentration shows that the chemical interactions within the network can be “transduced” by the QCM into a readable signal. Future work will focus on incorporation of peptide linking N-acryloxysuccinimide monomers into the NIPAAm films, covalent attachment of metal binding peptides to the N-acryloxysuccinimide moieties, and investigations between the LCST behavior of the NIPAAm layers and the conformational changes of the embedded peptides.

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