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Layered Xerogel Films Incorporating Monolayer Protected Cluster Networks on Platinum Black Modified Electrodes for Enhanced Sensitivity in 1st Generation Uric Acid Biosensing

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ABSTRACT

Amperometric uric acid (UA) biosensing schemes incorporating networks of alkanethiolate-protected gold nanoparticles, monolayer protected clusters (MPCs), and platinum black (Pt–B) electrode modification via layer-by-layer construction of xerogels are investigated. MPC doping and Pt-B augmentation are implemented within hydroxymethyl–triethoxy silane xerogel bilayers at platinum electrodes. The first xerogel adlayer is doped with an MPC network and houses uricase for the enzymatic reaction required for 1st generation schemes. Polyluminol-aniline and polyurethane are used as selective/stabilizing interfacial layers. Sensing performance with and without Pt–B and/or MPC doping is assessed via amperometry with standardized UA injections. The use of each individual material results in enhancement of UA sensitivity compared to analogous films without said materials. The use of Pt–B and MPC doping in concert results in a biosensor design with the highest observed UA sensitivity (0.97 $\mu A \cdot mM^{-1}$) and fast, linear responses over physiologically relevant UA concentrations. Enhancement is attributed to Pt–B providing increased electrode surface area and integration into the xerogel for greater electronic coupling of the MPC network and more efficient reporting of H_2O_2 oxidation. The findings have implications for advancing clinical *in vivo* sensing devices that require scalability or additional biocompatibility layering - both of which would benefit from signal enhancement strategies.

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1. Introduction

Biosensor research is gaining more attention due to its numerous applications that span many different fields. The simplicity and adaptability to different clinically relevant analytes and industrial applications allow electrochemical sensor research to continue at the forefront of this developing technology sector.[1] Enzyme–based amperometric biosensors offer effective selectivity in the detection of trace amounts of target molecules within complex matrices.[2] In particular, 1st generation amperometric biosensors employ the immobilization of oxidase enzymes which, catalyze the reaction of molecular oxygen with specific analyte to produce hydrogen peroxide (H_2O_2) by-product. The H_2O_2 is subsequently oxidized/reduced at a working electrode surface to yield a current response proportional to analyte concentration. Such biosensors have the advantage of affordability and simplicity while also allowing for relatively easy modification to a plethora of target analytes and possibility of miniaturization for *in vitro* and *in vivo* clinical applications.[1–2]

Uric acid (2,6,8–trihydroxypurine, UA), the main end product of purine nucleotide metabolism, is a critical factor in many medical conditions. Due to its poor solubility, UA exists in biological fluids such as human blood serum, plasma, urine and saliva as urate anion at physiological pH. Many diseases such as gout, hyperuricemia, Lesch–Nyhan syndrome, obesity, diabetes, high cholesterol, high blood pressure, kidney disease and heart disease are linked to abnormal levels of UA concentration. [3] Elevated levels of UA in the blood of late term, pregnant women suggest higher probability of pregnancy–induced hypertension (PIH), a condition that can lead to a disorder called pre–eclampsia and significant health risks for both mothers and their babies. Current UA testing requires time–consuming laboratory evaluation of blood/urine during which the PIH can remain undiagnosed with increasing risk of serious complications. Hence, effective UA sensors, capable of accurate, fast, and local monitoring at the bedside, are of interest in being able to detect and predict abnormal conditions. [3b]

One approach of biosensor design is to employ specific nanomaterials (NMs) into sensing schemes, taking advantage of their unique properties such as surface area, electronic conductivity, and the ability to interface with biomolecules.[2a, 4] NMs used as part of sensing devices and strategies have included, among others, carbon nanotubes (CNT)[5] and metallic nanoparticles (NPs)[6], including citrate–stabilized gold colloid nanoparticles (NPs).[2a, 4d, 7] In some cases, the NMs are incorporated into a scaffold material modifying an electrode, such as sol–gel or electropolymer,

that also serves to immobilize the enzyme required for 1st generation biosensing schemes. Despite their prominence in the literature, [4d, 7] monolayer–protected clusters (MPCs) are under–represented in this area of work. In 2013, a 1st generation amperometric glucose biosensor featuring a xerogel layer formed from 3–mercaptopropyl trimethoxy silane (3–MPTMS) and embedded with glucose oxidase (GOx) was presented with MPC doping to create a NP network within the xerogel.[2a] The MPC–doped sensor showed an order of magnitude increase in sensitivity, doubled linear range and 4–fold decrease in response times compared to similar films without MPC doping. A subsequent report established that the MPC network allowed for a decreased dependence on diffusion and a fast electron reporting system throughout the film, factors that significantly enhanced sensitivity. [1] While signal enhancement was ultimately achieved, the scheme suffered from less–than–optimal selectivity in discriminating against common interferents.

Leopold *et al*. recently developed a robust, functional layer–by–layer (LbL) system for a high performance, 1st generation amperometric glucose biosensor model. The sensing scheme included platinum (Pt) electrodes modified with GOx–doped and un–doped xerogel bi–layers and semipermeable membranes – a composite film with a collaborative and effective functionality. [8] While this scheme allows for well–defined dynamic/linear ranges, low response times, and significant discrimination against common interferents, the layering of the films does depress the sensitivity of the biosensing response. Both schemes, MPC–doped and 4–layer composite films, have advantages and disadvantages, but it would be ideal to combine the strategies and optimize both sensitivity and selectivity simultaneously. In this regard, the synergetic effect of modifying the underlying electrode and introducing a MPC network in such systems has not yet been explored.

In this work, two materials are explored in terms of their ability to enhance the performance of a LbL-constructed uric acid biosensing scheme: the incorporation of an MPC network within the xerogel layer and the electrochemical deposition of a platinum black (Pt-B) layer as a direct modification of the platinum electrode transducer. As previously mentioned, MPC doping has already been shown to enhance amperometric responses for glucose biosensors.[1,2a] Similarly, Pt–B, a layer of amorphous clusters of Pt nanoparticles, [9] has been successfully employed to increase the effective surface area of microelectrodes in certain biosensing schemes – a strategy resulting in a boosted current response.[9–10] To our knowledge, however, the use of Pt–B in conjunction with

MPC networks to counteract LbL signal depression in biosensing schemes has not yet been explored.

2. Experimental Section

2.1. Materials and Instrumentation.

All chemicals were purchased from Sigma–Aldrich unless specifically stated. Hydrothane AL25–80A polyurethane (HPU) was obtained from AdvanSource Biomaterials. A platinizing solution (3% chloroplatinic acid in water) was purchased from LabChem (Pittsburgh, PA). Hydroxymethyl–triethoxy silane (HMTES) was stored in a desiccated glovebox (Plas Laboratories, Inc.) and eventually transferred using a sealed micro–centrifuge to maintain the dry, N_2 environment and eventually deposited in a relative humidity (RH)–controlled chamber (Cole–Parmer) holding 50% RH. Ultra–purified water (H_2O, 18.3 MΩ·cm, Barnstead) was used to prepare all solutions. Uricase enzyme was created in–house using a previously described procedure,[11] which is provided in more detail in Supporting Information. An eight–channel potentiostat (CH Instruments, 1000B) was used to record amperometric current–time (i–t) curves to evaluate the analytical performance of the sensors, as described below. Electrochemical cells were comprised of a common Ag/AgCl (saturated KCl) reference electrode, a common platinum wire counter electrode (Sigma–Aldrich), and modified platinum working electrodes (2 mm diameter, CH Instruments).

2.2. MPC Synthesis

Alkanethiolate–based MPCs were synthesized using a variation of the well–established and widely used Brust reaction.[2a, 12] Briefly, aqueous $HAuCl_4$ was subjected to tetraoctylammonium bromide in toluene in order to transfer the gold from the aqueous portion to the toluene layer. Two molar equivalents of hexanethiol (C_6 thiol) was added to the separated organic layer, which was then stirred for a minimum of 30 minutes or until a color change from orange/red to translucent pale yellow solution was observed. The flask was chilled (0ºC) in an ice bath before chilled aqueous $NaBH_4$ was added as a reducing agent to produce metallic gold nucleation and growth in the presence of C_6 thiol, observed as a thick black solution of MPCs in the toluene layer. The

reaction was allowed to proceed overnight under these conditions before the organic layer was separated, rotary–evaporated to dryness, precipitated with acetonitrile, and isolated on a medium porosity glass frit with vacuum filtration and thorough washing with acetonitrile. As in previous reports, [24] the thiol–to–gold ratio of 2:1 produced MPCs with an average core structure of $Au_{225}(C_6)_{75}$ with a TEM–estimated diameter of ~2.0 nm.

2.3. Pt Black Synthesis

Platinum working electrodes were polished successively with 1.0, 0.3, and 0.05 μm Al_2O_3 powder (Electron Microscopy Sciences) and rinsed thoroughly with H_2O after each polishing steps. The electrodes were then electrochemically cleaned by cycling in 0.1 M H_2SO_4 between +1.2 and –0.25 V at 0.25 V/s until the characteristic voltammetry of clean platinum surface was observed. The cleaned platinum working electrode was platinized in 3% chloroplatinic acid (v/v in water) by cycling the potential from +0.6 to –0.35 V (vs Ag/AgCl) at sweep rate of 0.02 V/s using a CH Instruments 630B potentiostat to obtain platinum black (Pt–B) modified platinum (Pt–B/Pt) working electrode.[10]

2.4. Composite Film Fabrication

For fabrication of the biosensor, two centrifuge tubes, one with 3 mg of uricase (UOx) dissolved in 100 μL of H_2O and the other containing 25 μL of silane mixed with 100 μL of tetrahydrofuran (THF) and 7 mg of MPCs, were placed on a vortex mixer for ten minutes in a humidity controlled chamber. After the 10 min. mixing period, 50 μL of the aqueous UOx solution was transferred into the other centrifuge tube and mixed for another ten minutes. A 3 μL aliquot of the final mixture was placed on the electrochemically cleaned platinum electrode, either with or without the Pt–B treatment. A second sol–gel layer, which was prepared in the same manner with the exception of containing no MPCs or enzyme, was added on top of the dried layer after 5–30 minutes of waiting for the first layer to dry. Once both layers were deposited on the electrode surface, the electrodes were allowed to sit for 48 hours in a 50% RH controlled chamber.

The inner selective layer, 1:10 polyluminol:polyaniline (PL–A) layer, was applied based on a previously discovered procedure.[11,13] PL–A is known to provide selectivity for UA sensing.[11] The

xerogel–modified electrode described above (Scheme 1) was immersed in 25 mL stirred solution of a 5 mM aniline solution and 0.5 mM luminol (saturated) mixture (0.1 M H_2SO_4; N_2 degassed, 20 min.). The layer was electropolymerized while stirring using cyclic voltammetry (CV) at a sweep rate of 50 mV/s cycling between 0.0 and 1.0 V (vs Ag/AgCl reference electrode) for 12 cycles. Electrodes were rinsed with water and left to dry (ambient, 10 min.).

As used in other biosensing schemes for UA, a polyurethane semipermeable membrane was used as the last layer in the sensor design. A 100% HPU was prepared by adding 100 mg of HPU into a mixture of 2.5 mL of ethanol (EtOH) and 2.5 mL of THF solution and then stir overnight. Once the electrodes had been sitting for 48 hours, 10 μL of the dissolved polyurethane was deposited onto the electrode surfaces and allowed to dry for 30 minutes.

2.5. Film Characterization and Biosensor Performance Evaluation

Prior to testing, fabricated biosensors were soaked in 65.55 mM potassium phosphate buffer solution (pH = 7.00) for a period of one hour. To stabilize the sensor reading, all biosensors were subjected to +0.65 V vs Ag/AgCl in 25 mL of PBS for 20 minutes (Note: Additional pretreatment of 8000 s is necessary for biosensors constructed using MPCs and Pt–B to obtain a steady state background current). During testing, 50 μL aliquots of 50 mM UA stock solution were injected at 200 s intervals while stirring (1100 rpm) to obtain stair–step response to successive 100 μM UA increases. As in prior work, slopes of calibration curves (i.e., current response vs. UA concentration) corresponded to sensitivity while response times ($t_{R-95\%}$) were defined as the time required to reach 95% of the total change in current due to an increase in UA concentration.[2a] Each variable (e.g., the effect of MPC doping or the Pt-B effect on sensitivity) was tested with an individual experiment with multiple electrodes serving as experimental and control experiments, respectively. Each experiment was also repeated to ensure the observed trends were consistent. The multi-channel potentiostat allow for a number of variables to be controlled (e.g., film fabrication, solution conditions, reference electrode changes, injection/pipetting technique, electrode polishing) while largely isolating the variable to be tested.

3. Results and Discussion

Figure 1 is a general representation of a successful biosensing scheme previously developed using strategic LbL construction of xerogel and polymer films for glucose and uric acid detection [1, 2a, 8] The basic design comprises four primary layers of electrode modification: (a) an enzyme–encapsulating xerogel layer; (b) an un-doped xerogel (diffusional layer), (c) an inner selective electropolymer layer, and (d) a multi–functional adlayer of Hydrothane polyurethane (HPU). Taken collectively, the use of these layers as a composite film was shown to perform effectively with extended linear/dynamic rages of detection, adequate sensitivity, fast response times, and high selectivity.[2a] Stable xerogel layers are made via traditional sol–gel chemistry using hydroxymethyl–triethoxy silane (HMTES), a common silane with a hydroxyl functionality at the R group.[8] This particular sensing scheme represents the initial point for our current study focused on identifying and demonstrating modifications that amplify the amperometric signal that has be previously shown to be dampened with each layer of modification with the model glucose system.[8] Signal enhancement is critical to increasing sensitivity for the implementation of the strategy and materials toward target molecules of clinical relevance, like UA monitoring, for example. The two strategies explored here include (1) incorporation of a MPC network into the xerogel layer and (2) modification of the underlying electrode with platinum black (Pt–B) to increase electroactive surface area. To our knowledge, however, the incorporation of Pt–B into a biosensing scheme of this nature is unique. Particularly, the sol–gel is doped with hexanethiolate–stabilized gold clusters known as MPCs, for its well–established stability and versatility compared to other colloidal metal nanoparticles. [14]

3.1. MPC Network Incorporation – "The MPC Effect"

As previously mentioned, MPC doping of a MPTMS xerogel, a thiol-based sol-gel structure, has been shown to provide significant signal enhancement in glucose biosensing schemes of this nature.[2a] In preparation of the current study, the same type of enhancement through MPC doping of MPTMS xerogels was established for UA, a different target molecule. Calibration curves of this system with and without MPC doping, shown in Supporting Information (Fig. SI-1), demonstrate a similar MPC enhancement effect for UA. While the enhancement achieved with incorporating MPCs is easily reproduced, the linearity of the response is not sufficient and

prompted exploration of alternative xerogel material for UA biosensing schemes. Even though MPCs have not been incorporated into any other type of xerogel, prior development of glucose xerogel-based biosensors[8] suggested that HMTES xerogels may provide a promising alternative scaffolding for the current study. To incorporate MPCs into the first HMTES xerogel layer (**Fig. 1**, **inset**), the sol–gel was formed by doping an initial HMTES solution with MPCs as well as UOx. MPC incorporation into the xerogel was confirmed with TEM and has been well–explained in prior work. [2a, 8] After this initial layer, the other layers were deposited as in previous iterations of this strategy (**Fig. 1**). After the construction of the multi-layer film was completed, the analytical sensing performance of the UOx enzyme–doped and MPC–doped sol–gel biosensors, including the dynamic/linear range of the amperometric step response and sensitivity, were compared to analogous systems without MPCs. The systems with MPC–doped xerogels showed a significantly greater current response toward UA compared to sensors without MPCs (**Figure 2**) across physiological relevant UA concentration ranges (0.1 mM – 0.7 mM).[3c] As shown in **Fig. 2 (inset)**, the amperometric i-t responses of the respective films translate into highly linear calibration curves showing two–fold greater sensitivity (i.e., $0.80_{(\pm 0.15)}$ vs. $1.54_{(\pm 0.43)}$ $\mu A \cdot mM^{-1}$) toward UA when the films are doped with MPCs. This representative result was repeatable and additional examples of the effect of MPC incorporation into the HMTES xerogel system are included in Supporting Information (Fig. SI-2-3). The increase in sensitivity is attributed to the MPC network providing more efficient electronic pathways for reporting the peroxide oxidation throughout the film. Once generated by the enzymatic reaction with uric acid, the peroxide oxidation, the indirect signal of uric acid presence in 1st generation biosensing schemes, occurs at the MPC network, decreasing the system's diffusional dependence.[1, 2a]

The significance of this result is three–fold in the context of the current study. While the enhancement of an amperometric signal via the employment of a MPC network has been previously observed,[2a] it has only been reported for a glucose sensing model system. With its successful incorporation into a UA biosensing scheme, these results represent the first translation of the strategy to a new, clinically relevant target. Secondly, signal enhancement via the introduction of a MPC network within the xerogel was previously generated only for a single layer xerogel system (i.e., Pt modified with MPC–doped MPTMS and PU) whereas this work represents the first time the MPC–doped xerogel is used in conjunction with an additional, un-doped, diffusional xerogel layer (Fig. 1) – the xerogel bi–layer system which was so effective in model

glucose sensing systems.[2a, 8] Finally, in a prior study,[2a] MPCs have only been incorporated into xerogels formed from thiol–functionalized silanes (e.g., MPTMS), making this result the first time they have been utilized in other types of silane scaffolds. As in our prior work, [1] the signal enhancement induced with the MPC network is attributed to a decreased dependence of peroxide diffusion and heightened efficiency of reporting of peroxide oxidation throughout the film.

3.2. Platinum Black Modification – "The Pt–B Effect"

Platinized Pt electrodes have been previously employed to facilitate amperometric and voltammetric sensors with enhanced current response.[15] Modification of platinum electrodes with Pt–B was achieved with performing cyclic voltammetry (CV) of 3% chloroplatinic acid (v/v in water) via cycling the potential from +0.6 to –0.35 V (vs Ag/AgCl) at scan rate of 0.02 V/s, a process which visibly coats the electrode surface with a black adlayer. A representative example of the voltammetry during platinization and pictures of the modified electrodes are provided in Supporting Information (Fig. SI-4). CV, differential pulse voltammetry (DPV) and chronocoulometry (CC) of potassium ferricyanide at the modified electrode were used to determine changes to the electroactive surface upon modification with Pt–B. CV was used to qualitatively demonstrate alteration of the electrode interface while, CC, the measurement of charge as a function of time,[16] was employed as previously described[1] using the slope of Anson plots and Equation 1:

$$Q = 2nFACD^{1/2}\pi^{-1/2}t^{1/2} \quad (1)$$

where Q is the charge passed (C), n is the number of electrons transferred, F is Faraday's constant (96,500 C/mole), A is the electroactive surface area (cm^2), C is potassium ferricyanide concentration (5.00 mM in 0.50 M KCl), D is the diffusion coefficient for potassium ferricyanide (7.6×10^{-6} cm^2/s), and t is the time (s). The electroactive surface area calculated from the slope of Anson plots, examples of which are provided in Supporting Information (Fig. SI-5).

Figure 3 shows examples of potassium ferricyanide CV, DPV, and CC at electrodes modified with varying levels of Pt–B. The cyclic voltammetry shown in **Fig. 3A (inset)** is that of a bare Pt electrode (a) compared to Pt electrodes subjected to subsequent Pt–B treatments (b → e). The results indicate that Pt–B modified electrodes exhibit higher electrochemical activity towards

the redox reaction of potassium ferricyanide with progressively higher peak currents in direct correlation with increasing Pt–B deposition. Most notably from the CV results, however, is the increase in capacitive (i.e., charging) current with Pt-B adlayers, a result expected if electrode area increases. Given the significant levels of charging current in the cyclic voltammetry of the Pt-B modified electrodes, ferricyanide voltammetry was also assessed with more sensitive pulse voltammetry (DPV), a technique better able to discriminate Faradaic current from the charging current background. Increasing Faradaic current is observed in the DPV scans with each successive Pt-B treatment (**Fig. 3A**), though only a modest increase in current is noticed after for Pt-B exposures beyond the first.

Potassium ferricyanide CC results parallel the voltammetric measurements with increasing charge passed with each exposure of the electrode to Pt–B (**Fig. 3B)**. The CC results translate directly to measureable increases in electroactive surface area of the electrodes.[16] A summary of the DPV and CC results for Pt–B modified electrodes is shown graphically in **Fig. 3C**. The results indicate that the increasing current is likely related to an increase in surface area of the electrode, as expected with a Pt-B adlayer. A complete summary of measured current values for CV and DPV, as well as charge passed from CC experiments is provided in Supporting Information (Table SI–1) – all of which suggest that the Pt-B is increasing electroactive surface area.

Electrodes were modified with Pt–B prior to the deposition of the xerogel bi–layer and polymer layers (**Fig. 1**) and directly compared to a control group without a Pt–B underlayer. While both systems exhibited step responses across the relevant concentration range (**Figure 4**), Pt–B modified Pt electrodes exhibited a significant increase in the size and definition of the stepping, a nearly two–fold increase in sensitivity ($1.38_{(\pm 0.54)}$ vs. $0.8_{(\pm 0.15)}$ $\mu A \cdot mM^{-1}$). This representative result was repeatable and an additional example of signal enhancement from Pt-B modification of an electrode is included in Supporting Information (Figs. SI-6). The enhanced sensitivity suggests that the Pt–B facilitates an increase in electron transfer as compared to Pt electrodes without Pt–B, effectively increasing the electroactive area of the electrode without expanding its geometric footprint – an important aspect of developing miniature biosensing devices for *in vitro* or *in vivo* applications. This result is coherent with the aforementioned electrode response to potassium ferricyanide (**Fig. 3**). Similar systems were tested with additional layers of Pt-B applied to the electrode interface. The results of these experiments, the calibration curves included in Supporting

Information (Fig. SI-7) consistently show that first exposure of Pt-B results in the greatest increase in sensitivity whereas additional layers of Pt-B seem to decrease the sensitivity and show higher film-to-film variability. The reasons for these trends are not entirely understood but may be related to an inherent instability of Pt–B coatings, [17] in particular the frailty such material when additional adlayers are physically deposited on top of it.

3.3. Platinum Black and MPC Networks – "The Synergetic Effect"

With the effective demonstration of two different strategies that significantly enhance the sensitivity of the biosensing scheme, MPC–doping and application of a Pt–B underlayer, the two effects were combined in order to test for a beneficial synergetic effect. Pt–B was applied to electrodes before deposition of a HMTES xerogel bi–layer where the first layer was doped with MPCs and a capping layer of 100% HPU. Upon subjection of these films to UA injections as before, the performance of the films with Pt–B and MPC doping showed a striking increase in sensitivity compared to systems without these two factors, a nearly 3–fold increase in sensitivity ($2.06_{\pm 0.48}$ vs. $0.8_{(\pm 0.15)}$ $\mu A \cdot mM^{-1}$). **Figure 5** displays the comparison of typical amperometric i-t and calibration curves for each type of composite film: xerogel only (control), MPC-doped xerogel, Pt-B modified electrode, and the combination of incorporating MPCs into xerogels at a Pt-B modified electrode. The sensitivity of using both materials in concert is higher ($2.06_{\pm 0.48}$ $\mu A \cdot mM^{-1}$) than composite films featuring solely MPC doping or Pt-B modification, $1.54_{(\pm 0.43)}$ $\mu A \cdot mM^{-1}$ and $1.38_{(\pm 0.54)}$ $\mu A \cdot mM^{-1}$, respectively. A direct comparison of the film employing both of the materials simultaneously with a film that uses neither material is provided in Supporting Information (Figure SI-8). This representative result was repeatable and an additional example of the synergetic signal enhancement from Pt-B modification of an electrode coupled with MPC-doping enhancement is included in Supporting Information (Figs. SI-9). The combined strategy exhibits increased sensitivity across the clinically relevant range of UA[3c] and should serve to enhance signal within schemes that use layering of materials for selectivity or stability at the expense of dampening transducer signal. The results suggest that the observed enhancement can be attributed to a combination of increasing the electroactive surface area which, in turn, may electronically couple more reporting pathways through the MPC network to the electrode interface. [1]

3.4. Optimized LbL Uric Acid Biosensor – Full System

In order to complete a functional uric acid biosensor, a semipermeable selective membrane composed of a polyluminol–aniline (PL–A) electropolymer is added after the xerogel bi–layer and before the HPU capping layer (see **Figure 1**). Prior work has established that this electropolymerized layer is effective at increasing selectivity to uric acid via discrimination of common interferents.[11] The PL–A layer, however, is another physical layer that dampens the current response of the composite film and represents yet another justification for employing the signal enhancing supplemental materials of the MPC network and Pt–B layer. UA calibration curves and UA induced current signal are traced as a function of each adlayer and are provided in Supporting Information (Fig. SI-10) to show the depression of signal that accompanies the addition of each adlayer. The performance of the full uric acid biosensing system, formed with all four functional layers as well as Pt–B and the MPC network, is shown in **Figure 6** including a representative i-t curve and a typical calibration curve (**Figure 6A**). From this data, the sensitivity of the biosensor can be reported as 0.97 (± 0.11) $\mu A \cdot mM^{-1}$ with an effective linear range up to 0.8 mM UA, easily spanning the normal and abnormal (i.e., diagnostic) physiologically relevant range for UA. [3c] The sensor exhibits a typical response time ($t_{R-95\%}$), a conservative estimate of response time allowing for 95% of the total current change to be achieved, [2a] of ≤15 seconds. The IUPAC defined ($3\sigma_{blank}/\beta_1$) working limit of detection of the sensor is 0.015 mM, an order of magnitude below normal physiological levels of UA. The sensitivity and response time are generally stable for at least 5 days as well (Supporting Information, Fig. SI-11).

An assessment of the selectivity of the full biosensing scheme (**Figure 1**) is shown in **Figure 6B** where a complete film is subjected to injections of common interferents as well as UA injections of different concentrations. Injections of most interfering species (e.g., ascorbic acid, oxalic acid, glucose) resulted in insignificant current responses from the sensor relative to the observed UA response. Some interferents (e.g., acetaminophen and sodium nitrite) resulted in small responses that were still was significantly smaller than that of UA. Additionally, after exposure to interferents, the sensor maintained sensitivity to UA concentration with successive injections of UA at 100 μM and a proportional response to a 300 μM increase (Fig. 6B).

As in certain glucose biosensing reports, selectivity can be compared more quantitatively with the use of selectivity coefficients comparing the response of an interferent to that of the target species.[2a,8,10,20] While this approach is rarely included in literature reports, we have utilized the same

quantitative analysis (equations included in Supporting Information, Table SI-2) to calculate selectivity coefficients for each interferent and uric acid (300 μM), a result visually presented in Fig. 6B (inset). The figure emphasizes critical selectivity with a selectivity coefficient of 0.16 for UA compared to just 0.04 for sodium nitrite and negative selectivity coefficients for ascorbic acid, oxalic acid, and glucose. A direct comparison of selectivity coefficient values from this study are compared with glucose biosensing studies known to use the same parameter to establish selectivity in Supporting Information Table SI-2. With the exception of acetaminophen (see below) selectivity coefficient values for this UA sensing scheme are in line with selectivity deemed effective for other reported glucose biosensing schemes.[18] As with many other UA sensors,[19] acetaminophen, with a selectivity coefficient of 0.14, remains a problematic species during sensing, though we note its selectivity coefficient is lower here than in most studies. Because acetaminophen is an artificial interferent (i.e., not naturally occurring in the body) it can be managed with time and patient history to maintain the applicability of the sensing scheme for medical diagnosis.[11]

4. Conclusion

As the exploring of advantages and disadvantages of incorporating nanomaterials, [1–2] including metallic NPs, [4c, 4d] into LbL biosensor schemes increases in the scientific society, it is crucial to investigate signal enhancement. The use of modified electrodes is prominent in this field as is the inevitable signal depression that comes from blocking an electrochemical interface with any foreign material. This study has established two signal–enhancing strategies: the incorporation of an MPC network and the modification of the electrode interface with Pt–B. Both of these strategies, either individually or in concert with each other, allowed for an enhance signal for UA biosensing. The results suggest that the increased electrode surface area achieved with Pt-B coupled with the MPC network doped into the xerogel layer that also housing the enzymatic reaction allows for an optimized sensing mechanism where peroxide oxidation is readily reported via highly efficient electronic communication throughout the film.[1]

The performance of the complete biosensing scheme presented in this report rivals or exceeds that of existing UA sensing strategies, a comparison that can be found elsewhere.[11, 19] The significance of the presented findings however, are believed to be more expansive than simply

presenting another UA sensing scheme. First, this work represents the successful demonstration of using MPC networks from simple model glucose systems [2a] to other target species of clinical relevance, in this case UA for PIH detection and pre–eclampsia risk assessment. The results of these studies suggest that similar strategies using the same materials could be applied to numerous other target species and sensor development. Second, in terms of potential *in vivo* and *in vitro* sensors, the proposed sensing scheme demonstrates critical signal enhancing strategies that lend themselves to miniaturization of the system on to needle or wire microelectrodes. Similarly, with any implantable device, biocompatibility must be addressed and often is with additional electrode modification. Whether this modification involves NO releasing materials, [20] polymeric films, [21] or self–assembled materials,[22] the strategies presented in this study should prove useful for enhancing the analytical signal and moving biosensing technology forward.

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Captions (also listed under figures)

Figure 1. Schematic LbL representation of 1st generation UA biosensing scheme featuring platinum black modification of electrode and xerogel doping with MPCs (**Inset**).

Figure 2. Representative amperometric i-t curves and corresponding calibration curves (**Inset**) during successive 0.1 mM injections of uric acid at platinum electrodes modified with (**a**) UOx embedded HMTES xerogel and (**b**) UOx embedded HMTES xerogel doped with MPCs, each coated with undoped xerogel followed by HPU. Note: In some cases, standard error bars are smaller than markers for average value (n = 3-4).

Figure 3. **(A)** Differential pulse voltammetry (anodic sweep),cyclic voltammetry (**inset**), and **(B)** chronocoulometry (CC) of 5 mM potassium ferricyanide (0.5 M KCl) at **(a)** bare and platinum black modified platinum electrodes formed from **(b-e)** 1 to 4 voltammetric deposition scans; **(C)** summary of DPV current (n = 4) and CC-determined area (n = 8) as a function of the number of scans/layers of platinum black. Note: In some cases, standard error bars are smaller than markers for average value.

Figure 4. Representative amperometric i-t curves and corresponding calibration curves (**Inset**) during successive 0.1 mM injections of uric acid at platinum electrodes modified with **(a)** UOx embedded HMTES xerogel and **(b)** Pt–B and UOx embedded HMTES xerogel, each coated with undoped xerogel followed by HPU. Note: In some cases, standard error bars are smaller than markers for average value (n = 4).

Figure 5. Representative amperometric i-t curves and corresponding calibration curves (**Inset**) during successive 0.1 mM injections of uric acid at platinum electrodes modified with (**a**) UOx embedded HMTES xerogel, (**b**) UOx embedded HMTES xerogel doped with MPCs, (**c**) Pt–B and UOx embedded HMTES xerogel, and (**d**) Pt–B and UOx embedded HMTES xerogel doped with MPCs, each coated with undoped xerogel followed by HPU. Note: In some cases, standard error bars are smaller than markers for average value (n=3-4).

Figure 6. **(A)** Representative amperometric i-t curves and corresponding calibration curve (**Inset**) during successive 0.1 mM injections of uric acid at platinum electrodes modified Pt–B, UOx embedded HMTES xerogel doped with MPCs, undoped HMTES xerogel and 100% HPU; **(B)** typical amperometric i-t curve during injections of common interferent species and UA and a graphical summary (**Inset**) of selectivity coefficients for acetaminophen (AP), ascorbic acid (AA), $NaNO_2$, and glucose (Glu). Note: In some cases, standard error bars are smaller than markers for average value (n=4).