# Ultrathin Polypeptide Multilayer Films for the Fabrication of Model Liquid/Liquid Electrochemical Interfaces

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Ultrathin (<20 nm) polypeptide multilayer films are assembled by the electrostatic adsorption of alternating monolayers of poly(L-lysine) and poly(L-glutamic acid) onto carboxylic acid terminated alkanethiol-modified gold surfaces. These polypeptide multilayer films are hydrophilic, can bind electroactive anions such as ferri/ferrocyanide, and are stable when immersed in organic solvents such as 1,2-dichloroethane (1,2-DCE). A combination of ex situ polarization−modulation Fourier transform infrared reflection−absorption spectroscopy (PM-FTIRRAS) and surface plasmon resonance (SPR) measurements is used to characterize the film deposition and the incorporation of $D_2O$ and electroactive ions. Electrochemical cycling of the polypeptide films in 1,2-DCE is used to reversibly oxidize the ferrocyanide ions in the film, and in situ PM-FTIRRAS measurements demonstrate that more than 95% of the ferrocyanide ions can be converted to ferricyanide without loss to the organic phase. These ultrathin films will be used to study both ion and electron transport across the film/1,2-DCE interface.

## I. Introduction

The study of liquid/liquid (L/L) electrochemical interfaces has proven to be extremely valuable in the elucidation of ion and electron transport processes across L/L interfaces, potential distributions across the L/L and gel/liquid interfaces that are used in ion selective electrodes, and the mechanisms of phase transfer catalysis reactions.[1−3] Electrochemical measurements at the interface between two immiscible electrolyte solutions (ITIES) are also directly relevant to the study of the ion transport and photochemical processes that occur in biological and biomimetic membranes. To date, most L/L electrochemical studies have employed two bulk liquids such as water and an organic electrolyte solution (e.g., tetrabutylammonium tetraphenylborate in 1,2-dichloroethane). A great number of electrochemical methods have been used to investigate the structure and charge transport processes at the ITIES,[1−3] and recently, a variety of surface-sensitive spectroscopic measurements such as optical second harmonic generation have been employed to study these interfaces.[4−6] In general, the types of spectroscopic techniques that are applicable to the study of L/L systems are limited because there are problems with delivery of the light to the interface and an overwhelming background contribution in nonsurface selective spectroscopies from the two adjacent bulk phases. Moreover, the interpretation of the L/L electrochemical interfacial processes must account for the simultaneous diffusion of species from or to both bulk phases.

A solution to both the spectroscopic access and ion diffusion problems associated with L/L electrochemical interfaces would be to make one of the two phases an ultrathin film. Along these lines, some L/L electrochemical research has been performed previously in which one of the two phases was replaced by either agar or polymer gels.[7−9] Very recently, Shi et al.[10] have employed a trapped thin (ca. 30 $\mu$m) organic layer on a graphite electrode immersed in an aqueous solution for the study of electron transfer across this L/L (film/water) interface. Of course, there have been many studies of electroactive thin films at electrode surfaces, and the most common methods of making these thin films have been polymer spin coating and Langmuir−Blodgett (LB) multilayers.[11,12] A more recent method for the fabrication of thin films is to use sequential self-assembly to form multilayer structures. Two examples of this self-assembly process are the zirconium phosphonate multilayers employed by Lee et al.[13] and the electrostatic layer-by-layer (LbL) assembly of polyelectrolyte ions into thin films by Decher et al.[14−20] The electrostatic formation of LbL multilayers is a particularly versatile method for making ultrathin films that can include bioactive,[21−24] photoactive,[25,26] and electroactive species.[27]

In this paper, we demonstrate that by using the LbL self-assembly method with two hydrophilic polypeptides, the polycation, poly(L-lysine) (pLys), and the polyanion, poly(L-glutamic acid) (pGlu), we can fabricate ultrathin polypeptide films on alkanethiol-modified gold electrodes which can hold water and contain electroactive species. A combination of polarization−modulation Fourier transform infrared reflection−absorption spectroscopy (PM-FTIRRAS) and surface plasmon resonance (SPR) film thickness measurements is used to characterize the ultrathin films. Figure 1 shows a schematic diagram of the intended structure of the electroactive polypeptide multilayer. The polypeptide film is grown on a primer self-assembled monolayer of 11-mercaptoundecanoic acid (MUA), and can range in thickness from 1.5 to 20 nm (corresponding to 1−10 polymer layers). Ex situ PM-FTIRRAS measurements are used to determine that the films contain water and can have ferri/ferrocyanide ions incorporated into them. An additional set of in situ PM-FTIRRAS measurements during the electrochemical cycling of these films in 1,2-DCE shows that the ferrocyanide ions can be reversibly oxidized without any loss of ferri/ferrocyanide ions to the organic phase. This work is an extension of our previous studies of the electrostatic adsorption of pLys onto chemically modified gold surfaces[28] and the electrostatic adsorption of pGlu to the water/1,2-DCE interface.[29] These multilayer films will be used to study ion and electron transport

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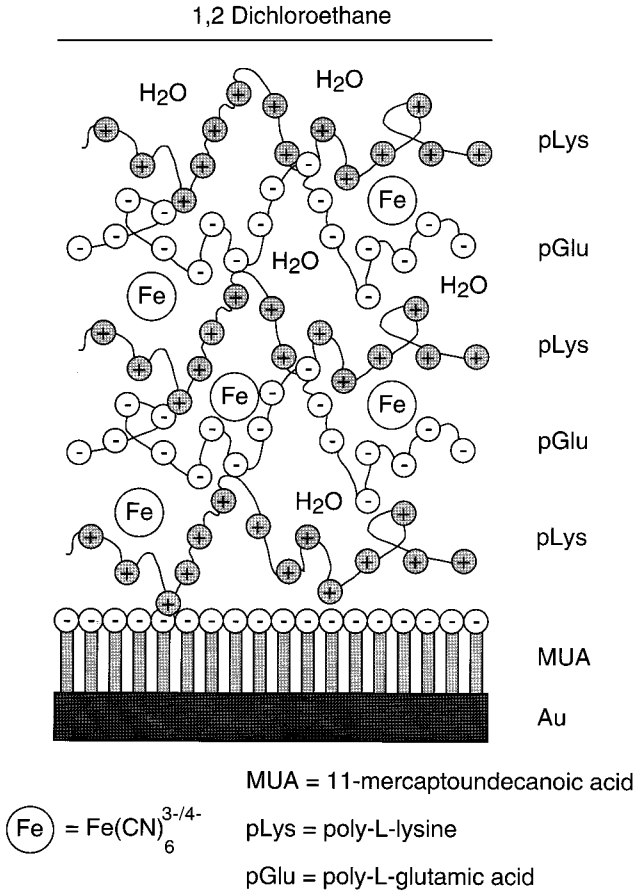


**Figure 1.** Schematic representation of a thin polypeptide multilayer film assembled on a negatively charged MUA-modified gold surface by the sequential adsorption of positively charged pLys and negatively charged pGlu. Water and ferri/ferrocyanide ions can be incorporated inside the film. Some small counterions are omitted for clarification. A L/L interface is formed when the multilayer film is immersed in 1,2-DCE.

across the ultrathin film/1,2-DCE interface with both spectroscopic and electrochemical methods.

## II. Experimental Considerations

Poly-L-lysine (molecular weight, 34 300) and poly-L-glutamic acid (molecular weight, 95 000) were purchased from Sigma. 11-Mercaptoundecanoic acid (MUA) (Aldrich), (3-mercaptopropyl)trimethoxysilane (MPS) (Aldrich), potassium ferricyanide ($K_3(Fe(CN)_6$) and potassium ferrocyanide ($K_4(Fe(CN)_6$) (Fluka), NaOH (Fluka), HCl (Fisher), $Na_2HPO_4 \cdot 2H_2O$ (Fluka), $NaH_2PO_4 \cdot H_2O$ (Fluka), tetrabutylammonium tetraphenylborate (TBATPB) (Fluka), tetrabutylammonium chloride (TBACl) (Fluka), 1,2-dichloroethane (1,2-DCE) (Aldrich), and absolute ethanol (Aaper) were all used as received. Other chemicals used were reagent grade. Millipore filtered water (>17 MΩ) was used for preparation of all aqueous solutions and rinsing.

Thin gold films (47 nm) were vapor-deposited onto BK7 microscope slide covers (Fisher no. 2, 18 × 18 $mm^2$) that had been silanized with MPS as described previously.[30] These samples were immersed into 1 mM ethanolic MUA solutions for at least 24 h before being rinsed with ethanol and water and then dried with a $N_2$ stream.

Solutions of pLys (2 mg/ml) and pGlu (2 mg/mL) were prepared using a 0.1 M phosphate buffer solution. The pH of the buffer solution was adjusted with dilute HCl or NaOH to a pH of 8.0. Multilayer polypeptide films were prepared by the repeated sequential dipping of a MUA-modified gold film into alternating solutions of pLys and pGlu for 30 min. Between each dip, the surface was rinsed with Millipore water and blown dry in a $N_2$ stream. Incorporation of ferri/ferrocyanide ions into the film was carried out by equilibrium of the multilayer film with 1.0 mM $K_3Fe(CN)_6$ and $K_4(CN)_6$ (1:1) solution in 0.1 M phosphate buffer (pH 5.6) for 30 min, followed by the same rinsing and drying procedure.

The chemical structure and thickness of the polypeptide films were monitored using PM-FTIRRAS and SPR angle shift measurements. PM-FTIRRAS spectra in the mid-IR region were obtained with 1000 scans at 4 $cm^{-1}$ resolution on a Mattson RS-1 spectrometer and a narrow-band HgCdTe detector.[31] As in previous papers,[28,31] the PM-FTIRRAS differential reflectivity spectra were converted to absorbance units to compare with standard reflectivity measurements. The thickness of the peptide films was determined from the shifts in the SPR angle in scanning SPR experiments[32,33] using thickness calculations that have been previously described in detail.[28,34] For these films, a five-phase complex Fresnel calculation (BK7 prism/gold/MUA/film/air) was employed using the following indices of refraction: BK7 = 1.515, Au = 0.153 + 3.554i, MUA = 1.45, and the polypeptide film = 1.52.[28]

Electrochemical measurements were conducted in a standard three-electrode cell configuration with the gold slide as the working electrode, a Pt wire counter electrode and a liquid junction built between a 10 mM TBATPB organic solution and a 10 mM TBACl aqueous solution containing an SCE reference electrode. The potential was controlled by an EG & G potentiostat (model 173, Princeton Applied Research) combined with a Universal Programmer wave generator (model 175, Princeton Applied Research). All potentials reported are referenced to an SCE after correction of a liquid junction potential. An in-situ PM-FTIRRAS cell was designed according to Porter et al.[35] with a $CaF_2$ equilateral prism tightly pressed against the Teflon cell body. The gold electrode was mounted in the cell with a screw on the back in order to push the electrode toward the prism, making a thin organic solution layer between the electrode and the prism. In these experiments, a narrow-band InSb detector was used. The reference electrode employed in the in-situ PM-FTIRRAS measurements was a AgTPB, which was prepared by the electrolysis of silver wire in a 10 mM TBATPB 1,2-DCE solution for more than 12 h.[36]

## III. Results and Discussion

**A. PM-FTIRRAS and SPR Characterization of Polypeptide Multilayer Films.** The first step in the fabrication of hydrophilic, electroactive ultrathin polypeptide films for L/L electrochemical studies is the deposition and spectroscopic characterization of the multilayer pLys/pGlu film. The multilayers were deposited onto a gold substrate that was first chemically modified with a self-assembled monolayer of the alkanethiol 11-mercaptoundecanoic acid (MUA). MUA monolayers have been characterized previously,[31] and when exposed to a pH 8.0 aqueous solution, the carboxylic acid groups ionize and form a negatively charged surface onto which a monolayer of pLys can be electrostatically adsorbed.[28] To deposit the multilayer film, the MUA surface was exposed in alternating 30 min immersions to pH 8.0 solutions of pLys and pGlu followed by a rinsing and drying procedure as described in section II. This process led to the formation of an electrostatically adsorbed pLys/pGlu multilayer (see Figure 1) similar to those described by Decher et al.[14−19] Films were created with up to 15 layers; odd-numbered multilayer films were exposed

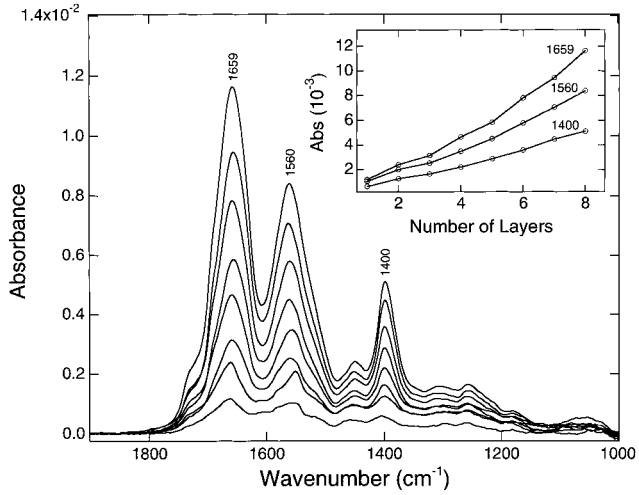


**Figure 2.** PM-FTIRRAS spectra of pLys/pGlu multilayer films assembled on a MUA-modified gold surface. The concentration of the pLys and pGlu solutions was 2 mg/mL in 0.1 M phosphate buffer (pH = 8.0), and the assembly time was 30 min. The inset shows the increase in absorbance of the amide I and II bands and the carboxylate symmetric stretch (1400 $cm^{-1}$) band with the number of layers deposited.

in the final deposition step to pLys, and even-numbered films had pGlu in the final deposition solution. Previous studies[37] have demonstrated that there is substantial interpenetration of the polymers into adjacent layers so that the surface of the multilayer is most likely neither pure pLys nor pure pGlu. However, the surface of a film that is terminated with a pLys adsorption step is expected to be predominantly amine-rich and positively charged, whereas the surface of a film that is exposed to a pGLu solution in the final deposition step is expected to be carboxylate-rich and negatively charged.

A combination of PM-FTIRRAS and SPR measurements was employed to characterize the structure and thickness of the polypeptide films. The ex situ PM-FTIRRAS spectra taken during the fabrication of an eight-layer polypeptide film is shown in Figure 2. Three prominent bands are observed in the spectra: the characteristic amide I and II bands of the polypeptide backbone at 1659 and 1560 $cm^{-1}$, respectively, and the symmetric carboxylate anion stretch of the glutamate residues at 1400 $cm^{-1}$. The positions of all of these bands remain unchanged throughout the deposition process, indicating that the film structure was independent of thickness. The presence of the carboxylate band at 1400 $cm^{-1}$, in lieu of the carbonyl stretch from a protonated glutamic acid residue (normally occurring at 1700 $cm^{-1}$), verifies that the pGlu is in the polyanionic state. Plotted in the inset of Figure 2 are the intensities of the vibrational bands as a function of number of layers deposited. The intensity of all of the bands grows with each additional layer in an increased manner, suggesting that more polypeptide is adsorbed in the later layer deposition steps. This effect is due to more interpenetrating of underlying polypeptide molecules into later deposited layers.

To determine the thickness of the polypeptide films, ex situ scanning angle SPR measurements were performed after the deposition of each layer in the fabrication process. A shift in the SPR angle was observed after each layer deposition, and a fit of the angle shift data with a five-phase complex Fresnel calculation (BK7 prism/gold/MUA/film/air) was used to determine a film thickness (see section II for more details). The results of these Fresnel calculations for the deposition of a 10-layer film are listed in Table 1; the total film thickness ranged from 1.5 nm (1 layer) to 20 nm (10 layers). Figure 3A plots the

**TABLE 1: Total Film Thickness of pLys/pGlu Multilayer Films Deposited at Three Different pHs on MUA-Modified Gold Surfaces**

| | total thickness of pLys/pGlu films (nm)[a] | | |
|---|---|---|---|
| number of layers | pH = 5.6 | pH = 8.0 | pH = 9.4 |
| 1 | 0.75 | 1.5 | 2.0 |
| 2 | 1.7 | 2.4 | 3.0 |
| 3 | 2.8 | 4.1 | 5.3 |
| 4 | 4.0 | 5.3 | 6.6 |
| 5 | 5.8 | 7.3 | 9.6 |
| 6 | 7.3 | 8.9 | 11.4 |
| 7 | 9.8 | 11.7 | 15.0 |
| 8 | 12.4 | 13.6 | 16.7 |
| 9 | 16.1 | 17.7 | 20.5 |
| 10 | 19.2 | 20.0 | 22.8 |

[a] The film thicknesses in this table are determined from SPR measurements and are accurate to ±10%.

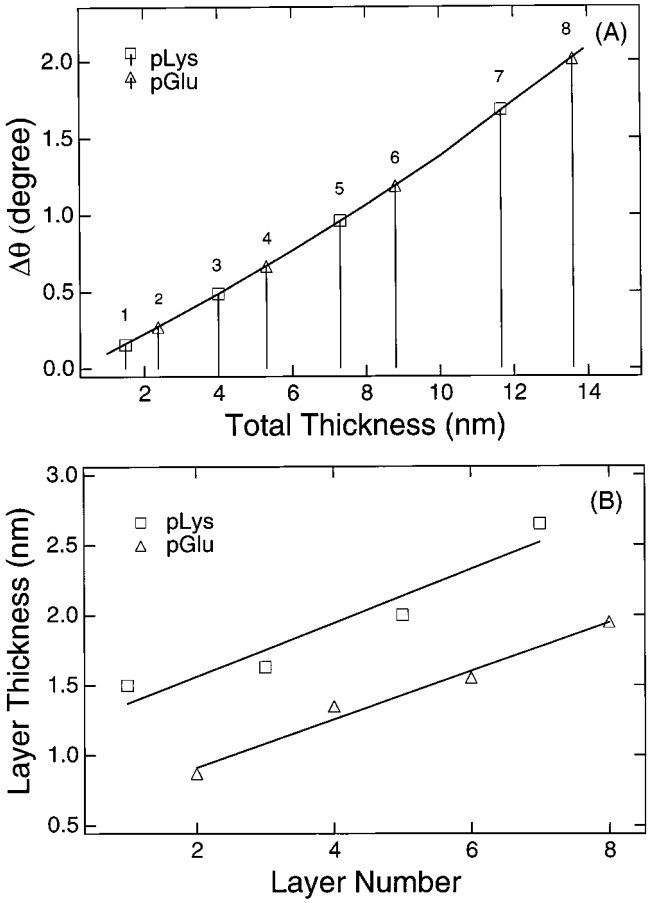


**Figure 3.** (A) SPR angle shifts vs the total film thickness for the assembly of an eight-layer pLys/pGlu multilayer film. The squares and triangles are experimental data, and the solid line is the result of a five-phase Fresnel calculation. (B) Individual layer thickness vs layer number. Notice that each pLys layer is thicker than the corresponding pGlu layer and the thickness of both layers increases with layer number.

shift in SPR angle observed after the deposition of each of the first eight polypeptide layers versus the total calculated film thickness, and Figure 3B plots the additional film thickness added with each new layer during the film deposition process. The following observations can be made from the SPR data in parts A and B of Figure 3: (i) in agreement with the PM-FTIRRAS data, the additional film thickness added with each new layer increases as the total number of layers increases; (ii) the pLys layers are thicker on average than the pGlu layers. A closer examination of the PM-FTIRRAS data reveals that

the difference between the thickness of the pLys and pGlu layers is also present in the FTIR data, and leads to the very slightly jagged appearance of the lines in the inset of Figure 2. The increase in layer thickness during deposition has been observed previously in other LbL multilayers[38] and was observed in all of the pLys/pGlu films that were prepared in the present studies.

Because the side chains of pLys and pGlu are weak bases and weak acids, respectively, the pH must be set correctly for film formation to occur. Rubner et al.[37] have shown that the thickness of weak acid films depends strongly upon solution pH. The polypeptide films described above were prepared from solutions buffered to a pH of 8.0. Also shown in Table 1 are SPR thickness measurements for films that were prepared from solutions buffered to pHs 5.6 and 9.4. The pH sensitivity of film thickness of weak polyelectrolytes adsorption arises from considerable variations of charge density of polyelectrolytes when a dipping solution pH changes around p$K_a$ values. The thickness of the eight-layer film at pH 9.4 was 24% greater than the film fabricated from pH 8.0 solutions, and the eight-layer film created with pH 5.4 solutions was 10% thinner. Since the operating pH values (5.6, 8.0, and 9.4) are within the p$K_a$ range of pGlu (4.9) and pLys (10.5), we expect that the vast majority of the residues on both of the polypeptides are charged and that the variations in the film thickness with solution pH in this range should not be very large. However, no LbL assembly was possible from solutions with a pH less than 4.4 or greater than 10.6. Moreover, for all pH solutions used, the additional thickness from a pLys layer was greater than that from a pGlu layer. The relative thickness of the pLys and pGlu layers could conceivably depend on several factors: residue size, molecular weight (34 kDa for pLys and 95 kDa for pGlu), and solution pH. We must conclude from our experiments at different pHs that the difference in pLys and pGlu layer thickness is due to the intrinsic structure of the pLys polycation relative to the pGlu polyanion and not the pH of the deposition solutions.

**B. Ex Situ PM-FTIRRAS Characterization of Water and Ion Incorporation.** The second step in the fabrication of the ultrathin electroactive polypeptide films was the incorporation of water and electroactive ions. Once again, the incorporation of these species was monitored with ex situ PM-FTIRRAS experiments. Figure 4 (inset) plots the OD stretching region of the PM-FTIRRAS spectrum of an eight-layer film after immersion into $D_2O$ for 1 h followed by drying with a nitrogen stream. The bands at 2468 $cm^{-1}$ and 2420 $cm^{-1}$ are assigned to the OD stretches of $D_2O$ and clearly indicate that water ($D_2O$) has been incorporated into the film. The hydrophilicity of these polypeptide films is consistent with hydration data of pLys and pGlu; the amount of water uptake by these two polymers can be as high as 20% (w/w) for solid pGlu and 50% (w/w) for solid pLys.[39] In addition, the intensity of the NH band at 3300 $cm^{-1}$ (not shown) of secondary amines in the polypeptide backbone decreased after the film was exposed to $D_2O$, demonstrating that the protons on the amide backbone are labile. Figure 4 also plots the OD stretching band intensity at 2420 $cm^{-1}$ as a function of total number of layers. The amount of water incorporated in the thin film increased with film thickness, demonstrating that the interior of the polypeptide multilayer is still accessible to aqueous solution after the multilayer assembly process.

Contact angle measurements verified that the polypeptide multilayers were very hydrophilic. For example, a seven-layer (pLys terminated) polypeptide film had a contact angle of 10.5° ± 1.6°, and an eight-layer (pGlu terminated) polypeptide film exhibited a contact angle of 28.1° ± 0.8°. These contact angles

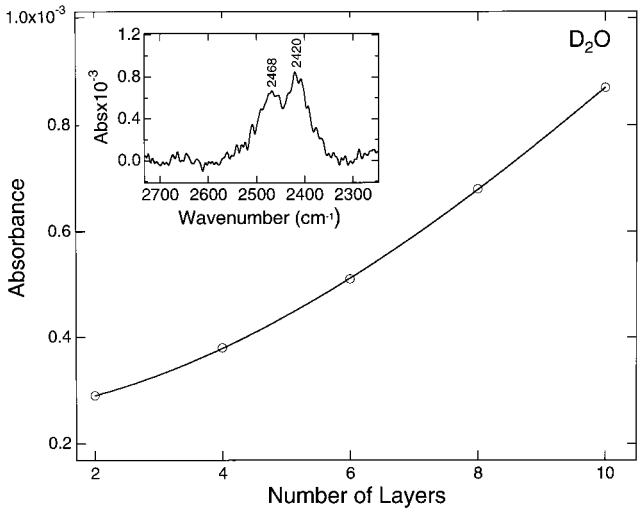


**Figure 4.** Incorporation of $D_2O$ into pLys/pGlu multilayer films consisting of 2, 4, 6, 8, and 10 layers as monitored by the absorbance of $D_2O$ at 2420 $cm^{-1}$. Notice that the amount of water ($D_2O$) incorporation into the film increases with the film thickness. The conditions used to assemble the pLys/pGlu film are described in the Experimental Section. The inset shows the PM-FTIRRAS spectrum obtained after a 10 layer film was immersed in pure $D_2O$ for 1 h.

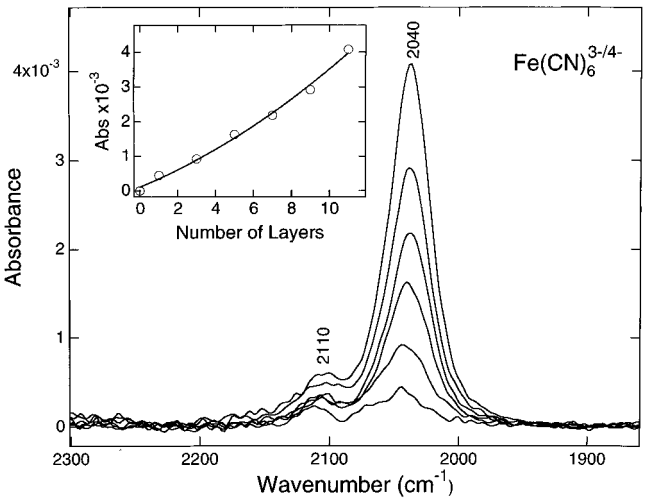


**Figure 5.** PM-FTIRRAS measurements of ferri/ferrocyanide incorporation into pLys/pGlu multilayer films consisting of 1, 3, 5, 7, 9, and 11 layers. Each film was immersed in a 1.0 mM ferri/ferrocyanide solution (0.1 M phosphate buffer, pH = 5.6) for 30 min, rinsed with water, and dried under a $N_2$ stream. The inset plots the change in absorbance of the CN stretch band (2040 $cm^{-1}$) with the number of layers adsorbed on the surface. The ferri/ferrocyanide incorporation was carried out after the corresponding numbers of pLys and pGlu layers were assembled at pH = 8.0.

did not change significantly with film thickness but consistently depended upon the nature of the outermost layer (pGlu or pLys). This behavior has been seen previously by Yoo et al.[37]

To create electroactive films, redox-active ions must be incorporated into the polyelectrolyte multilayer films. By immersion of the polypeptide multilayers into 1 mM ferri/ferrocyanide (1:1) solutions (0.1 M phosphate buffer pH = 5.6) for 30 min, the electroactive species were incorporated into the film. The uptake of electroactive ions into LbL multilayers has been observed previously by Lowy et al.[27] Figure 5 plots the PM-FTIRRAS spectra of six different polypeptide multilayer films of 1−11 layers after immersion into the ferri/ferrocyanide solution. The strong band observed at 2040 $cm^{-1}$ is assigned to the CN stretch of ferrocyanide ion. A weaker band due to

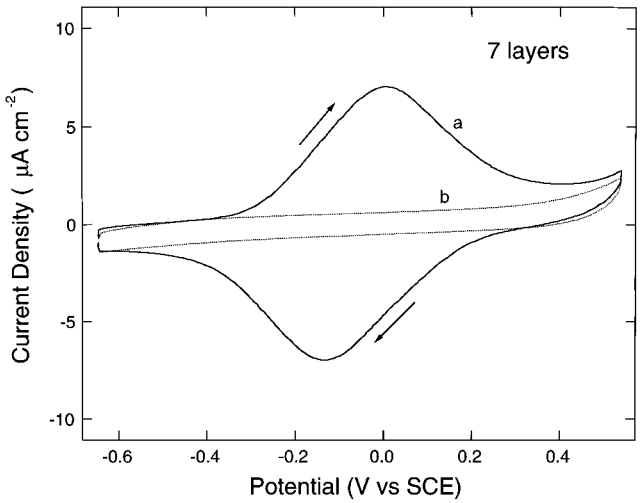


**Figure 6.** Cyclic voltammograms of a seven-layer Plys/pGlu film (12.4 nm) (a) with and (b) without loaded ferri/ferrocyanide ions obtained in 10 mM TBATPB 1,2-DCE solution. The potential scan rate was 50 mV $s^{-1}$.

the ferricyanide ion was observed at 2110 $cm^{-1}$. The greater incorporation of $Fe(CN)_6^{4-}$ into the polypeptide film compared to $Fe(CN)_6^{3-}$ ions is attributed to the stronger interaction of the more highly charged $Fe(CN)_6^{4-}$ ions with ammonium residues of the pLys. The intensity of these bands increased with the number of layers as plotted in the inset of Figure 5. For a one-layer polypeptide film with a thickness of 1.5 nm, the intensity of the ferrocyanide CN band at 2040 $cm^{-1}$ was $0.45 \times 10^{-3}$. For an 11-layer film with a thickness of 23 nm (15 times thicker than the one-layer film), an absorbance of $4.1 \times 10^{-3}$ was measured; this is about 9 times the CN band intensity observed for the one-layer film. These results show that ferri/ferrocyanide ions are incorporated into the majority of the bulk of the film (as opposed to just the outermost polymer layer), a conclusion that is consistent with similar observations of electroactive ions incorporation into other polyelectrolyte multilayers.[27]

**C. Electrochemistry and In Situ PM-FTIRRAS Characterization Experiments.** After fabrication of the electroactive polypeptide film, a L/L electrochemical interface was formed by immersion of the thin film in a 1,2-DCE solution of 10 mM TBATPB. Cyclic voltammetry was performed in a standard three-electrode configuration as described in section II. Electrochemical cycling of the ferri/ferrocyanide ions in the polypeptide film can only occur if there is interfacial ionic transport across the film/1,2-DCE interface. Figure 6 shows the cyclic voltammograms (CVs) of a seven-layer film (11.7 nm) with (solid line) and without (dotted line) ferri/ferrocyanide ions. Several points can be noted about these CVs: (i) virtually no faradaic current was observed in the absence of ferrocyanide; (ii) the faradaic current exhibited peaks on both positive and negative scans with approximately the same height and area, indicating the reversible reduction and oxidation of the ferri/ferrocyanide ions in the thin film; (iii) an integration of the peak areas resulted in a surface density of $4.0 \times 10^{-10}$ mol/$cm^{-2}$ of ferri/ferrocyanide ions in this seven-layer film; (iv) the cathodic and anodic peak potentials are not the same, indicating some solution resistance, diffusion, and possible electrode kinetics (preliminary results indicate that solution resistance is the primary factor in the peak potential asymmetry); (v) the shape of the CV was very stable for many potential cycles; (vi) a comparison of ex situ PM-FTIRRAS spectra recorded before and after the electrochemistry showed no changes in the intensity

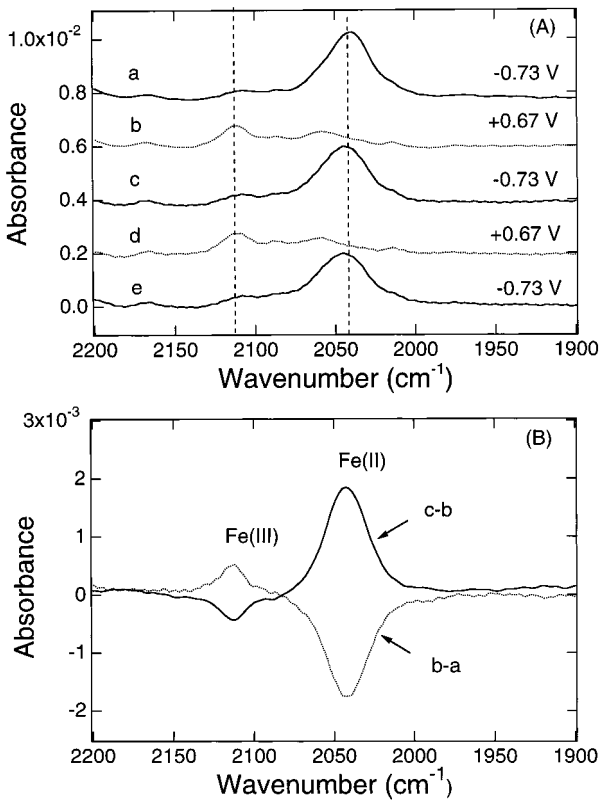


**Figure 7.** (A) In-situ PM-FTIRRAS spectra monitoring the oxidation and reduction of ferri/ferrocyanide ions incorporated into a nine layer pLys/pGlu film. The film was brought into contact with a 10 mM TBATPB 1,2-DCE solution and held at a potential of −0.73 and 0.67 V for 5 min, respectively, before the spectrum was taken. A total of 500 scans for each spectrum at 4 $cm^{-1}$ resolution was recorded. (B) The differential spectra obtained for reduction (c−b) and oxidation (b−a) of ferri/ferrocyanide ions.

of the ferrocyanide CN band at 2040 $cm^{-1}$. A full discussion of the electrochemical properties of this interface will be given in a subsequent paper.

In a final set of experiments, in situ PM-FTIRRAS experiments under potentiostatic control were performed in order to verify that the electrochemical cycling of the ferri/ferrocyanide ions in the film was complete. Figure 7 plots the in situ PM-FTIRRAS spectra obtained at two applied potentials, −0.73 V and +0.67 V, that are, respectively, well above and below the redox potential for the ferri/ferrocyanide ions. The electrode was held at each potential for 5 min after which the spectra were obtained. At −0.73 V (curve a), the ferrocyanide CN band at 2040 $cm^{-1}$ dominates, and at +0.67 V the ferrocyanide CN band is gone and is replaced by the ferricyanide CN band at 2110 $cm^{-1}$. The ferrocyanide and ferricyanide bands are not of equal intensity due to differences in their absorption cross sections, but since the PM-FTIRRAS spectra require no background subtraction (in contrast to potential difference FTIR measurements),[40] we can state unequivocally that more than 95% of the spectroscopically accessible ferrocyanide ions in the film are converted to ferricyanide in 5 min. This conversion process was repeated reversibly many times without a loss of ferri/ferrocyanide ions to the 1,2-DCE solution (see Figure 7). However, the total conversion of the redox couple from one oxidation state to another could not be achieved in the same time period when the concentration of the supporting electrolyte in the organic phase was decreased to 1 mM TBATPB. We attribute this incomplete conversion to increased solution resistance in the 1,2-DCE.

## IV. Conclusions

In this paper, it has been demonstrated that a polypeptide multilayer thin film can be prepared from pLys and pGlu using a sequential LbL electrostatic adsorption method. These ultrathin polypeptide films are very hydrophilic, and can incorporate electroactive ions such as ferrocyanide. Both electrochemical and in situ PM-FTIRRAS results confirm that the ferri/ferrocyanide ions in the film can be completely and reversibly reduced and oxidized when in contact with a 1,2-DCE solution. Such electrochemistry can only occur if there is interfacial ionic transport across the film/1,2-DCE interface. Future experiments will study both ion transport and electron transport across this interface with PM-FTIRRAS, EM-SPR, and other spectroscopic measurements.

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