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(8) DeAngelis, T. P.; Heineman, W. R. J. *Chem. Educ.* **1976**, *53*, 594.

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Controlled-Potential Electrolysis of Bulk Solutions at a Modified Electrode: Application to Oxidations of Cysteine, Cystine, Methionine, and Thiocyanate

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INTRODUCTION

Glassy-carbon electrodes modified by electrodeposition of a mixed-valence ruthenium oxide film stabilized by cyano cross-links (1, 2), mixed-valence ruthenium cyanide (mvRuCN), have been demonstrated to be highly stable (1, 3) and to promote the oxidation of several species that either are not electroactive or are oxidized only at very positive potentials (1-5). The physical-chemical stability and freedom from fouling during use relative to bare electrodes suggest that these electrodes can find application to investigations that require bulk-scale electrolysis. Although some electrode surfaces such as RuO₂ or IrO₂ on a titanium support, PbO₂, and NiO₂ are used for such purposes, systems that are generally considered as modified electrodes have not been used for bulk-scale electrolysis (6). Our primary interest in applying the mvRuCN electrode to bulk electrolysis is to test the hypothesis that this surface modification changes the course of certain electrochemical reactions by promoting both electron and oxygen transfer. A successful test of such a hypothesis can lead to new routes for electrosynthesis.

The test species in the present study were thiocyanate, cysteine, methionine, and cystine. The oxidation of thiocyanate at bare platinum and glassy-carbon electrodes caused passivation (7, 8). The reported products included trithiocyanide, (SCN)₃⁻, which was stabilized in the presence of a large excess of SCN⁻ in 0.1 M HClO₄ (9); CN⁻ and SO₄²⁻ (8, 10); and NO₂⁻, SO₄²⁻, and CO₂ (11). Using in situ infrared spectroscopy to monitor the process at a platinum electrode, Pons et al. (11) found no evidence of CN⁻. The passivating film on a glassy-carbon electrode fit the formula C₆N₄S₄ (8). Parathiocyanogen, (SCN)₂, was also reported to form on bare electrodes upon oxidation of SCN⁻ (10).

Cysteine oxidation at bare electrodes also yields passivating films (12-14). The formation of the disulfide dimer at a platinum electrode (12, 14) with adsorbed thiyl radicals (12) is indicated when the oxidation is performed in acid solution. The only suggestion of formation of an oxide of sulfur during electrolysis of cysteine is in a study by Reynaud et al. (15). X-ray photoelectron spectroscopy indicates that the adsorbed film in part comprises cystine sulfoxide and an S,S-dioxide from a Kolbe-type decarboxylation; however, even after exhaustive oxidative electrolysis, no evidence of a sulfur oxide as a solution species is seen (15).

Koryta and Pradac investigated the electrochemistry of cystine at platinum and gold electrodes, at which oxidation was observed at 1.3 and 1.45 V vs NHE, respectively (16, 17). In each case, the mechanism was reported as cleavage of the sulfur-sulfur bond and chemisorption of the resulting thiyl radicals.

In contrast to the above compounds, the electrochemical oxidation of methionine has not been reported at either bare or modified electrodes except for one analytical study in the

concentration range 0.1-100 ng of methionine/L (18). In this case, it was oxidized at 1.7 V vs Ag/AgCl at an anodically pretreated glassy-carbon electrode in a cell used as a detector for high-performance liquid chromatography.

The present study yields evidence that oxygen transfer is involved in the electrochemical process at the mvRuCN electrode. Equally important, it demonstrates that modified electrodes can possess the stability needed for large-scale applications in addition to the analytical applications that generally cause electrolysis of less than 10⁻¹⁰ equiv/experiment.

EXPERIMENTAL SECTION

An IBM Instruments EC/225 voltammetric analyzer was used without electronic filtering for all constant-potential measurements. Simultaneous output was made to a strip-chart recorder and an IBM XT computer (DASH 16 analog/digital interface, Metrabyte Corp.). Data acquisition and analyses were performed by using ASYSTANT+ (Macmillan Software). The electrochemical cells and electrodes (three-electrode configuration) were prepared in-house. Glassy carbon (Tokai Carbon) was purchased from Aimcor (Pittsburgh, PA). All experimental potentials reported are vs SCE. Indicator half-cells were separated from auxiliary half-cells by 4-8-μm (pore size) fritted-glass disks (8-mm diameter). For each of the oxidations reported, hydronium ion from 10⁻² M HCl in 1.0 M KCl was the cathodic depolarizer, and the auxiliary electrode was a platinum coil.

Unless otherwise noted, the chemicals were ACS reagent grade and were used without further purification. RuCl₃·3H₂O was obtained from Pfaltz and Bauer, and K₄Ru(CN)₆·3H₂O was obtained from Alfa Products. L-Cysteine hydrochloride hydrate, L-cysteic acid monohydrate, and sodium thiocyanate were purchased from Aldrich. L-Cystine hydrochloride, L-methionine, L-methionine sulfoxide, and L-methionine sulfone were obtained from Sigma Chemical Co. Primary standard arsenic trioxide was used to calibrate the cell. Supporting electrolyte solutions were 1.0 M in KCl, adjusted to pH 2.0 by using HCl. The water used was house-distilled and further purified by passing through a Barnstead NANOpure II system. Thin-layer chromatography was performed either with Macherey-Nagel Polygram Silica Gel-G plates or Macherey-Nagel 300 cellulose acetate plates, each with the fluorescing indicator at 254 nm (Alltech Associates, Inc., Deerfield, IL).

Glassy-carbon rods, 0.5-cm diameter × 2.0-cm length (geometric area = 3.3 cm²) for use with the half-cell with the 5.00-mL sample volumes and 0.3-cm diameter × 1.9-cm length (geometric area = 1.9 cm²) for use with the half-cell with the 3.50-mL sample volumes, were sealed in Pyrex glass tubing by using Torr-Seal epoxy (Varian Associates).

Prior to surface modification, the glassy-carbon rods were polished successively by using 1-, 0.3-, and 0.05-μm alumina (Mark V Laboratory, East Granbury, CT) on a metallographic polishing cloth (Buehler, Evanston, IL) with deionized water as the lubricant. The electrodes were thoroughly rinsed and sonicated with deionized water (sonication time, 10 min). The freshly prepared electrodes were immersed in 10.0 mL of plating solution containing 2 mM RuCl₃, 2 mM K₄Ru(CN)₆, and 0.5 M KCl with the pH adjusted to 2.0 by using HCl. The glassy-carbon indicators

were cycled between 500 and 1100 mV vs Ag/AgCl at a scan rate of 50 mV/s, for a total of 40 cycles; the initial and final potentials were 500 mV. Integration of the voltammetry peaks plotted in the current-time domain suggested an approximate film thickness of two monolayers (1, 2). The form of the film is primarily ruthenium oxide with some cyanide present as a cross-linking agent (2). Electrodes were then placed in contact with the supporting electrolyte solution and stored under this condition until needed.

Arsenic trioxide was dissolved by adding enough sodium hydroxide and deionized water to bring the solution pH to 13. This solution was then neutralized with HCl and diluted to volume with deionized water to yield 2.150 g of As_2O_3 in 250 mL. Cystine stock solutions were prepared by dissolving the solid in 0.1 N HCl. Stock solutions for cysteine and thiocyanate were prepared by dissolving the solids directly into 50 mL of the supporting electrolyte at pH 2.0. Dilutions of the stocks for each of the compounds were made by using the supporting electrolyte as a diluent. L-Cysteine hydrochloride hydrate was stored desiccated at 4 °C. All amino acid solutions were prepared immediately before use.

The applied potential for the arsenic(III), cysteine, and thiocyanate oxidations was 900 mV, whereas cystine and methionine were oxidized at 950 mV. Unless otherwise noted, solutions were sparged with prepurified nitrogen (water-saturated) for at least 10 min prior to the onset of the electrolysis potential and were blanketed with nitrogen throughout the electrolysis periods.

RESULTS AND DISCUSSION

Arsenic(III) oxidations were performed for three reasons. The first is the known diffusion-limited electrocatalytic behavior of the mixed-valence ruthenium cyanide (mvRuCN) film toward As(III) under pH 2 conditions by linear-scan voltammetry (1). Second, As(III) is not electroactive at bare glassy-carbon electrodes, so all data obtained reflect the electrocatalytic reaction. Third, it served as a preliminary test of the present film's physical-chemical stability during bulk-scale electrolysis of reactants at millimolar concentrations under stirred-solution conditions. Here, fouling by the electrolysis product is not a problem, but the catalyst must undergo repeated turnovers during the experiment. The film contains about 2×10^{-10} equiv cm^{-2} of catalysis (assuming $n = 1$), so it must turn over about 20 000–40 000 times (depending on its porosity) during each electrolysis experiment. Finally, the reaction



served to calibrate the electrochemical cells against a known value of $n = 2$.

Three electrolyses using a single preparation of mvRuCN were carried out with 1.74 mM As(III). An electrolysis time of 60 min, a 3.3- cm^2 indicator electrode, and a 5.00-mL sample were used. Integrated charges under these current-time curves yielded a calculated n value of 1.94 ± 0.01 (three trials). Thus, an apparent As(III) to As(V) conversion of 97% was observed. By use of the half-cell with the 1.9- cm^2 indicator electrode and 3.50-mL sample, 90% conversion was observed in 190 min; that is, n was 1.80 ± 0.02 (five trials).

The indicator half-cell pH was measured before and after bulk electrolysis. This was viewed both as a means to support the overall reaction stoichiometry and as a method to ensure that mass transport between the indicator and auxiliary compartments was not significant. The average and standard deviation in measured pH of the product solutions for a set of five experiments was 1.71 ± 0.02 . This value agrees with a calculated final pH of 1.69 with an initial pH 2 (unbuffered) solution. The calculated value was based on 90% conversion of As(III) to As(V), 38% dissociation of H_3AsO_4 at pH 1.7, and a yield of 2 mol of hydronium ion/mol of As(III) oxidized.

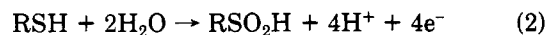
From our previous work, L-cysteine was known to oxidize at mvRuCN electrodes and to not passivate the surface during flow injection amperometry at the micromolar level (4). The present experiments were performed to elucidate the elec-

trolysis mechanism. It was of particular interest to compare the mechanism to that at a bare electrode. The electrolyses were performed on solutions with initial L-cysteine concentrations of 0.297, 0.279, and 0.271 mM. Higher concentrations of cysteine were not used because of solubility problems related to products; solubility also limited the concentration of methionine and cysteine in later experiments. In these initial trials, the solutions were not deaerated. The electrolysis potential, 0.900 V, was based on results of our previous linear-scan voltammetry and flow-injection amperometric (hydrodynamic voltammetry) measurements (4). The electrolyses were stopped when the currents reached values below 1% of that after 5 s. For example, for one current-time curve obtained, the current value at $t = 120$ min was 0.9% of that at $t = 5$ s.

Integrated charges under the i - t curves, measured against a background electrolysis, yielded n values of 4.9 ± 0.1 electrons/mol when the previously measured cell efficiency was considered. The known chemical oxidations yield sulfinic and sulfonic acids, which are respectively four-electron and six-electron reactions. Hence, the electrolysis value of $n = 5$ suggests a mixed product under the conditions employed.

We hypothesized that the experiments performed in the presence of dissolved oxygen either decreased the apparent n value through a contribution from chemical oxidation or increased the true n value through production of an electroactive intermediate; simple chemical oxidation of the $n = 4$ electrolysis product would not, of course, increase the number of coulombs passed; however, a two-electron oxidation of the $n = 4$ product could be initiated by reaction with oxygen but completed in part by the electrode reaction. In this case, the electrochemical path might terminate with the formation of the four-electron product in the absence of dissolved oxygen. Therefore, the experiments were repeated under nitrogen atmosphere. L-Cysteine present at 0.215, 0.307, and 0.448 mM levels for these electrolyses yielded an n value of 4.1 ± 0.3 when measured as described above. A Student's t test performed on the two data sets indicates that there is a significant difference (at the 99% confidence level) between the respective means.

The electrolysis results suggest that in the absence of dissolved oxygen, the sulfinic acid is the product. That is, unlike the case at a bare electrode, oxygen insertion to a solution-phase product is occurring. Further evidence for oxygen insertion at sulfur was obtained by thin-layer chromatography of the product solution. Aliquots were eluted by a method developed by Brenner and Niederwieser (19). This procedure made use of a mobile phase of 7:3 95% ethanol:water (saturated atmosphere) and a stationary phase of Silica Gel-G. Elution times were 35–45 min. Low molecular weight sulfinic acids are air-oxidized (20). This reaction occurs during the development of the chromatogram and complicates the results. Therefore, the product solution was exposed to air prior to initiating the chromatography and cysteine sulfinic acid (cysteic acid) was selected as an elution standard rather than the corresponding sulfinic acid. In one trial, the R_f values for the reaction product and sulfonic acid standard were identical at 0.75; in another trial, the values were 0.72 and 0.71, respectively. These results compare well with the literature value of 0.69 for cysteic acid using this method (19). The procedure used obviates detection of any sulfonic acid that may be present at the end of the electrolysis but is suitable for the goal of determining whether the electrolysis mechanism includes formation of an oxide of sulfur. The following stoichiometry appears to be supported for the electrocatalytic oxidation of cysteine:



The formation of an oxide of sulfur at the mvRuCN electrode

Table I. Effect of Thiocyanate Concentration on the Number of Electrons Transferred per Mole during Exhaustive Electrolysis^a

initial [SCN ⁻], mmol/L	<i>n</i>	initial [SCN ⁻], mmol/L	<i>n</i>
1.05	5.9	3.67	5.7
1.57	5.8 ± 0.1 ^b	5.76	5.8
2.36	5.8		

^a Conditions: electrolysis potential, 0.900 V; electrolyte, 1.0 M KCl at pH 2.0; mvRuCN area, 3.3 cm²; sample volume, 5.00 mL.

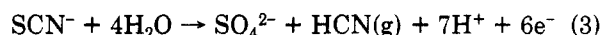
^b Average and standard deviation of four trials.

is in marked contrast to the results at bare surfaces (12, 14, 15).

No evidence of the dimer, cystine, was found by thin-layer chromatography of the product solution. A cystine standard gave an *R_f* value of 0.46, which is clearly different from the observed spot. This is not surprising since cystine itself is electroactive at mvRuCN electrodes. Electrolysis of 0.135 mM cystine solutions yielded *n* = 4.0 ± 0.5 on the basis of three trials.

Seven 5-mL samples of methionine in the concentration range 0.14–0.16 mM were oxidized at mvRuCN electrodes. A freshly prepared surface was used for each trial in this case. The electrolysis time was 85 min for each trial. The *n* value obtained was 3.8 ± 0.5 electrons/mol, which suggests an oxidation of the sulfide to a sulfone, possibly mixed with a lower *n* value product such as a sulfoxide. The presence of methionine sulfone was verified by thin-layer chromatography of the product solutions using the method reported by Bujard and Mauron (19, 21). Here, the mobile phase is 20:5:1 methanol:water:pyridine, and a cellulose acetate plate with a fluorescing indicator was the stationary phase. The *R_f* values of an electrolysis product and L-methionine sulfone standard solutions were both 0.49 with 45-min elutions.

Evidence that mvRuCN promotes oxygen transfer was also obtained from oxidations of thiocyanate. Four trials, which were performed as detailed above for cysteine as the analyte, using SCN⁻ at a fixed starting concentration of 1.57 mM yielded *n* values of 5.94, 5.76, 5.81, and 5.81. The calculated average and standard deviation was 5.83 ± 0.08. The proposed overall reaction at the mvRuCN electrode is



The presence of sulfate in the product solution was confirmed by thin-layer infrared spectroscopy. Peaks that were observed at 981, 1064, 1107, and 1208 cm⁻¹ were assigned to sulfate as barium sulfate (22), which had deposited as a film onto the BaF₂ cell window. A precipitate also formed on addition of BaCl₂ to the product solution.

Verification of hydrogen cyanide as a reaction product involved trapping the evolved gas in a receiver cell containing a basic solution. A highly selective fluorescence detection

method for cyanide ion developed by Guilbault and Kramer (23) was used for identification. Nitrogen was passed through the electrolysis chamber and exhausted through a 3.00-mL volume of 0.1 N NaOH. After the electrolysis, an aliquot from this receiver cell was neutralized with 0.1 N HCl and immediately added to 1.00 mL of 0.3 mM *p*-benzoquinone in dimethyl sulfoxide. The resulting solution emitted the blue-green fluorescence under UV light that serves as a positive spot test for cyanide (23).

Further electrolytic experiments, which are summarized in Table I, demonstrated that the value for *n* in the thiocyanate reaction was independent of concentration over the range 1–6 mM. The average and standard deviation for the entire set of eight trials was 5.8 ± 0.1, which agreed with the above average obtained for the fixed-concentration set.

The results of this study suggest that the freedom from surface fouling during amperometric measurements at this modified electrode relative to bare electrodes is related to differences in the oxidation mechanisms at these electrodes. Specifically, oxygen as well as electron transfer with the sulfur-containing compounds studied is occurring. The change in mechanism along with the stability of this modified electrode may open applications to electrosynthesis.

LITERATURE CITED

- (1) Cox, J. A.; Kulesza, P. J. *Anal. Chem.* **1984**, *56*, 1021.
- (2) Kulesza, P. J. *J. Electroanal. Chem. Interfacial Electrochem.* **1987**, *220*, 295.
- (3) Cox, J. A.; Gray, T. J.; Kulkarni, K. R. *Anal. Chem.* **1988**, *60*, 1710.
- (4) Cox, J. A.; Gray, T. J. *Electroanalysis* **1990**, *2*, 107.
- (5) Cox, J. A.; Gray, T. J. *Anal. Chem.* **1989**, *61*, 2462.
- (6) Couper, A. M.; Pletcher, D.; Walsh, F. C. *Chem. Rev.* **1990**, *90*, 837.
- (7) Nicholson, M. M. *Anal. Chem.* **1959**, *31*, 28.
- (8) Holtzen, D. A.; Allen, A. S. *Anal. Chim. Acta* **1974**, *69*, 153.
- (9) Itabashi, E. J. *Electroanal. Chem. Interfacial Electrochem.* **1984**, *177*, 311.
- (10) Rius, A.; Terol, S. *An. R. Soc. Esp. Fis. Quim., Ser. B* **1948**, *44*, 1234.
- (11) Pons, S.; Foley, J. K.; Smith, J. J. *Langmuir* **1985**, *1*, 697.
- (12) Koryta, J.; Pradac, J. J. *Electroanal. Chem. Interfacial Electrochem.* **1968**, *17*, 185.
- (13) Davis, D. G.; Bianca, E. J. *Electroanal. Chem.* **1966**, *12*, 254.
- (14) Kolthoff, I. M.; Barnum, E. J. *Am. Chem. Soc.* **1940**, *62*, 3061.
- (15) Reynaud, J. A.; Malfroy, B.; Canesson, P. J. *Electroanal. Chem. Interfacial Electrochem.* **1980**, *114*, 195.
- (16) Pradac, J.; Koryta, J. J. *Electroanal. Chem. Interfacial Electrochem.* **1968**, *17*, 167.
- (17) Koryta, J.; Pradac, J. J. *Electroanal. Chem. Interfacial Electrochem.* **1968**, *17*, 177.
- (18) Irimaya, K.; Yoshiura, M.; Iwamoto, T. J. *Liq. Chromatogr.* **1986**, *9*, 2955.
- (19) Pataki, G. *Techniques of Thin-Layer Chromatography in Amino Acid and Peptide Chemistry*, revised ed.; Ann Arbor Science Publishers, Inc.: Ann Arbor, MI, 1968; Chapter 4, pp 65–97.
- (20) Oae, S.; Kunieda, N. *Sulfonic Acids and Sulfonic Esters. In Organic Chemistry of Sulfur*; Oae, S., Ed.; Plenum Press: New York, 1977; Chapter 11, pp 603–648.
- (21) Bujard, E.; Mauron, J. J. *Chromatogr.* **1966**, *21*, 19.
- (22) Craver, C. D., Ed. *Coblentz Society Desk Book of Infrared Spectra*; Coblentz Society: Kirkwood, MO, 1977; p 475.
- (23) Guilbault, G. G.; Kramer, D. N. *Anal. Chem.* **1965**, *37*, 1395.

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