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DEVELOPMENT OF NITROGEN SENSOR FOR DETERMINATION OF PN(2) IN BO--ETC(U)

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Report N00014-82-C-0133

DEVELOPMENT OF NITROGEN SENSOR FOR DETERMINATION OF P_{N_2} IN BODY TISSUES

Lois S. Robblee and Maureen A. Parker

EIC Laboratories, Inc.
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July 1982

Final Report for Period 1 January 1982 - 30 June 1982
Approved for Public Release - Distribution Unlimited

Prepared for

OFFICE OF NAVAL RESEARCH
Department of the Navy
800 North Quincy Street
Arlington, Virginia 22217

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REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER N00014-82-C-0133

2. GOVT ACCESSION NO.

3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle)
DEVELOPMENT OF NITROGEN SENSOR FOR DETERMINATION OF P_{N_2} IN BODY TISSUES

5. TYPE OF REPORT & PERIOD COVERED
FINAL REPORT
1 Jan 82-30 June 82

6. PERFORMING ORG. REPORT NUMBER C-687

7. AUTHOR(s)
Lois S. Robblee and Maureen A. Parker

8. CONTRACT OR GRANT NUMBER(s) N00014-82-C-0133

9. PERFORMING ORGANIZATION NAME AND ADDRESS
EIC Laboratories, Inc.
111 Chapel Street
Newton, MA 02158

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
NR 201-563

11. CONTROLLING OFFICE NAME AND ADDRESS
Office of Naval Research
800 North Quincy Street
Arlington, VA 22217

12. REPORT DATE JULY 1982

13. NUMBER OF PAGES 28

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)

15. SECURITY CLASS. (of this report)
UNCLASSIFIED

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for Public Release; Distribution Unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Dinitrogen Complex; Electrochemical Sensor; Immobilized Ru; Modified Electrodes; Nitrogen Complex; Nitrogen-Sensing Electrode; P_{N_2} Measurement; Polymer; Poly-4-vinylpyridine; Ruthenium Ammines; Ru Dinitrogen Electrochemistry; Ru Pentaammine Dinitrogen; Transition Metals

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Experiments were performed to determine the feasibility of an electrochemical sensor for dissolved N_2 based on N_2-complexing activity of Ru ammines. The formation of $[Ru(NH_3)_5N_2]^{+2}$ by the reaction of $[Ru(NH_3)_5H_2O]^{+2}$ and $N_{2(aq)}$ was observed by voltammetry as a progressive increase in an anodic-current peak at +0.8V SCE due to the oxidation of $[Ru(NH_3)_5N_2]^{+2}$. Pseudo-first order rate constants for reaction times up to 140 min at

20. Abstract (Cont.)

pressures of 1 atm, ∿3 atm, and ∿6 atm at 22°C were 2.77×10^{-5}, 6×10^{-5}, and 9×10^{-5} sec^{-1}, respectively. The deviation from the expected proportionality to pressure was due to the long times required for equilibration of dissolved N_2. The feasibility of incorporating a Ru dinitrogen complex precursor onto an electrode surface was demonstrated with $[Ru(NH_3)_5H_2O]^{+2}$ attached to pyridine groups of poly-4-vinylpyridine (PVP) coated graphite. The electrochemical activity of the attached Ru(II) complex remained stable during >20 hours exposure to physiological saline containing protein and dissolved O_2. A model is proposed for a N_2-sensing electrode based on published data for reactions of cis-$[Ru(NH_3)_4(H_2O)_2]^{+2}$ with N_2 and pyridine, and the results of the present study on $[Ru(NH_3)_5H_2O]^{+2}$. The model consists of the complex cis-$[Ru(NH_3)_4(py)H_2O]^{+2}$ incorporated into a PVP polymer-coated electrode. The attached complex would react with dissolved N_2 to form an attached, electrochemically detectable N_2 complex, cis-$[Ru(NH_3)_4(py)N_2]^{+2}$ in proportion to the N_2 concentration. The release of N_2 from the oxidized complex and the reduction of Ru(III) back to the Ru(II) precursor will permit repetitive measurements of dissolved N_2.

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INTRODUCTION

Because nitrogen is not itself oxidizable or reducible in an aqueous solvent, the key to developing an electrochemical sensor to quantitate dissolved N_2 is to find a reaction involving molecular nitrogen whose product may be detected electrochemically. The findings that certain transition metal ions form complexes with molecular nitrogen[1] provided the impetus for this study. Thus the goal of the present research was to determine whether any of the transition metal-dinitrogen complexes might be detected electrochemically, and if so, whether the transition metal ligand might be immobilized at an electrode surface to form the basis for an electrode for quantitation of solution P_{N_2}.

The six month, Phase I effort was oriented along three main lines of investigation:

1. Evaluation of the electrochemical behavior of transition metal dinitrogen complex precursors to determine the most appropriate compound(s) for further study.
2. A study of the nitrogen complexation reaction using the transition metal ion(s) selected under 1 above and varying concentrations of both the metal ligand and N_2.
3. A study of immobilized transition metal ligands.

As the research evolved, it became clear that the ruthenium pentaammine and Ru tetraammine would be the most favorable candidates for a nitrogen detector. Both compounds form complexes with molecular nitrogen in aqueous solution[2,3]; both compounds have straightforward electrochemical behavior[4,5]; and immobilization of similar Ru compounds is reported in the literature.[6] Most of the work described here was carried out with the pentaammine due primarily to its availability at the start of the program.

---

[1] Khan, M. M. and Martell, A. E. in <u>Homogeneous Catalysis by Metal Complexes</u> (New York: Academic Press, 1974), pp. 181-291 and references cited therein.

[2] Allen, A. D., Bottomley, F., Harris, R. O., Reinsalu, V. P. and Senoff, C. V., J. Am. Chem. Soc. <u>89</u>, 5595 (1967).

[3] Elson, C. M., Itzkovitch, I. J. and Page, J. A., Can. J. Chem. <u>48</u>, 1639 (1970).

[4] Elson, C. M., Itzkovitch, I. J., McKenney, J. and Page, J. A., Can. J. Chem. <u>53</u>, 2922 (1975).

[5] Lim, H. S., Barclay, D. J. and Anson, F. C., Inorg. Chem. <u>11</u>, 1460 (1971).

[6] Oyama, N. and Anson, F. C., J. Am. Chem. Soc. <u>101</u>, 3450 (1979).

EXPERIMENTAL METHODS

Electrochemistry experiments were carried out using either a Bioanalytical Systems (W. Lafayette, Indiana) CV-1B potentiostatic control unit or an Amel Model 551 potentiostat controlled by a function generator. Current-potential curves were recorded on a Hewlett-Packard X-Y Recorder. The electrochemical cell was comprised of a 4-hole round bottom flask, 100 ml capacity, with ground glass connections for mounting electrodes and a gas dispersion frit. Vitreous carbon working electrodes (0.091 or 0.071 cm^2) were used for cyclic voltammetry. Potentials were measured with respect to a saturated Calomel reference electrode (SCE) and are reported relative to this reference. An auxiliary electrode consisting of a spiral of Pt wire completed the 3-electrode system.

Electrochemical studies were carried out in a sulfate electrolyte, 0.001M H_2SO_4-0.099M K_2SO_4 pH 3.3[3] unless indicated otherwise. Stability studies of immobilized Ru electrodes were carried out in a phosphate-buffered saline (PBS), 0.73% NaCl in 0.1M Sörensen phosphate buffer, pH 7.3, with or without the addition of 0.02% human serum albumin (Sigma Chemical Co.). All solutions were made from analytical grade chemicals and deaerated under Ar. Argon gas was freed of trace amounts of O_2 by passage through a 0.1M solution of $CrCl_2$ in 0.5M H_2SO_4 followed by passage through distilled water.

Preparation of Ru(II) Dinitrogen Complex

Ru(II) pentaammine dinitrogen, $[Ru(NH_3)_5N_2]^{+2}$, was prepared from Ru(II) aquopentaammine, $[Ru(NH_3)_5H_2O]^{+2}$ as follows: 10 mls of 10-50 mM $[Ru(NH_3)_5Cl]^{+2}$ in deaerated sulfate electrolyte was reduced to $[Ru(NH_3)_5H_2O]^{+2}$ with amalgamated Zn.[7] The reduction was carried out in a separatory funnel with a stopcock connection to one of the openings in the electrochemical cell. When the reduction was complete (about 75 min as determined in preliminary cyclic voltammetry experiments), the $[Ru(NH_3)_5H_2O]^{+2}$ was admitted to the electrochemical cell containing 90 ml sulfate electrolyte which had been presaturated with N_2. The formation of $[Ru(NH_3)_5N_2]^{+2}$ was followed by cyclic voltammetry measurements made directly in the reaction solution. Nitrogen gas was bubbled through the solution between cyclic voltammetry scans. In some experiments, aliquots of the reaction solution were removed with a syringe and transferred to an Ar-purged micro-UV absorbance cell to monitor the formation of N_2 complex by its characteristic absorbance at 222 nm.[8]

---

[3] Elson et al., loc. cit.

[7] Harrison, D. E. and Taube, H., J. Am. Chem. Soc. 89, 5706 (1967).

[8] Itzkovitch, I. J. and Page, J. A., Can. J. Chem. 46, 2743 (1968).

Reactions at pressures >1 atm were carried out in a Parr Pressure Reactor fitted with electrical feed-throughs for electrode connections. Electrode arrangements for the reactor are shown in Figure 1. Five ml of 0.04M $[Ru(NH_3)_5H_2O]^{+2}$ were prepared in a separatory funnel as described above. This funnel was then connected to the sample inlet tube on the pressure reactor with Tygon tubing. The pressure reactor contained 195 ml sulfate buffer, presaturated with N_2 gas. Immediately prior to admitting $[Ru(NH_3)_5H_2O]^{+2}$ to the reactor, the atmosphere above the $[Ru(NH_3)_5H_2O]^{+2}$ solution was purged with N_2. After 1-2 min, the $[Ru(NH_3)_5H_2O]^{+2}$ was admitted to the reactor; additional N_2 was bubbled through the sample inlet tube to insure that it was completely emptied of $[Ru(NH_3)_5H_2O]^{+2}$. A magnetic stirrer was used in the bulk electrolyte to provide thorough mixing. Control experiments at 1 atm pressure were carried out in the pressure reactor for comparison with those carried out in the 100 ml cell. In these experiments, N_2 gas was bubbled intermittently through the solution. For experiments carried out at higher pressures, the gas exit valve was closed as soon as the $[Ru(NH_3)_5H_2O]^{+2}$ was admitted and the N_2 pressure was raised to the desired level. The formation of $[Ru(NH_3)_5N_2]^{+2}$ was followed by cyclic voltammetry as described above.

The $[Ru(NH_3)_5N_2]^{+2}$ complex was also synthesized from $RuCl_3$ (Alfa Ventron) and hydrazine according to the procedure of Allen et al.[2] The UV spectrum and cyclic voltammetry of the resulting dinitrogen complex were compared with those of the dinitrogen complex prepared in solution from the reaction of $[Ru(NH_3)_5H_2O]^{+2}$ and N_2 gas.

Preparation of Electrodes with Immobilized Ru

Pyrolytic graphite electrodes, coated with poly-4-vinylpyridine (PVP), M.W. = 40,000, were prepared according to the procedure of Oyama and Anson.[6] Briefly, this consisted of exposing freshly cleaved graphite discs (basal planes parallel to the disc surface), sealed into glass tubing with heat-shrinkable polyolefin tubing, to a 0.5% (w/v) solution of PVP in methanol. In initial experiments, graphite electrodes were simply soaked in PVP solution for ∿15 min, but in later experiments, controlled quantities of PVP solution (0.5-3.0 μl) were applied to the graphite surface with a micropipet and air dried. PVP-graphite electrodes were soaked in 5 mM solution of $[Ru(NH_3)_5H_2O]^{+2}$ prepared in sulfate electrolyte as described above. After various times of exposure to $[Ru(NH_3)_5H_2O]^{+2}$, electrodes were rinsed with distilled water, dried and transferred to plain sulfate electrolyte for cyclic voltammetric measurements.

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[2] Allen et al., loc. cit.
[6] Oyama and Anson, loc. cit.

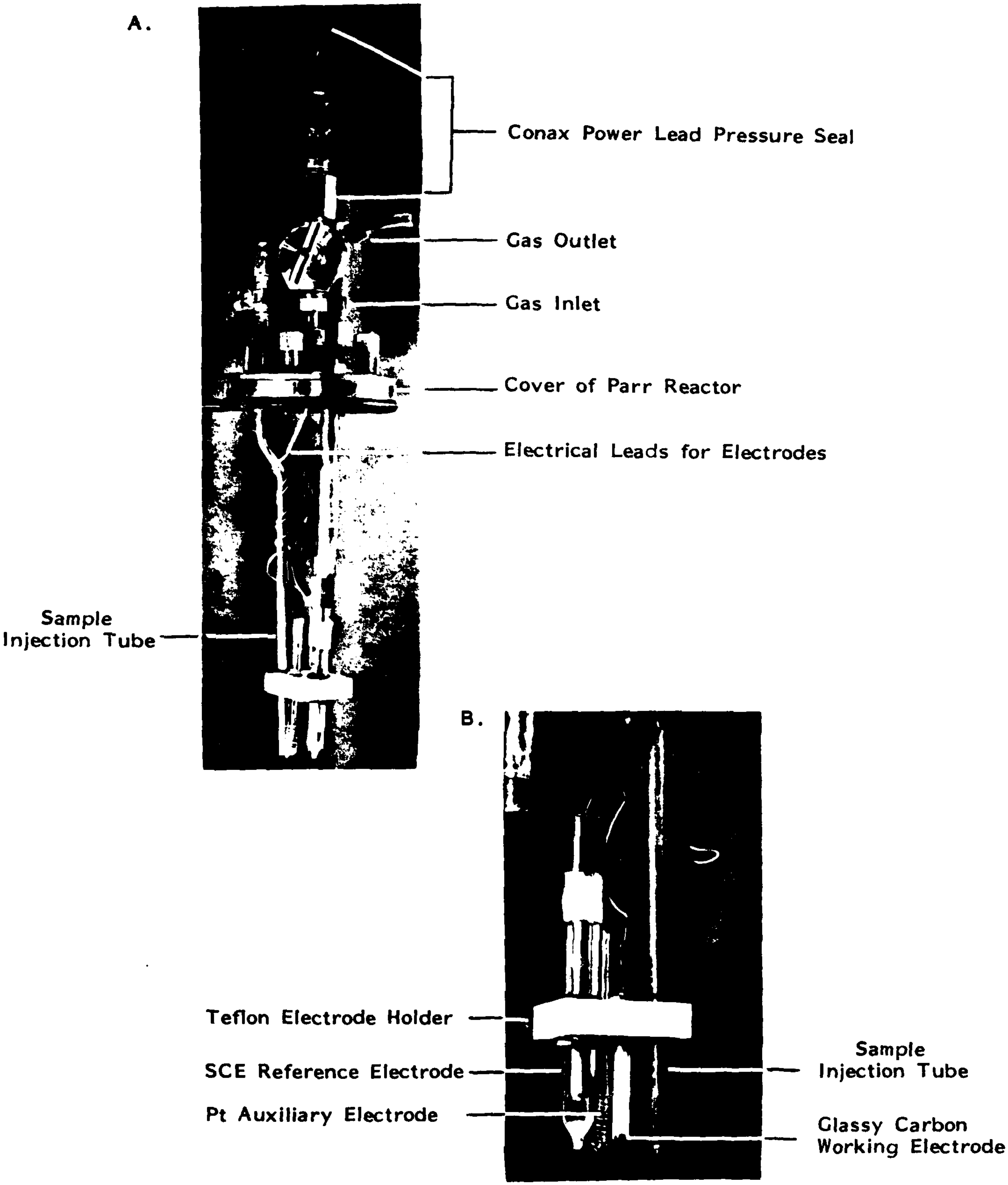

Figure 1. Electrode assembly for Parr Pressure Reactor. A. Entire assembly; B. Enlarged view of electrodes.

RESULTS AND DISCUSSION

Preliminary Studies

Electrochemical and UV spectral analyses were carried out on several Ru ammines obtained from commercial suppliers to compare with data reported in the literature and to establish criteria for monitoring the formation of Ru ammine dinitrogen complexes with these techniques.

$[Ru(NH_3)_6]Cl_3$ (Alfa Ventron). The Ru hexaammine does not form complexes with dinitrogen but is included here for comparison with other ammines which do form N_2 complexes. Cyclic voltammetry demonstrated a highly reversible redox couple with cathodic and anodic current peaks at -0.24V and -0.18V respectively in agreement with results published elsewhere.[4] The 60 mV separation of peak potentials and peak current ratio of ∿1.0 is consistent with a one electron charge transfer and a stable product:

$$[Ru(NH_3)_6]^{+3} + e^- \rightleftarrows [Ru(NH_3)_6]^{+2}$$

An absorption peak was observed at 275 nm. No effect of pH on this compound was observed by either cyclic voltammetry or UV analysis due probably to the stability of the NH_3 ligands in all coordinating positions.[9]

$[Ru(NH_3)_5Cl]Cl_2$ (Alfa Ventron). The electrochemical behavior and UV spectrum of the ruthenium(III) pentaammine was markedly sensitive to solution pH due to acid and base hydrolysis reactions leading to interconversion between chloro, aquo and hydroxo-species of the pentaammine.[10] A detailed discussion of these is beyond the scope of this work, but the changes in the UV spectrum and in the potentials of the redox reactions which accompany the interconversions are summarized in Figure 2. These are all in agreement with published data.[5,10,11]

cis-$[Ru(NH_3)_4Cl_2]Cl$. The electrochemical and UV spectral data of a commercial preparation of this compound did not agree in any respect with those published in the literature. Its UV spectrum showed a single absorbance maximum at 285 nm compared to twin maxima at 312 nm and 352 nm.[12] The cyclic voltammograms displayed cathodic peaks at +0.6V and

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[4] Elson et al., loc. cit.

[9] Endicott, J. F. and Taube, H., Inorg. Chem. 4, 437 (1965).

[10] Broomhead, J. A., Basolo, F. and Pearson, R. G., Inorg. Chem. 3, 526 (1964).

[11] Hartman, H. and Bushbeck, C., Z. physik. Chem. (Frankfurt) 11, 120 (1957).

[12] Movius, W. G. and Linck, R. G., J. Am. Chem. Soc. 92, 2677 (1970).

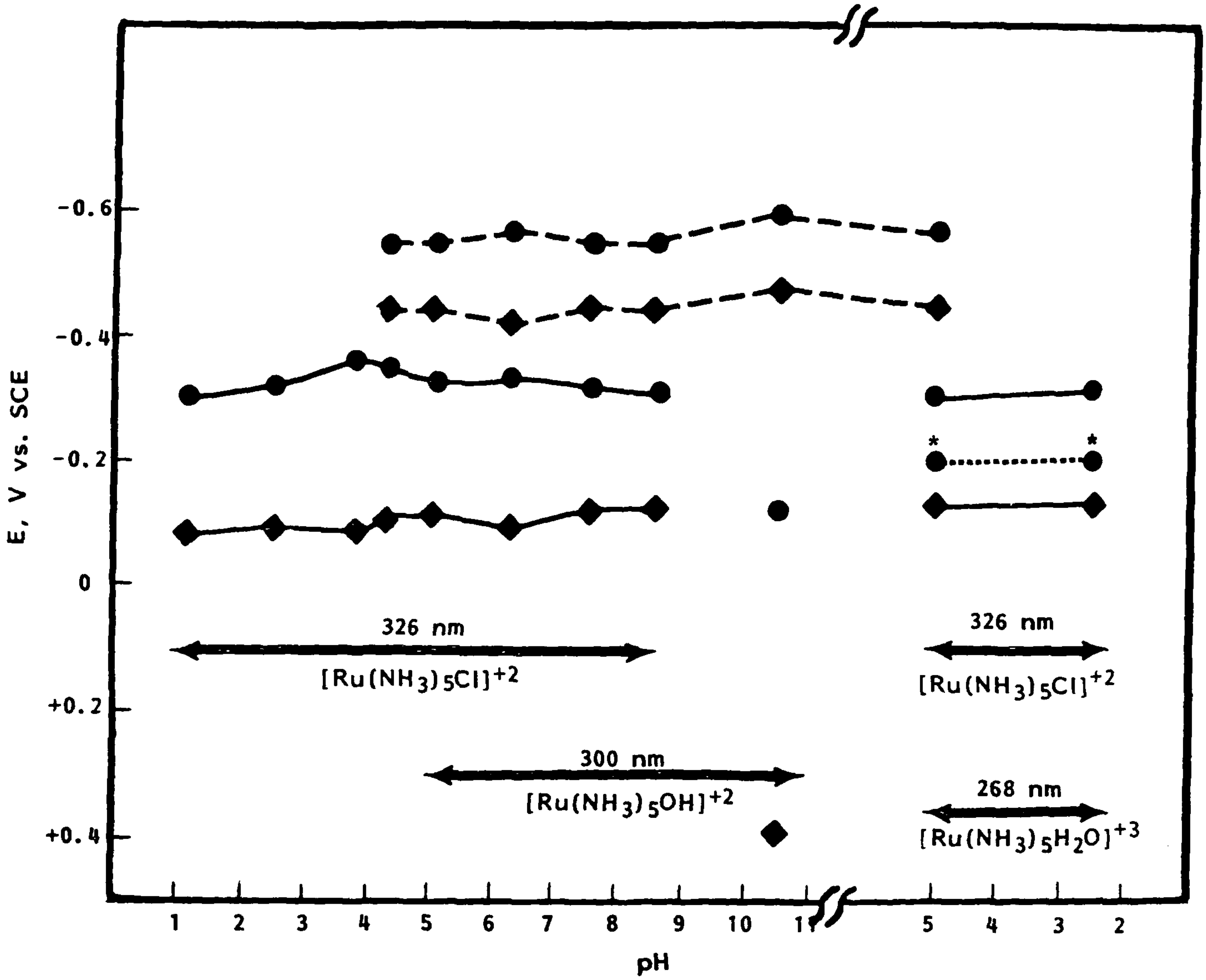

Figure 2. Effect of pH on cyclic voltammetry and UV absorbance of $[Ru(NH_3)_5Cl]^{+2}$. The pH of 1 mM $[Ru(NH_3)_5Cl]^{+2}$ in 0.1M HCl was slowly increased to 10.5 by the dropwise addition of conc. NaOH, after which it was acidified back to ∿2.5 by the addition of conc. HCl.

● Gives E_{p_c} on the forward scan

◆ Gives E_{p_a} on the reverse scan

*● Indicates E_{p_c} that was observed only the first scan. Scan rate: 100 mV sec^{-1}.

⟷ Indicates pH range over which the indicated UV absorbance maximum (λ_{max}) was observed. The Ru(II) species giving rise to that λ_{max} is indicated below the arrow.

-0.43V, and anodic peaks at +0.78V and -0.22V, again in disagreement with published results.* The commercial material was therefore rejected as a possible precursor for formation of a dinitrogen complex. An "in-house" synthesis of the cis-tetraammine was delayed due to unavailability of the starting material and therefore was not completed in time for inclusion in this report.

Na_2MoO_4. Cyclic voltammetry of the molybdate ion in 0.1M H_2SO_4 demonstrated two oxidation-reduction peaks at +0.18V and +0.3V anodically and +0.25V and +0.12V cathodically. The current peaks however were much smaller than those observed for Ru compounds suggesting that the electron transfer was somewhat more difficult. In view of the more straightforward electrochemistry of the Ru pentaammine, both as a nitrogen complex precursor and as an immobilized reactant, the major effort in the program was concentrated in that direction.

Formation of Ru Pentaammine Dinitrogen Complex from N_2

The sequence of reactions leading to the formation of the Ru pentaammine dinitrogen ion are as follows:

Reduction:

$$[Ru(NH_3)_5Cl]^{+2} + H_2O + e^- \rightleftarrows [Ru(NH_3)_5H_2O]^{+2} + Cl^- \qquad (1)$$

N_2 Complexation:

$$\text{Monomer-}[Ru(NH_3)_5H_2O]^{+2} + N_2 \rightarrow [Ru(NH_3)_5N_2]^{+2} + H_2O \qquad (2)$$

$$\text{Dimer-}[Ru(NH_3)_5N_2]^{+2} + [Ru(NH_3)_5H_2O]^{+2} \rightarrow [(Ru(NH_3)_5)_2N_2]^{+4} \qquad (3)$$

The progress of the reduction reaction (1) was followed by voltammetry. A single anodic potential sweep, starting from the open circuit potential, gave rise to a current peak at ~-0.16V, due to the oxidation of any $[Ru(NH_3)_5H_2O]^{+2}$ that might be present. The gradual increase in this current peak is in proportion to the concentration of $[Ru(NH_3)_5H_2O]^{+2}$ as the reduction proceeds (Figure 3). The reduction reaction was approximately 90% complete within 70-80 minutes.

The N_2 complexation reactions, (2) and (3), were also followed by voltammetry (Figure 4). The formation of $[Ru(NH_3)_5N_2]^{+2}$ was first observed within 10-20 min after introducing N_2, as an anodic wave at ~0.8V. After longer times of exposure to N_2, a second anodic wave due to the formation of the dimer was observed. The current in both waves increased with time, while the current at -0.16V decreased due to the decrease in $[Ru(NH_3)_5H_2O]^{+2}$.

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*Elson et al., loc. cit.

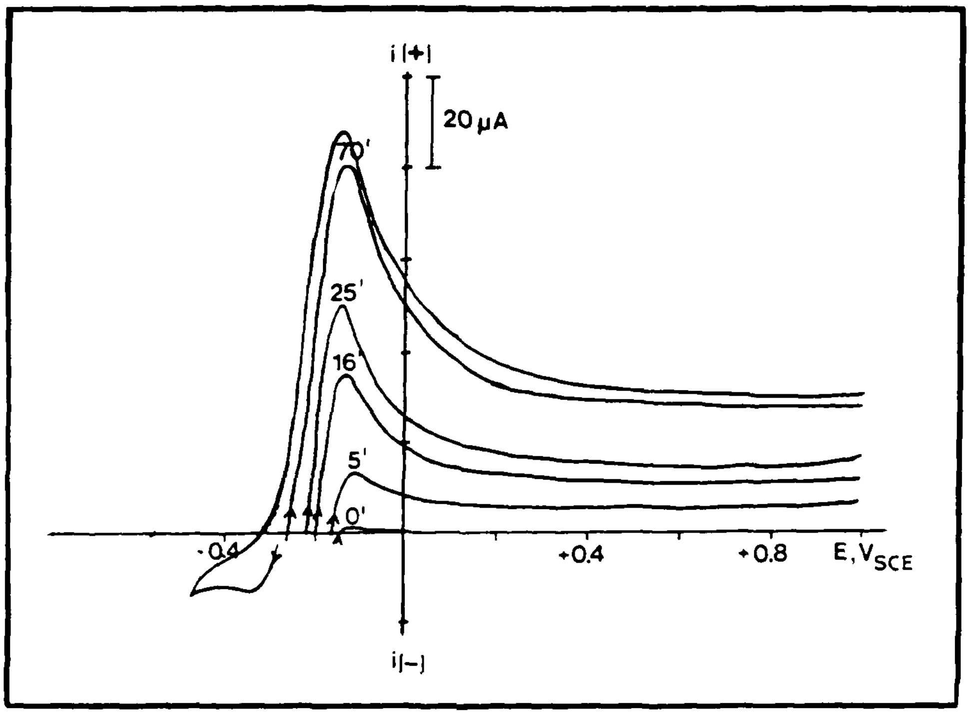

Figure 3. Reduction of 5 mM $[Ru(NH_3)_5Cl]^{+2}$. Current-potential curves were recorded at different times after the addition of Zn amalgam. Scan rate: 100 mV sec^{-1}.

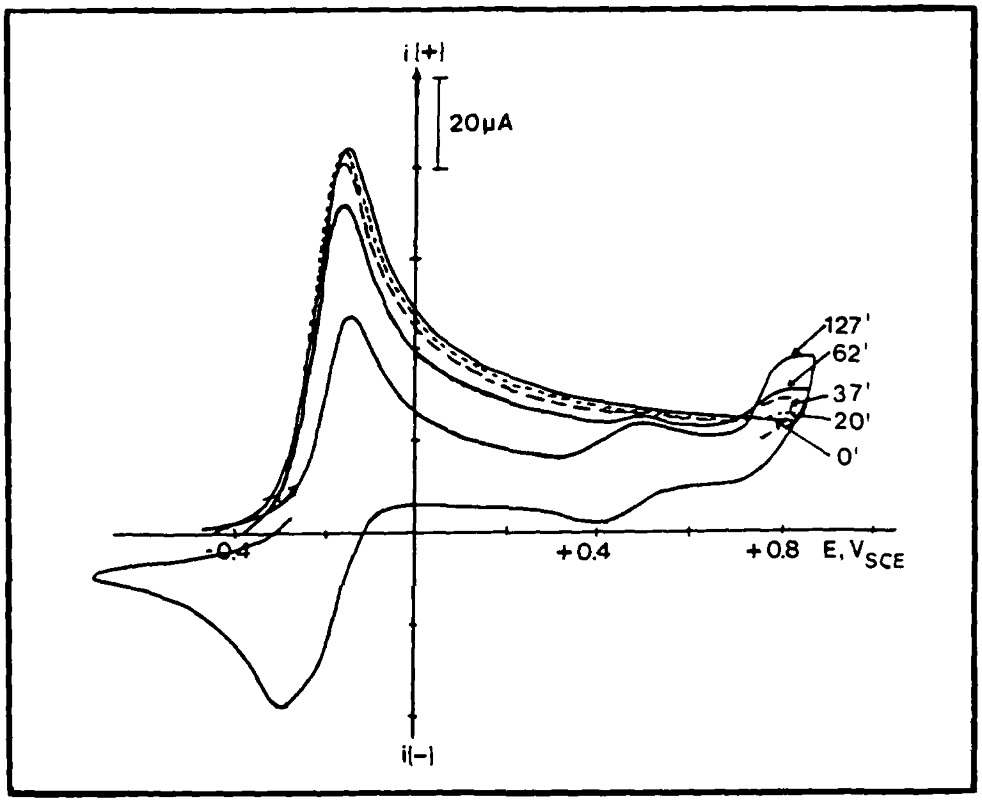

Figure 4. Formation of $[Ru(NH_3)_5N_2]^{+2}$. Current-potential curves were recorded at different times after exposure of 4.5 mM $[Ru(NH_3)_5H_2O]^{+2}$ to N_2. Scan rate: 100 mV sec^{-1}.

The oxidation at +0.8V of nitrogen monomer

$$[Ru(NH_3)_5N_2]^{+2} \rightarrow [Ru(NH_3)_5X]^{+3} + N_2 + e^-$$

(where X may be sulfate from the electrolyte) is not reversible, i.e., no reduction wave due to reduction of $[Ru(NH_3)_5N_2]^{+3}$ ion has ever been detected, even at very high scan rates.[13] Instead, a reduction wave is observed at -0.3V due to the reduction of $[Ru(NH_3)_5X]^{+3}$. This is consistent with a reaction in which the complexed N_2 is immediately displaced from the complex as soon as the Ru(II) is oxidized to the Ru(III) state. The dimer appears to undergo a reversible oxidation at +0.5V as indicated by the 60 mV peak separation for the reduction wave.

$$[Ru(NH_3)_5N_2Ru(NH_3)_5]^{+4} \rightleftarrows [Ru(NH_3)_5N_2Ru(NH_3)_5]^{+5} + e^-$$

However, this product is not stable indefinitely and dissociates as follows:

$$[Ru(NH_3)_5N_2Ru(NH_3)_5]^{+5} \xrightarrow{k\ =\ 0.1\ sec^{-1}} [Ru(NH_3)_5N_2]^{+2} + [Ru(NH_3)_5X]^{+3}$$

Therefore, the cathodic peak at +0.45V may not be seen at very slow scan rates.[13] With the scan rates used in the present program (100 mV sec^{-1}), the reverse wave f r dimer reduction was always observed. The appearance of current peaks at +0.8V and +0.5V and their increase with time of reaction with N_2 coincided with the appearance and increase of UV absorbance peaks at 222 nm and 262 nm, respectively, thus confirming that the current peaks were due to the presence of monomeric and dimeric N_2 complexes, respectively.[3] The linear relationship between the current peak at +0.8V and the absorbance at 222 nm is shown in Figure 5. The intercept on the X axis at 0.05 absorbance corresponds to the absorbance of $[Ru(NH_3)_5H_2O]^{+2}$.

For kinetic studies of monomer formation, the concentration of $[Ru(NH_3)_5Cl]^{+2}$ was decreased to 1 mM resulting in $\sim$0.8 M $[Ru(NH_3)_5H_2O]^{+2}$ at the termination of the reduction reaction. Rate constants were derived independently from electrochemical and spectral measurements. For the reaction

$$Ru(II) + N_2 \xrightarrow{km} Ru(II)\cdot N_2$$

$$\frac{d[Ru(II)\cdot N_2]}{dt} = km[Ru(II)][N_2] = km'[Ru(II)] \quad (N_2 \text{ saturated})$$

The pseudo-first order rate constant, km' was determined from plots of ln[Ru(II)] vs. time. The voltammetric peak (i_p) at -0.16V provides a

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[3] Elson et al., loc. cit.

[13] Elson, C. M., Gulens, J., Itzkovitch, I. J. and Page, J.A., Chem. Commun., 875 (1970).

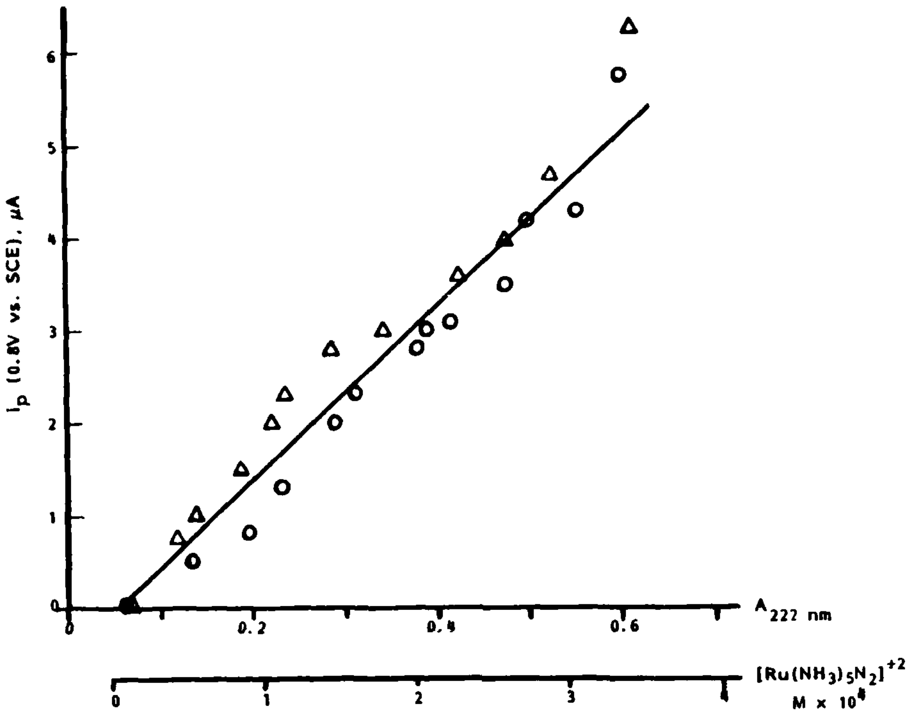

Figure 5. Correlation between i_p (+0.8V SCE) and A_{222} of $[Ru(NH_3)_5N_2]^{+2}$. Concentration of $[Ru(NH_3)_5N_2]^{+2}$ was determined from the molar extinction coefficient, 1.64 x 10^4. Electrode area = 0.091 cm^2.

direct measure of [Ru(II)] as the N_2 complexation reaction proceeds. Plots of ln $i_p(-0.16V)$ vs. time were linear up to 170 min with a pseudo-first order rate constant, km', of $3.63 \pm 0.15 \times 10^{-5}$ sec^{-1} ($n = 2$). The value of [Ru(II)] at various times during the reaction was also determined from the absorbance at 222 nm due to the formation of Ru(II)·N_2. Thus,

$$[Ru(II)]_t = [Ru(II)]_{initial} - [Ru(II)\cdot N_2]$$

[Ru(II)·N_2] was calculated using the molar extinction coefficient of 1.64×10^4 for the N_2 complex.[8] The value of km' derived from these spectral measurements was $3.66 \pm 0.05 \times 10^{-5}$ sec^{-1} ($n = 2$), in good agreement with that obtained from voltammetric data. Estimating the dissolved N_2 concentration at 1 atm, 25°C to be $6.2 \times 10^{-4}M$[14], the rate constant km, for the overall reaction was $5.9 \times 10^{-2}M^{-1}sec^{-1}$, in agreement with published values.[8]

Kinetic studies in the pressure reactor at elevated N_2 pressure were complicated by the relatively slow rate of N_2 equilibration between aqueous and gaseous phase. For these experiments, voltammetry was used exclusively to follow the formation of the nitrogen complex. Plots of ln $i_p(-0.16V)$ vs. time for 1, 3 and 6 atm pressure (0, 30 and 75 psig) are shown in Figure 6. The actual current at +0.8V vs. time curves are shown in Figure 7. At $P_{N_2} = 1$ atm in the pressure reactor, the ln i_p vs. time plot was linear over 100 min with km' = 2.77×10^{-5} sec^{-1}. At $P_{N_2} \approx 3$ atm, the ln i_p vs. time plot was linear up to 60 min when an increase in slope was observed. The km' values for the 0-60 and 60-160 min segments were 4.7×10^{-5} sec^{-1} and 6×10^{-5} sec^{-1}. At $P_{N_2} \approx 6$ atm, km' values were the same as those for the reaction at $P_{N_2} \approx 3$ atm up to 140 min. After that time, the km' (6 atm) increased to 9×10^{-5} sec^{-1} reflecting the further increase in dissolved N_2 concentration at the higher pressure. The reaction rates determined were not exactly in proportion to the increase in pressure. The rate at 3 atm was ∿75% of the expected rate, while that at 6 atm was only ∿55% of that expected.

The formation of the nitrogen complex has been demonstrated to be first order with respect to N_2 concentration using partial pressure $\overset{=}{<}1$ atm.[8] To our knowledge, this is the first time kinetic studies have been attempted at pressures >1 atm. The deviation from expected behavior is most likely an artifact of the experimental method and reflects the constantly changing N_2 concentration during the time required to reach a saturation level. If the $[Ru(NH_3)_5H_2O]^{+2}$ solution were introduced, under pressure, to solutions already presaturated with N_2 at elevated pressure, then we expect that the reaction rates would exhibit a direct proportionality to N_2 concentration. Similarly, if the $[Ru(NH_3)_5H_2O]^{+2}$ or some

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[8] Itzkovitch and Page, loc. cit.

[14] Stephens, H. and Stephen, T., Solubilities of Inorganic and Organic Compounds (New York: The Pergamon Press Ltd., 1963), Vol. I, Part 1, p. 85.

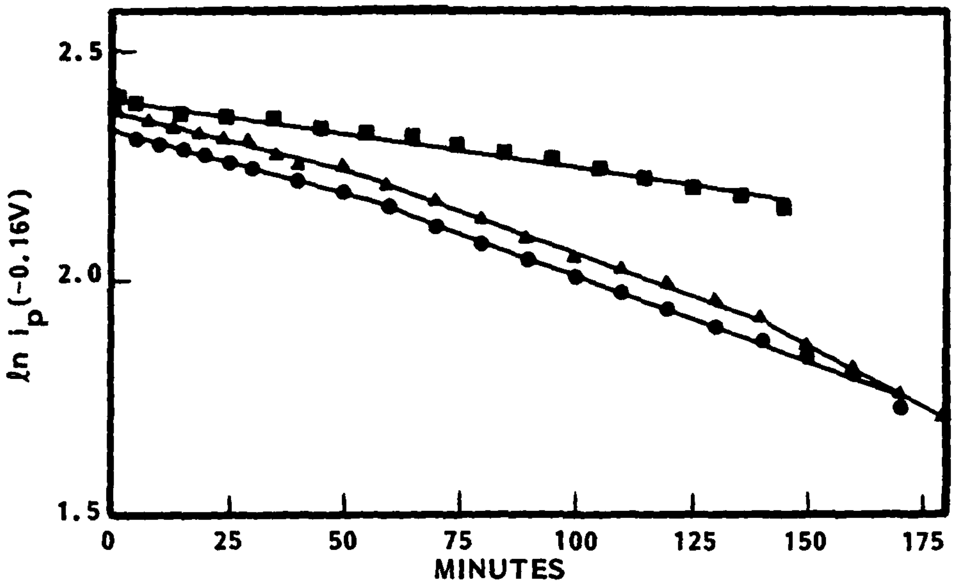

Figure 6. Rate of formation of $[Ru(NH_3)_5N_2]^{+2}$ at different P_{N_2}:

■ 1 atm
● 3 atm
▲ 6 atm

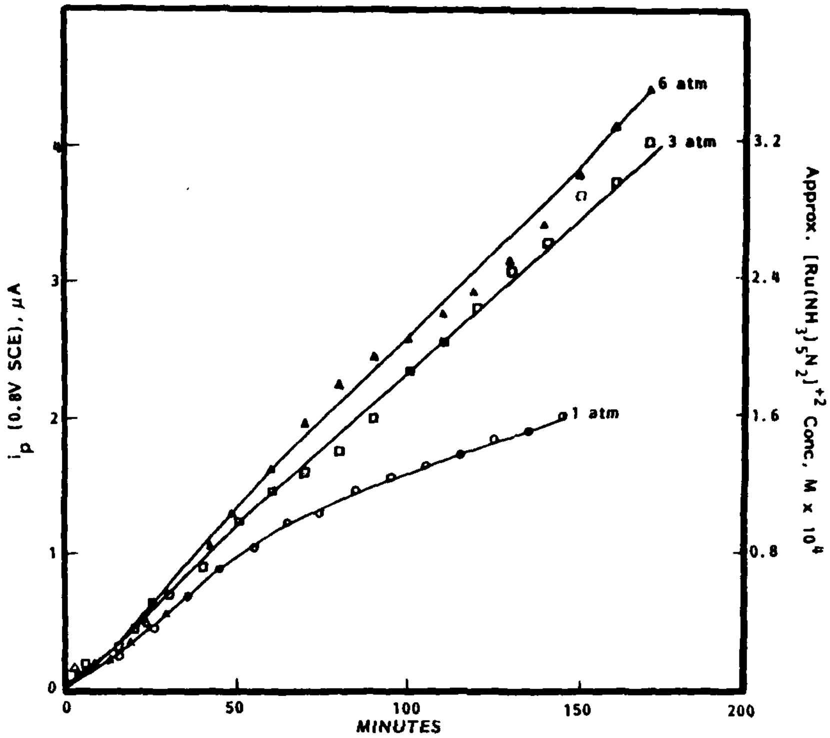

Figure 7. Increase in concentration of $[Ru(NH_3)N_2]^{+2}$ with different P_{N_2}. Concentration of $[Ru(NH_3)_5N_2]^{+2}$ estimated from Figure 5 with adjustment for electrode area of 0.071 cm^2.

other Ru(II) precursor were attached to an electrode surface, and the modified electrode introduced to the N_2 saturated solution, the reaction rate and amount of complex formed on the surface would be in direct proportion to P_{N_2}.

Properties of $[Ru(NH_3)_5N_2]^{+2}$ Prepared from Hydrazine

Cyclic voltammetry of the product obtained from the reaction of $RuCl_3$ with hydrazine[2] demonstrated an anodic wave at ∿0.8V as expected for $[Ru(NH_3)_5N_2]^{+2}$ (Figure 8). The UV spectrum also showed the characteristic absorbance peak at 222 nm from which it was possible to calculate that the product contained ∿16% $[Ru(NH_3)_5N_2]^{+2}$. The major contaminant was $[Ru(NH_3)_6]^{+3}$ as indicated by cathodic and anodic current peaks at -0.26V and -0.185V, respectively. These are the typical peaks seen on a cyclic voltammogram for the reaction

$$[Ru(NH_3)_6]^{+3} + e^- \rightleftarrows [Ru(NH_3)_6]^{+2}$$

The presence of the hexammine as a major contaminant produced during the hydrazine reaction was noted by Allen et al.[2]

Ru Immobilized at a Graphite Electrode

The progressive attachment of $[Ru(NH_3)_5H_2O]^{+2}$ to PVP-coated graphite electrodes was observed by cyclic voltammetry as an increase in anodic and cathodic current peaks at +0.05V and 0.0V, respectively. Figure 9 illustrates the dependence of current peak height on the duration of exposure of PVP-graphite to $[Ru(NH_3)_5H_2O]^{+2}$ and the quantity of PVP available on the graphite surface. The magnitude of these current peaks shows a fairly linear relationship to scan rate as expected for reactants attached to the surface (Figure 10). With the heavier PVP coatings, there is some deviation from linearity possibly due to slower electron transfer through the thicker PVP layer.[15]

Ru-PVP-graphite electrodes were stored dry for several days with no decrease in current peak in subsequent voltammograms. Increasing the pH of the sulfate electrolyte to pH 7.0 caused a slight decrease in current peak, but this was restored to its initial value when the electrode was again placed in pH 3.3 electrolyte. The current peak due to attached Ru remained stable during 20 hours or more of soaking in deaerated PBS (pH 7.3) and in air-saturated PBS containing protein, after an initial decrease during the first 1-2 hours (Figure 11). The Ru-PVP-graphite electrodes were held at -0.3V during the soaking period to maintain the Ru in the reduced state. This procedure was shown to improve the stability of the Ru-PVP-graphite electrodes. The slight decrease in current observed during

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[2] Allen et al., loc. cit.

[15] Oyama, N. and Anson, F. C., J. Electrochem. Soc. 127, 640 (1980).

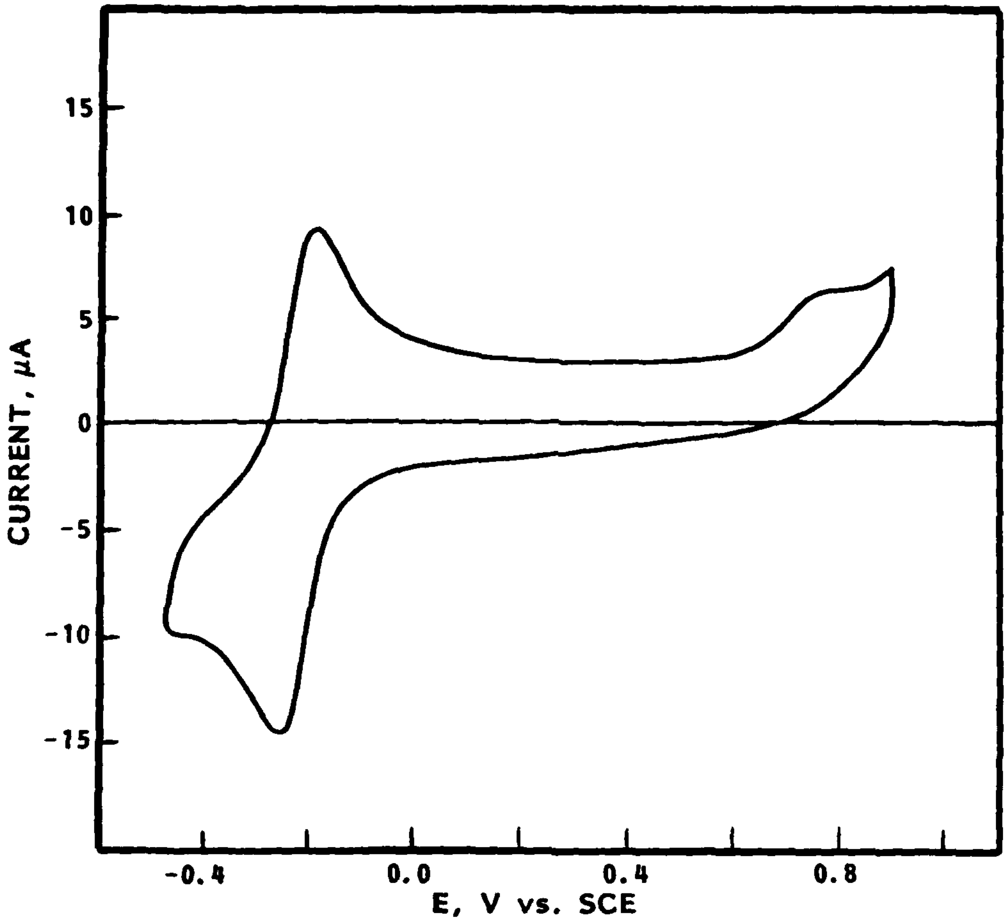

Figure 8. Cyclic voltammetry of $[Ru(NH_3)_5N_2]^{+2}$ prepared from reaction of $RuCl_3$ with hydrazine. Scan rate: 100 mV sec^{-1}.

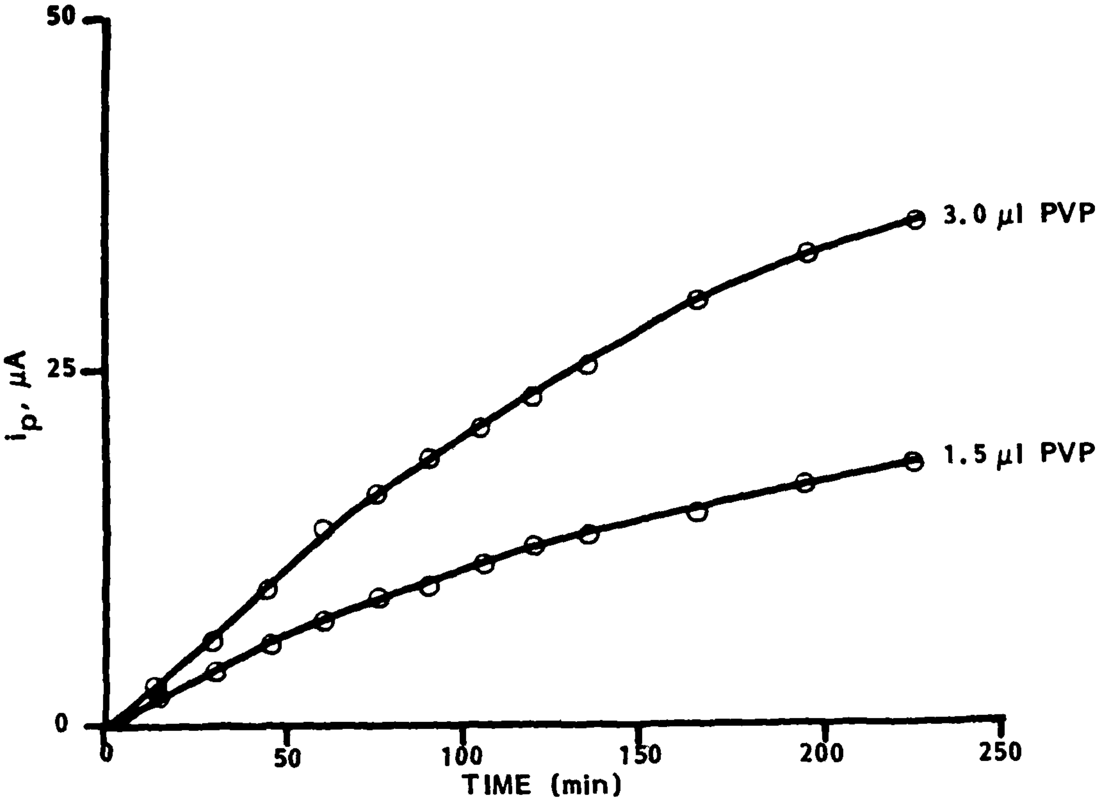

Figure 9. Attachment of $[Ru(NH_3)_5H_2O]^{+2}$ to PVP-coated graphite electrodes. Quantity of pyridine as PVP (MW = 40,000): A = 7 x 10^{-8} moles; B = 1.4 x 10^{-8} moles. Electrode area = 0.306 cm^2.

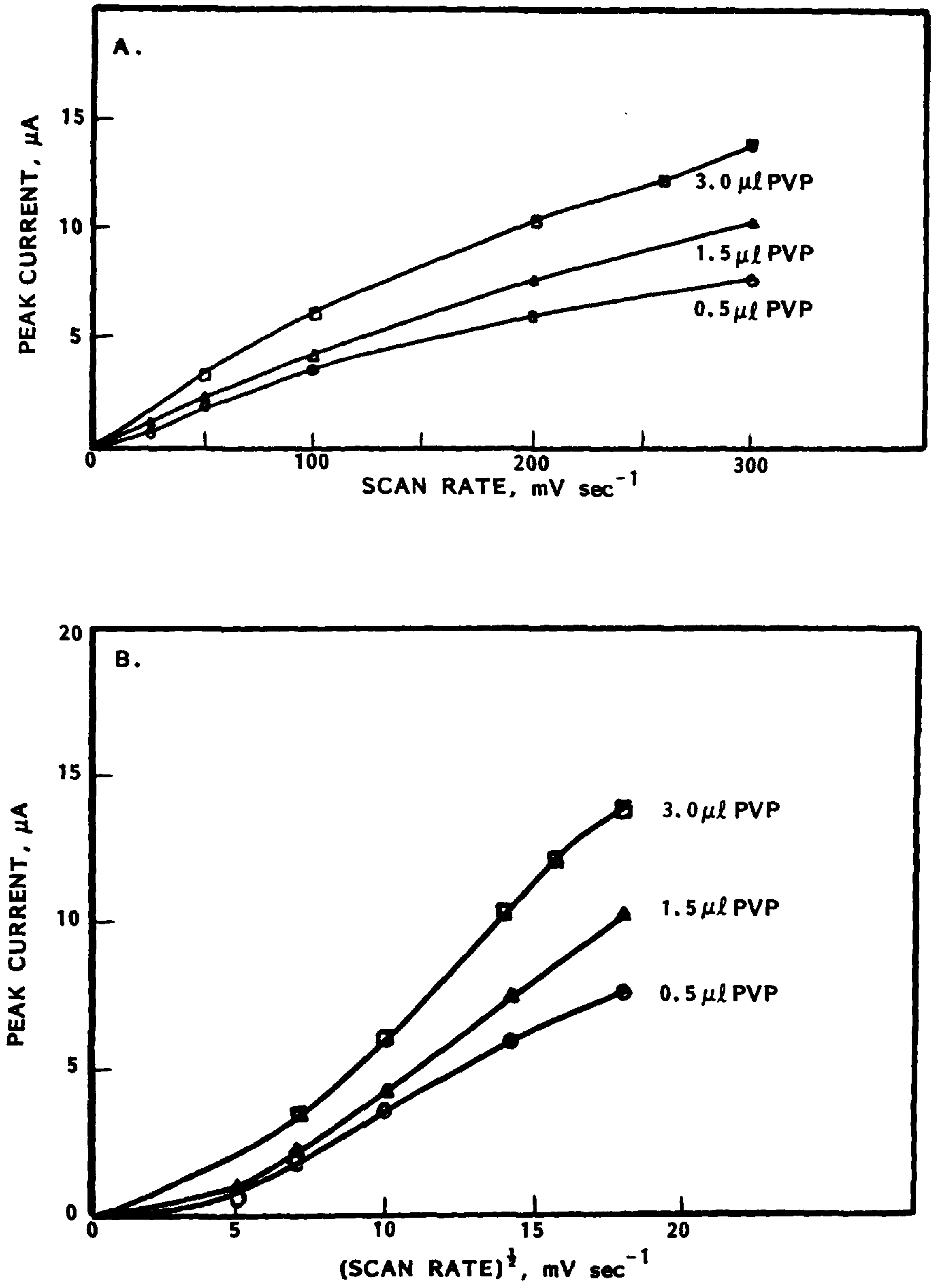

Figure 10. Peak currents of $[Ru(NH_3)_5H_2O]^{+2}$ attached to PVP-coated graphite electrodes as a function of scan rate (A) and (scan rate)$^{1/2}$ (B).

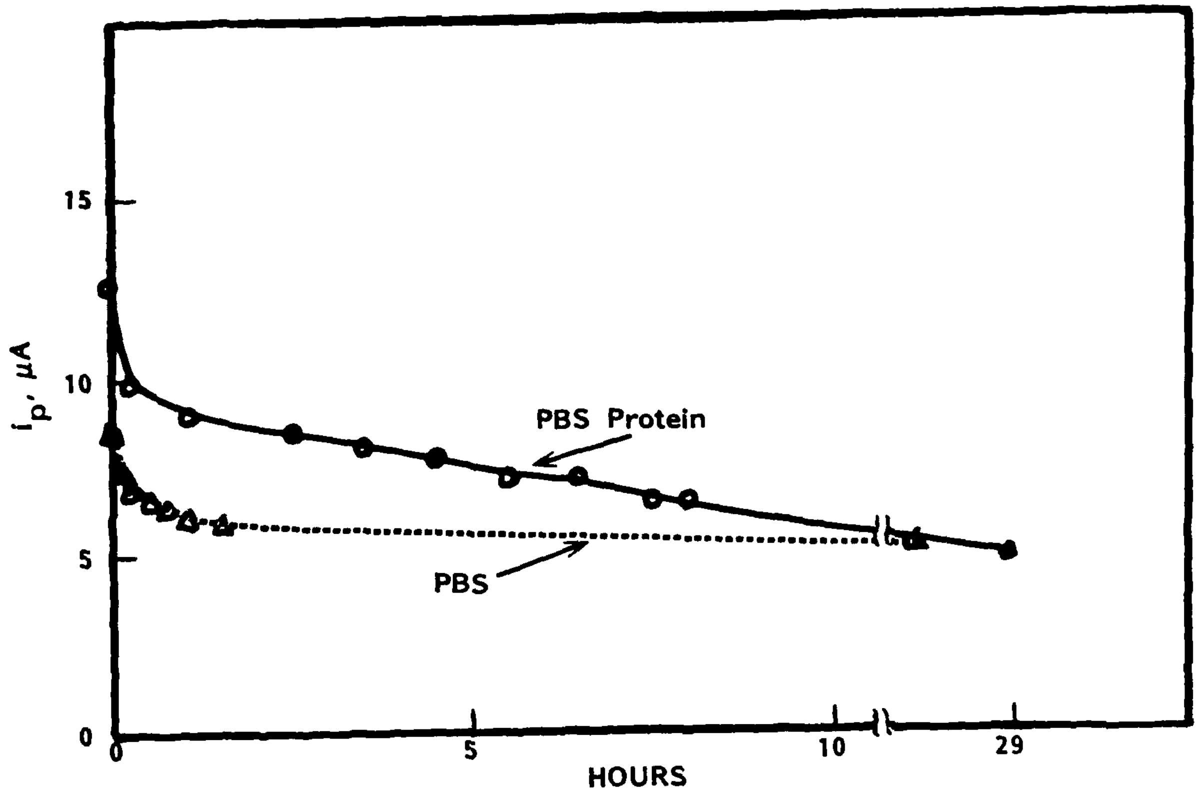

Figure 11. Stability of graphite-PVP-Ru(II) electrodes in deaerated PBS or air-saturated PBS containing protein.

the stability tests probably represents the loss of Ru from the PVP-coated surface as was found by Oyama and Anson.[6]

The mechanism by which $[Ru(NH_3)_5H_2O]^{+2}$ becomes attached to PVP-coated graphite probably involves the displacement of the H_2O ligand by pyridine-groups of PVP similar to the reaction between $[Ru(NH_3)_5H_2O]^{+2}$ and pyridine in solution,

$$[Ru(NH_3)_5H_2O]^{+2} + py \rightarrow [Ru(NH_3)_5py]^{+2} + H_2O$$

where py = pyridine.[6] The similarity between a cyclic voltammogram of a solution of $[Ru(NH_3)_5H_2O]^{+2}$ to which a small amount of pyridine was added, and a cyclic voltammogram of $[Ru(NH_3)_5H_2O]^{+2}$ after attachment to PVP-coated graphite is consistent with the formation of a $[Ru(NH_3)_5py]^{+2}$ complex with the PVP coating on the graphite surface (Figure 12).

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[6] Oyama and Anson, loc. cit.

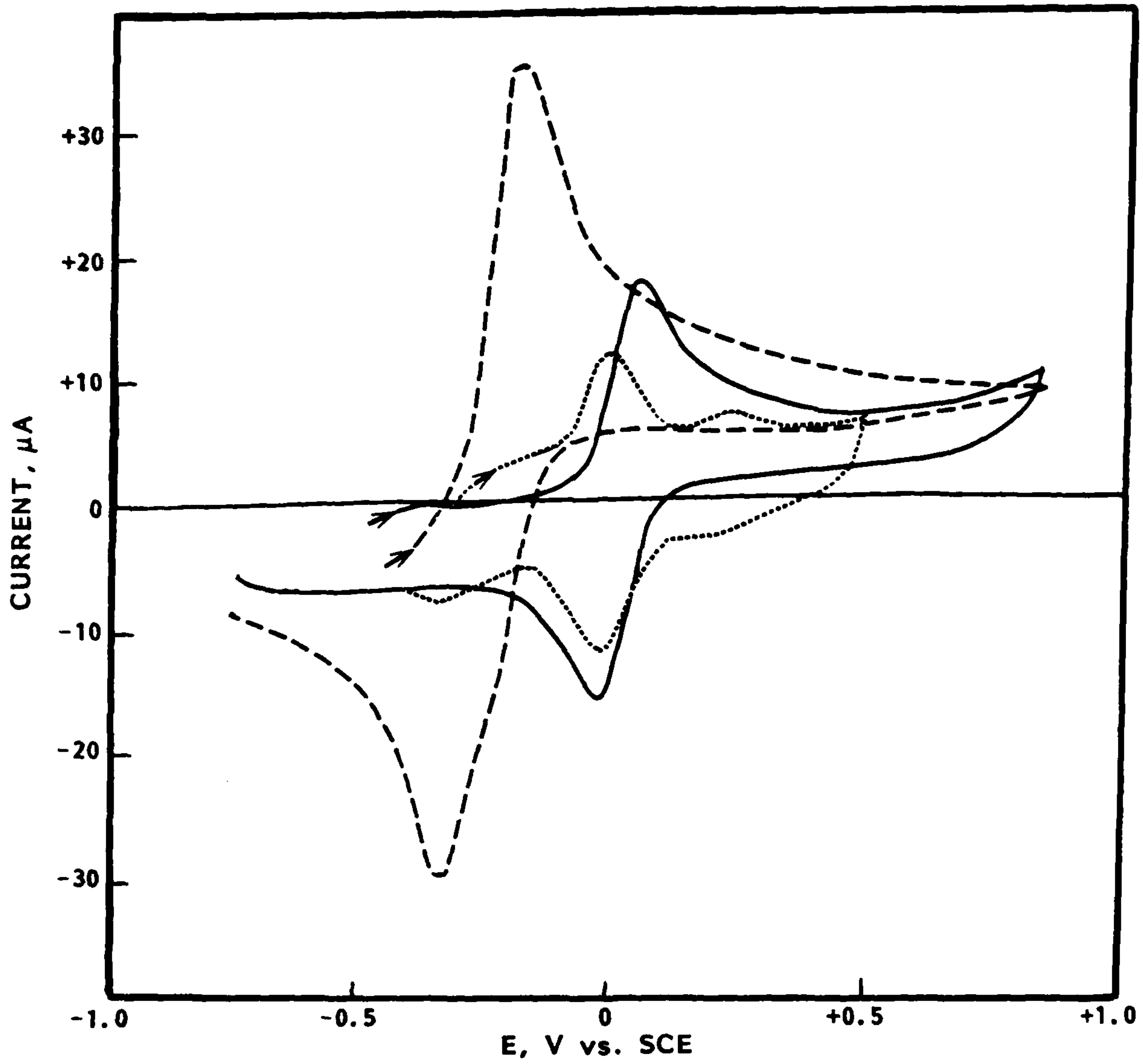

Figure 12. Cyclic voltammetry of $[Ru(NH_3)_5H_2O]^{+2}$ (— —); $[Ru(NH_3)_5H_2O]^{+2}$ + pyridine (——); $[Ru(NH_3)_5H_2O]^{+2}$ attached to PVP (---). Scan rate: 100 mV sec^{-1}.

CONCLUSIONS

It is evident from the results of this study that the formation of the dinitrogen complex $[Ru(NH_3)_5N_2]^{+2}$ can be monitored readily by voltammetry, and that the magnitude of the anodic current peak at +0.8V is proportional to the concentration of $[Ru(NH_3)_5N_2]^{+2}$. The reaction leading to the nitrogen complex was demonstrated, both in the present work and by others[8], to be first order with respect to the dissolved N_2 concentration. Therefore, the amount of complex formed in a given time, and the current peak at +0.8V due to its electrochemical oxidation, will be a direct measure of the solution N_2 concentration. The release of N_2 from the oxidized Ru complex, followed by electrochemical reduction of Ru(III) back to the Ru(II) precursor, will permit repetitive measurements of dissolved N_2 concentration with a N_2-sensing electrode based on these reactions.

The retention of electrochemical activity by $[Ru(NH_3)_5H_2O]^{+2}$ attached to PVP-coated electrodes, even during prolonged exposure to physiological saline, protein and dissolved O_2, demonstrates the feasibility of forming a stable Ru complex on a polymer-coated electrode. However, in order to utilize the immobilized Ru complex as a N_2-sensing electrode, the attached complex must retain its reactivity toward N_2. In the model systems studied here, the attachment of $[Ru(NH_3)_5H_2O]^{+2}$ to pyridine groups of PVP eliminates the H_2O ligand available for displacement by N_2. Other methods of immobilizing $[Ru(NH_3)_5H_2O]^{+2}$ which do not invoke the H_2O ligand, represents one area for further study.

A review of the literature on N_2-complexing Ru compounds indicates that the cis-Ru tetraammine, cis-$[Ru(NH_3)_4(H_2O)_2]^{+2}$ might have the properties needed for forming a N_2-sensing electrode with an immobilized complex. The cis-tetraammine reacts with dissolved N_2 to form the nitrogen complex cis-$[Ru(NH_3)_4(H_2O)N_2]^{+2}$ in the same manner as the Ru pentaammine.[3] The cis-tetraammine N_2 complex is electrochemically oxidized at +0.8V SCE, probably with release of N_2 from the oxidized Ru complex. The cis-Ru tetraammine also reacts similarly with pyridine as Ru pentaammine to form both mono- and disubstituted complexes, cis-$[Ru(NH_3)_4H_2O(py)]^{+2}$ and cis-$[Ru(NH_3)_4(py)_2]^{+2}$.[16] On the basis of these data, we would postulate that cis-$[Ru(NH_3)_4(H_2O)_2]^{+2}$ might attach to a PVP-coated electrode to form the monosubstituted complex, cis-$[Ru(NH_3)_4(py)H_2O]^{+2}$, which would then react with N_2 to form an attached, electrochemically detectable, N_2 complex, cis-$[Ru(NH_3)_4(py)N_2]^{+2}$. Since pyridine and N_2 are both π-acceptor

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[3] Elson et al., loc. cit.

[8] Itzkovitch and Page, loc. cit.

[16] Allen, R. J. and Ford, P. C., Inorg. Chem. 13, 237 (1974).

ligands, they can be expected to have similar reactivity toward Ru(II). Further study of the reactions of cis-$[Ru(NH_3)_4(H_2O)_2]^{+2}$ with N_2 and immobilizing ligands such as pyridine in PVP would be of great value in advancing the development of a N_2-sensing electrode.

We conclude that the unique reactions of Ru pentaammine and cis-Ru tetraammine toward molecular N_2, the electrochemical activity of the resultant N_2 complex, and the possibility for carrying out the N_2 complexation reaction with an immobilized Ru complex, might be effectively utilized to make in situ measurements of the dissolved N_2 concentration.

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