

Peroxidase-Like Behavior of Ni Thin Films Deposited by Glancing Angle Deposition for Enzyme-Free Uric Acid Sensing

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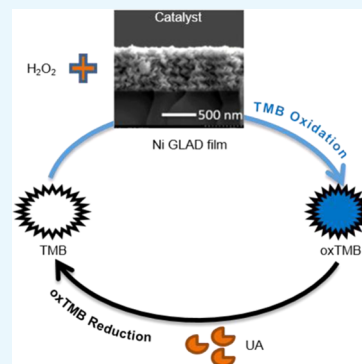
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ABSTRACT: We present a nanozyme-based biosensor fabricated from nanostructured Ni films deposited onto a silicon wafer by glancing angle deposition (GLAD) for enzyme-free colorimetric monitoring of uric acid (UA), a biomarker for gout, high blood pressure, heart disease, and kidney disease. The helically structured Ni GLAD nanozymes exhibit excellent peroxidase-like activity to accelerate the oxidation reaction of colorless 3,3',5,5'-tetramethylbenzidine (TMB) to a blue product, oxidized TMB (oxTMB), mediated by H_2O_2 . In the presence of UA, oxTMB is reduced, decreasing the optical absorbance by an amount determined by the concentration of UA in the solution. The nanozyme not only mimics peroxidase but also possesses the notable qualities of reusability, simple operation, and reliability, making it environment-friendly and suitable for on-demand analysis. We optimized essential working parameters (pH, TMB concentration, and H_2O_2 concentration) to maximize the initial color change of the TMB solution. The catalytic activity of this nanozyme was compared with conventional nanofilms using the Michaelis–Menten theory. Based on this, enzyme-free biosensors were developed for colorimetric detection of UA, providing a wide detection range and a limit of detection ($3.3 \mu M$) suitable for measurements of UA concentration in sweat. Furthermore, interference from glucose and urea was studied so as to explore the potential of the biosensor for use in the clinical diagnosis of UA biomarkers.



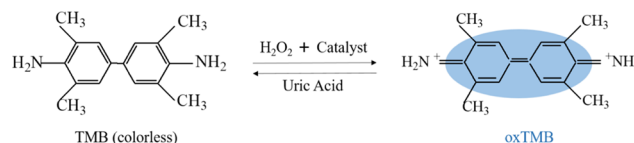
1. INTRODUCTION

Uric acid (UA) is an important biomarker of human health, given that it is implicated in a range of medical conditions such as gout, arthritis, kidney stones, heart diseases, and renal failure.¹ Gout, for example, is a prevalent and painful disease caused by uric acid buildup in joints.² At high concentrations, UA forms crystals, which deposit in the affected joints and cause tissue damage.³ The UA concentration in vivo is directly related to the incidence rate of gout (and the other conditions mentioned above), and thus, the assessment of UA levels in serum plays an important role in early diagnosis and therapy.^{4–6} Most current clinical methods are based on the analysis of blood samples, as the UA concentration in blood is relatively high: 155–357 μM in adult females and 208–428 μM in adult males.⁷ However, less invasive testing—such as the analysis of sweat—would reduce the need for collection of blood samples. As the UA concentration in sweat is much lower than in blood in the range of 18–35 μM , which requires quantification methods with high sensitivity and low operating range.⁸

In the literature, various methods for the detection and quantification of UA concentration in human serum samples have been described, including chemiluminescence, chromatographic, electrochemical, and spectroscopic/colorimetric techniques.^{3,9–12} Among these techniques, colorimetric detection has begun to attract massive attention over the last few decades due to its potential for simplified operation and user-friendliness. The convenience of colorimetric sensing could

ultimately lead to deployment outside a medical environment and/or operation by individuals with more limited training.¹³ Techniques for colorimetric detection of UA have previously been demonstrated. For instance, Mason et al. used horseradish peroxidase (HRP) to catalyze the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) with H_2O_2 . TMB is a chromogen (colorless in the reduced form and blue in the oxidized form), which can be easily observed with the naked eye or by UV–Vis spectroscopy.¹⁴ The TMB oxidation reaction and its distinctive color change are reversed in the presence of UA, and therefore, the reaction mechanism has been used to sense UA.^{3,5} In this sensing scheme (Scheme 1), the catalyst is a critical element

Scheme 1. Reaction Scheme for Colorimetric Detection of Uric Acid Using TMB and a Catalyst



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that facilitates the decomposition of hydrogen peroxide to generate active oxygen species or free radicals, which further react with colorless TMB to form the blue-colored oxTMB compound. When UA is added to this oxTMB solution, the oxTMB is reduced, and the blue color fades.

Throughout the literature, the initial oxidation of TMB is generally done through a natural peroxidase-based catalyst; however, these natural enzymes often require well-controlled storage conditions (temperature, pH, purity, etc.), which may result in poor stability and shelf-life.^{15–17} Nanoparticles used as “artificial enzymes” or “nanozymes” have also been investigated, and they have been shown to possess remarkably improved stability under harsh working conditions, and well as efficient catalytic activity at low concentrations.^{18,19} For instance, Kumar et al.³ used gold nanoparticles as a catalyst to accelerate the TMB oxidation reaction for UA sensing, and Kumar et al.⁵ expanded the catalytic surface area and reduced the limit of detection (LOD) by incorporating graphene oxide. These studies suggest that the extended surface area of the catalyst plays an important role in increasing the absorbance of oxTMB. As a result, metal–organic frameworks (MOFs) and nanocrystals (such as MIL-53 (Fe), ZnFe_2O_4 , etc.) are also being explored as peroxidase mimics, and some of these high-surface-area catalysts have been used for the colorimetric detection of UA. For example, Liu et al. used peroxidase-mimicking MOFs in the presence of TMB to colorimetrically detect H_2O_2 generated during the reaction of uric acid with uricase, and other nanoparticles and nanocrystals have also been used to mimic the behavior of peroxidase for UA biosensing.^{18,20–22} Among these high-surface-area nanozymes, Ni has begun to attract considerable attention due to its excellent catalytic activity, high stability, low cost, and ease of preparation.²³ One common drawback among these nanomaterial-based systems, however, is the difficulty in recovering the catalyst after use, which prevents the reuse of nanozyme catalysts.^{24,25}

The effectiveness of nanomaterials as catalysts results from their large specific surface area, which results in a high concentration of catalytically active sites.²⁶ One method of producing surface-anchored materials with a very high surface area is glancing angle deposition (GLAD). GLAD is a physical vapor deposition technique that exploits atomic shadowing and dynamic motion control to engineer nanostructures with high surface area and controlled porosity.^{27–29} During GLAD, vapor flux is incident onto the substrate at glancing angles ($>70^\circ$) with respect to the surface normal. The thin films fabricated by GLAD tend to be porous in nature with wide gaps separating nanosized columns, and as a result, they also tend to have high internal porosity. In the previous work, it has also been shown that the nanostructures may have a fine, fibrous structure, such as the Si helices deposited at 83° shown in ref 30. By employing dynamic substrate motion, the GLAD technique also enables the fabrication of peculiarly shaped nanostructures including vertical columns, zigzags, and helices. These GLAD films have been reported in a wide range of applications such as photocatalysis, solar cells, microfluidics, and electrical sensors, and NiO GLAD films, in particular, have been demonstrated previously as catalysts in a reagent-less, amperometric biosensor for urea.^{29–31}

For the first time, we report the TMB-based colorimetric detection of UA using helical Ni nanofilms deposited onto a silicon surface by GLAD as a catalyst. As these nanofilms consist of surface-anchored helical structures, we expect that

they can be easily recovered after use and reused. In this work, we first optimize the conditions (reactant concentration, pH, etc.) of the TMB oxidation reaction in the presence of the helical Ni catalyst. Next, we compare the catalytic activity of the nanostructured films to a flat Ni reference sample. Finally, we investigate the use of this system for the detection of uric acid at physiologically relevant concentrations. The selectivity, limit of detection, and reusability of the GLAD film are also characterized.

2. RESULTS AND DISCUSSION

2.1. Fabrication and Characterization of GLAD Films.

Two separate types of Ni thin films deposited on silicon surfaces were studied as peroxidase-mimicking catalysts for the oxidation of TMB: a nanostructured helical thin film deposited by the GLAD technique and, as a control sample, a flat Ni film deposited at a conventional, near-normal deposition angle ($\sim 0^\circ$). The GLAD film was deposited at an oblique angle of 78° , which was selected as a compromise between maximum surface area and maximum accessibility of the interior pore structure. The surface area generally peaks for lower deposition angles in the $60\text{--}70^\circ$ range, however, for this range of angles, the separation between neighboring nanocolumns can be quite small, which potentially limits diffusion of reactants into the interior.^{32,33} At greater deposition angles, the nanocolumns become increasingly separated reducing both the diffusion barrier and the surface area.³⁴

High-resolution SEM images of the two catalytic films, the GLAD film and the flat film, are presented in Figure 1. The

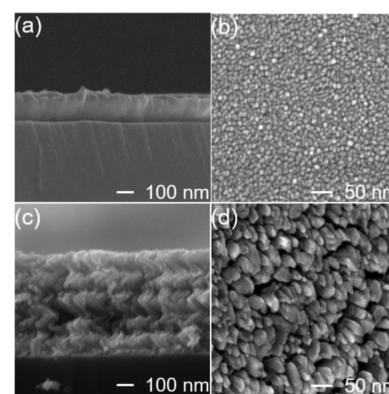


Figure 1. SEM images of the flat (a, b) and GLAD (c, d) catalytic Ni films. Cross-sectional views are shown in (a, c), and top views are shown in (b, d).

conventional flat Ni film (Figure 1a,b) is 150 nm thick, and while some surface roughness is apparent, there are no obvious voids present in the film. The GLAD structure (Figure 1c,d) is much different; analysis of the images leads to a thickness estimate of 610 nm, and the helical character of the film is clear. Spaces (10–30 nm) are observed between the individual columnar structures, which is significantly larger than all reactants and should allow diffusion into the interior of the GLAD structure.

To analyze the composition of the Ni GLAD film, X-ray photoelectron spectroscopy (XPS) was performed, and the binding energy regions associated with Ni and C are shown in Figure 2a,b. The Ni (2p) spectrum exhibits peaks for metallic Ni (at 852.2 and 869.5 eV), but higher-energy peaks are also observed for Ni^{2+} at 855 and 872.8 eV (together with their

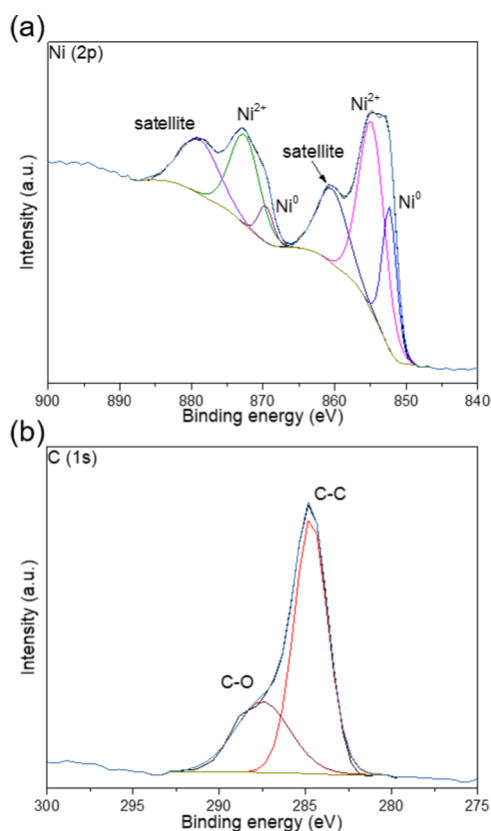


Figure 2. High-resolution XPS data for the Ni GLAD film in the binding energy regions associated with (a) Ni and (b) C.

associated satellite peaks at 860.7 and 879.2 eV).^{35,36} These higher-energy peaks likely arise as a result of spontaneous oxidation of the Ni surface following deposition, and they indicate that nickel oxide species may be present at the catalytically active surfaces.³⁷ In the C (1s) spectrum (Figure 2b), the peaks at 284.8 and 287.2 eV are, respectively, attributed to C–C and C–O from adventitious carbon adsorbed on the Ni surface.³⁸

2.2. Optimization of the TMB Oxidation Conditions.

Given the absence of information on Ni GLAD films as TMB oxidation catalysts, we first attempted to optimize the TMB oxidation conditions in the absence of uric acid to maximize the absorbance at 652 nm. We adjusted the TMB concentration, H_2O_2 concentration, and solution pH, and the results of these experiments are presented in Figure 3. In each case, the UV–Vis spectra were recorded after 15 min of contact with the catalytic GLAD Ni film.

In its oxidized form, the chromogenic reagent TMB appears blue in solution, and thus, increasing the TMB concentration in the reaction mixture was expected to lead to greater absorbance at 652 nm. This effect is observed in Figure 3a, where increasing the concentration was found to increase the light absorption. We also found that the standard deviation of absorption measurements decreased for greater TMB concentrations (Figure S1a), and, therefore, we fixed the TMB concentration to 1.6 mM in all subsequent experiments.

In Figure 3b, absorbance changes in response to variations in H_2O_2 concentration are shown. As hydroxyl radicals are known to oxidize TMB, greater H_2O_2 concentrations were expected to result in more strongly absorbing oxTMB solutions; this effect

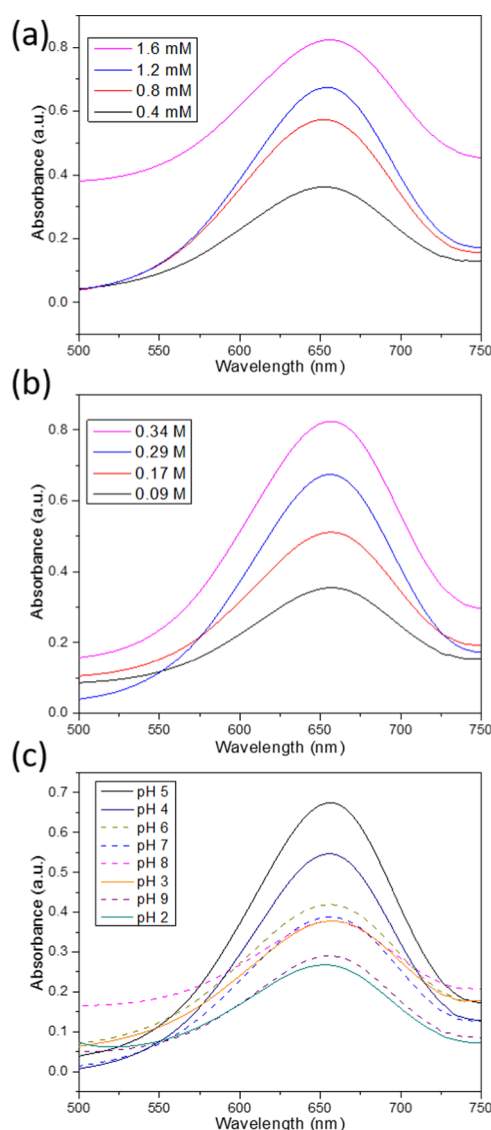


Figure 3. Representative UV–Vis absorbance spectra for TMB solutions oxidized in the absence of uric acid: (a) the TMB concentration is varied at a fixed pH (pH 5) and H_2O_2 concentration (0.29 M); (b) the H_2O_2 concentration is varied at a fixed pH (pH 5) and TMB concentration (1.2 mM); and (c) the pH is varied at fixed TMB and H_2O_2 concentrations (1.2 mM and 0.29 M, respectively). Spectra were recorded after 15 min of contact time with the Ni GLAD film, which was removed before measurement.

is observed in Figure 3b. The peak absorbance and standard deviation are shown in Figure S1b.

Fixing the TMB concentration at 1.2 mM and the H_2O_2 concentration at 0.29 M, we found that pH had a large influence over oxidation of the TMB. Figure 3c shows that lower pH values lead to dramatically increased absorption at 652 nm, with the highest absorbance measured at pH 5. The breakdown of hydrogen peroxide at low pH leads to the formation of $\text{OH}^\bullet$ radicals, which likely promote TMB oxidation and lead to increased absorbance.³⁹ Earlier studies of enzyme-catalyzed TMB oxidation have described a tendency of oxTMB to further oxidize into diimine at pH values below 4.⁴⁰ We also observed a reduced absorption for all pH values below pH 5, and because of this, we fixed pH 5 as our optimum and all subsequent experiments were performed at pH 5.

2.3. Peroxidase-Like Catalytic Activity. To demonstrate the increased catalytic activity of the GLAD film, the oxidation of TMB was compared for flat and GLAD Ni films as well as a catalyst-free solution. Figure 4 shows UV–Vis spectra recorded

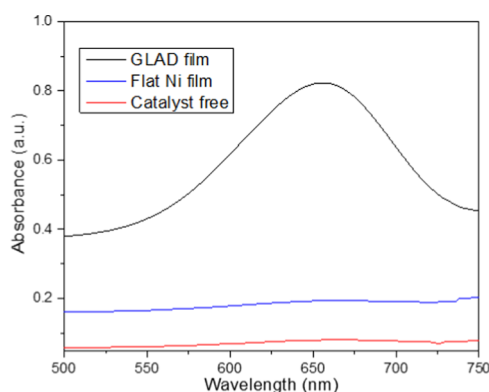


Figure 4. Comparison of absorbance spectra for aqueous solutions of 1.6 mM TMB as oxidized by a GLAD Ni film (black line), a flat Ni film (blue line), and a catalyst-free solution (red line) in the presence of 0.29 M H_2O_2 at pH 5. The areas of the catalytic films were both 0.5 cm^2 , and the total volume of solution was $515 \mu\text{L}$.

for optimized 1.6 mM TMB solutions at pH 5 after 15 min in the presence of 0.29 M H_2O_2 and the catalytic Ni films. The catalyst-free solution (without either Ni film) has a relatively featureless UV–Vis spectrum with a gentle curve; by eye, the solution appeared colorless. On the other hand, for the flat Ni film, a relatively small peak (0.19 a.u.) is observed, and a faint blue color could be seen in solution. For the nanostructured nickel GLAD films, a dramatically increased absorption peak is observed at 652 nm (0.82 a.u.), and the solution appeared blue. This enzyme-like activity of the Ni films may originate from the Ni^{2+} ions present at the surface of the films.⁴¹ Using the Beer–Lambert equation, we can estimate the oxTMB concentrations of the solutions oxidized in the presence of flat and GLAD catalytic films to be 4.8 and 21 μM , respectively. The concentration achieved using the flat film is considerably lower than that for the GLAD film, and thus we can conclude that the catalytic effect of the GLAD film is much stronger than that of the flat films for this reaction. This may be closely related to the high surface area of GLAD pillars and the associated high concentration of catalytically active sites.⁴² These qualities of GLAD films have previously been shown to promote enhanced analyte sensitivity in systems such as indium tin oxide (ITO) columns modified with toll-like receptors for pathogen identification or ITO columns modified with recombinant hemoglobin I for H_2S detection.^{43,44}

To further evaluate the performance of the catalytic films, the reaction kinetics were also studied and modeled using the Michaelis–Menten equation. Figure 5 describes the relationship between the rate of TMB conversion, V , and the TMB concentration, $[S]$, for GLAD and flat Ni films. At low concentrations, the reaction rate increases linearly with TMB concentration ($R^2 = 0.996$); however, beyond a certain TMB concentration, the density of catalytically active sites becomes the limiting factor and the reaction rate saturates. Using eq 1, the Michaelis–Menten constant, K_m , which describes the substrate concentration when the reaction rate is half of the maximum reaction rate, was estimated to be 1.07 mM for the Ni GLAD film and 2.38 mM for the flat Ni film. The lower

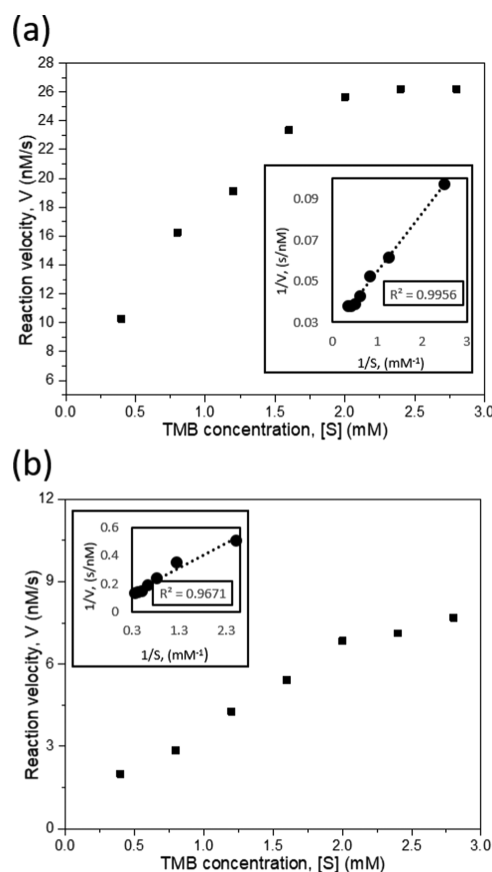


Figure 5. TMB conversion rate vs concentration for TMB in the presence of (a) the GLAD film and (b) the flat Ni film.

value is desirable as it indicates that the GLAD catalyst has a greater affinity for TMB than its flat counterpart. Comparing these kinetic parameters to values obtained for other catalysts (see Table 1), it is clear that GLAD films exhibit good catalytic behavior for the oxidation of TMB in the presence of H_2O_2 .

Table 1. K_m Values for Different Peroxidase-Mimicking Materials

catalyst	K_m (mM)	ref
hemin	5.23	45
HRP	0.43	12, 45
CuS nanoparticles	0.21	12
Ni GLAD film	1.07	this work
Ni flat film	2.38	this work

2.4. Reusability of the Catalytic GLAD Film. The reusability of the GLAD films was evaluated by recording absorbance spectra after consecutive TMB/ H_2O_2 reactions. Each reaction cycle was performed for 15 min in the presence of the same Ni GLAD film, and after every measurement, the catalytic film was removed from solution, rinsed, and immersed in a fresh TMB/ H_2O_2 solution before the next data point was collected. Precise handling and washing of the GLAD films with organic solvents after each use was important to ensure repeatability. As depicted in Figure 6, the absorbance of the oxTMB solutions after each reaction cycle was relatively constant (0.82 ± 0.03), indicating that the GLAD films can be reliably reused without a reaction terminating agent.⁴⁶ This suggests that the GLAD catalyst may be more environmentally

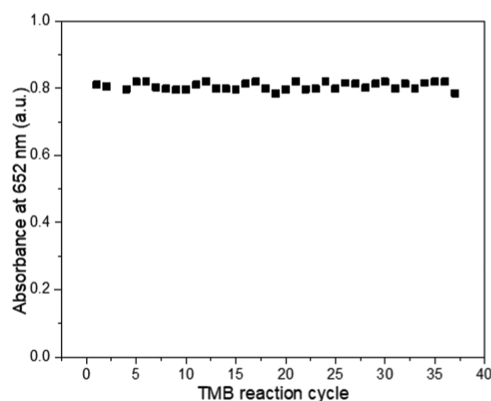


Figure 6. Absorbance of optimized oxTMB solutions at 652 nm. For every data point, a fresh solution (1.6 mM TMB, 0.29 M H_2O_2 , pH 5) was prepared and left in the presence of the same 0.5 cm^2 Ni GLAD film for 15 min. After 15 min, the absorbance was measured and the solution was discarded.

friendly and economically efficient than other technologies and possibly easier to implement in the field.

2.5. Colorimetric Sensing of Uric Acid. UA biosensors were comprised of 515 μL of oxTMB/ H_2O_2 solution, which was initially blue in color (absorbance of 0.82 a.u.). UA solution (100 μL) was then added at concentrations of 0–594 μM , resulting in a final volume of 615 μL . The biosensor operates based on the UA-activated suppression of the TMB color reaction; as shown in Figure 7a, the higher the concentration of UA that was added, the lighter in color the solution became. In the UV–Vis spectra shown in Figure 7b, the absorbance peak at 652 nm decreases with increasing UA concentration. Notably, UA concentrations as low as 1.5 μM were found to have an observable effect on the UV–Vis spectra. In the figures, the original concentration of the added UA is reported on the x -axis rather than the diluted values (which take into account the volume of the oxidized TMB and H_2O_2) because these represent the concentrations of UA in the actual test samples. For example, when solutions containing 0 μM UA were added to the oxTMB/ H_2O_2 , the absorbance decreased from 0.82 to 0.81 due to the effect of the dilution.

The effect of the UA concentration over absorbance at 652 nm is shown in Figure 7c, and a nonlinear response was observed. In the low concentration end of the curve (0–6 μM), however, a fairly linear response was noted ($R^2 = 0.98$). The achieved limit of detection (LOD), 3.3 μM , was calculated using the $3\sigma/\text{slope}$ within this low concentration range, and this value is lower than the UA concentration found in sweat (18–35 μM).⁸

To investigate the specificity of the biosensor, the response of the oxTMB solution to urea and glucose—which are also found in sweat—was characterized. In Figure 8, the GLAD Ni sensor's responses to equal concentrations (446 μM) of glucose, urea, and UA are presented. As depicted, the change in absorbance is the greatest in response to UA, however, the addition of either glucose or urea also strongly suppresses the absorbance at 652 nm. Because these substances are found in high concentrations in sweat (22 mM for urea and 1 mM for glucose), the data indicate that interference is likely to complicate the quantification of UA concentration based on the TMB mechanism.^{8,47} Because the TMB redox process is not unique to our sensing platform, other TMB-based sensor systems are also quite likely to suffer from these interference

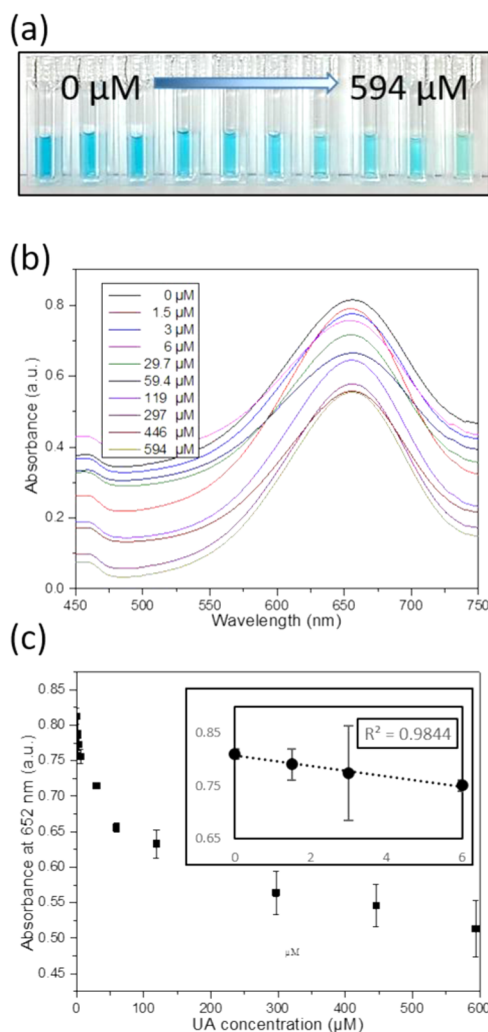


Figure 7. (a) Images of oxidized 1.6 mM TMB solutions, reduced via addition of UA solutions of various concentrations. (b) UV–Vis absorption spectra of the samples shown in (a). (c) Response curve for UA concentrations from 0 to 594 μM (inset: zoomed-in view of the low concentration region up to 6 μM).

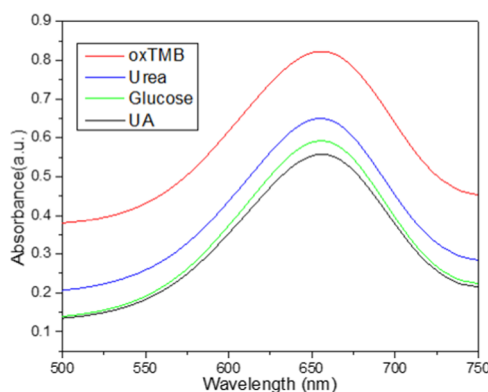


Figure 8. Comparison of absorbance spectra for oxTMB solutions before (red) and after the addition of 446 μM of urea (blue), glucose (green), or UA (black).

issues. One approach that could potentially be taken in the future to mitigate interference would be to use membrane separators to ensure that only UA reaches the sensor.

Additional interferents such as Na^+ , Cl^- , and Fe^{3+} should also be taken into account.

3. CONCLUSIONS

In summary, we have fabricated helically structured Ni nanofilms on silicon substrates using GLAD, and we found that these films exhibited peroxidase-like activity to catalyze the oxidation of TMB. We utilized this colorimetric TMB/oxTMB reaction to form uric acid sensors that were able to achieve a low LOD ($3.3\ \mu\text{M}$) and were sensitive throughout the entire clinically relevant range ($15\text{--}500\ \mu\text{M}$). The advantages of on-demand analysis, good recyclability, excellent analytical performance, and visual detection, as well as the lack of natural enzymes and terminating agents, suggest that GLAD-based sensors may be a promising tool for UA detection. However, we also conducted an interference study for TMB-based uric acid sensors, and we found that in addition to UA-sensitivity, the TMB-centered system also responds to several common metabolites (specifically, glucose and urea). The fact that the colorimetric nanozyme/TMB-based sensor for other analytes including glucose, cysteine, and ascorbic acid has been demonstrated underscores the lack of specificity of this process.^{48–51} Care must therefore be taken to mitigate interference effects, particularly when working with complex biological samples such as blood or sweat.

Despite this limitation, glancing angle deposition is a versatile technique that can be used to fabricate nanostructured thin films from a wide range of materials, including precious metals, transition metals, oxides, and carbon (i.e., materials that can be evaporated). In this work, we have shown that helical nickel films exhibit enhanced catalytic activity for TMB oxidation; nanostructured thin films deposited by GLAD could potentially be used as nanozymes in reactions beyond TMB oxidation. To realize the full potential of GLAD films as reusable catalysts, the effects of the many tailorable physical properties must be explored.

4. EXPERIMENTAL SECTION

4.1. Reagents and Materials. 3,3',5,5'-Tetramethylbenzidine tablets (TMB, T5525), 30% hydrogen peroxide (H_2O_2), phosphate citrate buffer, sodium hydroxide, dimethylformamide (DMF), uric acid (UA), urea, and glucose were purchased from Sigma-Aldrich (St. Louis, MO) and used as received. Deionized water of conductivity $0.055\ \mu\text{S}$ was used throughout this work.

4.2. Fabrication of GLAD Films and Characterization. The Ni GLAD films were deposited on clean silicon wafers by electron beam evaporation (see Taschuk et al. for an in-depth description of the process).²⁹ The base pressure prior to deposition was $<2 \times 10^{-6}$ Torr, and the pressure during deposition was roughly 1×10^{-5} Torr. The deposition angle between the incident vapor flux and the substrate normal was set to $\alpha = 78^\circ$, and during deposition, the substrate was rotated at a rate of one complete revolution for every 100 nm of film growth. The final thickness of the Ni GLAD film was 610 nm as measured on a cleaved sample in a cross-sectional view by scanning electron microscopy (SEM, Hitachi S-4800). X-ray photoelectron spectroscopy (XPS, Kratos AXIS Ultra) was used to analyze the surface composition of the GLAD film. In this technique, samples were irradiated with a monochromatic Al $K\alpha$ source ($h\nu = 1486.71\ \text{eV}$), and the pressure of the analysis chamber was below 5×10^{-10} Torr during all

measurements. XPS spectra were corrected for charge effects against the C (1s) peak at 284.8 eV.

4.3. Colorimetric Measurements. TMB solutions were prepared by dissolving the appropriate mass of TMB tablets in a 9:1 v/v mixture of phosphate citrate buffer and DMF followed by constant stirring at 700 rpm for 40 min at room temperature ($21\ ^\circ\text{C}$). Solutions of UA of various concentrations were prepared by dissolving UA powder in 0.01 M NaOH and stirring at room temperature ($21\ ^\circ\text{C}$) for 1 h.

UA-free test solutions (for the optimization of light absorption) were prepared by combining 500 μL of TMB solution (prepared at concentrations from 0.4 to 1.6 mM and pH values from 3 to 9) with H_2O_2 solution (at concentrations from 0.1 to 0.34 M). These solutions were then brought into contact with $0.5\ \text{cm}^2$ of the catalytic Ni film for 15 min, during which time the solution was stirred at 1100 rpm. (Care was taken to ensure that no contact occurred between the magnetic stir bar and the catalytic film.) The catalytic film was removed prior to UV–Vis measurement. UA-sensing test solutions were prepared by combining 100 μL of the appropriate UA solution, 500 μL of the 1.6 mM TMB solution, and 15 μL of the 0.29 M H_2O_2 solution and then adding one piece of the catalytic Ni film ($0.5\ \text{cm}^2$) and incubating at room temperature ($21\ ^\circ\text{C}$) for 15 min. The catalytic film was removed immediately prior to UV–Vis measurement, and the total volume of the test solutions (without the Ni film) was 0.615 mL. Prior to reuse, films were gently rinsed with deionized water, acetone, isopropyl alcohol, and then again with deionized water. To prevent damaging the surface of the film, the silicon substrates were always handled by the edges using plastic tweezers.

Light absorbance of both UA-free reference solutions and UA-sensing solutions was measured by UV–Vis spectroscopy (Jasco V-630 spectrophotometer) from 500 to 750 nm, at a scanning rate of 120 nm/min. A cuvette filled with only phosphate citrate buffer was used as a blank. The limit of detection (LOD) was calculated based on the formula $\text{LOD} = 3\sigma/m$, where σ is the standard deviation of the absorbance measurement of the blank (calculated based on four measurements of independent samples) and m is the slope of the absorbance vs UA concentration curve at low concentration.

4.4. Optical Measurement of Catalytic Activity. Catalytic activity was measured by monitoring the evolution of absorbance at 652 nm and fitting to the Michaelis–Menten equation⁵²

$$\frac{1}{V} = \left(\frac{K_m}{V_{\max}} \right) \left(\frac{1}{[S]} + \frac{1}{K_m} \right) \quad (1)$$

In this equation, V is the reaction rate, $V_{\max}$ is the maximal reaction rate, $[S]$ is the concentration of TMB, and K_m is the Michaelis–Menten constant. The concentrations and reaction rates were extracted from the absorbance data according to Beer–Lambert's law, $A = \epsilon l[S]$, where A is the absorbance at 652 nm, $\epsilon = 3.9 \times 10^4\ \text{M}^{-1}\ \text{cm}^{-1}$ is the absorbance coefficient for oxTMB, and l is the path length (i.e., 1 cm).⁵³

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.9b04071>.

Peak absorbance at 652 nm for TMB solutions oxidized in the absence of uric acid under different conditions; comparison of peak absorbance at 652 nm for aqueous

solutions of 1.6 mM TMB with different catalysts; plots of the peak absorbance at 652 nm in the presence of different analytes (PDF)

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Notes

The authors declare no competing financial interest.

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