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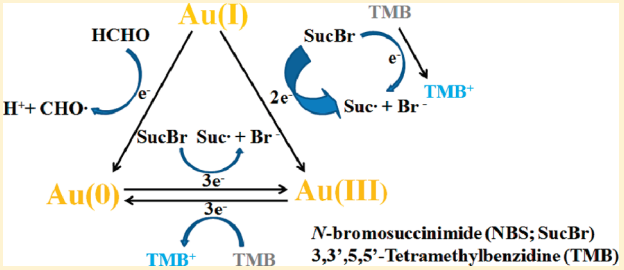
Balancing Redox Activity Allowing Spectrophotometric Detection of Au(I) Using Tetramethylbenzidine Dihydrochloride

Gyoung-Gug Jang and D. Keith Roper*

Ralph E. Martin Department of Chemical Engineering, 3202 Bell Engineering Center, University of Arkansas, Arkansas 72701, United States

Supporting Information

ABSTRACT: Aqueous, acid solutions containing balanced amounts of a strong reductant (formaldehyde, HCHO) and a strong oxididant (*N*-bromosuccinimide, NBS) allow the first sensitive spectrophotometric analysis of monovalent gold ion, Au(I), using oxidation of color reagent 3,3′,5,5′-tetramethylbenzidine dihydrochloride (TMB). This new method enables various oxidation states of Au ion to be quantified by balancing reduction potential in a Au solution. At low reductant levels, NBS oxidizes Au(I), which linearly suppresses subsequent oxidation of TMB by NBS to its blue charge-transfer complex of diamine and diimine to 2.00 mg L^{-1} of Au, resulting in reduced color formation. The linear range of Au(I) quantitation was increased substantially relative to existing methods: from 0.005 to 1.00 mg L^{-1} (R^2 = 0.988). For this range, the limit of detection was 0.0025 mg L^{-1}, which is comparable to the best reported spectroscopic method to analyze Au(III). At relatively high reductant levels, Au(I) is reduced to Au(0), then subsequently oxidized from Au(0) to Au(III) by addition of NBS. TMB is oxidized to its blue charge-transfer complex via the reduction of the reoxidized Au(III) to Au(0). Balancing redox conditions of HCHO/NBS at a molar ration of 22.7 allows quantitative measurement of Au(I) across a linear concentration range of 0.05−2.00 mg L^{-1} (R^2 = 0.997). This balancing redox condition could allow sensitive, quantitative, spectrophotometric analysis of other metal ions besides Au by targeting the metal ion's reduction potential with an associated redox-sensitive color reagent.



Au(I) compounds have attracted recent interest due to their unique biological activity[1] and their industrial utility as catalysts and as oxidation agents in electroless plating.[2] For instance, Au(I) phosphine complexes have been studied to treat breast cancer[3] and to catalyze oxidation of CO gas.[4] Electroless plating of Au(I) from aqueous solution has been used to fabricate solid-state islands, films, and particles of Au(0).[5−9] A method to measure Au(I) concentration in situ quantitatively and dynamically would be useful to characterize and control Au(I) content in electroless plating, catalysis, and in vivo therapies, just as several analytic methods have been employed to analyze Au(III) and Au(0) content in various applications. Instrumental methods to determine Au(III) content in aqueous and geological samples after thermo-chemical pretreatment were reviewed by Barefoot (1999).[10] These include atomic absorption spectroscopy (AAS), atomic emission spectroscopy (AES), inductively coupled plasma mass spectrometry (ICPMS), and voltammetry, as well as hyphenated (e.g., ICP-AES) approaches. Spectrophotometric methods to determine Au(III) in aqueous solution using color reagents were summarized by Zaijun (2003)[11] and by Kamble (2010),[12] who reviewed new synthetic reagents for spectrophotometry of Au(III). Techniques to measure Au(III) have also been used to quantatively determine Au(0) concentration in nanoparticles (NPs): MS,[13] direct amperometric methods,[14] fast-scan cyclic voltammetry,[15] ICPMS,[16] and UV−vis spectroscopy.[17,18] These techniques are supplemented by particle counting[19] to characterize distributions of NP populations. While it is possible to distinguish between various ionization states of Au using MS, extension of facile spectrophotometric methods to quantitatively measure Au(I) or to distinguish Au(0), Au(I), and Au(III) has not been reported.

This note introduces a direct spectrophotometric method to quantitatively determine Au(I) concentration in acidic aqueous solutions by balancing redox activities of a strong reductant (formaldehyde, HCHO) and a strong oxidant (*N*-bromosuccinimide, NBS). The reductant and oxidant are added successively, in varying amounts, to a solution of Au(I), which is then exposed to an oxidizable color reagent (3,3′,5,5′-tetramethylbenzidine dihydrochloride, TMB). At low reductant levels, NBS oxidizes Au(I), which linearly suppresses subsequent oxidation of TMB by NBS to its distinctive blue charge-transfer complexes of diamine and diimine.[20] At high initial HCHO reductant levels, Au(I) is reduced to Au(0)[21] and then is subsequently oxidized to Au(III) by NBS.[22] Upon addition of TMB, Au(III) is reduced to Au(0) accompanying oxidation of TMB to its spectrophotometrically detectable blue complexes, facilitated by the high HCHO

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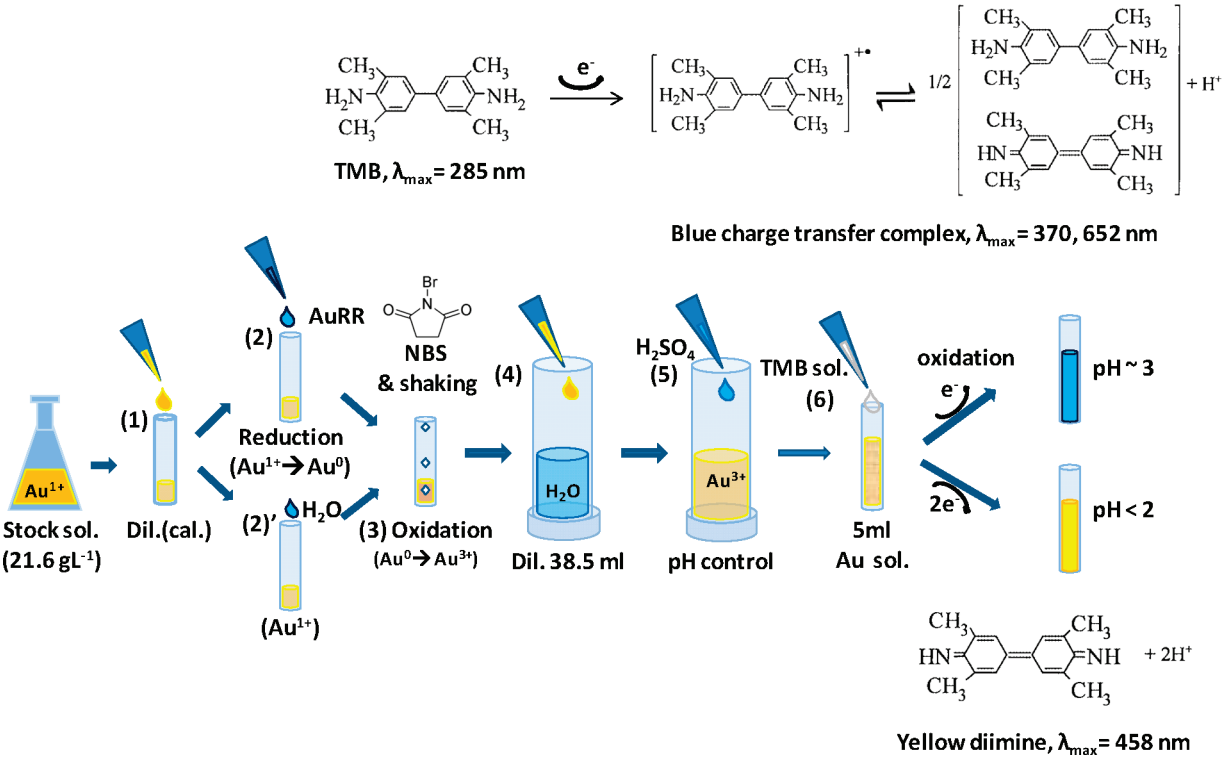
Scheme 1. Basic Procedure of Determination of Au(I) Content in TMB Aqueous Solution at High and Low Molar Ratio of Reductant HCHO and Oxidant NBS[a]



[a] Au reducing reagent (AuRR) is a mixture of HCHO and Na_2SO_3.

level. This note identifies levels of reductant, oxidant, and colorimetric agent which allow quantitative measurement of Au(I) to detection limits (LOD) of 0.0025 and 0.05 mg L^{-1} using low and high levels of reductant, respectively. Defining this straightforward spectroscopic method that uses commercially available redox reagents to measure Au(I) without surfactants or organic solvents permits rapid, accurate, and sensitive detection of Au ions for applications such as electroless plating, catalysis, and in vivo therapies.

EXPERIMENTAL METHOD

Apparatus. Absorption spectra were recorded with an AvaSpec-2048 spectrophotometer (Avantes Inc. CO) over the range of 200−1100 nm. A pH meter (Orion 920A) was used to record pH values.

Reagents. A stock standard solution of 21.6 g L^{-1} of Au(I) in commercial gold trisodium disulphite ($Na_3[Au(SO_3)_2]$, Oromerse Part B) was used to develop the assay. A stock solution of 1.00 g L^{-1} of Au(III) was prepared from $HAuCl_4 \cdot 3H_2O$ (99.9%, Sigma Aldrich) to use as a reference. A stock solution of Au(I) reducing reagent (AuRR) was prepared by mixing 1.90 mL of deionized H_2O, 23.0 mg of Na_2SO_3 (analytical reagent), and 0.10 mL of HCHO (solution reagent, ACS, 35.5−38.0 v/v %, Science Lab. com), following a procedure used to reduce Au(I) to Au(0) in electroless Au plating.[7] The *N*-bromosuccimide (NBS, 99.0%, Alfa Aesar) was purified by recrystallization from 90−95 °C hot water. The 3,3′,5,5′-tetramethylbenzidine dihydrochloride (TMB, 98.0%, Electron Microscopy Sciences) was diluted to 10.0% w/v of solution in deionized H_2O before being used as indicated in the procedure that follows.

Procedure. Scheme 1 shows the procedure to determine Au(I) content in aqueous solutions at high and low values of the reductant HCHO by subsequent addition of NBS, adjustment of pH to 3.00, and addition of TMB color indicator which is monitored spectroscopically. This procedure was used to generate data shown in Figures 1 and 2 in which spectral peak heights of TMB were measured at 370 nm.

To illustrate the procedure in Scheme 1, the specific steps used to produce a final Au concentration of 2.0 mg L^{-1} at a high reductant level of HCHO/NBS = 22.7 (identified by red circles in Figures 1 and 2) are summarized. In Step 1, 25.0 μL of transparent stock Au(I) solution was added to 75.0 μL of deionized H_2O in a borosilicate glass test tube to obtain 100 μL of Au(I) solution at 5.41 g L^{-1}. The volume of 100 μL was held constant as the volumes of stock Au(I) solution and deionized H_2O were adjusted proportionately to obtain lower target Au(I) concentrations shown in Figures 1 and 2. In Step 2, the diluted Au(I) solution was mixed with 600 μL of stock AuRR solution by swirling for 3 min to reduce Au(I) to Au(0) at ambient conditions (24−25 °C, 56−58% RH), yielding 700 μL of Au(0) at 0.77 g L^{-1}. The color of this mixture ranged from light blue to midnight blue as Au(I) content increased from 0.5 to 2.0 mg L^{-1} (at Step 6), concurrent with reduction of Au(I) to Au(0). The total volume of 600 μL of AuRR was held constant while the volumes of stock AuRR solution and deionized H_2O diluent were adjusted proportionately to obtain reductant levels of HCHO/NBS less than 22.7. In Step 3, three milligrams of recrystallized NBS was added to the reduced Au(0) solution container and mixed by swirling for 5 min. This value of 3 mg of NBS provided optimum colorimetric response across a wide

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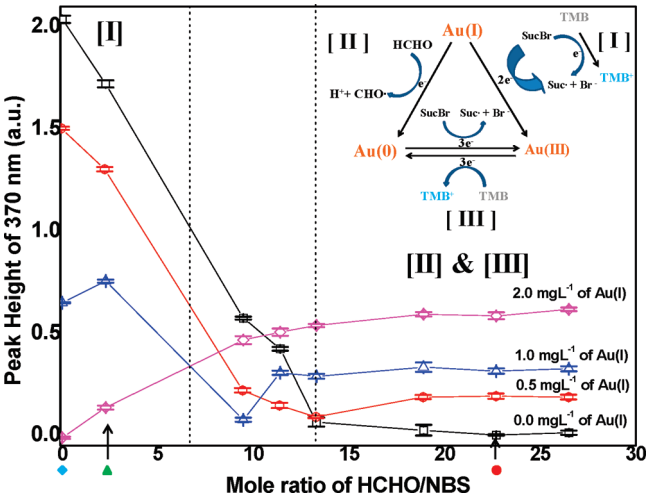

Figure 1. Au(I) is quantitatively detected by the effect of TMB oxidation in various mole ratios of HCHO/NBS and its association with Au(I). The error bars represent the standard deviation of peak variation (n = 8) after mixing from 30 to 60 min. The inset figures are suggested mechanisms of Au(I) detection in the matrix of Au, HCHO, NBS (SucBr), and TMB. (Solid blue diamond, solid green triangle, and solid red circle represent each calibration curve in Figure 2.)

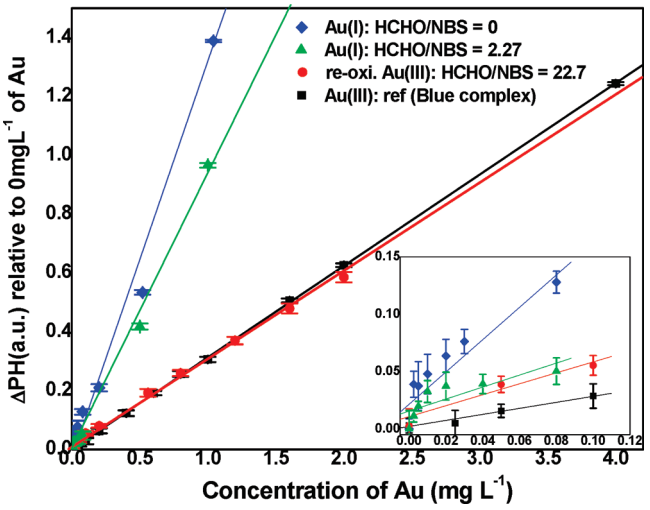

Figure 2. Balancing reduction potential improves the LOD of Au(I) determination. Comparison of calibration curves between Au(III) reference experimental result and reoxidized Au(III) are well matched. The two Au(I) determinations at low reluctant levels exhibit improved accuracy for detecting Au(I). The trend lines in the inset are in the range of 0.00−0.1 mg L^{-1}.

range of reductant levels. At low reductant levels, the added NBS oxidized Au(I) to Au(III), while at high reductant levels it oxidized Au(0) to Au(III), as will be discussed. When NBS was dropped into the blue reduced Au(0) solution, a yellowish color appeared near the solid NBS. This color change occurred in the presence of sodium sulfite alone, without Au(0), and appeared to be due to the sodium sulfite only. The solution was swirled until the NBS was completely dissolved. The final color of the Au(III) solution oxidized by NBS ranged from transparent to yellowish as the sodium sulfite concentration (and concurrently the Au(III) concentration) increased.

In Step 4, the 100 μL volume of what will be termed "re-oxidized" Au(III) solution was diluted into 38.6 mL of deionized water to achieve a final colorless Au(III) concentration of 2 mg L^{-1}. This total final volume of 38.6 mL of dilution amount was fixed in order to further optimize the assay. The reaction of the Au(III) solution with the color reagent, TMB, was then performed, following a method outlined by Fazli et al.[23] In Step 5, pH of the Au(III) solution was adjusted to 3.0 by dropwise addition of 0.50 M H_2SO_4. In Step 6, 5 mL of pH-adjusted Au(III) solution was reacted with 200 μL of TMB aqueous solution (10.0% w/v) by swirling for 30 s, to obtain a 3.85 μg mL^{-1} solution of TMB. Adding the TMB reagent to the Au(III) solution produced a color change from colorless to intense aqua blue in a time ranging from 5 s (high Au(III)) to 3 min (low Au(III)). The resulting solution was transferred into a polystyrene cuvette with a path length of 1.0 cm, and the absorbance of each solution was measured at a wavelength of 370 nm against a blank reference at room temperature. The blank reference was a mixture of AuRR solution, NBS, and TMB with no Au ion. For low reductant levels (HCHO/NBS = 2.27 and 0), each step in the assay was the same except for Step 2 in which the respective volumes of HCHO reductant solution added to the Au(I) were replaced by equivalent volumes of HCHO diluted with deionized H_2O diluent to the desired level (Step 2′). To generate the calibration curves in Figure 2, each assay was performed at increasing values of Au concentration. The UV−vis spectra were measured every 3 min for at least 1 h after TMB reaction with Au ion. Detailed procedures for generating calibration curves for Au(I) are available as Supporting Information (Table S1).

RESULTS AND DISCUSSION

Spectrophotometric Methods for Different Oxidation States of Au. Spectroscopic analysis of Au(III) to date has utilized various chemical reactions with Au(III), whose reactivity derives from its high positive reduction potential and three valence electron state. Table 1 summarizes recent spectrophotometric methods for Au(III), which can be classified into four types: (1) complexation of dye, metal-binding ligand, and Au(III);[24] (2) oxidation of a color reagent by another oxidant facilitated by Au(III) as a catalyst;[25,26] (3) formation of a color complex with Au(III);[11,12,27,28] and (4) redox reaction of color reagent with Au(III).[23,29] Compared with the more complex interactions in methods of Types 1 and 2, methods of Types 3 and 4 (color complex formation and redox reaction of a color reagent) are based on direct reaction between Au(III) and a colorimetric reagent in a relatively straightforward manner. Improvements in color complex formation have been made by introducing synthetic reagents that provide higher sensitivity and selectivity, allowing the LOD for Type 3 to approach nanomolar levels (1.8 nmol L^{-1} = 0.00035 mg L^{-1}).[11] Disadvantages of Type 3 methods that rely on color complex formation include complicated synthesis procedures, low purities, short storage lifetimes, and low production yields. Reported values of sensitivity for methods that rely on Type 4 redox reaction of a color reagent with Au(III) are not as low, as a result of low molar extinction coefficients and poorly developed color stability in these approaches. Redox reaction methods often require organic solvents, surfactants, and additional treatments such as separation and extraction.

Spectroscopy has also been used to determine concentration and size of Au(0) and Au NPs.[17,18] LOD for these methods have not been reported for three primary reasons: (1) different

Table 1. Comparison of the Present Method with Other Spectrophotometric Methods for the Determination of Au(III) Ion

reagent	λ_{max} (nm)	Beer's law validity (mg/L);(LOD)	ε (L mol^{-1} cm^{-1})	medium; (pH)	comments	ref
phloxine and thiamine	570	0.02−0.8	2.1×10^5	methylcellulose, EDTA3·Na, citric acid; (4.6)	acidic dye and metal binding ligand with Au(III), surfactant, thermal treatment (40 °C)	24
variamine blue B and KIO_3	546	0.1−12 (0.03)		*N*,*N*-dimethylformamide, CH_3COONa, HCl; (0.8)	kinetic oxidation, catalytic effect of Au(III), organic solvent, flow injection system	25
5-(2-hydroxy-5-nitrophenylazo) rhodanine	480	0.001−0.048 (0.00035)	2.0×10^6	cetyltrimethylammonium bromide, Trition X-100, H_3PO_4	color complex with Au(III) synthetic reagent, surfactant, high sensitivity	11
methylene blue B and ammonium peroxide-disulfate	662	0.09−2.9 (0.0055)	3.6×10^4	sodium citrate, HCl (1−5)	kinetic oxidation, catalytic effect of Au(III)	26
morin	291	0.2−12 (0.2)	2.02×10^4	methanol, HCl (2−3)	redox reaction by Au(III), thermal treatment (70 °C), organic solvent, low sensitivity	29
5-(2-hydroxy-5-nitrophenylazo) thiorhodanine	520	0.01−3	1.37×10^5	*N*,*N*-dimethylformamide, emulsifier-OP, H_3PO_4	color complex with Au(III), synthetic reagent, surfactant, solid-phase extraction	27
sodium 8-aminoquinoline-5-azobenzene-4′-sulfonate	611	0.05−0.5		cetyltrimethylammonium bromide, HCl, and NaOH	color complex with Au(III), adsorption, synthetic reagent, separation and preconcentration, surfactant, solid-phase extraction	28
1-(2′,4′-dinitro aminophenyl)-4,4,6-trimethyl-1,4-dihydropyrimidine-2-thiol	445	2.5−20	8.7×10^3	pyridine, 1,2-dichloroethane, HCl, NaOH (1.7−2.4)	color complex with Au(III), synthetic reagent, liquid−liquid extraction, organic solvent, low sensitivity	12
3,3′,5,5′-tetramethylbenzidine dihydrochloride	458	0.1−2.0 (0.05)	1.1×10^4	H_2SO_4	redox reaction by Au(III), ambient condition, low sensitivity	23
3,3′,5,5′-tetramethylbenzidine dihydrochloride	370	0.05−4.0 (0.025)	6.17×10^4	H_2SO_4(3)	redox reaction by Au(III), improved sensitivity	P[a]
	370	0.05−2.0 (0.025)	6.08×10^4	NBS and HCHO, H_2SO_4(3),	redox reaction by Au(III); (Au(I) →Au(0) → Au(III))	
	370	0.005−1.0 (0.0025)	2.75×10^5	NBS, H_2SO_4(3)	interference effect by Au(I), high sensitivity, and low LOD	

[a] P: present work.

aqueous matrixes produce different effects; (2) sensitivities of UV/vis assays in detecting dilute Au NP dispersions are modest; and (3) the tendency of Au NP to aggregate impacts the size-dependent spectral features which are used for analysis. Surfactants and polymer coatings added to disperse Au NPs in aqueous solutions can change the refractive index of the matrix, especially at low Au(0) concentrations, resulting in changes in intensity and wavelength of the peak used for characterization.

In spite of the utility of these four types of spectroscopic methods for characterizing Au(III), Au(0), and Au NPs, neither direct nor indirect spectrophotometric procedures to determine Au(I) have been reported. One possible alternative to the approach examined in this note could be direct spectrophotometric detection of Au(I) using fluorescence measurement, since luminescence of Au(I) complexes range from brilliant yellow[30] to purple.[31] In particular, Au(I) phosphine has a reported emission at 547 nm in aqueous solution.[32] However, use of Au(I) phosphine as a spectrophotometric reagent is complicated by the fact that it requires hours-long synthesis from an Au(III) reagent like $HAuCl_4$. In addition, it is difficult to obtain stable spectrophotometric results from short fluorescence emission lifetimes (μs).[33,34]

pH-Dependent Oxidation States of the Colorimetric Reagent TMB. The modest oxidation potential of TMB and its unique fluorescence and colorimetric features have made it valuable to analyze nitrophenols,[35] various heavy metals such as Cr(VI)[36] that have high reduction potentials, and trace Au(III) levels in natural seawater.[23] Two differently colored oxidation products of TMB may be obtained in aqueous solution, depending on pH,[20] as illustrated in Scheme 1. The single-electron oxidation product obtained at pH∼3 consists of a blue charge-transfer complex of the parent diamine and diimine which exists in rapid equilibrium with the radical cation. This blue charge-transfer complex exhibits two peaks at 370 and 652 nm, respectively, in the UV−visible wavelength range. The two-electron oxidation product obtained at pH < 2 is a diimine complex that exhibits yellow color at λ_{max} = 458 nm. It has been reported that oxidation of TMB by Au(III) forms a yellow diimine product which absorbs strongly at 458 nm (1.1×10^4 cm^{-1} mol^{-1} L).[23] In this note, the blue charge-transfer complex of oxidized TMB is detected, rather than the previously reported yellow oxidation product[23] in order to improve the sensitivity and range of Au ion detection. In this work, it was found that the first charge-transfer complex at λ_{max}= 370 and 652 nm was stable at pH 2.0−3.5 with a molar absorption coefficient of 6.17×10^4 cm^{-1} mol^{-1} L at 370 nm, which is approximately 6 times higher than the yellow diimine state which was previously used as a reference. Figure 2 shows that using the pH-stabilized blue charge-transfer complex extended the linear Au(III) detection

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range to between 0.025 and 4.0 mg L^{-1}. The previous range reported using the yellow diimine complex was from 0.1 to 2.0 mg L^{-1}.[23] Furthermore, the LOD obtained by detecting the blue complex was 0.025 mg L^{-1}, lower than the value of 0.05 mg L^{-1} reported for the diimine reference complex formed at pH < 2.[23]

Effect of Redox Reagents on Oxidation of TMB by Au(I). The redox-dependent ability of Au(I) to affect spectroscopically detectable oxidation of TMB is the basis for the proposed method. Au(I) alone does not oxidize TMB to a blue charge-transfer complex in aqueous solution in spite of its large reduction potential (E^0 = +1.68 V) relative to that of Au(III), E^0 = +1.50 V. This happens in spite of modest reduction potentials reported for TMB in acetonitrile (~0.1), in methanol/benzene mixtures (~0.7), and in voltammogram simulation (0.254) which suggest that TMB should be oxidizable via reduction of Au(I) alone. However, in the presence of a stronger oxidizing agent NBS (E^0 > 1.68 V), Au(I) reduces oxidation of TMB by NBS due to preferential oxidization of Au(I) in solutions with low to moderate reducing power. This is illustrated by the decreasing absorbance values at 370 nm in section I of Figure 1, where the mole ratio of HCHO to NBS is less than 7. In solutions where reducing and oxidizing power is balanced, low to moderate levels of Au(I) result in oxidation of TMB in a time-dependent manner after Au(I) has been reduced to Au(0) and subsequently oxidized to Au(III). This is illustrated by the increasing absorbance values at 370 nm in section II/III of Figure 1, where the mole ratio of HCHO to NBS is greater than 13.

The method described herein identifies molar ratios of strong reducing agent HCHO and strong oxidizing agent NBS which are balanced with levels of Au(I) to allow quantitative, spectroscopically detectable oxidation of color reagent TMB to its blue charge-transfer complex. The capacity of Au(I) to kinetically modulate oxidation of TMB in the presence of selected values of HCHO and NBS was quantified by examining colorimetric products obtained from combining mixtures of each redox reagent alone and in various combinations with an aqueous solution of the TMB color reagent, as detailed in the Supporting Information. Table S1 in the Supporting Information shows concentrations and volumes of redox reagents used to form each mixture. Table S2 (Supporting Information) summarizes the reported reduction potential of each redox reagent. Figure S1 (Supporting Information) and the accompanying description shows the effect of each mixture of redox reagents on the oxidation of color reagent TMB. Figure S2 (Supporting Information) and the accompanying description shows time-dependence of the oxidation states, which stabilizes after ~30 min at low to moderate values of Au(I). For example, Spectrum 5 in Figure S1 (Supporting Information) shows that Au(I) alone, even at concentrations up to 8.0 mg L^{-1}, could not oxidize the TMB in aqueous solution to produce a colorimetric response in spite of its strong positive standard reduction potential, but adding HCHO and NBS at a molar ratio of HCHO/NBS = 6.82 to the Au(I) solution allowed detectable kinetic modulation of TMB oxidation by Au(I) in a linear absorption range, as illustrated by Spectra 9 of Figure S1 (Supporting Information). Related details are found in Supporting Information.

Effect of Molar Ratio of HCHO to NBS on Determining Au Ion Content. The quantitative effect of Au ions on spectrophotometric oxidation of TMB depends on the HCHO/NBS mole ratio. NBS is strong enough to completely oxidize both Au(0) in NPs to Au(III) ions[22] and TMB to the blue charge-transfer complex due to its strong positive reduction potential of succinimidyl radical (E^0 > +1.83 V (NHE)). The strong reducing reagent, HCHO (E^0 = −1.07 to −3.00 V; −1.07 (SHE), ~−3.00 (KOH/KCl)), can suppress the oxidation of TMB by NBS. Figure 1 shows that, at a mole ratio of HCHO/NBS = 0, 3 mg of NBS oxidized TMB to its blue complex resulting in a peak height (PH) at 370 nm above 2.0. As the HCHO/NBS mole ratio increased, the PH of 0 mg L^{-1} Au decreased, ultimately reaching 0 above a mole ratio of 18.9.

A value of 3 mg of NBS was selected after observing that, at a level of 0.0 mg L^{-1} of Au(I) in Step 1 of the procedure, when 600 μL of stock HCHO solution was added at Step 2, both 6 and 9 mg of NBS oxidized TMB to the blue complex (observed spectroscopically), whereas neither 1 nor 3 mg of NBS oxidized TMB to a measurable extent, which resulted in a transparent final solution at Step 6. This suggested that 3 mg of NBS oxidant was just balanced by 600 μL of stock HCHO reductant solution, so that the final mixture yielded no measurable oxidation of TMB.

The optimal molar ratio of HCHO/NBS = 22.7 for reoxidized Au(III) was identified by holding 3 mg of NBS constant and varying the HCHO/NBS ratio from 0 to 26.5. Adding Au(I) oxidant at this defined HCHO/NBS ratio of 22.7 resulted in oxidation of TMB to its blue charge-transfer complexes with a monotonic, essentially linear response function. Whereas, adding Au(I) under nonreducing conditions depleted the availability of NBS to oxidize the TMB completely. These PHs decrease monotonically as Au(I) content increases at molar ratios of HCHO reductant relative to NBS oxidant less than 7, as illustrated by a blue diamond (HCHO/NBS = 0.0) and a green triangle (HCHO/NBS = 2.27) in Figures 1 and 2. In contrast, at molar ratios of HCHO reductant relative to NBS oxidant greater than 13.3, these PHs increase monotonically, as illustrated by red circles (HCHO/NBS = 22.7) in Figures 1 and 2. At HCHO/NBS > 22.7, the range of values over which 370 nm peak height increased in proportion to added Au(I) ceased to become larger.

In Steps 2 and 3 of the procedure, Au(I) ions were reduced to Au(0) by adding HCHO and then were oxidized to Au(III) by subsequent addition of NBS. This occurred before the solution was introduced to aqueous TMB. Interestingly, Au ion changed from a reducing agent to an oxidizing agent, depending on the HCHO content. In the nonreducing environment produced by no HCHO addition, Au was preferentially oxidized by NBS, which interfered with oxidation of TMB in a proportional fashion as initial Au(I) content increased from 0.0 to 2.0 mg L^{-1}. The range over which this low-reductant level mechanism appears valid is identified as region I on the left-hand side of Figure 1, and the suggested redox Mechanism I is shown in the inset of Figure 1. It is useful that the blue color of the TMB charge-transfer complex is quantitatively regained with decreasing Au(I) ion, because this permits sensitive detection of dilute Au ion at an improved LOD. Increasing mole ratio of HCHO/NBS to 11.4 decreases TMB oxidation at Au(I) ≤ 0.5 mg L^{-1} but increases it at Au(I) ≥ 1.0 mg L^{-1}. Further increasing the molar ratio of HCHO/NBS to 13.3 or above permits monotonically increasing oxidation of TMB by Au(I). This appears due to reduction of Au(III), since a constant, approximately proportional relationship between the amount of NBS oxidized and Au content occurs, beginning at ca. 18.9 mol ratio of HCHO/NBS. The range over which this high-reductant level mechanism appears valid is identified as region II/III on the right-hand side of Figure 1, and the suggested redox steps II and III are shown in the inset of Figure 1. Between HCHO/NBS molar ratios from 7 to 13.3, the role of Au ion shifts from being preferentially oxidized,

to that of an oxidizing agent, depending on Au concentration. This feature is attributed to the moderate reduction potential of TMB color reagent (E^0 = +0.22−0.7 V) relative to HCHO (E^0 = −1.07 ∼ −3.00 V), Au(III) (E^0 = +1.50 V), Au(I) (E^0 = +1.68 V), and NBS (E^0 > +1.83 V). The dual-mechanism behavior illustrated in Figure 1 allowed two distinct calibration curves to be employed to detect Au(I) using either low (0< 2.27 < 7) or high (13 < 22.7) molar ratios of HCHO/NBS.

Effect of Reaction Time and Au Concentration. The optimal reaction time for obtaining stable, full color development at Au(I) ≤ 2.0 mg L^{-1} in both low and high reducing environments was determined to be 30 min by monitoring absorption peak variations from 0 to 6 h (see Figure S2 in Supporting Information). Concentrations of Au(I) above 2.0 mg L^{-1} at the 0 mol ratio of HCHO/NBS could not be determined due to the saturation evident in the curve for 2.0 mg L^{-1} Au in Figure 1. The blue complex obtained by NBS reduction was stable for 6 h. The optimal full color development reaction time for detecting stable reoxidized Au(III) as an oxidant to TMB at the 22.7 mol ratio of HCHO/NBS was only applicable to a low Au(III) concentration range (0−2.0 mg L^{-1}) because at high concentration (4−14 mg L^{-1}) the absorption PH was persistently time variant. Additional details are available as Supporting Information (Figure S2). After 24 h, the blue complex transformed to a transparent TMB solution. This could be concurrent with oxidation of Au(0) to Au(III). It has been reported elsewhere that Au ion oxidized to Au(III) by NBS exhibits kinetic reduction back to Au(0) in solution.[22]

Calibration Curve. Adjusting the redox potential balance in determining Au(I) content allows enhancement of linear range of detection at high reductant levels and improved LOD at low reductant levels. Figure 2 shows two Au(I) calibration curves at low reductant levels (blue diamonds, green triangles), one curve at high reductant level (red circles), and a reference curve obtained using stock Au(III) solution (black squares). To ensure a valid comparison, the absorption data were collected every 3 min from 30 to 60 min after reaction commenced. Direct measurement of Au(I) via interference with TMB oxidation by NBS at 0 mol ratio of HCHO/NBS (blue diamonds) yielded a Beer's law relationship over the concentration range of 0.005−1.00 mg L^{-1} (R^2 = 0.988) with a molar absorption coefficient of 2.75 × 10^5 cm^{-1} mol^{-1} L calculated by subtracting the peak observed at a particular concentration from the peak observed at 0.0 mg L^{-1} Au(I). The LOD was 0.0025 mg L^{-1}, a value lower than all but one previously reported method for colorimetric detection of Au(III). The RSD calculated from 8 repetitions for determining 0.52 mg L^{-1} of Au(I) was 0.60%. The standard deviation of absorption values at low Au(I) concentrations from 0.005 to 0.08 mg L^{-1} were relatively higher than values in other systems. This was attributed to peak noise that occurred at low Au(I) concentrations which produced spectra near the maximum spectrophotometric UV absorption limit of 2.0. Increasing reducing agent HCHO to a mole ratio of HCHO/NBS = 2.27 (green triangles) decreased the measured UV absorption at 0.0 mg L^{-1} Au(I) from 2.0 to 1.70 and improved peak symmetry, both of which reduced the standard deviation. The linear concentration range in this case was from 0.01 to 1.00 mg L^{-1} (R^2 = 0.995) with a molar absorption coefficient of 1.91 × 10^5 cm^{-1} mol^{-1} L.

At high reductant levels typified by the optimized molar ratio of HCHO/NBS = 22.7, the reoxidized Au(III) system obeyed Beer's law over a wider concentration range than at lower reductant levels: 0.05−2 mg L^{-1} (R^2 = 0.998). The molar absorption coefficient was 6.07 × 10^4 cm^{-1} mol^{-1} L. Interestingly, as shown in Figure 2, the calibration curve of the reoxidized Au(III) matched well with the stock solution of Au(III). This supports a mechanism at this optimized high molar ratio in which initial Au(I) ions are reduced to Au(0) and then reoxidized to Au (III).

Future Work. Plans are underway to evaluate why Au(I), with a higher measured reduction potential than Au(III), appears unable to oxidize TMB in the absence of HCHO or NBS at the conditions studied. Fazli et al. reported interference of foreign ions in Au(III) determination associated with TMB.[23] The extent to which such interferences impact the use of this method for analyzing Au(I) as reoxidized Au(III) is the subject of future work. Rigorous statistical validation of the method, including influence of foreign ions, interferences, matrix effects, sensitivity analysis, spike testing, and recovery testing is also the subject of future work. This would allow, for example, Au(I) determination in catalyst fabrication and electroless gold plating and applications in cancer biology.

■ CONCLUSIONS

Adjusting the balance of redox activity in acid solution allows sensitive spectrometric analysis of Au(I) via oxidation of the color reagent TMB. Methods were developed at both low and high reductant conditions which were simple, reproducible, and sensitive. The promising features of this novel approach are (i) improved LOD of 0.0025 mg L^{-1} at low reductant levels, which is comparable to more elaborate analytical instruments; (ii) improved linear detection range of 0.05−2.00 mg L^{-1} at high (balanced) reductant levels; (iii) ability to cross-check Au(I) content in different redox environments; (iv) extension to detect other metal ion concentrations spectrophotometrically may be possible; and (v) the method is simple, straightforward, and economical, since all key redox reagents are commercially available and readily available spectrophotometric determination obviates the need for expensive capital equipment. The proposed approach could also allow Au(I) to be distinguished quantitatively from Au(III) in solutions since the latter (but not the former) can be reduced in acidic aqueous solutions to oxidize TMB to its blue charge-transfer complex.

■ ASSOCIATED CONTENT

Supporting Information. Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +1 479 575 6691. Fax: +1 479 575 7926. E-mail: dkroper@uark.edu.

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