Homogeneous, sub-micron thick bioactive films containing redox hydrogels and the enzyme laccase were prepared on gold-coated glass slides via convective self-assembly. Sub-micron film thickness (10–50 nm) allowed higher mediator and enzyme utilization by lowering transport limitations. This study demonstrates control of redox hydrogel film morphology by manipulating hydrophilicity, spreading and wetting properties of the hydrogel/electrode interface. Use of a non-ionic surfactant (Triton X-100) enabled spreading of enzyme containing precursor solutions with no negative impact on enzyme activity. Ellipsometry was used to measure film-swelling properties of these systems, square wave voltammetry was employed to estimate the amount of electroactive redox polymer, and cyclic voltammetry enabled estimation of apparent electron diffusivity. This study impacts the design of thin, catalytic films for bioelectronic applications.

© The Author(s) 2014. Published by ECS. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 License (CC BY, http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse of the work in any medium, provided the original work is properly cited. [DOI: 10.1149/2.0121413jes] All rights reserved.

Manuscript submitted September 9, 2014; revised manuscript received October 24, 2014. Published November 6, 2014. This paper is part of the JES Focus Issue in Recognition of Adam Heller and His Enduring Contributions to Electrochemistry.

Thin-film technologies enable increased sensitivity and selectivity in electrochemical sensors and biosensors, novel engineered nanoscale materials and micro-engineered catalytic materials. Fabrication of thin films eliminates mass transfer limitations leading to fast response and high utilization. Efforts to prepare homogeneous films with high electro-active species utilization have included various approaches; these include co-immobilization of biocatalyst and redox mediators on the electrode surface using Nafion, a sol-gel entrapment, a cross-linking agent such as poly(ethylene glycol diglycidyl ether) (PEGDGE), electrodeposition or via layer-by-layer technique. The choice of immobilization technique depends on the enzyme, the electrode surface, and the specific operational requirements.

Biomimetic approaches toward encapsulating proteins in well-defined meso-porous structures have marked new avenues for synthesis and assembly of nanomaterials. Convective self-assembly, first implemented by Denkov et al., and improved by Prevo and Velev, is a well-established approach particularly to cast templated meso-porous films especially for applications such as membrane-based separators, selective catalysis and sensors. This technique relies on the principle of solvent evaporation leading to the formation of well-defined micro-patterned films, which could be implemented in device fabrications. The mechanism of controlled casting of these films using this technique is discussed in detail by Yuan et al. The film formation velocity, porosity of the deposited films, the volume fraction of particles in suspension and convective contribution to molecular transport largely control the thickness of the films cast. Sub-micron sized thin films are ideal as both practical and analytical electrodes. High utilization of electro-active species (enzyme and mediator), ability to accurately estimate electron transport properties and electrode kinetic parameters, and controllable film morphology make these electrodes a promising candidate for practical bioelectronic devices. The thickness of these redox hydrogel films impacts estimation of electron transport and kinetic parameters. Various well-established techniques are available for determination of dry film thickness with precision, such as quartz crystal microbalance (QCM), profilometric techniques, scanning electron microscopy (SEM), atomic force microscopy (AFM), confocal microscopy, and ellipsometry. Of these available techniques, confocal microscopy is used to determine thickness measurements of sub-micron sized films.

Estimation of dry film thickness is often of limited value, because these charged redox hydrogels undergo swelling (or de-swelling) when exposed to ionic aqueous media, typical of experimental environments. The degree of film swelling depends on several properties of the film, including the extent of counterion flux into or out of these films in order to maintain the charge electro-neutrality. Since the difference between the wet and the dry film thicknesses could be large, it is essential to estimate the wet film thickness, for which only few techniques are appropriate. Profilometric techniques and AFM cannot be used for these estimations because these swollen hydrogels are too soft to be measured directly. Ellipsometry, QCM, and environmental scanning electron microscopy are some of the techniques that allow such thickness determinations of swollen hydrogels. In this work, ellipsometric techniques have been adapted to directly measure film swelling of these thin-film electrodes on gold-coated glass substrate.

Electron transfer between the electrode and enzyme active sites can be achieved by either direct electron transfer (DET) or mediated electron transfer (MET). DET requires specific orientations of the enzymes with their active sites close to the electrode to facilitate electron transfer through the electron tunneling distance. MET utilizes small redox active centers to facilitate electron transfer to the enzyme active site eliminating orientation dependency and enabling multi-layer catalyst activation. This work explored and demonstrated DET as well as MET for oxygen reduction reaction (ORR) using laccase enzyme from Trametes versicolor (TvL).

The objective of this work is to prepare uniform sub-micron scale redox hydrogel films for bioelectronic applications. We demonstrate the preparation uniform sub-micron scale redox hydrogel films, of thickness 10–50 nm, using convective self-assembly. This is the first report where the convective self-assembly technique is used to cast sub-micron thick redox hydrogel films where co-immobilization is achieved using a chemical cross-linking agent (PEGDGE). Control on redox hydrogel film morphology and thickness is achieved by manipulating substrate hydrophilicity, spreading and wetting ability of the precursor solutions and its drying rate. Use of a non-ionic surfactant (Triton X-100) enables improved spreading of enzyme-containing precursor solutions with no negative impact on enzyme activity. Thickness of the resulting films is obtained via ellipsometry,

*Electrochemical Society Active Member.
'E-mail: DChakraborty@ dow.com
and mediator utilization, apparent electron diffusivity and overall activity are obtained by electrochemical techniques.

**Experimental**

**Chemicals and reagents.—** Laccase from *Trametes versicolor* (TvL) was purchased from Sigma-Aldrich (St. Louis, MO) and purified as previously reported. 41 Sodium citrate and citric acid were obtained from Fisher Chemical (Suwanee, GA). Poly (ethylene glycol) diglycidyl ether (PEGDGE, Polysciences Inc., Warrington, PA) was used as received. All solutions were made with ultra filtered DI water (18 MΩ·cm). Three redox polymers A, B and C (structures reported in Supporting Information, Figure S1) were synthesized in-house according to reported procedures.22,27 Their structures and properties are tabulated in Table I. These sets of PVI-Os based redox polymers were chosen because of their differences in physical and electrochemical properties, primarily the redox potential. Fluorescein isothiocyanate (FITC) and Triton X-100 were purchased from Sigma Aldrich (St. Louis, MO) and used as received. Ultra-pure oxygen, air and nitrogen were obtained from Air Gas Great Lakes Inc. (Lansing, MI). Gold-coated glass slides and plain microscope glass slides were obtained from Fisher Chemical (Suwanee, GA) and were used as the substrate for thin film electrodes.

**Coating solution.—** Solutions containing redox polymers (no enzymes).— 16 μL of Redox polymer solution (RP, 10 mg/ml) was mixed with 3.5 μL of PEGDGE crosslinker (5 mg/ml) to form a solution with RP: crosslinker = 90:10 by weight. To enhance spreading and drying rate, this solution was then diluted with either 3.8 μL pure ethanol and 3.2 μL Millipore water or 7.0 μL pure ethanol, to yield solutions of either 2.5 M or 4.6 M ethanol.

**Solutions containing enzymes and redox polymers.—** 16 μL of RP solution (10 mg/ml), 4.0 μL of laccase solution (21 mg/ml), 3.0 μL of 1.0 wt% Triton X-100 was mixed with 3.5 μL of crosslinker solution (5 mg/ml) to prepare the aliquot for casting the films containing enzymes and redox polymers. Weight percentages of RP: Crosslinker: Triton-X (1% solution) were 54:6:10:29.

**Coating procedure.—** A small drop (1–5 μL) of the precursor solution was placed at the junction of the gold substrate surface and the applicator slide, to form a thin continuous meniscus along the line of contact. Films were coated on the gold electrode by dragging this small volume of liquid confined in the meniscus between two plates at a linear rate of 10–30 μm s⁻¹. For the purpose of this study, the slide angle, θ, was optimized to be 20° ± 2°. Low θ generated a broader liquid meniscus at the glass slide-electrode juncture resulting in thin and long films, while high θ generated thick and short films. The optimal coating velocity for this study was found to be ∼20 μm s⁻¹. Linear velocity > 25 μm s⁻¹ led to retention of the precursor solution at the meniscus. Lower values of v lead to thick films of small area due to relatively fast drying.

Drop sizes of 1 to 5 μL were used for almost all the studies involving redox polymers only. Drop-sizes more than 5 μL resulted in flooding of the meniscus at the applicator-substrate interface. For enzyme-containing systems, 3 μL drop volume was used for all the studies. After formation, all films were dried under ambient conditions in controlled relative humidity (RH < 10%, controlled by flow of dry air) for at least 12 hours before testing.

**Film thickness measurements.—** Atomic force microscopy (AFM) was used to determine the film homogeneity. The measurements in the dry state (scan size: 1 μm, scan rate: 0.50 Hz) were performed in the tapping mode on a Digital Instrument Nanoscope 4 (Santa Barbara, CA) in air using NSC 15 tip (Umasch). A multi-wavelength, fixed angle (75°) spectroscopic ellipsometer (EC270, J. A. Woollam Co., Inc.) with a 75 W Xenon light source (LPS 300) was used to measure the dry and buffer soaked film thicknesses under ambient conditions. To obtain the film thickness, a three layer
model (air-film-gold) was applied to the measured reflectivity spectra as described below.

**Ellipsometry model.**—For translucent films, such as films containing Os redox centers or Cu active sites, uncertainty of film thickness measurements arise due to incomplete knowledge of film refractive index. In this system, refraction can be considered in terms of the refraction of the surrounding medium ($n_1$), the film ($\tilde{n}_2$), and the gold substrate ($n_3$), where the tilde ($\sim$) indicates complex quantities. The film refractive index, $\tilde{n}_2$, depends on the wavelength of the plane polarized light, concentration of the absorbing sites, film inhomogeneity and thickness and ambient conditions. Translucent films have a complex refractive index given by:

$$\tilde{n}_2(\lambda) = n_2(\lambda) + ik_2(\lambda)$$

where $n_2$ and $k_2$ are the real and imaginary components, respectively, of the film refractive index, both of which are a function of wavelength ($\lambda$). The extinction coefficient, $k_2$, is zero for transparent films, but is significant for the present materials. The Cauchy model$^{34,35}$ was used to fit the ellipsometric data for these redox polymer thin films:

$$n_2(\lambda) = A_n + \frac{B_n}{\lambda^2} + \frac{C_n}{\lambda^4}$$

$$k_2(\lambda) = \alpha \exp \left[ \beta \left( \frac{1}{\lambda} - \frac{1}{\gamma} \right) \right]$$

where $A_n$, $B_n$, and $C_n$ are constants of refraction, $\lambda$ is the wavelength, $\alpha$ is the extinction coefficient, $\beta$ is the exponent factor, and $\gamma$ is the band edge.

The measured ellipsometric parameter, $p$, represents the complex reflectivity ratio of in-plane and out-of-plane polarized light. It is composed of an amplitude ratio, $\tan(\psi)$, and phase shift ratio, $\Delta$:

$$p = \tan(\psi) \exp(-i \Delta)$$

These parameters, $\psi$ and $\Delta$, in turn depend on the measuring wavelength ($\lambda$), refractive indexes of the layers ($n_1$, $\tilde{n}_2$, $n_3$), film thickness, $d$, and the angle of incidence ($\theta_0 = 75^\circ$).

Initial estimates of $A_n$ were obtained from the reported values in the work by Larsson (Table S1, Supporting Information)$^{32}$ for both thick and thin films, with the assumption that the refractive index would not be much affected by replacing the poly(vinylpyridine) polymer backbone with poly(vinylimidazole) (PVI). Larsson demonstrated that for poly (4-vinylpyridine Os(bpy)$_2$Cl)$_2$ with varying PVP:Os ratio, $n_2$ remains fairly constant while $k_2$ is sensitive to Os content, both for the thin and thick films (dry or in 0.1 M KCl).$^{32}$ The polymer backbone (PVI or PVP) being fairly transparent, relative proportion of the absorbing Os redox centers in these films primarily controls molar absorptivity, $k_2$. For a swollen film, Os content is dilated as compared to the dry films. During this initial estimate, only the film thickness ($d$) was fitted at 44 different wavelengths. A representative plot of the experimental and model data for redox hydrogel film with polymer C, estimated with the assumed values of $A_n$ and $\alpha$ from literature for the films in both dry and buffer soaked states is represented in Figure 1a and 1c. The values of $B_n = 0.01$, $C_n = 0$, $\gamma = 4000$ and $\beta = 1.5$ was fixed for all measurements. Once the preliminary estimate of the film thickness ($d$) was obtained, $n_2$ and $k_2$ were fitted at all wavelengths to have a better fit of the data and obtain the film refractive index for the various cases under consideration (Fig. 1b and 1d). Ellipsometric calculations were done using WVA32 software.

**Electrochemical studies.**— Electrochemical studies were conducted in 100 mM citrate buffer (pH 4, 40°C). Thin film electrodes on gold-coated glass slides or drop deposited films on glassy carbon RDEs were used as working electrodes. The counter electrode was platinum wire, with an Ag/AgCl reference electrode (BAS, West Lafayette, IN). Data were collected with a VSP Potentiostat and EC-Lab software (Bio-Logic USA, LLC, Knoxville, TN). All measurements, including the RDEs were taken at 0 rpm under constant sparging of N$_2$ and/or O$_2$. Electroactive mediator loading was estimated in N$_2$-saturated buffer using square-wave voltammetry (SWV). Pulse height of 25 mV and a pulse width of 50 ms were used for SWV. The mediated bioelectrodes were scanned in the potential range of 0.2 to 0.75 V SHE for RP-A and 0.4 to 1.05 V SHE for systems containing RPs B and C. Apparent electron diffusion was analysed by cyclic voltammetry at 50 to 1000 mV s$^{-1}$. Working electrode potentials were corrected for uncompensated resistance by measuring the real component of the impedance spectra, $R$, measured at high modulating frequency (200 kHz).$^{12}$ Catalytic activities of TVL toward ORR in mediated and unmediated systems were evaluated under varying O$_2$ fractions under atmospheric pressure (diluted with N$_2$) using chronoamperometry and slow-cyclic voltammetry (1 mV s$^{-1}$).

**Results and Discussion**

**Film thickness estimates.**— Ellipsometry experiments were conducted for the purpose of measuring film thickness as a function of redox polymer (RP) structure, loading, water saturation, and enzyme content. Ellipsometry provided additional data on the index of refraction. Figure 1 provides example ellipsometry spectra for an RP C film under dry and water saturated (wet) conditions. Here, the $\psi(\lambda)$, $\Delta(\lambda)$ spectra are fit the model described above with $A_n$, $\alpha$, and the film thickness, $d$, as fit parameters. The instrument used white light with an unfocussed beam diameter of 1–10 mm. The film thickness measurements were taken at least at 8 to 10 different points on the electrode surface to ensure 2D film thickness homogeneity. It is evident from Fig. 1 that this approach yields excellent fit to the experimental data, under both dry and wet conditions. Estimated refractive index at 631 nm for the redox polymer systems as evaluated in both dry state and in presence buffer are tabulated in Table II. These estimates also demonstrate that the real part of the refractive index does not vary significantly with increasing concentration of the absorbing species (Os); however the absorption term, $k_2$, is sensitive to Os concentration and film thickness (dry/swollen state). The values of $k_2$ as estimated for buffer swollen films are an order of magnitude higher than that reported by Larsson.$^{32}$ This could be directly related to ~10 times higher Os concentration (~2500 mM) in these submicron-sized thin films as compared to the films Larsson reported (~200 mM for the same Os:PVP ratio).$^{32}$

**Comparison of dry and wet film thickness.**— The thickness of films formed by convective self-assembly depend on such factors as the material loading, solvent used, the structure of the redox polymer, and the water content. Figure 2 gives an estimate of film thickness for three different redox polymers, in both wet and dry conditions, as a function of drop volume. Ellipsometric measurements indicate a
Table II. Estimated refractive index of redox hydrogel films as determined by ellipsometry.

| Redox Polymers | PVI:Os | Film thickness (nm) | Dry:2* | Wet:2*×10^2 |
|----------------|--------|---------------------|--------|-------------|
| A             | 12:1   | 9 ± 2               | 1.5 ± 0.01 | 3.9 ± 1     |
| B             | 20:1   | 6 ± 0.3             | 1.6 ± 0.01 | 0.9 ± 0.1   |
| C             | 15:1   | 16 ± 3              | 1.6 ± 0.03 | 3.7 ± 0.5   |

*Reported n and k are estimated values at 631 nm wavelength.

swelling factor of 1.8 ± 0.14, independent of polymer type or loading. 
This may be compared to confocal microscopy results for thicker films 
(1 to 3 µm) on RDEs, where the observed swelling factor was 2.7 ± 0.2.16 A decrease in swelling ratio for sub-micron thick films is consistent with the work of Larsson, who reported swelling factors of 1.9 to 2.7 for thin films of ~100 nm thickness and 2.3 to 3.4 for films of ~500 nm thickness.32 Increased effects of interfacial tension may explain decreased swelling at low thickness.

**Effect of incorporation of enzyme on film swelling.**— For enzyme containing films, a swelling factor of ~1.5 was estimated using ellipsometry, while confocal microscopy gives a factor of ~ 2.15 A comparison of the relative swelling factor of these films with or without enzyme and redox polymers is depicted in Figure 3, using films prepared with Os-free poly(vinylimidazole) (PVI) and with three redox polymers. It is evident that films containing enzymes swell to relatively lesser extent, possibly due to electrostatic interactions between the negatively charged enzymes and positively charged redox polymers. The presence of enzyme in the PVI film reduces swelling to a greater extent than in the RP films. The redox complex may screen electrostatic interactions, resulting in increased relative swelling with laccase incorporation. The presence of enzyme itself may also increase the degree of chemical cross-linking. Table III lists the dry and wet film thicknesses of the enzyme containing films for the redox polymers.

**Direct electron transfer vs. Mediated electron transfer.**— TvL has been shown to exhibit direct electron transfer (DET) on gold.11,46–52 Polarization of the laccase-catalyzed, thin-film electrodes in the presence of O2-saturated electrolyte allowed for the comparison of catalysis via the DET and MET mechanisms. Figure 4a displays oxygen reduction polarization curves for TvL incorporated thin films prepared with RP A and with mediator-free PVI, where it is demonstrated that DET is negligible in the potential range where MET generates significant oxygen reduction current density. At more negative potential, TvL catalyzes O2 reduction by DET in the absence of mediator (Fig. 4b). However, the area-specific activity only modestly exceeds that of bare gold.

Using controlled immobilization strategies, the onset potential for DET may be shifted higher,39,40,53 but DET is not observed under the present, less controlled conditions. This may be further explained by the relatively small fraction of laccase that resides within electron

Figure 2. a) Film thickness estimates redox hydrogel films (RP: Crosskinker:: 90:10 wt%) in dry and buffer soaked (wet, 0.1 M citrate buffer, pH 4) conditions, determined by ellipsometry. The films were obtained through convective self-assembly technique with varying drop size of the precursor solution containing 4.5 M ethanol, and b) Relative swelling of the films obtained for the three different redox polymer systems.

Figure 3. Effect of enzyme incorporation on thin-film polymer hydrogel swelling for three polymer types. 3 µL loading, TvL content: 40 wt% (PVI), 32 wt% (RP A, B, C). Crosslinker content: 10 wt%.

Table III. Film thickness of enzyme containing films (3 µL drop size, \( \theta = 20^\circ, v = 20 \mu \text{m/s} \)).

| Redox Polymers | Area (cm²) | Dry: Film thickness (nm) | Wet (0.1 M Citrate Buffer): Film thickness (nm) | Swelling Ratio |
|----------------|------------|-------------------------|-----------------------------------------------|----------------|
| A             | 3.6 ± 0.48 | 32 ± 5                  | 47 ± 5                                       | 1.5 ± 0.1      |
| B             | 3.2 ± 0.33 | 39 ± 2                  | 52 ± 2                                       | 1.3 ± 0.03     |
| C             | 2.3 ± 0.21 | 16 ± 3                  | 28 ± 5                                       | 1.7 ± 0.1      |
increased mediator utilization. For example, films made with RP B due to electron transport, thin films electrodes display significantly improved mediator utilization in thin film electrodes relative to drop deposited films on RDEs for the three mediated systems. (RP: Crosslinker:: 90:10 wt%)

Figure 5. Comparison of the % active Os content of a thin film electrode and a drop deposited film electrode on a glassy carbon RDE as obtained from SWV (de-aerated 100 mM citrate buffer, pH 4, 40 °C, 0 rpm) for a) RP A, b) RP B, c) RP C. d) demonstrates improved mediator utilization in thin film electrodes compared to drop deposited films on RDEs for the three mediated systems. (RP: Crosslinker:: 90:10 wt%)

S4). Such high utilization limits the achievable improvement obtained in comparing thick and thin films, and RP B films show relatively small improvement compared to RP A and C.

Figure 6 shows the comparison of catalytic current (per mg of TVL) for thin-films and drop-deposited films. By virtue of eliminating film resistance to electron transport and significantly improving mediator utilization, thin films display dramatically increased enzyme utilization in all the three mediated systems.

Note that the thin-film reduction current depends nearly linearly on oxygen concentration, and does not appear to be saturated in a Michaelis-Menten sense. This is likely due to the lack of stirring in these experiments, leading to oxygen mass transfer limitation. However, the average slope of this curve, \( m = 93 \pm 3 \text{ mA m}^{-2} \text{mg}^{-1} \text{TVL} \text{mM}^{-1} \text{M}^{-1} \text{L}^{-1} \text{s}^{-1} \), may be used to assess utilization. Based on a reported value of the bimolecular rate constant, \( k = k_{\text{cat}}/K_s = 200 \text{ s}^{-1} \text{mM}^{-1} \text{M}^{-1} \text{L}^{-1} \text{s}^{-1} \), and assuming the enzyme is saturated in mediator, utilization, \( u \), may be calculated according to

\[
u = m \cdot \frac{MW_{\text{TVL}}}{nFk}
\]

Assuming the enzyme molecular weight, \( MW_{\text{TVL}} = 65 \text{ kDa} \), the calculated utilization, \( u = 7.8\% \). This utilization is low due to oxygen mass transfer limitation, and may be reduced by loss of enzyme from the film, and deactivation at interfaces, possibly due to denaturation, but is significantly greater than that observed at the thick-film RDE.

Estimation of electron transport properties.— In redox polymer electrodes, charge transport via the redox mediator is often one of the rate determining steps, where charge may migrate by self exchange or electron hopping between adjacent redox active sites. 

Electro-active species (mediator and enzyme) utilization.— For both thin-film electrodes and drop deposited films on glassy carbon RDEs, mediator utilization was estimated using square-wave voltammetry (SWV). 

Mediator utilization refers to fraction of redox sites electrically wired. Square-wave voltammetry is a powerful electrochemical tool for estimating charge transferred due to faradaic reactions alone and hence gives a precise estimate of electro-active species present at the electrode surface. 

The integration of the voltammogram provided an estimate of the electrically wired electroactive species at the electrode surface. SWV measurements were conducted in de-aerated 100 mM citrate buffer (pH 4, 40 °C, 0 rpm). Figure 5 represents the relative Os utilization in a thin film as compared to that of thicker films prepared by drop depositing RP precursor solution on a rotating disk electrode. Experiments were conducted under identical conditions. It is demonstrated that by avoiding limitations due to electron transport, thin films electrodes display significantly increased mediator utilization. For example, films made with RP B achieve utilization in excess of 90% at drop loading below 2 μL. This is primarily attributable to the sub-micron film thickness as well as improved film uniformity (Supporting Information, Figure S3 and
$D_m$, via cyclic voltammetry with scan rates from 50 to 1000 mV s$^{-1}$. The peak current densities ($i_{\text{peak}}$) from the cyclic voltammograms were found to vary linearly with the square-root of scan rate indicating semi-infinite diffusion (Figure 7) as expressed by Randles-Sevčík equation:

$$i_{\text{peak}} = 0.4463 \left( \frac{F^3}{RT} \right)^{1/2} n^{3/2} C_m^{1/2} D_m^{1/2} v^{1/2}$$

where, $F$ is Faraday’s constant, $R$ is the universal gas constant, $T$ is the temperature, $n$ is the number of electrons transferred in the redox reaction, $C_m$ is the bulk mediator concentration and $v$ is the scan rate. Table IV tabulates an estimate of $D_m$ as a function of drop size. The estimates of $D_m$ for thin film systems were found to be three orders of magnitude lower than that of the previously reported data for RDEs. The most probable explanation for such low electron diffusion coefficients in these sub-micron sized films could be the fact that these films swell to a relatively lower extent than the drop deposited films, which leads to restricted mobility of the redox active sites and hence lower diffusion coefficients. No significant difference in the film thickness measurements (in buffer) was observed before and after the experiments indicating negligible mass loss from the electrode surface due to the presence of buffer during the course of the experiments.

**Conclusions**

The convective self-assembly technique was applied to cast redox hydrogel films incorporating enzymes as bioactive electrodes of uniform sub-micron thickness. These thin film electrodes have high mediator and enzyme utilization by eliminating transport limitations. Film thickness in both dry and electrolyte-saturated conditions was estimated by ellipsometry. Film refractive index for all the three systems has been estimated for ellipsometric measurements. Enzymes could be incorporated within the redox hydrogel using a non-ionic surfactant, Triton X-100, with no negative impact on enzyme activity. Transport properties of these films could also be estimated; however, low estimates of apparent diffusion coefficient could be attributed to limited swelling of the thin films leading to restricted mobility of the redox centers. Direct electron transfer to the laccase enzyme was negligible except at very negative potential.

Such enhanced mediator and enzyme utilization presents an opportunity for enhanced design of bioelectronic devices. For example, knowledge of diminished transport properties at low electrode thickness may provide guidance on optimal material loading on high-surface area porous electrode. Similarly, the ability to control spreading on two-dimensional surfaces would allow precise control for the manufacture of analytical arrays.

**Acknowledgments**

The authors gratefully acknowledge support from the University of New Mexico under contract FA9550-06-1-0264 from the Air Force Office of Scientific Research.
