

Electrochemical analysis of ruthenium nitrosyl complexes of the ethylenediaminetetraacetic acid ligand



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ABSTRACT

Ruthenium nitrosyl complex has been studied recently for its potential as NO donor. However, much of its chemistry is not yet well understood. Synthesis and spectroscopic characterization of the [Ru(Hedta)Cl]K and [Ru(Hedta)NO] complex were conducted. The complex [Ru(Hedta)NO] was prepared from the simple reaction of [Ru(Hedta)(H₂O)] with NO gas in acid solution. Characterization of the complex was done using UV-vis spectrophotometer through scanning method. The ruthenium complexes were investigated using FT-IR spectroscopy. The $\nu(\text{N-O})$ for the [Ru(Hedta)NO] complex absorbs at 1884 cm⁻¹. The absence of the counteranions suggest that the edta ligand was monoprotonated which was confirmed at $\nu_{\text{COOH}} = 1716 \text{ cm}^{-1}$. The electrochemical property of [Ru(Hedta)NO] was conducted in aqueous and nonaqueous solvent. The complex undergoes electrochemically reversible one-electron reduction at $E_{p,c} = -203\text{mV}$, followed by a second multi-electron reduction, which is irreversible. The reversibility of the first one-electron process suggests greater stability of [Ru(Hedta)NO]⁻ complex than previously reported, which could decrease its ability to release NO.

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1. Introduction

Nitric oxide (NO) is an important messenger molecule (Snyder and Bredt, 1992), and an essential component in many physiological and pathophysiological process (Malinski, 2005). It is recognized as a highly toxic gas which is the major constitute of car exhaust emission and as a potent ozone-destroying pollutant but it is astonishing that the molecule plays a role in mammalian physiology as one of the most versatile and ubiquitous molecule. NO has been shown to have biodirecting functions including cardiovascular control (Calver et al., 1993), neuronal signaling (Garthwaite and Boulton, 1995), and as an agent for defense mechanisms against microorganisms (Fang, 1997). NO also plays a role in the immune system's ability to kill tumor cells (Jenkins et al., 1995). Any dysfunction in NO metabolism causes a number of diseases such as epilepsy (Beckman, 1991), arthritis (Connor et al., 1995), hypertension (Huang et al., 1995) and septic shock (Nava et al., 1991). The role played by NO in

many biological processes has stimulated the study of its interaction with transition metal complexes, with the aim of modeling compounds potentially usable as NO carrier drugs (Hofseth et al., 2003).

The study of transition metal NO complexes are effective nitrovasodilators *in vitro* and *in vivo* through the release of NO since they are generally water soluble (Ford and Lorkovic, 2005), bind NO rapidly and tightly under biological conditions (Stamler et al., 1992), low toxicity (Wink and Roberts, 1998) and generally inert with respect to other biological substrates (Ford and Lorkovic, 2002). Several metal complexes (Zhang et al., 2003) have been studied as a new class of NO donors, which includes ruthenium nitrosyl complexes that possesses low toxicity and quite stable in aqueous solution (Marmion et al., 2004). There are compounds that promote fast and slow NO release, which are both useful depending on the situation. Fast release of NO is suitable in uses that require high concentration of NO in a short period of time, while slow release of NO is useful when small but continuous flow of NO is needed. Moreover, the distribution of NO concentration within the body must be controllable to avoid damages in the tissue mainly when there's a very high concentration applied. The potential of a ruthenium nitrosyl complex as NO donor can be used for possible

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therapeutic application. It can also be used as an effective NO scavenger through manipulation of the ligand framework (Mukaida et al., 1965).

Studies done by Tfouni et al. (2012) showed that ruthenium nitrosyl complexes have the ability to donate NO. [Ru(Hedta)NO] has been evaluated for its potential to release NO catalytically (Zanichelli et al., 2006). This [Ru(Hedta)NO] is a water-soluble complex and is air-stable at room temperature (Zanichelli et al., 2004). It is also soluble in nonaqueous solvent such as Me₂SO. It has been suggested that one-electron reduction of a [Ru(Hedta)NO] complex may lead to NO labialization (Fricker et al., 1997), but this has yet to be fully evaluated, particularly on the potential of multi-electron reduction, which may lead to products other than NO.

This study focuses on the electrochemical property of [Ru(Hedta)NO]. From this perspective, further evaluation of the potentials of ruthenium nitrosyl complex is done, which answers the fundamental question of whether this compound is suitable as NO donors.

2. Materials and methods

All chemicals were of reagent grade and used as purchased. The experiments were performed under argon atmosphere unless otherwise noted.

All weighings were done using a Mettler Toledo AT21 Comparator analytical balance. The area was kept clean and moisture-free. UV-Vis spectroscopic analysis was done using Lasany LI 2800 UV-Vis spectrophotometer in a 1 cm quartz cuvette. The Nicolet iS-50 Analytical FT-IR spectrometer equipped with diamond ATR sample cell was used to acquire the FT-IR spectra of NO gas and ruthenium

complex. The pH was measured using Fisher Accumet pH meter model 610A.

2.1. Modified set-up for NO production

The modified set-up (Aga, 2008) for the nitric oxide generator was performed under the laboratory hood. Sodium nitrite (360 mg) dissolved in water (600 mL), in a buret was added dropwise to a 1 L Buchner flask containing sulfuric acid (600 mL, 6 M). Clear plastic tubing was attached to the side arm and leads into a sequence of six Buchner flasks that serves as traps (Fig. 1). The entry tube to the Buchner flask was a Pasteur pipette. All glass-to-plastic tubing connections were wrapped with Teflon and secured with nylon cable tie. Prior to the reaction, ultra-high purity argon gas was introduced to the set-up for at least 30 min.

The first Buchner flask is empty to collect any solution that was resorbing from the second and third flask. The second and third flask contained 0.1 M NaOH which traps any nitrogen oxides other than NO, e.g. nitrogen dioxide, and neutralizes the acid that might have been carried from the reaction flask. The next two flasks contained distilled water, intended to trap droplets of NaOH solution. The last flask contained the desiccant, to dry the outgoing gas, which was then connected to the three-necked flask through a needle and a Suba-Seal septum. The three-necked flask serves as a reaction vessel for the synthesis of the ruthenium complex.

The NO produced from the disproportionation of HNO₂ was analyzed through FT-IR immediately after collecting the NO from the set-up. The IR spectrum shows the $\nu_{NO} = 1915\text{-}1840\text{ cm}^{-1}$ and $1385\text{-}1340\text{ cm}^{-1}$ (strong, two bands).

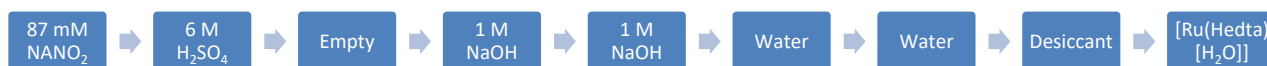


Fig. 1: Schematic set-up of NO gas generator

2.2. Synthesis of [Ru(Hedta)Cl]K

The synthesis of [Ru(Hedta)Cl]K was performed using a published procedure (Mukaida et al., 1965). Briefly, [Ru(Hedta)Cl]K·2H₂O was prepared by dissolving [RuCl₅H₂O]K₂ (1 g) in water (40 mL) followed by addition of concentrated HCl (2 mL). The solution was concentrated through heating until the solution volume is reduced to 20 mL. To this, a solution of Na₂(H₂edta)·2H₂O (1.4 g in 30 mL H₂O) was added. The resulting mixture was then transferred to an evaporating dish and was concentrated through evaporation until a film formed on the surface of the solution just before it solidified. Appearance of the black precipitate which is the ruthenium oxide will disappear with thorough evaporation process. The concentrate was then dissolved in water (30 mL) and again concentrated by heating. This was done for at least three times and the concentrated solution was placed in a cool, dry

place overnight at room temperature and a crystalline material with yellow-brown color was deposited. The precipitate was washed several times with ethyl alcohol and dried under reduced pressure at room temperature. The synthesis of [Ru(Hedta)Cl]K yield 77.42%.

2.3. Synthesis of [Ru(Hedta)NO]

[Ru(Hedta)NO] was prepared from the reaction of [Ru(Hedta)(H₂O)] and NO in acidic solution following the procedure of Zanichelli et al. (2004). The [Ru(Hedta)Cl]K (1 g) was dissolved in HCl (20 mL, 0.1 M) in a three-necked round-bottom flask sealed with a Suba-seal septum and was kept under Ar for 30 min. The flask was connected directly to the NO gas generator set-up through a needle and a rubber septum. NO was bubbled to the solution for 3 h and 45 min. The resulting solution was stored in cool, dry place overnight at room temperature. The

solution was then freeze-dried. Addition of ethyl alcohol to the dried product gave a pale pink precipitate, which was filtered and dried under vacuum at room temperature. The synthesis gave a 78.2% yield.

2.4. Cyclic voltammetry

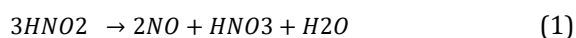
Cyclic voltammetry experiments were performed with Metrohm 797 VA Computrace voltammetric analyzer. All solutions were deaerated with ultra-high purity argon for at least 10 min prior to each measurement. Buffer solution of pH 4.3 (0.1 M CH₃COOH/NaCH₃COO at 25°C) was used as the supporting electrolyte for aqueous experiment. The supporting electrolyte for nonaqueous experiment used was 0.2 M Bu₄NPF₆ dissolved in DMSO. The three-electrode system employed consist of a glassy carbon working electrode, a platinum wire counter electrode and silver/silver chloride (Ag/AgCl, 3 M KCl) reference electrode for aqueous measurement and a silver/silver ion (Ag/Ag⁺, 0.01 M AgNO₃ dissolved in anhydrous Me₂SO) reference electrode for nonaqueous experiment. Prior to the addition of the sample in the cell, the solvent containing the supporting electrolyte was first run and was deaerated with ultra-high purity argon gas for at least 10 min for aqueous solution and at least 20 min for nonaqueous solution. The working electrode was polished using BASi PK-4 Polishing Kit MF-2060 and tapped dry with Kim Wipes[®] prior to each experiment.

The [Ru(Hedta)NO] solid sample was added directly in the sample cell after the analysis of the blank solution. The cyclic voltammetry experiments were conducted at several scan rates of 100 mV/s, 30 mV/s and 10mV/s. Ferrocene was used as standard for CV experiment.

3. Results and discussion

3.1. NO gas production

The modified set-up (Fig. 2) for the nitric oxide generator was performed under the laboratory hood. Several attempts in producing a high purity NO gas was successfully delivered. The production of NO gas used in this study is based on the disproportion of nitrous acid in equation 1. Although the set-up was deaerated with ultra-high purity argon for 30 min, brown fumes of NO₂ was seen as it is difficult to fully deoxygenate the sulfuric acid solution. However, the NO₂ produced from the reaction flask was removed in the NaOH traps,



3.2. IR and UV-Vis characterization

The [Ru(Hedta)Cl]K and [Ru(Hedta)NO] complexes were characterized using UV-vis and IR spectrophotometry. The [Ru(Hedta)Cl]K was

dissolved in deionized water which rapidly aquates to the aqua complex and exist predominantly in its most labile form (Shoukry et al., 2005). The [Ru(Hedta)(NO)] was dissolved in deionized water without forming into aqua complex. The UV-vis spectrum of [Ru(Hedta)Cl]K agrees well with the literature (Fig. 3). The complex was also verified by calculating its molar extinction coefficient which was $2.81 \times 10^3 \text{ mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$.



Fig. 2: Set-up for NO generator

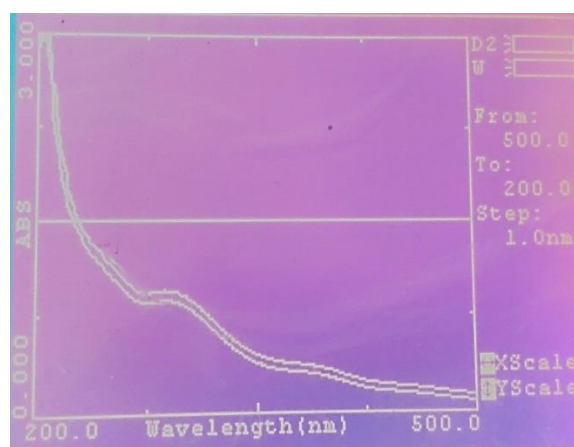


Fig. 3: UV-vis spectrum of [Ru(Hedta)H₂O] at 280nm

The [Ru(Hedta)NO] absorbs at 322 nm which agrees well with the literature, confirming the formation of the [Ru(Hedta)NO] (Fig. 4). The Ru-NO bond is stable which explains why the [Ru(Hedta)(H₂O)] complex easily forms to aqua complex. This can also be explained with equation 2 and 3; also with the result of cyclic voltammetry.

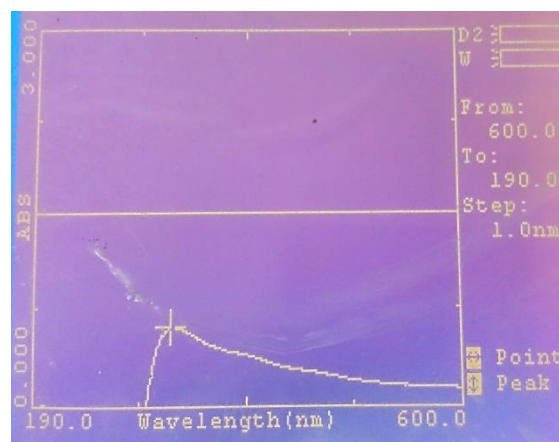


Fig. 4: UV-vis spectrum of [Ru(Hedta)NO] at 322 nm

The IR analysis of [Ru(Hedta)Cl]K and [Ru(Hedta)NO] were shown in stack spectra (Fig. 5). The NO absorbs at 1884 cm⁻¹ which agrees well in the literature (Kim et al., 1999). The carboxyl and

carboxylic group absorbs at 1635 and 1716 cm^{-1} , respectively, which corresponds to the protonated complex.

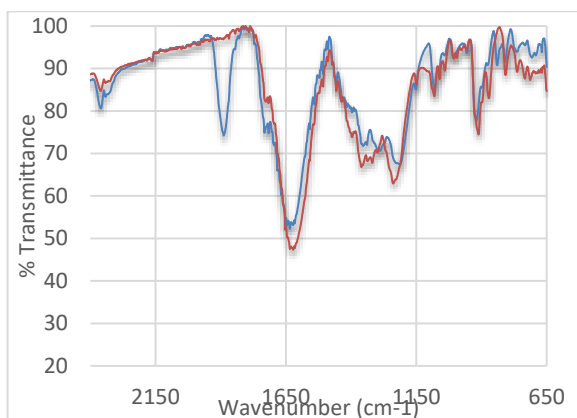
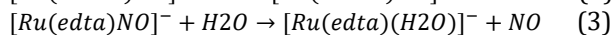
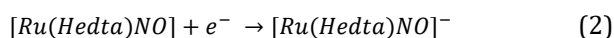


Fig. 5: IR spectra of [Ru(Hedta)NO] (blue) and [Ru(Hedta)Cl]K (red)

Due to the uncertainty of the procedure from the previous journals, many attempts in synthesizing the complex leads production of other products, particularly the formation of a black precipitate, which presumably is ruthenium oxide. Further modification of the procedure of Mukaida et al. (1965) for the preparation of [Ru(Hedta)Cl]K leads to a more precise result of the [Ru(Hedta)Cl]K which agrees well in the literature. The two trials of [Ru(Hedta)Cl]K were synthesized separately in different days in order to figure out the consistency of its product since previous experience in synthesizing [Ru(Hedta)Cl]K resulted to unexpected result.

3.3. Cyclic voltammetry

The [Ru(Hedta)NO] has been evaluated for its potential to release NO catalytically (Zanichelli et al., 2004). It has been suggested that [Ru(Hedta)NO] undergo one-electron reduction followed by aquation to release NO and forming [Ru(Hedta)H₂O]⁻ according to equation 2 and 3:



The experiment with the two solvent were done using a modified cell (Fig. 6) in order to minimize the use of the sample.

Aqueous experiment: The cyclic voltammogram of the [Ru(Hedta)NO] (Fig. 7) shows a one-electron electrochemically reversible process at $E_{p,c} = -203$ mV with an oxidative component at $E_{p,a} = -393$ mV. The reversibility of this process indicates greater stability of the complex, which could decrease its ability to release NO that corresponds to the equation 1 in which the complex was reduced. The current ratio is one parameter to consider the chemical reversibility of the electrode reaction that shows the stability of the electrogenerated species

during reduction in the cyclic voltammetric timescale (Fig. 8).



Fig. 6: Cell for cyclic voltammetry analysis

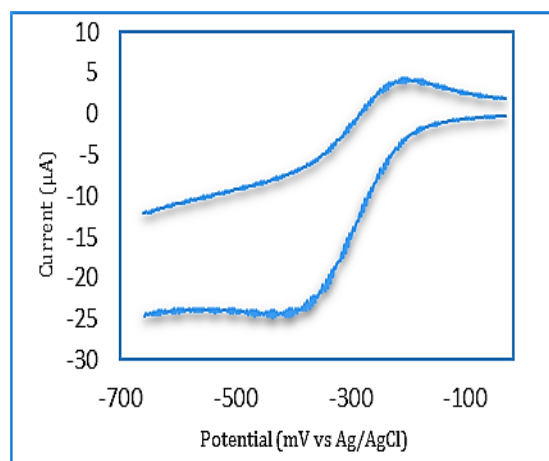


Fig. 7: Cyclic voltammogram of [Ru(Hedta)NO] (5 mM) in 0.1 M CH₃COOH/NaCH₃COO, pH 4.3; 25°C. Scan rate = 100 mV/s at a glassy carbon electrode

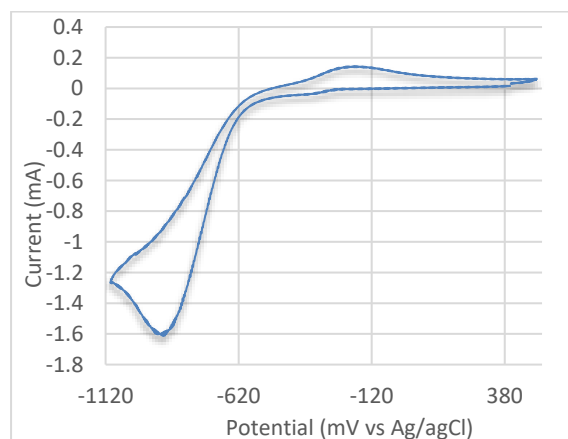


Fig. 8: Cyclic voltammogram of [Ru(Hedta)NO] (5 mM) in 3.0 M KCl solution, 0.1 M CH₃COOH/NaCH₃COO, pH 4.3; 25°C. Scan rate = 10 mV/s at a glassy carbon electrode

Scanning past -700 mV shows a multi-electron reduction process at -901 mV (Fig. 8). This second reduction process of [Ru(Hedta)NO] is not reversible due to the absence of anodic peak current. Furthermore, the possibility of a second reduction may lead to products other than NO.

Nonaqueous experiment: The cyclic voltammogram of [Ru(Hedta)NO] at 100 mV/s scan rate in DMSO (Fig. 9) shows a one-electron reduction process at $E_{p,c} = -560$ mV and $E_{p,a} = -620$ mV. The peak separation and the peak height ratio suggest an electrochemical reversible process. This is followed by a second one-electron reduction process at $E_{p,a} = -940$ mV. This second reduction process is irreversible, similar to what was observed in aqueous solution (Fig. 6). As in the aqueous solution case, the reversibility of the first reduction step indicates stability of the [Ru(Hedta)NO]⁻. This, combined with the possibility of a second reduction could limit the use of [Ru(Hedta)NO] as an efficient NO donor.

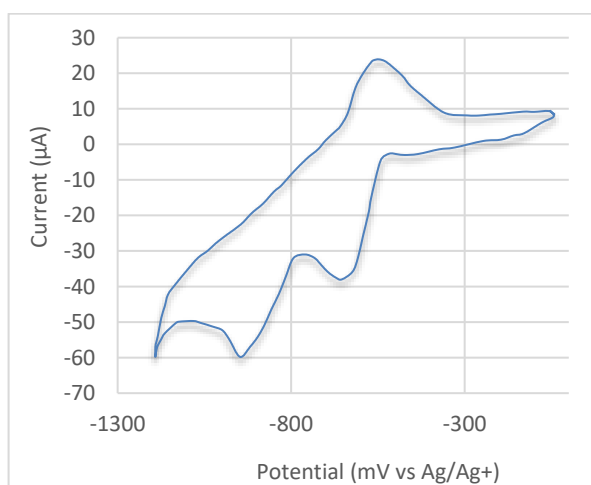


Fig. 9: Cyclic voltammogram of [Ru(Hedta)NO] (5 mM) in 0.2 M Bu₄NPF₆ in DMSO at 25°C. Scan rate = 100 mV/s at a glassy carbon electrode

The cyclic voltammogram of aqueous and nonaqueous appears to have same result in which one-electron reduction occurs then a multi-electron reduction of the complex. In this manner, there is no differences in the [Ru(Hedta)NO] neither aqueous nor nonaqueous solvent. In other case, the use of aqueous solvent is preferable since oxygen easily dissolves in DMSO that would contribute other effect in [Ru(Hedta)NO], henceforth, Ar was purged in the solution for at least 20 min.

4. Conclusion

The cyclic voltammetry analysis of [Ru(Hedta)NO] complex in both aqueous and nonaqueous solution showed two reduction processes: a reversible one-electron reduction, followed by a second irreversible reduction step. The first reduction process is electrochemically reversible for the while the second reduction resulted to a multi-electron reduction since the height of the second anodic peak was in high difference than the first anodic peak. The second reduction may lead to other products than NO. Hence, the complex may possibly be not a suitable NO donor. Further studies using other ruthenium nitrosyl complexes are being conducted in our lab.

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