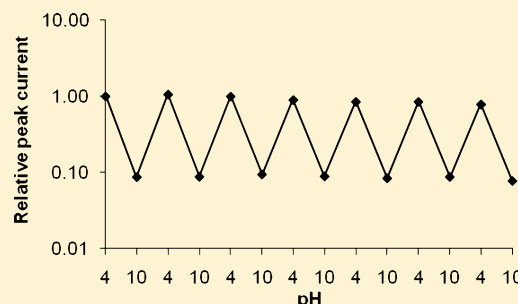


pH- and Voltage-Responsive Chitosan Hydrogel through Covalent Cross-Linking with Catechol

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ABSTRACT: A new method for covalently cross-linking chitosan is developed by chemically oxidizing catechol to *o*-quinone which subsequently reacts with and cross-links chitosan through Michael addition and Schiff base formation. The cross-linked chitosan film shows a pH-responsive, switchlike behavior toward the negatively charged redox probe, $\text{Fe}(\text{CN})_6^{3-/4-}$, and withstands harsh acidic conditions. The negative $\text{Fe}(\text{CN})_6^{3-/4-}$ is found to be trapped and enriched in the catechol-cross-linked chitosan film under acidic conditions and released into solution by either increasing pH or applying a negative voltage. Chitosan films made with different techniques, i.e., solvent evaporation (simple deposition), electrodeposition, and covalent cross-linking, are examined using cyclic voltammetry and electrochemical impedance spectroscopy (EIS), and the results demonstrate that fabrication methods greatly affect the properties of the chitosan films.



INTRODUCTION

Stimuli-responsive polymer films and coatings offer exciting potential for a range of applications.^{1–9} For instance, polymer films that can provide protection for labile components but release these components in response to external stimuli could provide novel methods to deliver therapeutic agents, agricultural chemicals, cosmetic ingredients, or food components. In addition, coatings and films that change properties in response to external components (e.g., by swelling) may enable new approaches for sensing and actuation. These opportunities have spurred extensive efforts to identify polymeric materials and fabrication methods that yield “smart” films and coatings that can be ingested, injected, implanted, topically applied, or released into the environment.

Chitosan is a biopolymer that has been extensively studied in recent years because of its biocompatibility, low toxicity, and versatile properties and reactivities. Several reviews have concentrated on its biodegradability and low toxicity,¹⁰ drug delivery ability,^{11–16} as well as molecular imaging.¹⁷ The large quantity of amine groups on the chitosan backbone confers the pH-dependent solubility, which has been utilized to fabricate biosensors and other devices,¹⁸ and its properties can be conveniently altered by derivatizing chitosan through the hydroxyl and amino groups.^{19–22} Fabrication of chitosan films can be achieved in several ways including the following: (1) casting that employs the natural film forming ability of chitosan and its pH-dependent solubility, (2) ionic cross-linking and complexation, (3) covalent cross-linking. Protonation of the chitosan amines under acidic conditions (usually pH < 6) yields a chitosan solution, which can be cast with other components on a solid support, and evaporation of the solvent generates a chitosan film with other components embedded inside the film.

Electrodeposition of chitosan is achieved by applying a reducing potential to a chitosan solution which causes the local pH to rise due to either the electrochemical reduction of H^+ to H_2 or the reduction of water (to H_2 and OH^-) thus leading to the gelation of chitosan from the solution to the electrode surface.^{23–27} Electrodeposition allows the rapid and reversible fabrication of chitosan films with temporal and spatial selectivity. Ionic cross-linking is based on the polycationic nature of chitosan at low pH and uses anions to electrostatically form a chitosan network.²⁸ Stronger ionic cross-linking or complexation will form when polycationic chitosan is mixed with polyanionic polymers, proteins, polysaccharides, or nucleotides. Since chitosan films made using the above methods generally redissolve at low pH, in applications where more robust chitosan constructs are needed, covalent cross-linking may be the only option. The most widely used covalent cross-linkers are dialdehydes such as glutaraldehyde.^{15,28} However, these chemicals are known to be toxic. Therefore, low toxicity cross-linkers are being sought after.

It has been shown that in nature tyrosine residues of proteins can be oxidized by tyrosinase and the oxidized *o*-quinone residues can undergo cross-linking with proteins and polysaccharides,^{29–33} either through Michael addition or Schiff base formation (Scheme 1A). Here, we report that even without the use of the enzyme tyrosinase, catechol can be chemically oxidized to *o*-quinone which subsequently will undergo Michael addition and/or Schiff base formation with

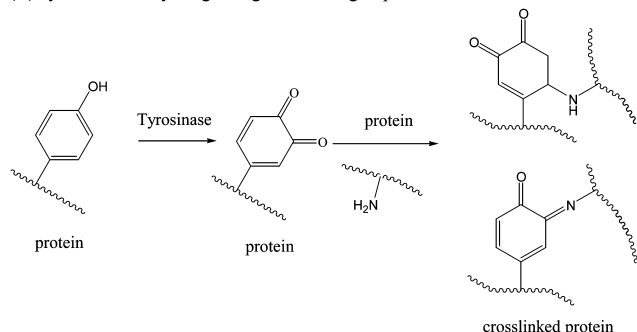
Received: October 19, 2011

Revised: December 19, 2011

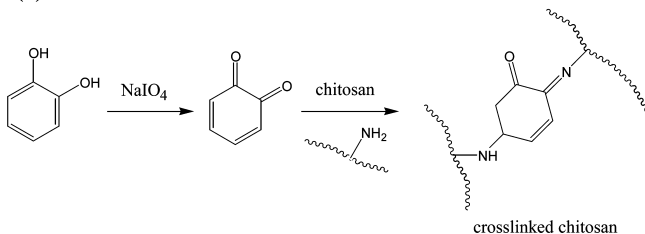
Published: January 9, 2012

Scheme 1. (A) Tyrosinase-Catalyzed Grafting/Cross-Linking of Proteins; (B) Catechol-Cross-Linked Chitosan

(A) Tyrosinase-catalyzed grafting/crosslinking of proteins



(B) Catechol-crosslinked chitosan



the chitosan amines and will effectively cross-link chitosan covalently.

We used several approaches to fabricate chitosan-based films and explored their ability to respond to changes in pH. Chitosan films should swell and deswell in response to pH changes. In fact, chitosan can completely dissolve at low pH unless the chains are interconnected through covalent or physical (e.g., multivalent anions) cross-links. A swollen polycationic chitosan network may concentrate anionic species through electrostatic attractive interactions. We report that the

response of these chitosan films to pH is markedly affected by the fabrication method. We also report that a catechol cross-linked chitosan film can undergo repeated pH-induced switching. We further report that the catechol cross-linked chitosan film can enrich the negative redox species, $\text{Fe}(\text{CN})_6^{3-/4-}$, to an unusually high extent, and the release of the trapped $\text{Fe}(\text{CN})_6^{3-/4-}$ can be effectively controlled by pH and voltage.

EXPERIMENTAL SECTION

Materials. Medium molecular weight chitosan was purchased from Aldrich (St. Louis, MO). All other chemicals were from either Aldrich or VWR and were used as received. Platinum counter electrode (EE011) and Ag/AgCl miniature reference electrode (EE008) were purchased from Cypress Systems (Chelmsford, MA). Glassy carbon electrodes (CHI 104) (3 mm) were purchased from CH Instrument, Inc. (Austin, TX).

The chitosan solution was prepared by dissolving chitosan in 1% acetic acid with overnight stirring. The concentration of the resultant solution was diluted to 10 mg/mL by adding deionized water, and the pH was adjusted to 5 by adding dilute NaOH.

Electrode Modification. Glassy carbon electrodes (GCEs) were modified with chitosan films as follows.

ED-Chit. Electrodeposition of chitosan was performed by immersing a GCE in a pH 5 solution containing 2 mg/mL of chitosan and applying a potential of -2.5 V (vs Ag/AgCl) for 2 min. The resultant ED-chit modified GCE was rinsed extensively with water and phosphate buffer (pH 7) and was stored in water or buffer before use. Measurements were usually carried out within 10 min.

Chit. Simple deposition of chitosan was performed by placing 15 μL of chitosan solution (2 mg/mL, pH 5) on the top of a GCE. The solution was allowed to dry and cure at room

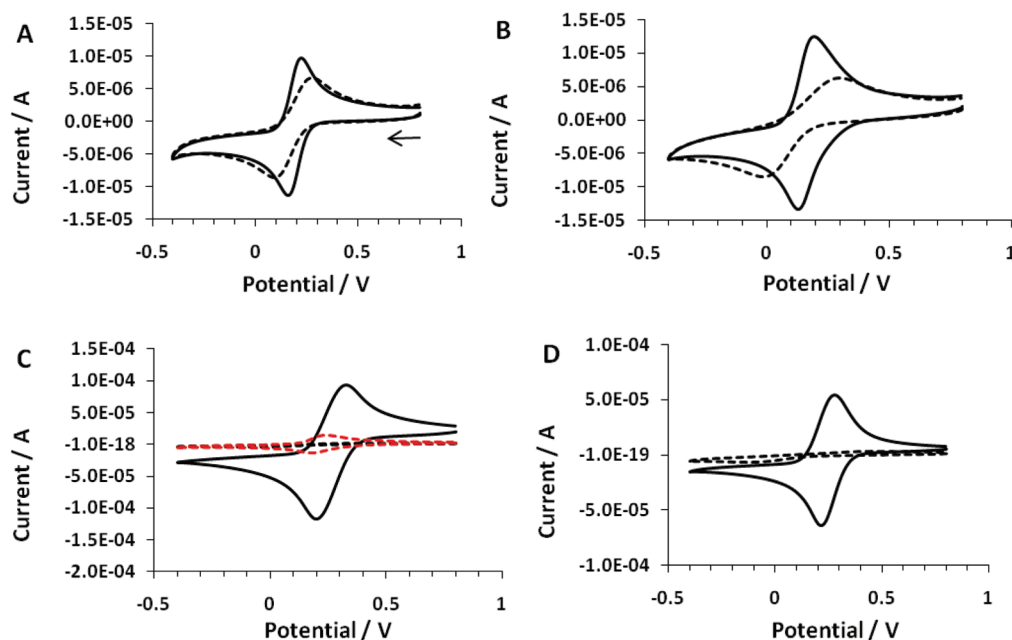


Figure 1. Cyclic voltammograms of 1.0 mM $\text{Fe}(\text{CN})_6^{3-}$ at (A) a bare glassy carbon electrode (GCE), (B) a GCE modified with chitosan film by electrodeposition (ED-chit), (C) a GCE modified with chitosan film by simple deposition (chit), (D) a GCE modified with catechol-cross-linked chitosan film (cat-chit). Solid (—), pH 4; dashed (---), pH 10; red dashed (red ---), pH 4 after HCl treatment. Scan rate: 50 mV s^{-1} . Reference: Ag/AgCl.

temperature overnight. The chit-modified GCE was stored in air at room temperature and was rinsed with water and/or buffer before use.

Cat-Chit. The deposition solution was made by mixing 100 μL of chitosan (10 mg/mL, pH 5), 10 μL of catechol (20 mM), 290 μL of water, and 100 μL of NaIO_4 (0.2 M, dissolved in 1 M HCl). Fifteen microliters of the mixture was placed on the top of a GCE and was allowed to dry and cure at room temperature overnight. The cat-chit modified electrode was stored in air at room temperature and was rinsed with water and/or phosphate buffer before use.

Electrochemical Measurement. All electrochemical measurements were performed using a CHI 660C Electrochemical Workstation (CH Instruments, Austin, TX), connected to a conventional three-electrode cell with a 3 mm glassy carbon electrode (CHI 104, CH Instruments, Inc., Austin, TX) as the working electrode, a miniature Ag/AgCl electrode (EE008, Cypress Systems, Chelmsford, MA) as the reference electrode, and a Pt wire as the counter electrode. All experiments were carried out at room temperature.

RESULTS AND DISCUSSION

Electrode Modified with Chitosan by Electrodeposition and Simple Deposition. The various modification methods were examined by cyclic voltammetry using 1.0 mM $\text{Fe}(\text{CN})_6^{3-}$ as a redox probe. Figure 1A shows the cyclic voltammograms of $\text{Fe}(\text{CN})_6^{3-}$ at a bare glassy carbon electrode at pH 4 and pH 10, respectively. At pH 4 a pair of redox waves are centered at a formal potential of 0.192 V, corresponding to the reversible redox process between $\text{Fe}(\text{CN})_6^{3-}$ and $\text{Fe}(\text{CN})_6^{4-}$. At pH 10, the peak intensity becomes lower and the separation is significantly larger (183 mV), indicating a more sluggish redox process. When the electrode is modified with chitosan by electrodeposition (ED-chit) as shown in Figure 1B, slight changes are observed in the CV compared to Figure 1A: at pH 4 the peak intensity increases slightly while the peak separation remains the same; at pH 10 the peak intensity is about the same as that at the bare electrode but the separation increases (305 vs 185 mV). The slight increase in peak current at the ED-chit-modified electrode at pH 4 can be attributed to the protonation of the chitosan film at this pH where the chitosan backbone becomes cationic and the concentration of the negatively charged $\text{Fe}(\text{CN})_6^{3-/4-}$ in the chitosan film is slightly increased due to electrostatic attraction, as reported by Zangmeister et al.³⁴ At pH 10 the chitosan film is neutral, and the film itself may hinder the diffusion of the redox probe to a certain degree, thus the slower redox process (larger peak separation). It is noteworthy though that the changes are small and thus the impact of the electrodeposited chitosan film on the diffusion of the redox probe is not significant. In both cases the peak current at pH 10 is about 65–75% of that at pH 4.

Figure 1C shows the cyclic voltammograms of 1.0 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ at an electrode modified with chitosan by simple deposition (chit); i.e., an aliquot of chitosan solution was delivered to the electrode surface, the solvent gradually evaporated at room temperature, and the electrode was cured overnight, yielding a uniform chitosan film. At pH 4 the peak intensity is about 10 times higher than that at the bare electrode, and at pH 10 the peak is much smaller than that at the bare electrode. The unusually high peak current at pH 4 may be explained by the swelling of the chitosan hydrogel and the electrostatic attraction between the protonated amines and the negatively charged $\text{Fe}(\text{CN})_6^{3-/4-}$. With a pK_a of ~ 6.5 ,^{35–37}

the chitosan amines are fully protonated at pH 4, the large amount of positive charges on the chitosan backbone makes the chitosan chains fully extended and hydrated (swelling), and the swelling of the chitosan hydrogel exposes more amine groups; the multianionic $\text{Fe}(\text{CN})_6^{3-/4-}$, on the other hand, may replace some of the Cl^- and H_2PO_4^- as the counterions due to its strong attraction to the chitosan backbone and be trapped and enriched in the chitosan hydrogel, leading to the unusually high peak current.

Since simple deposition and electrodeposition rely on the pH-dependent solubility of chitosan, the deposition process is generally reversible. It has been shown that chitosan films generated by electrodeposition can be conveniently removed from the electrode by treatment with acid.²⁴ Figure 1C (red trace) shows that after soaking the chit-modified electrode in 0.2 M HCl, the cyclic voltammogram of $\text{Fe}(\text{CN})_6^{3-}$ is essentially the same as that at the bare electrode (Figure 1A), suggesting the chitosan film has been removed.

Electrode Modified with Cross-Linked Chitosan. As shown in Scheme 1A, it has been demonstrated that tyrosinase can oxidize the tyrosyl residue of a protein to *o*-quinone which then serves as a cross-linker to graft another protein, either via Michael addition or Schiff base formation. We propose that this principle can be used to effectively cross-link chitosan (Scheme 1B). A mixture of chitosan, catechol, and NaIO_4 was cast on the top of a glassy carbon electrode and was cured at room temperature overnight. NaIO_4 oxidized catechol to *o*-quinone (as evidenced by the fact that the solution turned slightly yellow after mixing catechol and NaIO_4) which then reacted with the amines of chitosan to form cross-linked chitosan (cat-chit). Figure 1D shows the cyclic voltammograms of 1.0 mM $\text{Fe}(\text{CN})_6^{3-}$ at the cat-chit electrode. Much like the case of simple deposition (chit), the peak current at pH 4 is significantly higher than that at the bare electrode, ~ 6 times higher, while at pH 10 the peak is barely visible as in the case of the chit-modified electrode. Similar to simple deposition, this catechol-cross-linked chitosan film seems to be highly flexible and have the ability to swell significantly at pH 4 resulting in high peak current.

The greatly diminished peak intensity of the redox process of $\text{Fe}(\text{CN})_6^{3-/4-}$ at pH 10 for the chit-modified electrode (Figure 1C) and the cat-chit modified electrodes (Figure 1D) is very intriguing. Since the chitosan film is neutral at pH 10, electrostatic forces should not be a factor. One may think this is because the chitosan film shrinks to a high extent and physically blocks the diffusion of the redox probe. Figure 2 shows the CVs of $\text{Ru}(\text{NH}_3)_6^{2+/3+}$ at a cat-chit-modified

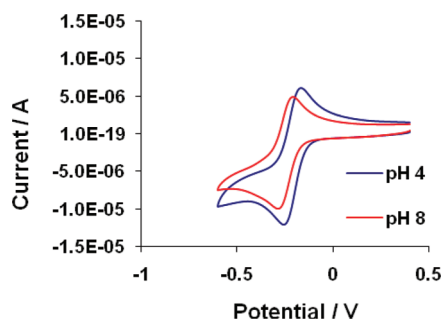


Figure 2. Cyclic voltammograms of 1.0 mM $\text{Ru}(\text{NH}_3)_6\text{Cl}_3$ at different pH values. The electrode was modified with catechol-cross-linked chitosan film (cat-chit). Scan rate: 50 mV s^{-1} . Reference: Ag/AgCl.

electrode at pH 4 and pH 8, where the positive redox species is not trapped in the chitosan film. The peak current is slightly higher at pH 4 than at pH 8, suggesting that diffusion may be negatively impacted at high pH to a small degree. More studies are needed to understand this unusual effect.

An interesting finding of Figure 1 is that cross-linking is not the determining factor of whether the chitosan film is pH-responsive. Instead, other conditions of the fabrication methods may be more important. As shown in Figure 1B, ED-chit is not as pH-responsive as chit and cat-chit. A possible explanation is that in ED-chit a negative potential (-2.5 V) is applied to the chitosan solution to increase the local pH above 6.5 so that chitosan will deprotonate and deposit on the electrode; deprotonation of amines may lead to the formation of a large number of intra- and interchain hydrogen bonds, and numerous associations between the neutral chitosan chains may yield a robust and rigid network. It is well established that hydrogen bonding is one of the main reasons for the insolubility of chitin as well as chitosan in neutral and basic conditions,^{38–40} and reports have shown that disruption of hydrogen bonding significantly improves the solubility of chitin and chitosan.^{39,41–43} It is possible that when chitosan is electrodeposited hydrogen bonding prevails resulting in a rigid chitosan network which does not swell well and thus is not pH-responsive. In the cases of chit and cat-chit, acidic chitosan solutions are cast on the electrode surface and the solvent is gradually evaporated; however, the ions remain in the whole process and the chitosan chains remain charged, which may prevent the formation of hydrogen bonding. As a result, these dried chitosan films may hydrate easily, remain highly flexible, and respond to pH more dramatically.

Effect of Chitosan-to-Catechol Ratio. Since catechol is the precursor of the cross-linker, it is conceivable that the amount of catechol plays a critical role in the properties of the cross-linked chitosan film. Figure 3 shows the peak current of 1 mM $\text{K}_3\text{Fe}(\text{CN})_6$ at pH 4 at electrodes modified with catechol-

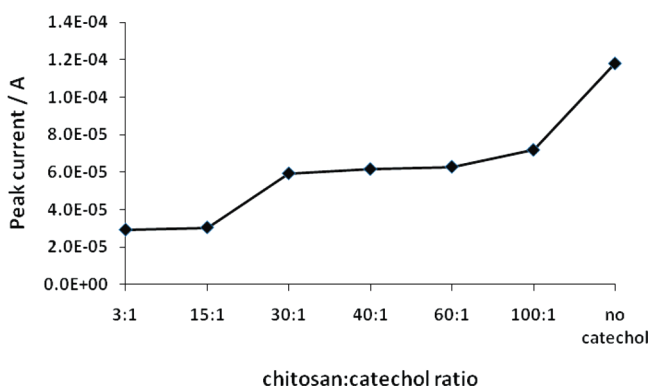


Figure 3. Peak current of 1 mM $\text{K}_3\text{Fe}(\text{CN})_6$ at electrodes modified with catechol-cross-linked chitosan of different chitosan-to-catechol ratio (represented by the mole ratio of the glucosamine unit to catechol), pH 4. The amount of chitosan was kept the same for all electrodes.

cross-linked chitosan of different chitosan-to-catechol ratio (represented by the mole ratio of the glucosamine unit to catechol), while the amount of chitosan remained the same. As expected, with high cross-linker content (3:1 and 15:1 chitosan-to-catechol ratio) the peak current is ~ 30 μA , significantly lower (\sim half) than the peak current observed in Figure 1D where the ratio is 30:1; as less catechol is used (30:1, 40:1, and

60:1 chitosan-to-catechol ratio), the peak current becomes greater than 60 μA , and when 1 catechol is used for every 100 glucosamine units the peak current increases to more than 70 μA . This confirms that catechol indeed plays a critical role in determining the properties of the chitosan film, probably through covalent cross-linking as shown in Scheme 1B. A possible explanation is that as more cross-linkers are used the chitosan hydrogel network becomes more rigid⁴⁴ and less able to swell; thus, fewer amino groups will be accessible for $\text{Fe}(\text{CN})_6^{3-}$ to be attracted and trapped in the film leading to much lower current.

We thus arbitrarily choose 30:1 chitosan-to-catechol ratio as the preferred recipe for making cat-chit electrodes based on the fact that this ratio gives a high peak current and the assumption that more cross-linking yields more stable and robust chitosan film.

pH Effect. The pH effect on the chitosan film was investigated by measuring the $\text{Fe}(\text{CN})_6^{3-}$ peak current at a cat-chit-modified electrode at different pH values (Figure 4). The peak current is at its highest at pH 4 and decreases with

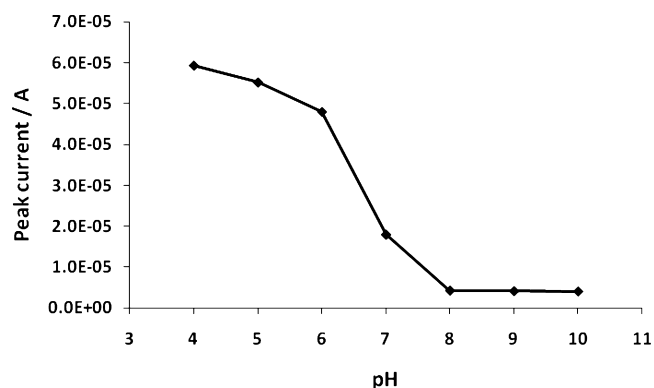


Figure 4. Peak current of 1 mM $\text{K}_3\text{Fe}(\text{CN})_6$ at an electrode modified with catechol-cross-linked chitosan (30:1 chitosan-to-catechol ratio) as a function of pH.

pH; when the pH is above 8 the peak current drops to the lowest. Between 6 and 7 the decrease is the steepest with the midpoint around pH 6.5 which correlates perfectly with the pK_a of the chitosan amine.^{35–37} This observation further supports that the increased peak current of $\text{Fe}(\text{CN})_6^{3-}$ at low pH is due to the protonation of the chitosan film.

Electrochemical Impedance Spectroscopy (EIS). The chitosan films were also investigated with electrochemical impedance spectroscopy using $\text{Fe}(\text{CN})_6^{3-}$ as the probe. Figure 5 shows the total impedance at four different electrodes as a function of frequency at pH 4 and pH 10. In the high frequency range, for both pH 4 and pH 10, all four electrodes have similar impedance. This is conceivable because at a high frequency the impedance simplifies to solution resistance. It is in the low frequency end where the four electrodes show significantly different characters, proving that chitosan coating drastically alters the diffusion behavior. In the low frequency region, at pH 4 (Figure 5A), the bare electrode has the highest impedance, followed by the ED-chit-modified electrode, the cat-chit-modified electrode, and the chit-modified electrode. This is in agreement with the findings in the CV study where $\text{Fe}(\text{CN})_6^{3-}$ shows greatly increased peak current at the chit-modified electrode and the cat-chit-modified electrode (Figure 1). As discussed above, a coating of flexible chitosan hydrogel carries a

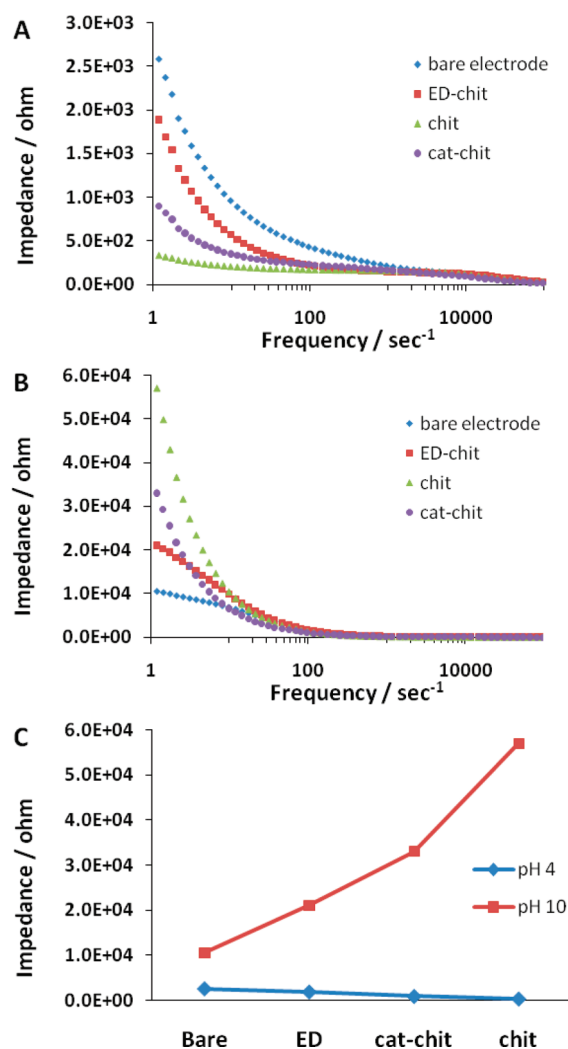


Figure 5. Impedance as a function of frequency at various electrodes at (A) pH 4 and (B) pH 10. (C) Impedance at 1 Hz for all electrodes at pH 4 and pH 10. Initial potential: 0.2 V. Low frequency: 1 Hz. High frequency: 100 kHz. Amplitude: 5 mV.

large quantity of positive charges at pH 4 and attracts and concentrates the negatively charged $\text{Fe}(\text{CN})_6^{3-/4-}$ in the film, leading to increased conductivity. At pH 10 (Figure 5B) the trend is completely reversed; the bare electrode has the lowest impedance, followed by the ED-chit-modified electrode, the cat-chit-modified electrode, and the chit-modified electrode. Since all three chitosan coatings showed increased impedance compared with the bare electrode, it suggests that neutral chitosan films (at pH 10) indeed may deter the diffusion of $\text{Fe}(\text{CN})_6^{3-/4-}$ to some extent; further, the chit coating has lower impedance than the cat-chit coating at pH 4 but higher impedance at pH 10, suggesting that the chit coating is more flexible and not only swells more than the cat-chit coating at pH 4 but also shrinks more and becomes more tightly packed at pH 10. This is in agreement with previous discussions that more cross-linking leads to more rigid structure. The low frequency (1 Hz) impedances for the four electrodes at pH 4 and pH 10 are summarized in Figure 5C.

pH-Responsive Switching. Stimuli-responsive materials or “smart” materials that respond to external stimuli such as electric signals, pH, temperature, pressure, etc., have emerged as one of the most exciting topics of scientific research.^{3–6,45–49}

In this work, the catechol-cross-linked chitosan film and the deposited chitosan film show a unique and interesting behavior; i.e., the redox process of negatively charged redox probes such as $\text{Fe}(\text{CN})_6^{3-/4-}$ is significantly enhanced at pH 4 and is virtually shut down at pH 10. This phenomenon is reversible and can be repeated over and over like a pH-dependent switch, as illustrated in Figure 6. This property can be potentially used

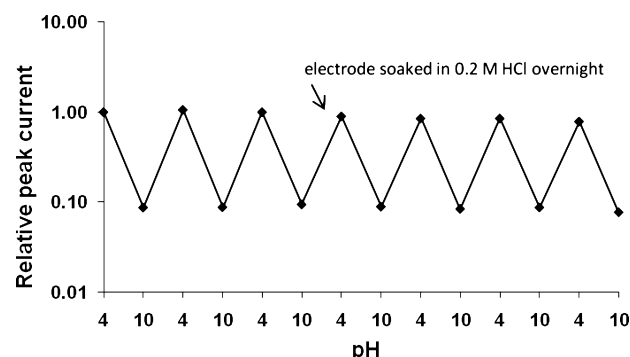


Figure 6. pH-responsive switching. The electrode was modified with catechol-cross-linked chitosan (30:1 chitosan-to-catechol ratio). The pH of the buffer solution containing 1.0 mM $\text{K}_3\text{Fe}(\text{CN})_6$ was alternated between pH 4 and pH 10, and the peak current of the cyclic voltammogram was measured (as shown in Figure 1). The initial peak current at pH 4 was used as the reference, and all other peak currents were calculated as fractions. All other conditions are the same as in Figure 1.

in fabricating bioelectronic devices as it provides a unique control over the redox process at the electrode.

It is important to note that after a few cycles the cat-chit-modified electrode was soaked in 0.2 M HCl overnight, a condition that dissolves uncross-linked chitosan films (ED-chit and chit); yet, the cat-chit-modified electrode shows no sign of diminishing current. This shows that the cat-chit film is robust and proves indirectly that the film is covalently cross-linked.

pH-Responsive Releasing of $\text{Fe}(\text{CN})_6^{3-/4-}$. As discussed above, we hypothesize that the greatly enhanced peak current of $\text{Fe}(\text{CN})_6^{3-/4-}$ for chit- and cat-chit-modified electrodes at pH 4 is due to the protonation of the amine groups leading to the swelling of the chitosan hydrogel which exposes much of the $-\text{NH}_3^+$, resulting in the enrichment of $\text{Fe}(\text{CN})_6^{3-/4-}$ in the chitosan hydrogel. Support for this hypothesis is provided by the experiment in Figure 7. A cat-chit-modified electrode was soaked in a pH 4 buffer solution containing 1.0 mM $\text{Fe}(\text{CN})_6^{3-}$ for 20 min or until the CV stabilized (Figure 7A, curve a). The electrode was then taken out and rinsed with water and immersed in a PBS (pH 4) containing no $\text{Fe}(\text{CN})_6^{3-/4-}$, and the CV was recorded again (Figure 7A, curve b). The CV in PBS is only slightly smaller than the CV in the $\text{Fe}(\text{CN})_6^{3-/4-}$ solution, indicating that the redox current is due to the trapped and enriched $\text{Fe}(\text{CN})_6^{3-/4-}$ in the chitosan hydrogel. The peak current of b is used as the initial peak current (100%). The electrode was then immersed in a pH 4 buffer solution containing no $\text{Fe}(\text{CN})_6^{3-/4-}$, and the peak current was monitored with time. As shown in Figure 7B, when soaked in a pH 4 buffer solution, over 90% of the peak current of $\text{Fe}(\text{CN})_6^{3-/4-}$ maintained in the first 20 min, suggesting the trapped $\text{Fe}(\text{CN})_6^{3-/4-}$ in the chitosan hydrogel did not leak out quickly. A similar experiment was performed except the cat-chit-modified electrode was immersed in a pH 10 buffer. Figure

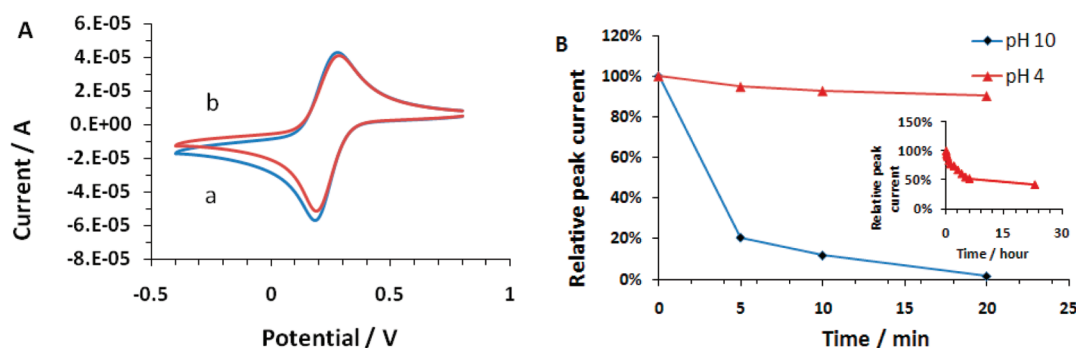


Figure 7. pH-responsive releasing of $\text{Fe}(\text{CN})_6^{3-}$. The cat-chit-modified electrode was soaked in a pH 4 buffer solution containing 1.0 mM $\text{Fe}(\text{CN})_6^{3-}$ for 20 min. (A) Cyclic voltammograms of the electrode in 1.0 mM $\text{Fe}(\text{CN})_6^{3-}$ (a), and after the electrode was taken out, rinsed with water, and immersed in a pH 4 buffer without $\text{Fe}(\text{CN})_6^{3-}$ (b). The peak current of b was used as the initial peak current (100%). (B) The electrode in (A) was immersed in pH 4 and pH 10 buffer solutions, respectively, and the peak current was monitored with time. Inset: Releasing of $\text{Fe}(\text{CN})_6^{3-}$ in pH 4 buffer in extended study. Scan rate: 50 mV s^{-1} .

7B shows that the peak current quickly dropped to 1% of the initial value after only 20 min which suggests that the $\text{Fe}(\text{CN})_6^{3-/4-}$ had diffused from the film. The inset in Figure 7B shows that even after 24 h of soaking the film in pH 4 buffer the peak current had dropped by only 60%, which suggests that much of the $\text{Fe}(\text{CN})_6^{3-/4-}$ was retained in this film.

Voltage-Responsive Releasing of $\text{Fe}(\text{CN})_6^{3-}$. Stimuli-controlled releasing of $\text{Fe}(\text{CN})_6^{3-}$ can also be achieved by electrical signals, as illustrated in Figure 8. As in Figure 7, a cat-

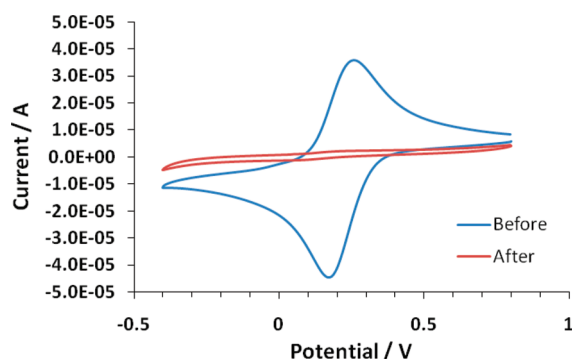


Figure 8. Voltage-responsive releasing of $\text{Fe}(\text{CN})_6^{3-}$. The cat-chit-modified electrode was soaked in a pH 4 solution containing 1.0 mM $\text{Fe}(\text{CN})_6^{3-}$ for 20 min. The electrode was then taken out and rinsed with water and immersed in a PBS (pH 4) containing no $\text{Fe}(\text{CN})_6^{3-}$, and the CV was recorded immediately (before). A negative potential of -2.5 V was then applied for 5 min, and the CV was recorded again (after). Scan rate: 50 mV s^{-1} . Reference: Ag/AgCl .

chit-modified electrode was soaked in a pH 4 buffer solution containing 1.0 mM $\text{Fe}(\text{CN})_6^{3-}$ for 20 min or until the CV stabilized. The electrode was then taken out and rinsed with water and immersed in a PBS (pH 4) containing no $\text{Fe}(\text{CN})_6^{3-/4-}$, and the CV was recorded (Figure 8, before). A negative potential of -2.5 V was then applied for 5 min, and the CV was recorded again in the same PBS (pH 4) solution (Figure 8, after). As demonstrated in Figure 7, soaking in pH 4 buffer does not release the trapped $\text{Fe}(\text{CN})_6^{3-}$ quickly, or at least in the first 20 min more than 90% should remain. However, application of the negative potential effectively and immediately released the $\text{Fe}(\text{CN})_6^{3-}$. The applied negative potential results in electrolytic reduction reactions (as evidenced by the evolution of bubbles in the solution) which results in a localized pH increase in the vicinity of the electrode

surface. Under these conditions chitosan's amines are expected to be deprotonated eliminating the film's positive charge and the electrostatic interactions that trap the $\text{Fe}(\text{CN})_6^{3-}$ in the film.

The pH- and voltage-responsiveness of the catechol-cross-linked chitosan hydrogel demonstrated above suggests that it may be potentially used as a stimuli-responsive drug delivery/releasing system for biomedical devices.

CONCLUSIONS

We identified fabrication conditions that yielded a stable chitosan-based film that can be repeatedly switched in response to changes in pH. Fabrication is based on a biomimetic oxidative cross-linking mechanism involving catechol. There are numerous examples in which biology enzymatically oxidizes phenols to generate reactive intermediates (quinones or free radicals) that initiate cross-linking processes (e.g., lignification, quinone tanning, and the setting of mussel glue).^{32,50} When we exposed the catechol-cross-linked chitosan films to different pH values, we observed an order-of-magnitude change in mediator peak currents. Negatively charged redox mediators such as $\text{Fe}(\text{CN})_6^{3-/4-}$ were found to be trapped and enriched in the catechol-cross-linked chitosan films under acidic conditions and released into the solution upon increasing the pH or applying a negative potential. These results illustrate the potential for enlisting biobased materials and mechanisms to generate "smart" films that could be employed for sensing, actuation, and controlled release.

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ACKNOWLEDGMENTS

Y.Z. gratefully acknowledges financial support from NSF and QEM (Quality Education for Minorities network). G.F.P. and E.K. gratefully acknowledge financial support from Deutsch Foundation and the National Science Foundation (EFRI-0735987).

REFERENCES

- (1) Hoffman, A. S. J. *Controlled Release* **1987**, *6*, 297–305.
- (2) Miyata, T.; Uragami, T.; Nakamae, K. *Adv. Drug Delivery Rev.* **2002**, *54*, 79–98.

- (3) Alvarez-Lorenzo, C.; Concheiro, A. *Mini-Rev. Med. Chem.* **2008**, *8*, 1065–1074.
- (4) Bawa, P.; Pillay, V.; Choonara, Y. E.; du, T. L. C. *Biomed. Mater. (Bristol, U. K.)* **2009**, *4*, 022001/1–022001/15.
- (5) Nandivada, H.; Ross, A. M.; Lahann, J. *Prog. Polym. Sci.* **2010**, *35*, 141–154.
- (6) Liu, F.; Urban, M. W. *Prog. Polym. Sci.* **2010**, *35*, 3–23.
- (7) Roy, D.; Cambre, J. N.; Sumerlin, B. S. *Prog. Polym. Sci.* **2010**, *35*, 278–301.
- (8) Kirsebom, H.; Galaev, I. Y.; Mattiasson, B. *J. Polym. Sci., Part B: Polym. Phys.* **2011**, *49*, 173–178.
- (9) Bajpai, A. K.; Bajpai, J.; Saini, R.; Gupta, R. *Polym. Rev.* **2011**, *51*, 53–97.
- (10) Kean, T.; Thanou, M. *Adv. Drug Delivery Rev.* **2010**, *62*, 3–11.
- (11) Agnihotri Sunil, A.; Mallikarjuna Nadagouda, N.; Aminabhavi Tejjraj, M. *J. Controlled Release* **2004**, *100*, 5–28.
- (12) Mao, S.; Sun, W.; Kissel, T. *Adv. Drug Delivery Rev.* **2010**, *62*, 12–27.
- (13) Park, J. H.; Saravanakumar, G.; Kim, K.; Kwon, I. C. *Adv. Drug Delivery Rev.* **2010**, *62*, 28–41.
- (14) Amidi, M.; Hennink, W. E. *Adv. Drug Delivery Rev.* **2010**, *62*, 1–2.
- (15) Bhattarai, N.; Gunn, J.; Zhang, M. *Adv. Drug Delivery Rev.* **2010**, *62*, 83–99.
- (16) de la Fuente, M.; Raviña, M.; Paolicelli, P.; Sanchez, A.; Seijo, B.; Alonso, M. J. *Adv. Drug Delivery Rev.* **2010**, *62*, 100–117.
- (17) Agrawal, P.; Strijkers, G. J.; Nicolay, K. *Adv. Drug Delivery Rev.* **2010**, *62*, 42–58.
- (18) Krajewska, B. *Enzyme Microb. Technol.* **2004**, *35*, 126–139.
- (19) Sashiwa, H.; Aiba, S.-i. *Prog. Polym. Sci.* **2004**, *29*, 887–908.
- (20) Bhattarai, S.; Kc, R.; Kim, S.; Sharma, M.; Khil, M.; Hwang, P.; Chung, G.; Kim, H. *J. Nanobiotechnol.* **2008**, *6*, 1.
- (21) Dowling, M. B.; Kumar, R.; Keibler, M. A.; Hess, J. R.; Bochicchio, G. V.; Raghavan, S. R. *Biomaterials* **2011**, *32*, 3351–3357.
- (22) Laudenslager, M. J.; Schiffman, J. D.; Schauer, C. L. *Biomacromolecules* **2008**, *9*, 2682–2685.
- (23) Wu, L. Q.; Gadre, A. P.; Yi, H.; Kastantin, M. J.; Rubloff, G. W.; Bentley, W. E.; Payne, G. F.; Ghodssi, R. *Langmuir* **2002**, *18*, 8620–8625.
- (24) Fernandes, R.; Wu, L.-Q.; Chen, T.; Yi, H.; Rubloff, G. W.; Ghodssi, R.; Bentley, W. E.; Payne, G. F. *Langmuir* **2003**, *19*, 4058–4062.
- (25) Wu, L.-Q.; Lee, K.; Wang, X.; English, D. S.; Losert, W.; Payne, G. F. *Langmuir* **2005**, *21*, 3641–3646.
- (26) Pang, X.; Zhitomirsky, I. *Mater. Chem. Phys.* **2005**, *94*, 245–251.
- (27) Boccaccini, A. R.; Keim, S.; Ma, R.; Li, Y.; Zhitomirsky, I. *J. R. Soc., Interface* **2010**, *7*, S581–S613.
- (28) Berger, J.; Reist, M.; Mayer, J. M.; Felt, O.; Peppas, N. A.; Gurny, R. *Eur. J. Pharm. Biopharm.* **2004**, *57*, 19–34.
- (29) Hansen, D. C.; Corcoran, S. G.; Waite, J. H. *Langmuir* **1998**, *14*, 1139–1147.
- (30) Yu, M.; Deming, T. J. *Macromolecules* **1998**, *31*, 4739–4745.
- (31) McDowell, L. M.; Burzio, L. A.; Waite, J. H.; Schaefer, J. J. *Biol. Chem.* **1999**, *274*, 20293–20295.
- (32) Chen, T.; Small, D. A.; Wu, L.-Q.; Rubloff, G. W.; Ghodssi, R.; Vazquez-Duhalt, R.; Bentley, W. E.; Payne, G. F. *Langmuir* **2003**, *19*, 9382–9386.
- (33) Anghileri, A.; Lantto, R.; Kruus, K.; Arosio, C.; Freddi, G. *J. Biotechnol.* **2007**, *127*, 508–519.
- (34) Zangmeister, R. A.; Park, J. J.; Rubloff, G. W.; Tarlov, M. J. *Electrochim. Acta* **2006**, *51*, 5324–5333.
- (35) Vårum, K. M.; Ottøy, M. H.; Smidsrød, O. *Carbohydr. Polym.* **1994**, *25*, 65–70.
- (36) Anthonsen, M. W.; Smidsrød, O. *Carbohydr. Polym.* **1995**, *26*, 303–305.
- (37) Rinaudo, M.; Pavlov, G.; Desbrières, J. *Polymer* **1999**, *40*, 7029–7032.
- (38) Marguerite, R. *Prog. Polym. Sci.* **2006**, *31*, 603–632.
- (39) Pillai, C. K. S.; Paul, W.; Sharma, C. P. *Prog. Polym. Sci.* **2009**, *34*, 641–678.
- (40) Nishimura, S.; Kohgo, O.; Kurita, K.; Kuzuhara, H. *Macromolecules* **1991**, *24*, 4745–4748.
- (41) Li, Z.; Zhuang, X.; Liu, X.; Guan, Y.; Yao, K. J. *J. Appl. Polym. Sci.* **2003**, *88*, 1719–1725.
- (42) Park, I. K.; Park, Y. H. *J. Appl. Polym. Sci.* **2001**, *80*, 2624–2632.
- (43) Park, J. H.; Cho, Y. W.; Chung, H.; Kwon, I. C.; Jeong, S. Y. *Biomacromolecules* **2003**, *4*, 1087–1091.
- (44) Wu, L. Q.; McDermott, M. K.; Zhu, C.; Ghodssi, R.; Payne, G. F. *Adv. Funct. Mater.* **2006**, *16*, 1967–1974.
- (45) Bernaerts, K. V.; Fustin, C.-A.; Bomal-D'Haese, C.; Gohy, J.-F.; Martins, J. C.; Du Prez, F. E. *Macromolecules* **2008**, *41*, 2593–2606.
- (46) Brun-Graeppe, A. K. A. S.; Richard, C.; Bessodes, M.; Scherman, D.; Merten, O.-W. *Prog. Polym. Sci.* **2010**, *35*, 1311–1324.
- (47) Du, F.-S.; Wang, Y.; Zhang, R.; Li, Z.-C. *Soft Matter* **2010**, *6*, 835–848.
- (48) Ionov, L. *J. Mater. Chem.* **2010**, *20*, 3382–3390.
- (49) Motornov, M.; Roiter, Y.; Tokarev, I.; Minko, S. *Prog. Polym. Sci.* **2010**, *35*, 174–211.
- (50) Chen, T.; Embree, H. D.; Wu, L.-Q.; Payne, G. F. *Biopolymers* **2002**, *64*, 292–302.