

Spectroelectrochemical Sensing Based on Multimode Selectivity Simultaneously Achievable in a Single Device. 20. Detection of Metal Ions in Different Oxidation States

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Spectroelectrochemical sensing a metal in two different oxidation states, both of which are weakly absorbing in the visible wavelength range, was demonstrated with ferrous and ferric ion. The sensor consisted of an indium tin oxide optically transparent electrode (ITO OTE) coated with a thin film of Nafion preloaded with the ligand 2,2'-bipyridine (bipy). Fe^{2+} in the sample partitioned into the film where it reacted with bipy to form $\text{Fe}(\text{bipy})_3^{2+}$, which absorbs strongly at 520 nm. The change in absorbance (ΔA) at 520 nm associated with the accumulation of $\text{Fe}(\text{bipy})_3^{2+}$ complex in the film was measured by attenuated total reflectance spectroscopy and was proportional to the concentration of Fe^{2+} in the sample. Iron in the Fe^{3+} form can also be determined, but it has a more complex coordination chemistry involving formation of $[\text{Fe}_2(\text{bipy})_4\text{O}(\text{H}_2\text{O})_2]^{4+}$ as well as $\text{Fe}(\text{bipy})_3^{3+}$ in the film. Fe^{3+} was detected indirectly by reducing the nonabsorbing Fe^{3+} -bipy complexes that accumulated in the film to absorbing $\text{Fe}(\text{bipy})_3^{2+}$ and monitoring ΔA at 520 nm. The effects of film thickness and ligand concentration in the film on sensor sensitivity and response time for Fe^{2+} were evaluated. Detection limits of 0.6×10^{-6} M for Fe^{2+} and 2×10^{-6} M for Fe^{3+} were obtained with 300 nm thick films after 30 min of exposure to a quiescent sample. Careful manipulation of the potential applied with simultaneous optical detection enables Fe^{2+} to be distinguished from Fe^{3+} , which is the first step in developing a sensor for speciating the two oxidation states in a mixture.

A spectroelectrochemical sensor combines optical spectroscopy, electrochemistry, and selective partitioning into a single device.^{1–4} The selectivity of the sensor benefits from the three requirements of an analyte for detection: (1) preconcentration into a chemically selective film, (2) electrochemical oxidation or

reduction, and (3) a difference in spectroscopic properties between oxidized and reduced forms at a selected wavelength. The change in optical response that accompanies the electrochemically changed oxidation state of the analyte that has partitioned into the film is the analytical signal. Sensor sensitivity depends in part on the magnitude of the difference in the optical properties of the two oxidation states being electrochemically modulated. Thus far, the spectroelectrochemical sensor has been demonstrated mainly for detection of species that strongly absorb or fluoresce in only one oxidation state, which gives good sensitivity.^{1–4} However, all species of interest will not have these ideal properties. Consequently, we have explored strategies for detecting species that exhibit poor optical characteristics in all electrochemically accessible oxidation states. One strategy was to use a redox mediator with appropriate optical properties to detect a nonabsorbing organic analyte indirectly by virtue of its homogeneous electron exchange with the mediator.⁵ This was demonstrated with the determination of ascorbate with the mediator $\text{Ru}(\text{bipy})_3^{2+}$. In a second strategy a metal cation whose oxidation states all absorb poorly in the visible region was rendered detectable by coordinating it with a ligand that significantly improves the optical properties of one oxidation state.⁶ The work discussed here is a continuation of this strategy of using in situ coordination chemistry to improve sensor performance for weakly absorbing metal ions.

Sensing a metal ion using spectroelectrochemistry in the absorbance mode can have poor sensitivity due to a small difference in the molar absorptivities ($\Delta\epsilon$) between the metal ion in its oxidized and reduced states, resulting in weak optical signals associated with electrochemical modulation. However, when a coordinating ligand that serves as a strong chromophore for one of the oxidation states is used to complex with the metal ions, a much larger $\Delta\epsilon$ value is associated with electrochemical modulation, and the optical signal is improved significantly. This approach is illustrated by the general scheme in Figure 1 for metal ion M^{a+} and ligand L. M^{a+} partitions from the sample into film preloaded with L where the strongly absorbing complex $\text{M}(\text{L})_x^{a+}$ forms. $\text{M}(\text{L})_x^{a+}$ is then electromodulated with weakly absorbing $\text{M}(\text{L})_x^{b+}$ to provide a large ΔA for sensitive sensing. Alternatively, the metal

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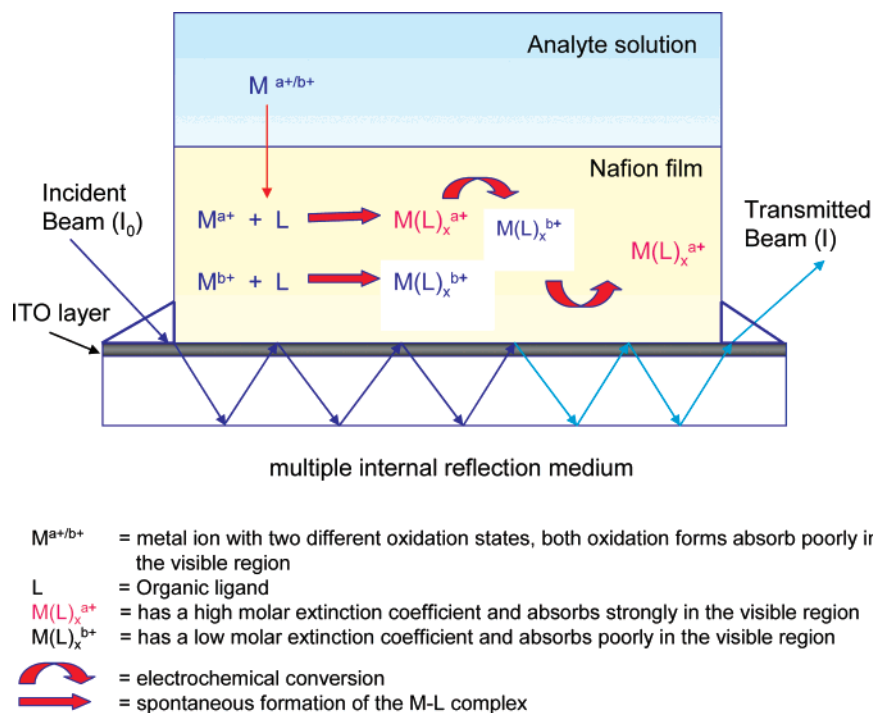


Figure 1. Schematic diagram of spectroelectrochemical sensor for detection of a metal ion in two different oxidation states.

ion in its other oxidation state M^{b+} can be determined by using the electrode potential to convert the initially formed weakly absorbing (or colorless) $M(L)_x^{b+}$ into optically detectable $M(L)_x^{a+}$, as shown in Figure 1. Even a third possibility exists—to determine a mixture of M^{a+} and M^{b+} . In this case metal ions in both oxidation states would partition into the film, both forming their respective complexes. This step would be done under open circuit to not allow electrochemical interconversion of the two forms. M^{a+} would be determined directly by measuring the optical signal associated with $M(L)_x^{a+}$. The potential would then be stepped to convert the $M(L)_x^{b+}$ to optically observable $M(L)_x^{a+}$, allowing M^{b+} to be determined by the change in optical signal associated with the potential step.

Only the first of these three possibilities has been reported—determination of M^{a+} by its formation of $M(L)_x^{a+}$. The concept was demonstrated with ferrous ion (Fe^{2+}) by incorporating the ligand 2,2'-bipyridine (bipy) into a thin film of the cation-exchange polymer Nafion on an optically transparent electrode (OTE) consisting of indium tin oxide (ITO).⁶ The dual nature of Nafion, which results from the hydrophobic domains of fluorocarbon and the hydrophilic anionic sulfonate groups, plays an important role in sensor function. Bipy can be preloaded in the film by its attraction to the hydrophobic domains. Ferrous ion from the sample to be analyzed is then attracted into the bipy-loaded film by cation exchange with the sulfonate groups. The $Fe(bipy)_3^{2+}$ complex that rapidly forms in the film is intensely colored ($\epsilon = 7.70 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ in 0.1 M NaCl, $\lambda_{\text{max}} = 520 \text{ nm}$), whereas the oxidized form $Fe(bipy)_3^{3+}$ is essentially nonabsorbing at 520 nm. The sensitivity of the sensor is enhanced by the larger $\Delta\epsilon$ for the $Fe(bipy)_3^{2+/3+}$ couple compared to that of the hydrated $Fe^{2+/3+}$ species that both have small ϵ values. For example, $Fe-$

$(H_2O)_6^{2+}$ has a $\epsilon < 1 \text{ M}^{-1} \text{ cm}^{-1}$, and various hydroxo species of Fe^{3+} such as $[Fe(OH)(H_2O)_5]^{2+}$ are pale yellow.⁸ Although not yet demonstrated, the coordinating ligand could provide a fourth mode of selectivity by virtue of its selective reaction with the target metal ion. A more fundamental study of the in situ complexation reaction between Fe^{2+} and bipy was done using spectroscopic ellipsometry.⁷ Bipy was found to be retained strongly in the Nafion film with only about 13% leaching out after 1000 min of soaking in supporting electrolyte. Complexation of Fe^{2+} with bipy in the film occurred efficiently forming chemically stable $Fe(bipy)_3^{2+}$. However, the complex leached out of the film more rapidly ($\sim 30\%$ after 1000 min) than bipy.

Here we describe continued development of the sensor for ferrous ion⁶ by obtaining a calibration plot and evaluating the effects of film thickness and ligand concentration on sensor sensitivity and response time. The extension of this sensor concept to the detection of iron as ferric ion, which does not form a strongly absorbing complex with bipy as does ferrous, is demonstrated. Also, we explore the possibility of using a single sensor for detecting mixtures of ferrous and ferric ions, i.e., speciation of the two oxidation states. The difference in optical properties of $Fe(bipy)_3^{2+}$ and $Fe(bipy)_3^{3+}$ makes this possible through judicious use of spectroelectrochemical modulation.

EXPERIMENTAL SECTION

Chemicals and Materials. The following chemicals were used: ferrous ammonium sulfate hexahydrate [$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, Mallinckrodt], ferric ammonium sulfate dodecahydrate [$FeNH_4(SO_4)_2 \cdot 12H_2O$, Baker], 2,2'-bipyridine (bipy, Eastman), sodium chloride (Fisher), and Nafion (5% solution in lower aliphatic alcohols and 10% water, Aldrich). All chemicals were used

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as received without further purification. The supporting electrolyte, 0.1 M NaCl, was prepared with deionized water from a D2798 Nanopure water purifications system (Barnstead, Boston, MA). Solutions for analyses of Fe^{2+} and Fe^{3+} were prepared by dissolving the appropriate amount of ferrous ammonium sulfate or ferric ammonium sulfate into 0.1 M NaCl electrolyte solution; solutions for speciation were prepared in exactly the same manner, but mixing both oxidation forms together. ITO tin float glass (11–50 Ω/\square , 150 nm thick ITO layer over tin float glass) was obtained from Thin Film Devices (Anaheim, CA) and diced into 1 \times 3 in. slides.

Preparation of Nafion Sensing Films. Nafion films were prepared with different thicknesses by spin-coating Nafion solution onto ITO glass slides using a spin-coater (model 1-PM101DT-R485 photo-resist-spinner, Headway Research, Inc.) at different spin rates for 30 s. Thin films were prepared using 2% Nafion solution (5% Nafion stock solution diluted with 1:4 water/propanol mixture), and thick films were prepared using 5% Nafion stock solution directly. Prior to spin-coating, the ITO glass substrates were cleaned as described previously.⁶ In all experiments ITO glass substrates prepared using 5% Nafion at 2000 rpm (284 nm) were used unless otherwise stated. Both ends of the ITO side of the glass slide were masked with tape to a width of \sim 1 cm prior to spin-coating to provide bare area for electrical contact and prism coupling. Nafion-coated ITO glass slides were dried in air and soaked in the appropriate concentration of bipy in 0.1 M NaCl overnight prior to use in all experiments.

Instrumentation. Film thickness measurements were obtained using ellipsometric methods on a J. A. Woollam variable angle spectroscopic ellipsometer. Transmission spectroscopic measurements were made using a Hewlett-Packard 8453 diode array spectrophotometer. ATR spectroelectrochemical measurements were done using an instrumental setup that has been described before.^{1,2} Absorbance values were obtained by using the light intensity when the sensor is first exposed to pure supporting electrolyte (I_0) and the light intensity after being exposed to an analyte solution (I), where the sensor absorbance in the attenuated multiple reflection mode is $A = \log(I_0/I)$.

Spectroelectrochemical Cell. A plastic cell that has been described previously^{1,2} was used in the ATR spectroelectrochemical measurements. ITO glass slides served as the working electrode where electrical contacts were made with four copper alligator clips attached to each exposed corner. The reference electrode was Ag/AgCl, 3 M KCl (Cypress Systems, Inc.), and a platinum mesh served as the auxiliary electrode.

RESULTS AND DISCUSSION

Ferrous Ion (Fe^{2+}). We have used ferrous ion to first demonstrate the concept of in situ coordination to enhance the optical properties for spectroelectrochemical detection.⁶ Proof of concept was demonstrated using two concentrations of Fe^{2+} , but parameters important to sensor performance were not studied. Here the effects of film thickness and concentration of bipy loaded into the film on sensor response are reported together with a calibration plot for the determination of Fe^{2+} .

Relationship between Film Thickness and Sensor Response to Fe^{2+} . Film thickness affects sensor characteristics in several ways. The time required to prepare the sensor by preloading the film with bipy increases with film thickness. However, this is not so

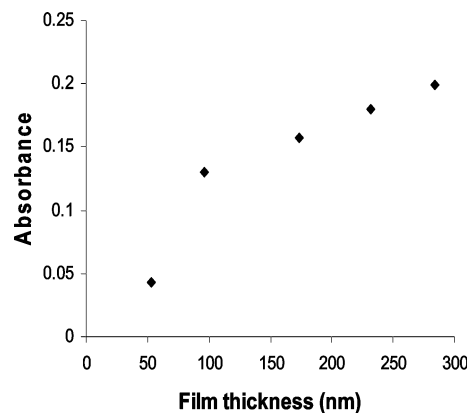


Figure 2. Effect of increasing film thickness on sensor response. Nafion-coated ITO OTE sensors were exposed to 300 μM bipy in 0.1 M NaCl overnight prior to use. The ATR absorbance for each sensor was measured after 30 min of exposure to a solution of 100 μM Fe^{2+} in 0.1 M NaCl.

important because a sensor is loaded prior to use, and so this step is not a part of sensor response time. The time required to analyze a sample is affected by film thickness in two ways: the time to load the film with Fe^{2+} from the sample and the time required for spectroelectrochemical modulation, if it is required. Both of these times increase with increasing film thickness.

The effect of film thickness on sensor response to 100 μM Fe^{2+} was investigated using 53, 96, 173, 232, and 284 nm thick films. Films of different thicknesses were obtained by varying the spin-coating speed during preparation. Nafion film thickness has been shown to exhibit an inverse exponential relationship with spin rate.^{5,9} Figure 2 shows the effect that film thickness has on the optical signal for a film preloaded with bipy after exposure to Fe^{2+} . As expected, thicker films effectively increase the optical path of the sensor, giving larger absorbance values because more $\text{Fe}(\text{bipy})_3^{2+}$ is concentrated in the evanescent waves at the reflection points of the ATR beam. The sensitivity improvement can be substantial; the optical signal recorded for the 284 nm film was about 3 times greater than that for the 53 nm film. However, the gains in sensitivity diminish with each incremental increase in film thickness because the intensity of the evanescent wave decreases exponentially in the film. When the film thickness exceeds the penetration depth of the evanescent waves, the optical signal becomes insensitive to further increases in film thickness.

The time required for the thinnest film (53 nm) to achieve equilibrium during uptake of Fe^{2+} ions was substantially shorter (\sim 10 min) than for the thickest film (284 nm), which took \sim 30 min. Thus, faster response can be achieved with thinner films, but with a sacrifice in sensitivity.

Most of the following experiments were done with \sim 284 nm thick films as used in our previous work.⁶ By using this thickness, we compromised response time to gain sensitivity.

Effect of Ligand Concentration in the Film. The concentration of bipy in the film is important for the stoichiometric conversion of Fe^{2+} from the sample to $\text{Fe}(\text{bipy})_3^{2+}$ in the film. At least a 3:1 excess of ligand to Fe^{2+} in the film is needed to satisfy the stoichiometry of the complex, and an excess is preferable to drive the reaction to completion. Also, some of the bipy that partitions

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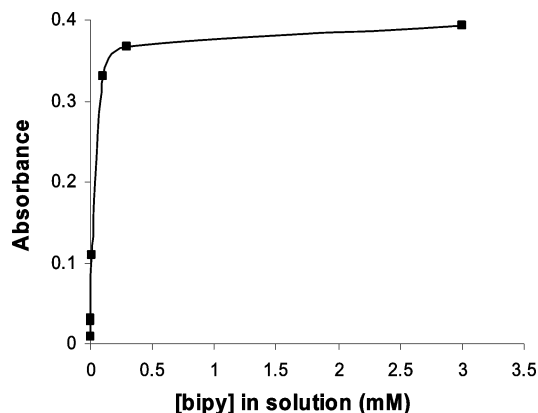


Figure 3. Effect of concentration of bipy in solution used to preload sensors for uptake of Fe^{2+} . Nafion-coated ITO OTEs were exposed to a series of bipy solutions (0.1, 1, 5, 10, 100, and 300 μM and 3 mM) overnight prior to use. Each sensor was exposed to 100 μM Fe^{2+} in 0.1 M NaCl, and each data point represents the ATR absorbance at 30 min.

into the film might not be available for coordination with Fe^{2+} . Thus, a high concentration of bipy in the film is desirable and becomes increasingly necessary as Fe^{2+} concentration in the sample increases.

The effect of bipy concentration in the film was studied by soaking Nafion-coated ITO OTEs in a series of different concentrations of bipy in 0.1 M NaCl overnight to obtain a homogeneous distribution of ligand throughout the film. Each OTE sensor was then immersed in 100 μM Fe^{2+} solution, and the optical response was recorded in the ATR mode as $\text{Fe}(\text{bipy})_3^{2+}$ partitioned into the film. Figure 3 depicts the increase in absorbance due to $\text{Fe}(\text{bipy})_3^{2+}$ formed in the film with respect to the ligand concentration in the soaking solution used to preload the film with bipy. A sharp increase in absorbance was obtained as bipy concentration in the film was increased by soaking in more concentrated solutions of bipy followed by an abrupt plateauing at around 100 μM . No significant difference in the absorbance values is seen for the sensors that were exposed to bipy concentrations above 100 μM in solution. To ensure a maximum concentration of bipy in the film for optimal sensor response 300 μM bipy was used to pre-equilibrate Nafion-coated ITOs in the studies that follow.

In a spectroscopic ellipsometry study of the reaction of Fe^{2+} with bipy in Nafion, we reported the concentration of bipy loaded into Nafion film that had been exposed to 3 mM bipy overnight and then dried for 24 h.⁷ The concentration was determined by measuring the absorbance of bipy at 290 nm on 300 nm thick films using a molar absorptivity of $1.33 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$. The concentration of bipy in the film was estimated to be $0.33 \pm 0.06 \text{ M}$ by assuming the Beer–Lambert law, which corresponds to a partition coefficient of 110 (concentration of $\text{bipy}_{\text{film}}/\text{bipy}_{\text{solution}}$). Such strong partitioning of bipy into a thin Nafion film is to be expected. Nafion is a perfluorinated ion-exchange polymer that contains hydrophilic ionic clusters and hydrophobic perfluorocarbon chains. Strong hydrophobic interaction occurs between the hydrophobic domains of Nafion and the somewhat hydrophobic bipy. The acidic environment in Nafion– H^+ increases the incorporation of bipy, which is an N-donor ligand whose basicity plays a role in partitioning into Nafion by protonation to form a cation.¹⁰

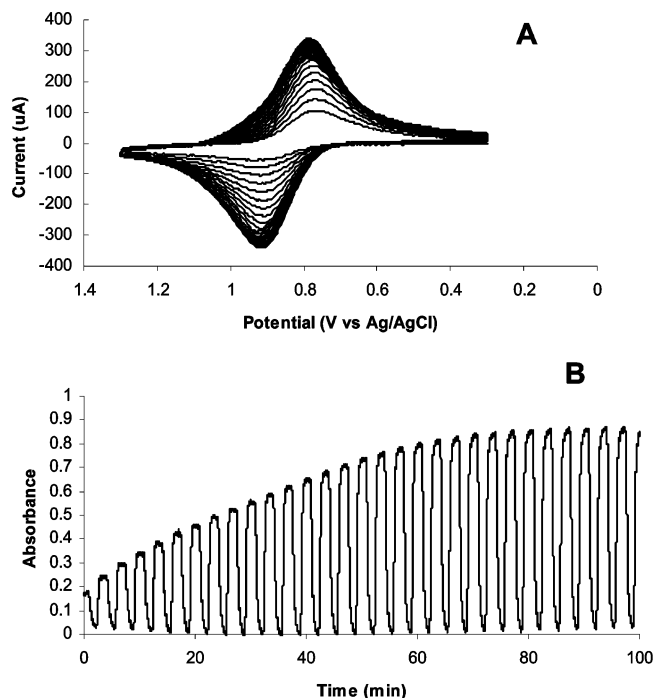


Figure 4. Spectroelectrochemical modulation during uptake of Fe^{2+} from a sample of 100 μM Fe^{2+} in 0.1 M NaCl. The sensor was prepared by soaking a Nafion-coated ITO OTE in 300 μM bipy overnight. Panel A: cyclic voltammograms recorded at 0.01 V/s. Panel B: the optical modulation measured by ATR at 520 nm during uptake of Fe^{2+} by the sensor used in panel A.

Determination of Fe^{2+} . Partitioning of Fe^{2+} into a bipy preloaded Nafion ITO OTE to form $\text{Fe}(\text{bipy})_3^{2+}$ can be followed either electrochemically by cyclic voltammetry or optically by monitoring absorbance at 520 nm as shown for 100 μM Fe^{2+} in Figure 4. The cyclic voltammograms (panel A) exhibit a regular increase in peak current as Fe^{2+} partitions into the film and reacts with bipy to give a voltammogram with an E° of 0.7 V, which is characteristic of $\text{Fe}(\text{bipy})_3^{2+/3+}$. Fe^{2+} must react reasonably rapidly with bipy to form $\text{Fe}(\text{bipy})_3^{2+}$, as there is no evidence of a wave for free Fe^{2+} in the voltammograms. The accompanying spectroelectrochemical modulation at 520 nm (panel B) shows an increase in ΔA that corresponds to the increasing current in the voltammograms as more Fe^{2+} partitions into the film to form $\text{Fe}(\text{bipy})_3^{2+}$ up to about 80 min when they both level off as the film equilibrates with Fe^{2+} in the sample.

Figure 5A shows the optical responses with no modulation for sensors in different Fe^{2+} concentrations. Each Fe^{2+} concentration was measured using a different sensor, all identically prepared Nafion ITO OTEs with preloaded bipy. As the concentration of Fe^{2+} increases the absorbance also increases due to the formation of more $\text{Fe}(\text{bipy})_3^{2+}$ in the film. A representative calibration plot for Fe^{2+} is shown in Figure 5B. As is typical of the spectroelectrochemical sensor in general,^{1–4} a linear region exists at low concentrations with deviation from linearity setting in at higher concentration. Perhaps bipy concentration in the film becomes a limiting factor for coordinating the incoming Fe^{2+} . Under these conditions (284 nm film and 30 mL sample volume) the sensor detected Fe^{2+} at 5 μM and exhibited a rather limited linear range

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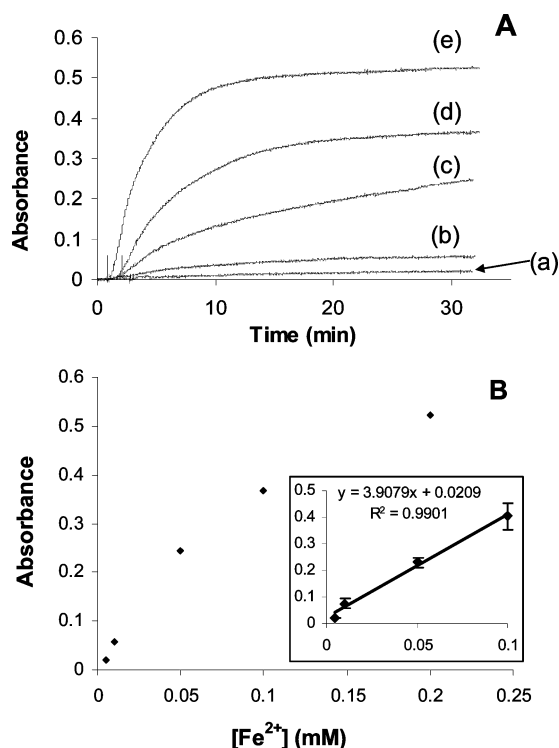


Figure 5. Panel A: ATR optical response curves recorded at 520 nm at open circuit potential. Different concentrations of Fe²⁺ in 0.1 M NaCl were exposed to identically prepared Nafion-coated ITO OTEs that has been preloaded by exposure to 300 μM bipy solution. The curves are for (a) 5, (b) 10, (c) 50, (d) 100, and (e) 200 μM Fe²⁺, respectively. Panel B depicts the calibration curve for Fe²⁺ constructed using the absorbance values at 30 min for each Fe²⁺ concentration in panel A. The inset represents the linear portion of the calibration curve for concentrations ranging from 5 to 100 μM ($n = 2$).

up to about 100 μM (inset of Figure 5B). A detection limit of 0.6 μM was calculated from the ratio ($3 \times$ standard deviation of the lowest detected concentration)/(slope of the calibration curve).

In our initial paper on this sensor we reported a proof of concept in the form of a change in optical response with concentration of Fe²⁺ based on only two concentrations and estimated the detection limit to be 5 μM. The response time for a concentration of 100 μM of Fe²⁺ was 80 min. Here we report a calibration plot for a sensor with a detection limit of 0.6 μM and with a faster response of about 20 min for 100 μM Fe²⁺. It should be noted that a lower limit of detection could be achieved with longer equilibration times as the sensor had not completely equilibrated with the sample in the 20 min used for the plots in Figure 5B. Also, faster response could be achieved by stirring the sample during partitioning to enhance mass transport.

Ferric Ion (Fe³⁺). Since Fe³⁺ partitions into the cation-exchange film and complexes with bipy forming nonabsorbing Fe(bipy)₃³⁺, it must be detected by electrochemical reduction to Fe(bipy)₃²⁺, which can be monitored at 520 nm. However, when the sensor was exposed to a Fe³⁺ solution and the potential scanned during accumulation of Fe³⁺, the cyclic voltammograms had not only the expected reversible couple for Fe(bipy)₃^{3+/2+} at $E^\circ = 0.7$ V that was obtained during accumulation of Fe²⁺ but the additional reduction wave at 0.24 V shown in Figure 6A. Thus,

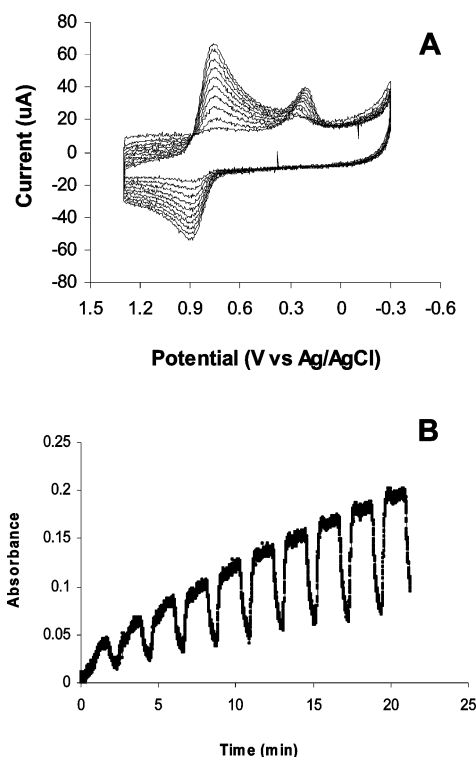
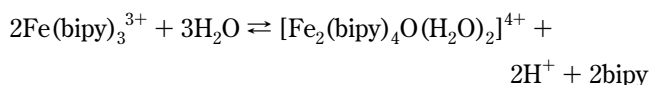
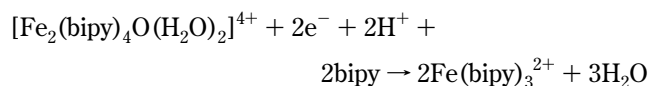


Figure 6. Spectroelectrochemical modulation of 100 μM Fe³⁺ solution in 0.1 M NaCl. Panel A: cyclic voltammograms recorded at 0.025 V/s for a Nafion-coated ITO OTE sensor prepared by soaking in 300 μM bipy overnight. $E_{\text{initial}} = 1.2$ V, and $E_{\text{final}} = -0.3$ V vs Ag/AgCl. Panel B: the optical modulation at 520 nm during uptake of Fe³⁺ by the sensor used for panel A.

different voltammetry was obtained depending on the oxidation state of iron in the sample. This behavior can be attributed to the large difference in hydrolysis of ferric ion compared to ferrous ion ($pK_h = 10.1$ and 2.19 for Fe³⁺ and Fe²⁺, respectively¹¹) that also extends to the Fe(bipy)₃^{3+/2+} system.^{12,13} Fe(bipy)₃³⁺ electro-generated by oxidation of Fe(bipy)₃²⁺ has been shown to undergo hydrolysis according to the following reaction.¹²



The reported cyclic voltammogram¹² exhibits a wave near 0 V for reduction of $[\text{Fe}_2(\text{bipy})_4\text{O}(\text{H}_2\text{O})_2]^{4+}$ at a bare electrode, and the overall shape of the voltammogram is similar to the one in Figure 6A at Nafion-coated ITO. We conclude that some hydrolyzed Fe(bipy)₃³⁺ complex forms in the Nafion film during accumulation of iron. In Figure 6A, the anodic peak at 0.92 V is due to the oxidation of Fe(bipy)₃²⁺ in the film to Fe(bipy)₃³⁺, the cathodic peak at 0.78 V to the reduction of Fe(bipy)₃³⁺ to Fe(bipy)₃²⁺, and the second cathodic peak at 0.24 V to the reduction of $[\text{Fe}_2(\text{bipy})_4\text{O}(\text{H}_2\text{O})_2]^{4+}$ complex ion to Fe(bipy)₃²⁺:



The peak currents for all three waves increase as more Fe³⁺ partitions into the film and forms $[\text{Fe}_2(\text{bipy})_4\text{O}(\text{H}_2\text{O})_2]^{4+}$ and Fe(bipy)₃³⁺.

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The corresponding changes in absorbance during potential cycling are shown in Figure 6B. Neither of the Fe^{3+} –bipy complexes absorb at 520 nm; however, by electrochemical conversion into the $\text{Fe}(\text{bipy})_3^{2+}$ form, the overall absorbance is increasing as more iron partitions into the film. The absorbance maxima during cycling correspond to the more negative potential region where the iron exists as absorbing $\text{Fe}(\text{bipy})_3^{2+}$ in the film. The absorbance minima correspond to the more positive potentials where the iron exists in the 3+ oxidation state, which is nonabsorbing. It should be noted that the absorbance does not drop to zero because the scan rate was too fast for all $\text{Fe}(\text{bipy})_3^{2+}$ to be oxidized as evidenced by the substantial anodic current after the peak at the positive switching potential in Figure 6A compared to that shown in Figure 5 for Fe^{2+} . This phenomenon has been observed with other systems.¹⁴ Another contributing factor could be formation of a precipitate in the film, which would block the light. Freshly prepared ferric ion solutions are almost colorless but they turn pale yellow with time and can form brown precipitates (e.g., $\text{Fe}(\text{OH})_3$). Any precipitation in the film would contribute to the increasing absorbance minima in Figure 6B.

The optical response of the sensor to different Fe^{3+} concentrations is shown in Figure 7A. This experiment is analogous to the one shown in Figure 5A for Fe^{2+} , but the potential of the ITO was held at -0.3 V to reduce the iron in the ferric form to form $\text{Fe}(\text{bipy})_3^{2+}$ which could be monitored at 520 nm. Similar to Fe^{2+} detection, the sensor absorbance increased as more Fe^{3+} partitioned into the film. However, the response time of the sensor when operating in this mode is slower than for Fe^{2+} . The absorbance values for the Fe^{3+} samples are lower than those for equivalent Fe^{2+} samples at a given time (e.g., the absorbance at 30 min for $100 \mu\text{M}$ Fe^{2+} is 0.36 in Figure 5 compared to 0.28 for Fe^{3+} in Figure 7). Everything equal, the sensitivities should be identical since the optically detectable species is the same for both analytes, namely, $\text{Fe}(\text{bipy})_3^{2+}$. This difference is attributed to one or more of these factors: the more complex coordination chemistry for ferric ions caused by the hydrolysis, the need to electrochemically reduce the ferric forms to ferrous to obtain a measurable optical signal, and differences in the partition rates and partition coefficients. We also found it very important to prepare fresh sample solutions of ferric ion. Ferric ions form various hydroxo species in aqueous media that precipitate. Since precipitates do not partition into the sensing film, their formation effectively lowers the concentration of iron in the sample, causing lower absorbance signals.

Figure 7B shows a calibration plot for aqueous Fe^{3+} performed using the spectroelectrochemical sensor with preloaded bipy using experimental conditions identical to those used for detecting Fe^{2+} except for the applied potential of -300 V. The plot is similar to that obtained with Fe^{2+} . The sensor detected Fe^{3+} at $5 \mu\text{M}$ and exhibited the same rather limited linear range up to about $100 \mu\text{M}$ (inset of Figure 7B). The calculated detection limit was $2 \mu\text{M}$, which is a bit higher than that obtained for Fe^{2+} .

Speciation of Fe^{2+} and Fe^{3+} . A potentially important application of a sensor for iron is the determination of each oxidation state of iron in samples that are mixtures of both, i.e., speciation

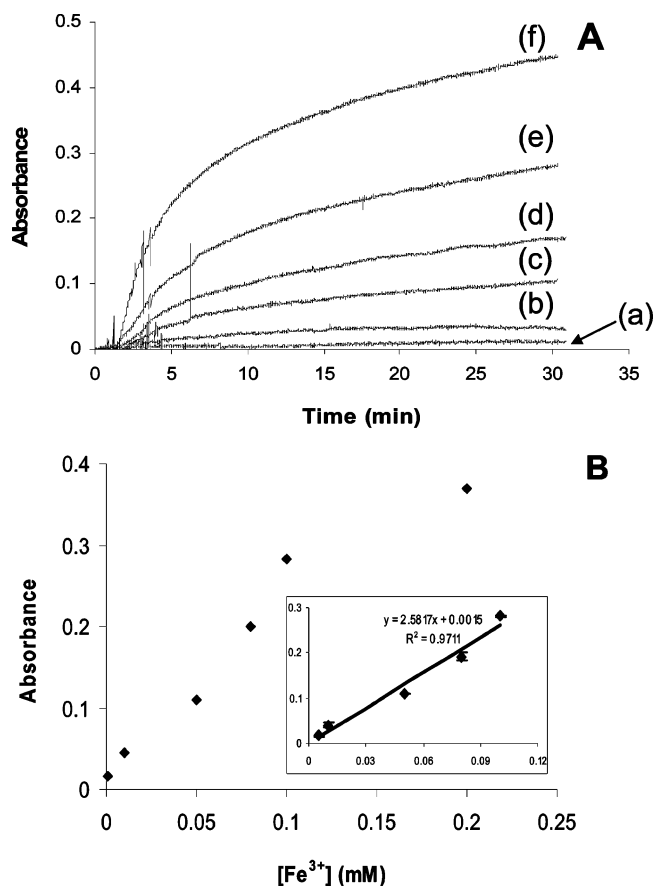


Figure 7. Panel A: ATR optical response curves measured at 520 nm with an applied potential of -0.3 V vs Ag/AgCl for different concentrations of Fe^{3+} in 0.1 M NaCl exposed to identically prepared Nafion-coated ITO OTEs that had been preloaded by exposure to $300 \mu\text{M}$ bipy solution. The curves are for (a) 5 , (b) 10 , (c) 50 , (d) 80 , (e) 100 , and (f) $200 \mu\text{M}$ Fe^{3+} , respectively. Panel B depicts the calibration curve for Fe^{3+} constructed using the absorbance values at 30 min for each iron concentration in panel A. The inset represents the linear portion of the calibration curve for concentrations ranging from 5 to $100 \mu\text{M}$ ($n = 2$).

of ferrous and ferric iron. Here we explore the potential of the spectroelectrochemical sensor to speciate iron. Since only the reduced state of the complex is strongly absorbing, we wanted to see if careful manipulation of iron that partitions into the film by the applied potential would enable Fe^{3+} to be distinguished from Fe^{2+} .

Scheme 1 represents an adaptation of the general scheme shown in Figure 1 to the speciation of iron with the added complication of the hydrolysis reaction.

Spectroelectrochemical detection of a mixture of Fe^{2+} and Fe^{3+} ($20 \mu\text{M}$ each) in which the potential was controlled to enable the two oxidation states to be distinguished by their difference in spectral properties is shown in Figure 8. The sensor was exposed to a solution containing the mixture under the condition of open circuit to avoid any interconversion of the oxidation states during the film loading step. The curve shows a continuous rise in absorbance until 50 min as Fe^{2+} partitions into the film and forms $\text{Fe}(\text{bipy})_3^{2+}$. Fe^{3+} was simultaneously partitioning into the film but made no contribution to the optical response since its bipy complexes do not absorb at 520 nm. This is clearly shown by comparison with curve b, which is an identical experiment,

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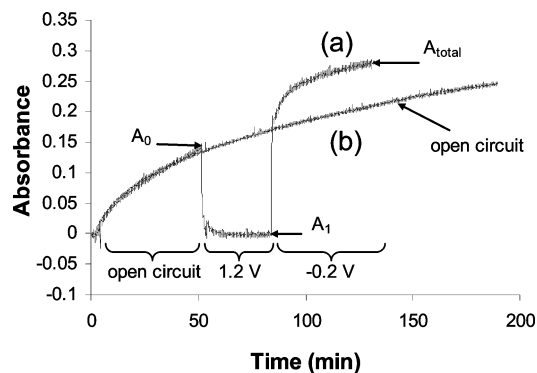
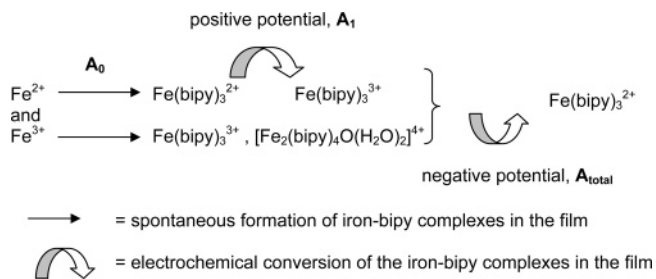


Figure 8. ATR optical response to a mixture of Fe^{2+} and Fe^{3+} . The sensor preloaded with bipy was exposed to a solution of $20 \mu\text{M}$ Fe^{2+} and Fe^{3+} in 0.1 M NaCl . (a) Initial gradual increase in ATR optical response to Fe^{2+} uptake at open circuit, sharp decrease in optical response with the electrochemical oxidation of Fe^{2+} to Fe^{3+} by applying a potential step of 1.2 V vs Ag/AgCl , and finally, sharp increase in optical response with the electrochemical reduction of total Fe^{3+} to Fe^{2+} after applying a more negative potential step of -0.2 V vs Ag/AgCl . (b) Uptake from a $20 \mu\text{M}$ solution of Fe^{2+} and Fe^{3+} without electrochemical modulation showing the increase in absorbance that corresponds only to Fe^{2+} in the solution.

Scheme 1



but without Fe^{3+} in the sample solution. The two curves are barely distinguishable up to a time of 50 min. However in the curve a experiment, the potential was stepped to 1.2 V at 50 min, which oxidized all $\text{Fe}(\text{bipy})_3^{2+}$ to $\text{Fe}(\text{bipy})_3^{3+}$ and $[\text{Fe}_2(\text{bipy})_4\text{O}(\text{H}_2\text{O})_2]^{4+}$, causing the absorbance to drop to zero. During the time that the potential was held at 1.2 V , Fe^{2+} and Fe^{3+} would be expected to continue to partition into the film, but $\text{Fe}(\text{bipy})_3^{2+}$ formed would be oxidized to undetectable $\text{Fe}(\text{bipy})_3^{3+}$, and so the absorbance held at about zero. The potential was then stepped at 80 min to -0.2 V to reduce all complexes in the film to optically detectable $\text{Fe}(\text{bipy})_3^{2+}$. The contribution from Fe^{3+} , which was unobservable up to this point, can now be seen as the difference in absorbance between the curves for the two experiments. These results on a mixture of Fe^{2+} and Fe^{3+} demonstrate proof of concept for this strategy for speciating iron with the spectroelectrochemical

sensor. However, the magnitude of the optical signal for Fe^{3+} ($A_{\text{total}} - A_{\text{open circuit}}$) is less than expected for this equimolar mixture, suggesting that separate calibration curves may be needed for Fe^{2+} and Fe^{3+} . This difference in response may be due to the more complex coordination chemistry for Fe^{3+} described above and differences in the partition rates and partition coefficients of Fe^{2+} and Fe^{3+} .

CONCLUSIONS

Quantitative sensing of ferrous and ferric ions using a spectroelectrochemical sensor has been demonstrated. Sensor response is affected by film thickness and concentration of bipy preloaded into the Nafion film. The relationships among film thickness, sensitivity, and response time will need consideration for any application. Detection limits of $0.6 \times 10^{-6} \text{ M}$ for Fe^{2+} and $2 \times 10^{-6} \text{ M}$ for Fe^{3+} were achieved with relatively thick films (300 nm) and a 30 min sampling time from a quiescent solution. However these limits can probably be pushed farther down by circulating the sample through the ATR cell, as demonstrated by our group recently for other systems.¹⁵ This would also improve response time. Also, replacing bipy with a ligand that acts as a stronger chromophore for Fe^{2+} would improve sensitivity. However, replacement ligands would also need the important property of strongly partitioning into a film on the sensor in order to be preloaded. These improvements would be important for those applications involving nanomolar and lower iron concentrations such as in some samples of seawater and fresh water.^{16,17}

Careful manipulation of the potential applied with simultaneous optical detection enables Fe^{2+} to be distinguished from Fe^{3+} , which is the first step in developing a sensor for speciating the two oxidation states in a mixture. However, the sensor response needs further studies in order to be optimized with respect to the analysis of iron mixtures.

Another important factor in the application of the sensor for the detection of iron in real samples such as natural water is its selectivity with respect to other metal ions in the sample. Other metal ions that partition into the film and coordinate with bipy would need to be selected against by their difference in electrochemical and spectral properties from those of $\text{Fe}(\text{bipy})_3^{2+}$.

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