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Electropolymerized Layers as Selective Membranes in First Generation Uric Acid Biosensors

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ABSTRACT

Electropolymerized films that can serve as semi-permeable membranes and provide selectivity within a xerogel-based, 1st generation biosensor assembly are explored in this study. Layered biosensing schemes of this nature rely primarily upon an electropolymerized ad-layer to supplement the xerogel and provide effective selectivity for detection of a targeted analyte. While effective electropolymers have been established for glucose sensing, the adaptation of the strategy to other analytes of clinical importance hinges upon the systematic evaluation of electropolymerized films to identify a selective film. Uric acid is a key species in the diagnosis/monitoring of a number of diseases and conditions. An effective uric acid biosensor, exhibiting high selectivity against common interferent species while maintaining uric acid sensitivity across physiologically relevant concentrations, would represent significant sensor development. Cyclic voltammetry allows for initial electropolymerization as well as the verification of polymer-modified electrodes. By forming electropolymerized films at glassy carbon electrodes, the sensitivity and permeability index toward uric acid and other interferents is readily measured via amperometric current responses. Of the significant number of polymer films examined in the study, only those films formed from luminol/aniline and luminol/Nafion mixtures showed positive selectivity coefficients for uric acid when incorporated into the layered xerogel schemes. The use of these specific mixed polymer films within the biosensing scheme resulted in well-defined amperometric responses to uric acid, linear calibration curves across clinically relevant uric acid concentrations, and effective selectivity for uric acid with discrimination against all major interferents except acetaminophen. The demonstrated and systematic evaluation of a specifically selective electropolymerized film is an important advancement for uric acid biosensor development as well as further adaptation of biosensing strategies involving polymer interfaces to other targeted analytes.

Keywords: Uric acid, xerogel, biosensor, electropolymerization, polyluminol, polyaniline, Nafion

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Introduction

Electropolymerized films remain popular material for use as semi-permeable membranes and/or media for immobilizing biomolecules within biosensor designs [1-4]. For enzyme-based amperometric biosensing schemes, the immobilization of sufficient amount of enzyme at the electrode is critical to the analytical signal. Materials serving to immobilize enzyme at an electrode include electropolymerized films[1, 3, 4], sol-gels[5], nanoporous gold[6], and hydrogels[7]. Electropolymerized films have been used to confine enzyme at an electrode in order to harness enzyme selectivity to indirectly signal the presence of a specific substrate (analyte). Examples of the use of electropolymerized films as a scaffold in biosensing schemes of this nature include the biosensing of glucose[8-12], hydrogen peroxide [13][14], as well as uric acid[15, 16]. In each of the systems electropolymerized films have been utilized as a three-dimensional electroconductive matrix.

First generation amperometric biosensors rely on analyte detection via an indirect measurement of hydrogen peroxide (H_2O_2), a by-product of a highly selective enzymatic reaction. One of the most common examples of these enzymatic reactions has been the detection of glucose using immobilized glucose oxidase for biosensor designs relevant for diabetic patients[17]:

$$\text{glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2 \qquad (1)$$

where H_2O_2 from this reaction is further oxidized by the following reaction to release electrons that produce an electrical signal:

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \qquad (2)$$

A common issue in such strategies is eliminating problematic interferents that also give rise to amperometric responses. Endogenous physiological interferents, such as glucose, and synthetic interferents that are introduced to the body, such as acetaminophen and ascorbic acid, are particularly problematic for 1st generation biosensing[18]. A particularly challenging interferent is acetaminophen, whose oxidation potential is low enough to be oxidized with the electrode poised at a potential for immediate oxidation of H_2O_2 [12, 17]. Electropolymerized layers have been successfully incorporated into 1st generation biosensing schemes as semi-permeable membranes. In particular, the electropolymerization of phenol has been shown to be an effective strategy for discriminating against acetaminophen interference within glucose biosensing systems[17, 19, 20]. One such successful strategy reported recently used sol-gel based layered materials to immobilize GOx at an electrode and employed polyphenol as a semi-permeable membrane [12]. The strategy showed sensing response toward glucose highlighted with large linear/dynamic ranges, fast response times, and superior interferent discrimination. The major attribute of the system described in that

work is that it could, in theory, be adapted to sense any other species that has an associated enzymatic reaction. A critical part of adapting this sensor scheme, however, remains the design of an effective strategy for the identification and characterization of semi-permeable electropolymerized layers that provide specific selectivity for a targeted analyte.

In this work, we present a systematic evaluation of electropolymerized films to identify a specific film that effectively functions as a semi-permeable membrane within a layer-by-layer (L-B-L), xerogel-based, 1st generation amperometric biosensor featuring immobilized uricase oxidase (uricase, UOx) for the measurement of uric acid. The detection and real-time monitoring of uric acid levels in blood and plasma can be beneficial to diagnosing a wide range of conditions and diseases. Serum uric acid concentration has been identified as a significant indicator of such conditions as atherosclerotic cardiovascular disease[21], peripheral artery disease[22], non-alcoholic fatty liver disease[23], and pregnancy induced hypertension (PIH)[24]. Electropolymers used for uric acid biosensors were identified from the literature and subsequently synthesized, characterized, and assessed via a series of microscopy, voltammetry, and permeability experiments. Based on permeability measurements, certain films were then incorporated into full uric acid sensing schemes and evaluated for overall performance. The inclusion of an effective electropolymer layer optimizes the sensing scheme, establishing a successful ensemble for uric acid biosensing. The approach outlined by this work and used to successfully identify and characterize an appropriate electropolymer layer can serve as a template strategy for expanding the xerogel-based biosensor design that incorporates electropolymer selective membranes to other species of interest.

2 Experimental Details

2.1 Materials and Instrumentation.

All chemicals were purchased from Sigma-Aldrich unless specifically stated. All aqueous solutions were prepared using 18.2 MΩ·cm ultrapurified (NP) water. Electrochemical experiments were performed using multi-channel potentiostats (CH Instruments, 1000B or 1030C) and a single-channel potentiostat (CH Instruments, 630B). Electrochemical cells were composed of working electrodes of either modified glassy carbon working electrodes (GCE, 3 mm dia., CH Instruments) or modified platinum (2 mm dia., CH Instruments), a common Ag/AgCl (satrd. KCl) reference electrode (CH Instruments), and a common platinum wire counter electrode (Sigma-Aldrich). A Mettler Toledo MP225 pH Meter was used to assess pH of solutions and an Asylum MFP-3D Bio atomic force microscope (AFM) equipped with an extended z-range and a Zeiss Axio Observier optical microscope was used to characterize polymerized films.

2.2 Electropolymerization Procedures.

Glassy carbon and platinum working electrodes were polished successively with 1.0, 0.3, and 0.05 μm Al_2O_3 powder (Electron Microscopy Sciences), each time rinsed with nanopure (NP) water (18 MΩ·cm). Platinum electrodes were then cycled in 0.1 M H_2SO_4 until voltammetry of platinum oxide oxidation/reduction was achieved. Specific electropolymerized films were formed as described below or with similar modified procedures.

2.2.1 *Aniline*. For application of the polyaniline (PANI) selective membrane, a previously reported preparation was modified and adapted[25]. In brief, glassy carbon electrodes (GCEs) were immersed in 0.08 M aniline in 0.1 M KNO_3 (pH ~9). Electropolymerization of aniline was achieved using cyclic voltammetry between -0.255 V and +0.945 V (vs. Ag/AgCl, satrd. KCl) for 7 cycles (50 mVs^{-1}). PANI-coated electrodes were rinsed with NP water and allowed to dry (30 min.) at ambient conditions until further use. AFM estimated film thicknesses for typical films formed with protocol of ~200 nm.

2.2.2 Luminol. In order to apply the polyluminol (PLUM) layer, an established procedure was used[26]. GCEs were immersed in a 0.5 mM luminol solution in 0.1 M H_2SO_4 (N_2 degassed for 20 minutes while stirring). The luminol solution was then stirred while performing cyclic voltammetry (50 mVs^{-1}) between 0.0 and +1.0 V (vs. Ag/AgCl, satrd. KCl) for 12 cycles. The luminol-coated electrodes were rinsed with NP water and allowed to dry (15 min.) at ambient conditions until further use. AFM estimated film thicknesses for typical films formed with protocol of ~2-10 nm.

2.2.3 Pyrrole. For the application of the polypyrrole selective membrane, a previously determined procedure was altered to fit this experiment[27]. In brief, GCEs were immersed in a solution of 0.1 M pyrrole in 0.1 M H_2SO_4. The solution was degassed with nitrogen (20 min.) to remove the oxygen before electropoloymerization of pyrrole was achieved using cyclic voltammetry, cycling between 0.0 V and +0.9 V (vs. Ag/AgCl, satrd. KCl) for 5 cycles (50 mVs^{-1}). The pyrrole-coated electrodes were rinsed with NP water and allowed to dry (15 min) at ambient conditions until further use. AFM estimated film thicknesses for typical films formed with protocol of ~100 nm.

2.2.4 Nafion®. Nafion was electropolymerized on GCEs using a previously described method [28]. The GCEs were placed in 5% Nafion solution (LIQion, Ion Power Inc.), and ran for 30 seconds at a constant potential of +1.0 V (vs. Ag/AgCl satrd. KCl). After drying in air for 10 seconds, the electrodes were cured

in an oven (70 ºC; 10 minutes) or allowed to sit at room temperature for an extended time. In a separate procedure, an adaptation from the luminol procedure,[26] Nafion was also electropolymerized on GCEs using cyclic voltammetry. In this particular method, the GCE were dipped in the Nafion solution, and allowed to cycle 12 times between 0.0 V and +1.0 V (vs. Ag/AgCl satrd. KCl) at 50 mVs^{-1}. Nafion-coated electrodes were rinsed with NP water and allowed to dry (30 min.) at ambient conditions until further use.

2.2.5 Tyramine. In order to apply the polytyramine layer, a previously discovered procedure was used[29]. GCEs were immersed in 0.1 M tyramine solution (0.3 M NaOH-containing methanol). Tyramine was electropolymerized, without stirring, using cyclic voltammetry at a sweep cycle of 500 mVs^{-1} cycling between -0.1 V and +1.7 V (vs Ag/AgCl satrd. KCl) for 40 cycles. Electrodes were rinsed with NP water and left to dry for 10 minutes. An alternative procedure [30] used to apply polytyramine involved immersing the GCE in 30 mM tyramine (0.1 M KCl, N_2 degassed while stirring) and using cyclic voltammetry at a sweep cycle of 500 mVs^{-1} cycling between 0.0 V and +1.4V (vs Ag/AgCl satrd. KCl) for 40 cycles. Tyramine-coated electrodes were rinsed with NP water and allowed to dry at ambient conditions (10 min.) until further use. AFM estimated film thicknesses for typical films formed with protocol of ~10-15 nm.

2.2.6 Phenol. In order to apply the polyphenol layer, a previously reported preparation was adapted[12]. In brief, GCEs were immersed in 0.04 M phenol solution (66.55 mM potassium phosphate buffer solution (PBS) at pH 7, degassed with N_2 for 20 min). Electropolymerization of phenol was achieved by holding the working electrode at a constant potential of +0.9 V (vs Ag/AgCl, satrd KCl) for 900 seconds. Polyphenol-coated electrodes were rinsed with NP water and allowed to dry (30 min.) at ambient conditions until further use. Typical film thicknesses of this self-limiting electropolymerization have been reported at 10-100 nm.[20]

2.2.7 Mixed Polymer Films. Two types of mixed polymer films were explored: sequential layered (seq) and mixed monomers (mix) films. In a typical sequential film procedure, a polymer film was formed from a single monomer solution according to the procedure described above but for half the number of voltammetric cycles. The polymer-modified electrode was then immersed in a second single monomer solution for half the cycles of its procedure. For polymer films formed from a mixture of monomers, electrodes were immersed in a solution of two different monomers at a specific ratio (e.g., 10:1, 50:50, 1:10) during electropolymerization. For *polyaniline-polyluminol mixed films*, for example, a procedure adapted from De Robertis et al.[31] was utilized. For, the polyluminol-polyaniline (1:10) mixed film, the GCE was immersed in a stirred solution of 0.5 mM luminol (saturated) and 5 mM aniline (0.1 M H_2SO_4, N_2 degassed, 20 min.) before cyclic voltammetry was performed between 0.0 and +1.0 V (vs. Ag/AgCl, satrd.

KCl) for 12 cycles (50 mVs^{-1}). Electrodes were rinsed with NP water and allowed to dry (10-15 min.) at ambient conditions until further use. In some cases, films were formed with nucleation and growth steps. The solution explained above was electropolymerized on the electrode surface using cyclic voltammetry that was performed with stirring between -0.105 V and +1.245 V (50 mVs^{-1}) for 3 cycles (nucleation), followed by 10 additional cycles in a smaller window of -0.105 V to 0.995 V (growth).[31] Electrodes were rinsed with NP water and left to dry (10-15 min.) at ambient conditions until further use.

2.2.8 Nafion-luminol mixed films were formed using modifications of a previously-reported method.[26] For example, in creating a 10:1 Nafion-luminol mixed film, the GCE was immersed in a 10:1 Nafion-luminol mixed solution (0.125 g of luminol in 25 mL of as received Nafion solution) and cyclic voltammetry between 0.0 V and +1.0 V was performed for 12 cycles. The electrodes were rinsed with NP water and allowed to dry (30 min.) at ambient conditions until further use. Mixed films with pyrrole as a component were formed from a previously reported procedure as well with the details provided in Supporting Materials.

2.3 Characterization and Assessment of Electropolymer Films

Cyclic voltammetry electrochemical probing experiments and film permeability measurements were used to assess the successful application of electropolymerized films and their properties. For probing experiments, working electrodes were immersed in 65.55 mM PBS for current-time (I-t) experiments where the working electrode potential was held at +0.65 V (vs Ag/AgCl, satrd KCl) during injections in the sequence of 40 μM acetaminophen, ascorbic acid, sodium nitrite, and oxalic acid, 100 μM glucose and 100 μM uric acid. From the resulting amperometric current–time (I-t) curves, the permeability index of each interference species *j* can be calculated by comparing the current passed at the bare working electrode to that passed at the electropolymerized film modified electrode, using the following equation:

$$PI = \frac{\Delta I_j}{\Delta I_{j\ bare}} \qquad (3)$$

where ΔI_j and $\Delta I_{j\ bare}$ are the measured currents for a specific interferent species (*j*) at a working electrode and at a bare electrode, respectively.[32]

As previously described in the literature[12, 20, 32], amperometric selectivity coefficients (K^{amp}) were used to evaluate interferent responses of sensors assembled using electropolymerized films from amperometric I-t curves. To achieve this, the following equation was used:

$$K^{amp}_{uric\ acid,j} = \log\left(\frac{\Delta I_j / C_j}{\Delta I_{UA} / C_{UA}}\right) \qquad (4)$$

where ΔI_j and ΔI_{UA} are the measured currents for a specific interferent species (*j*) and uric acid and C_j and C_{UA} are concentrations of the interferent species and uric acid, respectively. Film thicknesses were measured via AFM as a profilometry-type measurement assessing the difference in height between on and off film positions.

The electropolymerized films were ultimately tested for selectivity effectiveness by incorporating the polymer film within the L-B-L assembly as previously reported[12] and evaluating the biosensing performance. In brief, platinum electrodes were coated with a layer uricase (UOx)-doped octyl-trimethoxy xerogel (OTMS) and allowed to dry for 5 minutes before a second layer of OTMS, not doped with UOx, was deposited. The OTMS xerogel bi-layer was allowed to age in a humidity-controlled environment (50% relative humidity, 48 hours) before the electropolymerized layer was added via the procedures described above. A Hydrothane polyurethane layer (100% HPU) was deposited and allowed to dry (30 min.) under ambient conditions. Prior to uric acid and interferent injections, the sensor was pre-conditioned by soaking in PBS (65.55 mM) for 1 hr. and then held under potential control (+0.65V vs. Ag/AgCl) for 20 minutes.

3 Results and Discussion

In 2015, the L-B-L construction of a xerogel-based biosensing scheme was introduced as model system for the 1st generation electrochemical detection of glucose[12]. The general design featured an electrode modified with a bi-layer of xerogels, one doped with glucose oxidase (GOx) and the other un-doped, followed by semi-permeable layers of electropolymerized polyphenol and blended polyurethane. The performance of the sensor design was shown to be very effective, achieving extended linear/dynamic ranges of responses to increasing glucose concentrations as well as superior glucose selectivity. A critical component of the system's selectivity, the ability to discriminate against common interferents, was shown to be the polyphenol layer that is electropolymerized after the two xerogel are deposited.[12] A major attribute of the sensing strategy and materials that was suggested by that work was the ability to adapt the approach away from the glucose model and toward the specific design of other clinically relevant biosensors such as uric acid sensing. A uric acid biosensor adaptation (**Scheme I**) would still employ xerogel layers as a base and a corresponding enzyme catalyzed reaction[33]:

$$\text{uric acid} + O_2 \xrightarrow{\text{uricase (UOx)}} \text{allantoin} + H_2O_2 + CO_2 \qquad (5)$$

One of the key challenges of the uric acid adaptation, however, remains the identification of an appropriate electropolymerized layer for effective uric acid selectivity. The following study is a systematic evaluation,

characterization, and identification of an optimal electropolymerized layer for layered, xerogel-based uric acid biosensing schemes.

Unlike the glucose model, the target analyte, uric acid, is electroactive and undergoes oxidation and reduction at an electrode[34]. **Figure 1** shows the cyclic voltammetry of uric acid in phosphate buffer solution at both a glassy carbon electrode (GCE) as well as a platinum electrode (Fig.1, inset). In each case, the oxidation potential is consistent with that observed for the oxidation of uric acid.[34] For additional confirmation that the voltammetry observed is from uric acid electrochemistry, the solutions were spiked with uric acid and a corresponding increase in the oxidation current was observed. The voltammetry of uric acid at the GCE versus the platinum electrode does differ significantly. At the platinum electrode only an oxidation (anodic) wave is observed whereas the voltammetric response at the GCE reveals both a larger, more well-defined oxidation wave as well as a small cathodic signal. While 1st generation sensor constructs (Scheme I) traditionally feature a platinum electrode base for the detection of hydrogen peroxide, the voltammetry of uric acid at GCEs and platinum electrodes suggests that GCE will provide a more sensitive evaluation of the electropolymerized layer in terms of permeability to uric acid and common interferents.

An extensive literature search revealed a number of polymer films that were incorporated into uric acid sensing schemes including polyaniline (PANI)[25], polyluminol (PLUM)[26], polypyrrole[27], Nafion[28], polytyramine[29, 30], and polyphenol[12, 20]. For the current study, each of the established electropolymerization procedures for these identified films was followed in order to form the film from a monomer solution at a GCE (Experimental details). After each electropolymerization, cyclic voltammetry was used in two ways in order to verify the success of the electropolymerization procedure and show the presence of an actual polymer film at the electrode interface. First, the polymer-modified electrodes were immersed in their respective, monomer-free electrolyte solutions and cyclic voltammetry was performed in the same potential window as the electropolymerization step. Often, the voltammetry of the modified GCE revealed signature voltammetric responses characteristic of the polymer film. Secondly, the voltammetry of potassium ferricyanide (FeCN), a common redox probe molecule, was recorded at both a polymer film-modified and bare GCE. Successfully polymerized electrodes should effectively block FeCN voltammetry compared to the freely diffusing, reversible cyclic voltammetry observed for the probe at a bare GCE. As an example, **Figure 2** summarizes these experiments for a PANI-modified GCE, showing the electropolymerization voltammetry, the voltammetry of the PANI-modified and bare GCE in aniline-free background electrolyte, and the FeCN voltammetry comparison of PANI-modified and bare electrodes. The electropolymerization cyclic voltammetry shows oxidation-reduction peaks that progressively grow with each scan (Fig. 2A) – evidence that the redox activity necessary for electropolymerization is occurring. After electropolymerization, the modified electrode was immersed in a monomer-free solution and the

voltammetry was repeated with the result compared to the same voltammetry of a bare GCE (Fig. 2B) to reveal peaks characteristic of a PANI film at the electrode[25]. The voltammetry of FeCN at the PANI-modified GCE is completely blocked compared to that of a bare GCE (Fig. 2C). These electrochemical results suggest the presence of a PANI film at the electrode and verify the electropolymerization procedure used. In addition to this electrochemical characterization, atomic force microscopy was used to estimate the thicknesses of some of the single component polymer films deposited using the electropolymerization procedures (see Experimental details and Supporting Materials). In each case, the electropolymerization proceeded as expected from the literature.

After establishing the presence of the polymer film, the permeability of the film towards uric acid and certain common interferents was assessed using amperometric current-time (I-t) experiments. In these experiments, the polymer-modified electrode was immersed in PBS and poised at +0.65 V (vs. Ag/AgCl) while the amperometric responses to injections of physiological relevant concentrations of uric acid and common interferents were recorded. The potential of +0.65 V, common for 1st generation biosensing schemes for the oxidation of the peroxide by-product of the enzymatic reaction (Scheme I)[17], is also sufficiently more positive than the anodic peak potential for the oxidation of uric acid (+0.3V vs. Ag/AgCl) (Fig. 1). For this study, the interferents examined are well known and include both endogenous species and medicinal species (e.g., acetaminophen) that are well-known to oxidize at the operating potential and conflict with the analytical signal of the biosensor. **Figure 3** shows permeability experiments, the injection of uric acid and interferents, at PANI (Fig. 3A) and PLUM (Fig. 3B) modified GCEs in comparison to unmodified GCEs. As shown by the respective I-t responses, the PANI film can be considered extremely *blocking* toward both the uric acid and interferents - nearly negligible responses compared to the bare GCE. In contrast, the PLUM films (Fig. 3B) exhibit significant amperometric responses (i.e., *non-blocking*) to certain species, similar current to the responses at the bare GCE for acetaminophen, ascorbic acid, and uric acid. Both of these film responses are non-ideal as they both either completely block (Fig. 3A) or allow (Fig. 3B) signal from both uric acid and major interferents.

The permeability of each of the individual polymer films formed from various procedures was assessed using I-t responses to uric acid and interferents in the manner described. Because of the prominence of both *blocking* and *non-blocking* (i.e., nonselective) behaviors for most of the films tested, combination films formed from mixtures of different monomers were also evaluated. These mixed films were formed using procedures either specifically described for mixed films or derived from the monomer procedures described in the literature. **Figure 4** represents an example of voltammetry-based electropolymerization (Fig. 4A) and characterization of a polymer film formed from a mixture of aniline and luminol (10:1) following the procedures of De Robertis et al.[31] The results again exhibit clear

evidence of successful film deposition with both signature voltammetry of the polymer film (Fig. 4B) as well as blocked voltammetry of FeCN by the PLUM:PANI-modified electrode (Fig. 4C). The amperometric response (I-t curve) of uric acid and interferents at the PLUM:PANI-modified GCE is shown in **Figure 5**. Notably, this result establishes that the mixture is more effective at allowing uric acid to permeate the film to produce a significant signal (last injection) while simultaneously allowing for smaller signals of problematic interferents (e.g., ascorbic acid; 2nd injection). The results also show that acetaminophen remains a problematic interferent even with the mixed film.

Specific permeability indices (PI) of each type of polymer film explored in this study are summarized and provided in the Supporting Materials (Table SM-1), including films electropolymerized from single monomers as well as those formed from mixtures of different monomers. Additionally, the sequential layering of two different polymer films was also explored. For example, the table includes entries where PANI was deposited first by itself and then was subsequently coated with PLUM. This type of "mixed" polymer film is distinguished from those formed from solutions of mixed monomers as sequential (seq) and mixed (mix), respectively. We note that for nearly every film studied, no significant amperometric response toward sodium nitrite, oxalic acid, and glucose was recorded. These species, therefore, effectively registered 0% permeability, and were not included in Table SM-1. Thus, the critical signals that required more evaluation are uric acid, acetaminophen and ascorbic acid.

Figure 6 shows a visual comparison of PIs for polymer films allowing the most significant uric acid signals. In addition, the ratio of PIs for uric acid-to-acetaminophen and uric acid-to-ascorbic acid are compared for different films (Fig. 6B). In examining these comparisons, the results indicate that uric acid selectivity against acetaminophen is not achieved successfully with these polymer films. The permeability of acetaminophen typically ranges from 40 to 100% for these select films and the corresponding PI ratio of uric acid-to-acetaminophen is lower, on average, for each film compared to the uric acid-to-ascorbic acid ratio. Based on permeability of uric acid-to-ascorbic acid for these select films, the ones with the most potential to prescribe the necessary selectivity in a biosensing scheme appear to include polyphenol, PLUM, Nafion, PLUM:PANI (1:10 and 10:1), Nafion:PLUM and PLUM:polytyramine (seq) - all the shaded entries in Table SM-1.

The biosensing strategy reported by Poulos et al. established that, while each layer of the system (Scheme I) could be evaluated separately, it was the layers working in concert with each other that ultimately created effective selectivity and biosensing performance[12]. As a final step of identifying an effective polymer membrane for uric acid biosensing, the polymer films with the most sensitivity toward uric acid were incorporated into the full xerogel-based biosensing scheme (Scheme I) and tested for both

uric acid and common interferent responses. I-t curve examples of systems showing both effective and poor selectivity performance are included in the Supporting Materials. From these I-t responses, selectivity coefficients (K_j^{amp}) were calculated (Experimental Details) to assess and compare the full systems' selectivity for uric acid. A positive selectivity coefficient for uric acid with smaller or negative selectivity coefficients for major interferents constitutes a system selective for uric acid. **Figure 7** shows a visual representation of the comparison of selectivity coefficients between systems for uric acid and each major interferent, most of which exhibit significantly negative selectivity coefficients. Unfortunately, a prominent feature of these results remains the inability of the layered systems to exclude acetaminophen. While not optimal, acetaminophen is, at least, an interferent that in most medical applications can be addressed prior to biosensing by not administering the drug or waiting for it to clear the body before testing. Of the films explored, only two of the luminol-aniline mixtures (10:1 and 1:10) and the Nafion-luminol mixture ($10:1_{CV-N}$) show positive selectivity coefficients for uric acid and negative selectivity coefficients for interferents other than acetaminophen.

Given the low selectivity coefficient for uric acid for layered films with these three particular electropolymer films incorporated (Fig. 7e, 7h, and 7i), the viability of getting a reliable uric acid response from a biosensor featuring this polymer film was questionable. To address this concern, OTMS-biosensors with a PLUM:PANI semi-permeable layer and polyurethane coating were constructed and I-t responses for uric acid across the physiological relevant range of uric acid (200-500 μM)[24] as well as the response of an major interferent panel were collected. **Figure 8** shows the amperometric I-t curves for injections of common interferents compared to uric acid at these biosensor constructs. For all three films, nearly negligible responses were recorded for all major interferents other than acetaminophen while concentration proportional signals for 100 μM versus 300 μM were recorded for uric acid. Similarly, all three sensors yielded well-defined current steps for increasing concentrations of injected uric acid (Fig. 8B), responses that translated to linear calibration curves (Fig. 8B, inset). The calibration curve is extremely linear and easily spans concentration ranges meaningful to medical conditions where uric acid levels are monitored for diagnosis[24].

4 Conclusions

A xerogel-based, layered biosensing scheme featuring an electropolymerized film for selectivity has been established for glucose sensing[12]. The successful adaptation of that strategy to another analyte is heavily dependent on identifying a corresponding electropolymerized layer that can act as a semi-permeable membrane and effectively select for the desired analyte. Basic electrochemical techniques such

as cyclic voltammetry and amperometry can be used to successfully form the polymer films at electrode interfaces as well as evaluate their presence and permeability, respectively. In the case of a biosensing scheme for uric acid, a clinically relevant target, luminol-aniline and luminol-Nafion mixed polymer films provided effective selectivity for uric acid while simultaneously excluding common interferents like ascorbic acid. The luminol-aniline and luminol-Nafion mixed films were unable to eliminate the signal from acetaminophen, an ingested, pharmaceutical or "artificial" interferent that, in theory, can be controlled. The establishment of the methodology in this study for identifying effective electropolymer films is an important step for moving forward with the optimization of the specific xerogel uric acid biosensing scheme as well as adaptation of the L-B-L approach to other target molecules. The development and performance assessment of 1st generation uric acid biosensors utilizing the electropolymer films identified in this study are currently being explored in our laboratory. Ultimately, a biosensing strategy that lends itself to a relatively easy, systematic adaptation to an array of analyte species represents a very important step in the development of biosensing strategies.

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Captions

Figure 1. Cyclic voltammetry (50 mV·sec^{-1}) of an unmodified glassy carbon electrode immersed in **(a)** 65.55 mM PBS (pH = 7); **(b)** 1 mM uric acid (65.55 mM PBS; pH = 7), and **(c)** the uric acid solution in (b) spiked with additional uric acid. **Inset:** Identical experiments at a platinum electrode.

Figure 2. Cyclic voltammetry for **(A)** 0.08 M aniline (0.1 M KNO_3, pH ~9) during electropolymerization at a GCE (50 mV·sec^{-1}); **(B)** bare **(a)** and polyaniline (PANI) modified **(b)** GCEs in 0.5 M H_2SO_4 (no monomer) solution (50 mV·sec^{-1}) and; **(C)** 5 mM ferricyanide (0.5 M KCl) at bare **(a)** and PANI-modified **(b)** GCEs to confirm the presence of the electropolymer film (250 mV·sec^{-1}).

Figure 3. Amperometric I-t curves during successive injections of different interferent species and uric acid at (a) unmodified GCE and (b) GCE modified with **(A)** polyaniline and **(B)** polyluminol and held at +0.65 V vs. Ag/AgCl. Note: Injection of interferents (acetaminophen at 300 sec.; ascorbic acid at 400 sec.; sodium nitrite at 500 sec.; oxalic acid at 600 sec.; glucose at 700 sec.; uric acid at 800 sec.) resulted in a concentration of 40 μM (in the case of acetaminophen, ascorbic acid, sodium nitrite, and oxalic acid) and 100 μM (in the case of glucose and uric acid) (65.55 mM PBS; pH=7).

Figure 4. Cyclic voltammetry for **(A)** 0.5 mM luminol and 5 mM aniline (1:10) during electropolymerization at a GCE (50 mV·sec^{-1}) in 0.1 M degassed H_2SO_4; **(B)** bare **(a)** and polyluminol-aniline (PLUM:PANI) modified **(b)** GCEs in 0.1 M H_2SO_4 (no monomer) solution (50 mV·sec^{-1}) and; **(C)** 5 mM ferricyanide (0.5 M KCl) at bare **(a)** and PLUM-PANI-modified **(b)** GCEs to confirm the presence of the electropolymer film (250 mV· sec^{-1}).

Figure 5. Amperometric I-t curves during successive injections of different interferent species and analyte at (a) unmodified GCE and (b) GCE modified with PLUM:PANI (1:10) mixed polymer layer. Note: Injection of interferents (acetaminophen at 300 sec.; ascorbic acid at 400 sec.; sodium nitrite at 500 sec.; oxalic acid at 600 sec.; glucose at 700 sec.; uric acid at 800 sec.) resulted in a concentration of 40 μM (in the case of acetaminophen, ascorbic acid, sodium nitrite, and oxalic acid) and 100 μM (in the case of glucose and uric acid) (65.55 mM PBS; pH=7).

Figure 6. (A) Permeability indices and **(B)** permeability index ratios for representative polymer systems including mixed polymer systems with ratios parenthetically reported (e.g., 10:1) and sequential mixed layering of polymer films (Seq).

Figure 7. Selectivity coefficients comparison for uric acid (100 μM and/or 300 μM) and common interferent species (40 μM) at OTMS xerogel biosensor (Scheme I) featuring various electro-polymerized layers acting as the semi-permeable membrane between the xerogel and polyurethane layers. Electropolymerized layers including **(a)** Phenol **(b)** Aniline **(c)** Luminol and **(d)** Nafion (CV) as well as mixed-electropolymerized films including **(e)** Nafion-Luminol ($10{:}1_{CV\text{-}N2}$ **(f)** Luminol-Tyramine **(g)** Pyrrole-Luminol **(h)** Luminol-Aniline (1:10) **(i)** Luminol-Aniline (10:1) **(j)** Luminol-Aniline (1:10) with nucleation and growth steps and **(k)** Luminol-Aniline (10:1) with nucleation and growth. Note: Glucose was tested at 100 μM.

Figure 8. (A) Amperometric I-t curve for successive injections of common interferent species (40 μM) and uric acid (100 μM and/or 300 μM) and **(B)** successive 50 μM injections of uric acid with corresponding calibration curves (inset) for platinum electrodes modified with layers of UOx-doped OTMS xerogel, undoped OTMS xerogel, electropolymerized layers of **(a)** Nafion-luminol, **(b)** luminol-aniline (1:10), or **(c)** luminol-aniline (10:1) formed with no nucleation and growth, hydrothane polyurethane capping layer. Solution conditions include 65.5 mM PBS, pH = 7.0; μ = 150 mM; Error bars represent standard error; Glucose was tested at 100 μM.