Electrochemical Oxidation of Amines Using a Nitroxyl Radical Catalyst and the Electroanalysis of Lidocaine

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Abstract: The nitroxyl radical of 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO) can electro-oxidize not only alcohols but also amines. However, TEMPO has low activity in a neutral aqueous solution due to the large steric hindrance around the nitroxyl radical, which is the active site. Therefore, nortropine *N*-oxyl (NNO) was synthesized to improve the catalytic ability of TEMPO and to investigate the electrolytic oxidation effect on amines from anodic current changes. Ethylamine, diethylamine, triethylamine, tetraethylamine, isopropylamine, and *tert*-butylamine were investigated. The results indicated that TEMPO produced no response current for any of the amines under physiological conditions; however, NNO did function as an electrolytic oxidation catalyst for diethylamine, triethylamine, and isopropylamine. The anodic current depended on amine concentration, which suggests that NNO can be used as an electrochemical sensor for amine compounds. In addition, electrochemical detection of lidocaine, a local anesthetic containing a tertiary amine structure, was demonstrated using NNO with a calibration curve of 0.1–10 mM.

Keywords: sensor; nitroxyl radical; electrocatalytic oxidation; lidocaine; TEMPO

1. Introduction

Electrochemical analysis has the advantages of easily created probes (being an inexpensive process), the ability to miniaturize the probes, and real-time measurement [1]. In addition, biosensors can be constructed with high selectivity, sensitivity, and time resolution using enzymatic reactions [2–5]. Biosensors then can be optimized according to its purpose (e.g., food, medical, industrial, or environmental analysis use). However, the use of enzymes for sensors has encountered barriers of high cost, low stability, and lack of homogeneity of catalytic activity. Therefore, an electrochemical catalyst of 2,2,6,6-tetramethylpiperidine *N*-oxyl (TEMPO) has been investigated for chemical sensor probes [6–8]. 2,2,6,6-tetramethylpiperidine *N*-oxyl is a stable free radical and an organic molecular catalyst for the oxidation of alcohols [9–15]. In electrochemistry, TEMPO catalyzes the oxidation of alcohols by application of electric potential in aqueous solutions and organic solvents [16–21]. Since the response current obtained is proportional to alcohol concentration in the solution, alcohol concentration can be quantified [6,7]. However, TEMPO has low reactivity due to large steric hindrance

around the nitroxyl radical active site, and an adequate response current could not be obtained under physiological conditions.

Iwabuchi and colleagues [22] reported that 2-azaadamantane *N*-oxyl (AZADO), which lacks steric hindrance around the active site, exhibited greater activity than TEMPO in organic synthesis reactions. Less-hindered nitroxyl radicals with various steric environments have been synthesized, and those with less bulky functionality exhibited greater activity [23–26]. Therefore, nortropine *N*-oxyl (NNO), which was modeled on AZADO, was synthesized in one step as a novel nitroxyl radical compound [27]. The NNO was capable of electrolytic oxidation of alcohols in neutral aqueous solutions. The NNO could be used in place of enzymes, allowing non-enzymatic analysis of glucose under physiological conditions [27]. Phenylboronic acid derivatives have been investigated as glucose sensors [28–30]. Phenylboronic acid (PBA) spontaneously binds with a moiety containing a diol [31]. The structural and electrical changes in PBA derivative probes when PBA bonds with the diol moiety can be used for glucose detection [32–34]. However, these probes had low specificity for glucose and did not respond under physiological conditions. Superiority of NNO was demonstrated from this report [27]. In contrast, TEMPO has catalytic oxidation ability toward amines as well as alcohols [35–37]. Therefore, the present study examined the electrolytic oxidation effect of NNO on amines under physiological conditions and compared the results with those obtained with TEMPO. The results demonstrated that NNO was a good electrochemical analysis probe for secondary and tertiary amines and for isopropylamine under physiological conditions (Figure 1). The oxoammonium ion, which is the active species, reacts with the amine to form hydroxylamine. The catalyst regenerates by reoxidation of hydroxylamine. Furthermore, electrochemical detection of lidocaine, a general local anesthetic, was accomplished using NNO to show the applicability of this method to therapeutic drug monitoring (TDM).
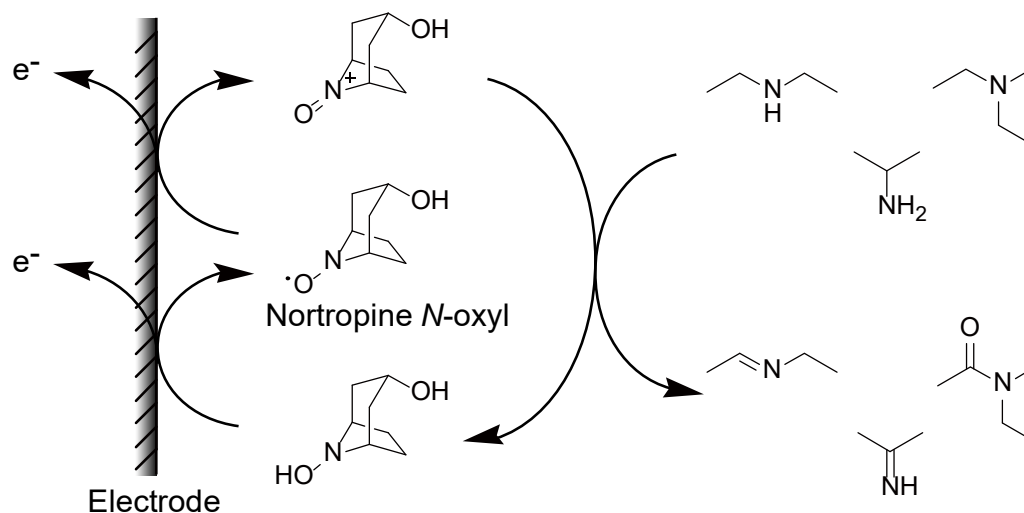
Figure 1. Electrochemical reactions of nortropine *N*-oxyl (NNO) for secondary amines, tertiary amines, and isopropylamine.

2. Results and Discussion

2,2,6,6-tetramethylpiperidine *N*-oxyl is widely known as an oxidation catalyst for alcohols, but it can also electrochemically oxidize amines [35]. However, the reaction is limited to organic solvents and basic aqueous solutions. The NNO, a nitroxyl radical compound which improved the functionality of TEMPO, confirmed that the reaction proceeded even under physiological conditions. Amines with different structures were added to a phosphate buffer solution (pH 7.4, 100 mM) containing 1 mM NNO to obtain the cyclic voltammetry (CV) measurements. If NNO electrolytically oxidizes amines at a sufficiently fast reaction rate, the anodic current should increase and the cathodic current disappear, depending on the concentration of the amine, resulting in a voltammogram. The oxoammonium ion is expected at first to react with amines, that is, to eliminate a proton from the amines, then to change

them to hydroxylamines. The disproportionation of hydroxylamine and oxoammonium ion produces NNO, which is re-oxidized electrochemically to complete a catalytic cycle. Addition of diethylamine and triethylamine resulted in an increase in anodic current, depending on the concentration (Figure 2). For example, ΔIp values upon addition of 10 mM of diethylamine and triethylamine were 5.5 and 6.9 μA, respectively, indicating the ability to use the system as an electrochemical sensor. The NNO responded electrochemically to compounds containing secondary amines and tertiary amines.
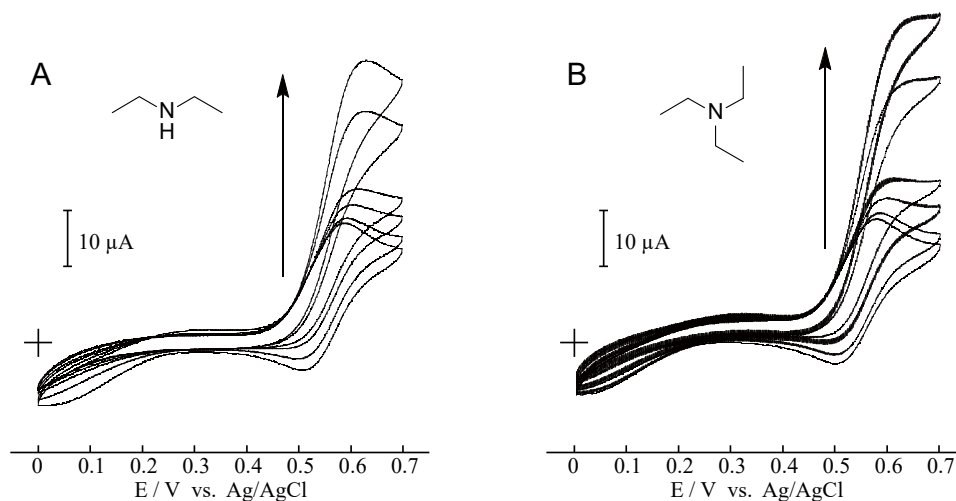
Figure 2. Cyclic voltammograms of NNO (1 mM) in the absence and presence of 1, 5, 10, 50, and 100 mM (**A**) diethylamine and (**B**) triethylamine in 100 mM phosphate buffer solution (pH 7.4). Scan rate was 100 mV s^{-1}.

In contrast, no increase in current with NNO was observed for ethylamine or tetraethylammonium (Figure 3). A significant reduction in cathodic current of NNO was observed upon addition of ethylamine. The nitroxyl radical moiety was oxidized at the electrode surface to an oxoammonium ion, which is the active form. The oxoammonium ion reportedly binds to a primary amine in aqueous solution resulting in the disappearance of catalytic activity [38–40] (Note: TEMPO oxidizes primary amines in acetonitrile [35]). Likewise, the oxoammonium ion of NNO bonds with ethylamine in aqueous solution causing the catalytic activity to disappear (Figure 4). However, the results confirmed that tetraethylammonium does not change and does not react with NNO.
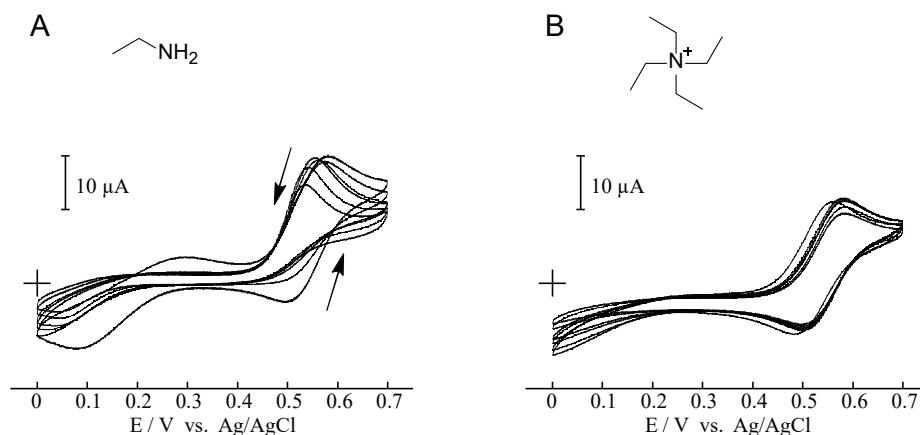
Figure 3. Cyclic voltammograms of NNO (1 mM) in the absence and presence of 1, 5, 10, 50, and 100 mM (**A**) ethylamine and (**B**) tetraethylammonium in 100 mM phosphate buffer solution (pH 7.4). Scan rate was 100 mV s^{-1}.
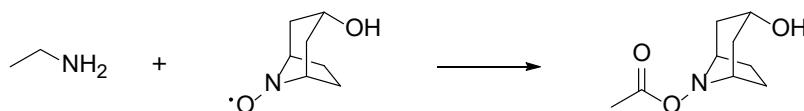
Figure 4. Reaction of NNO and ethylamine.

Similarly, for isopropylamine and *tert*-butylamine, the oxidizing ability of NNO was investigated using CV (Figure 5). For isopropylamine, the oxidation current increased depending on the concentration, indicating that electrolytic oxidation was performed by NNO. The ΔIp value was 2.4 μA when 10 mM of isopropylamine was added. No increase in response current could be confirmed with *tert*-butylamine, which cannot assume an imine structure as tetraethylammonium can. These results showed that NNO can act as an electrochemical analysis probe for compounds with secondary, tertiary, and isopropyl amine structures. The peak potential for generation the radical and oxidation of amines was not observed at the reach from 0 V to + 0.7 V.
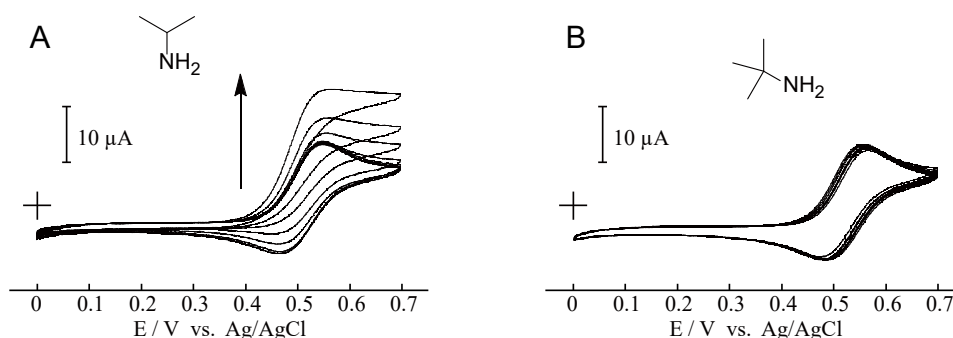
Figure 5. Cyclic voltammograms of NNO (1 mM) in the absence and presence of 1, 5, 10, 50, and 100 mM (**A**) isopropylamine and (**B**) *tert*-butylamine in 100 mM phosphate buffer solution (pH 7.4). Scan rate was 100 mV s^{-1}.

An investigation was carried out to determine whether this electrochemical reaction could be accomplished for TEMPO under physiological conditions (100 mM PBS, pH 7.4), using 10 mM ethylamine, diethylamine, triethylamine, tetraethylammonium, isopropylamine, and *tert*-butylamine in a solution containing 1 mM TEMPO for cyclic voltammetry (Figure 6). The anodic peak current of TEMPO was observed at approximately +0.6 V vs. Ag/AgCl. Even upon addition of each amine, this anodic peak did not increase in response current (ΔIp) compared with that without amine. Although TEMPO has sufficient oxidation catalytic ability in organic solvents [35], it could not oxidize amines under physiological conditions and was difficult to use for electrochemical analysis under these conditions.
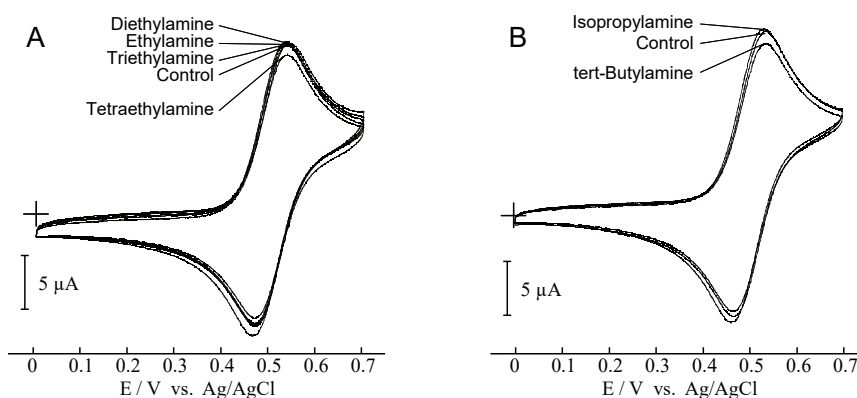
Figure 6. Cyclic voltammograms of TEMPO (1 mM) in the absence and presence of 10 mM (**A**) ethylamine, diethylamine, triethylamine, and tetraethylammonium, and (**B**) isopropylamine and *tert*-butylamine in 100 mM phosphate buffer solution (pH 7.4). Scan rate was 100 mV s^{-1}.

Nortropine *N*-oxyl can be an electroanalysis probe for chemical compounds having secondary or tertiary amines or isopropylamine structures under physiological conditions. Here, to show the utility of NNO, electrochemical analysis of lidocaine was conducted. Lidocaine (2-diethylamino-*N*-(2,6-dimethylphenyl)acetamide) is a common amide drug with high efficacy as a local anesthesia and anti-arrhythmia medication [41]. Since lidocaine overdose can damage the heart and nervous system, highly sensitive and reliable monitors for lidocaine are extremely important for clinical purposes [41]. For this reason, lidocaine has been analyzed using techniques such as LC/MS [42], HPLC [43–45], and GC-MS [46,47]. Previous experiments have shown that determining the amount of a tertiary amine is possible using NNO. Since lidocaine has one tertiary amine in its molecular structure, electrochemical analysis using NNO was possible. Figure 7 shows a plot of change in anodic peak current upon addition of lidocaine to phosphate buffer solution (100 mM PBS, pH 7.4) containing 1 mM NNO, with an anodic current change (ΔIp) of +0.6 V vs. Ag/AgCl. A good calibration curve was drawn between 0.1 and 10 mM. Although the sensitivity at μmol/L level is necessary to perform serum TDM of lidocaine [48], this paper shows the detection principle of new lidocaine. Further sensitivity increase can be expected by electrode materials and electrochemical measurement equipment. Electrochemical analysis of lidocaine using modified electrodes has been reported [49,50]. This method is simple as it allows measurements with unmodified electrodes and the response principle is simple.

Enzyme reactions and antigen–antibody reactions are used for construction of electrochemical biosensors for reasons of sensitivity and specificity. However, constructing these biosensors for lidocaine is challenging because antibodies and enzymes for lidocaine are difficult to obtain. However, rapid electrochemical analysis for molecules such as lidocaine were thought to be possible using NNO.
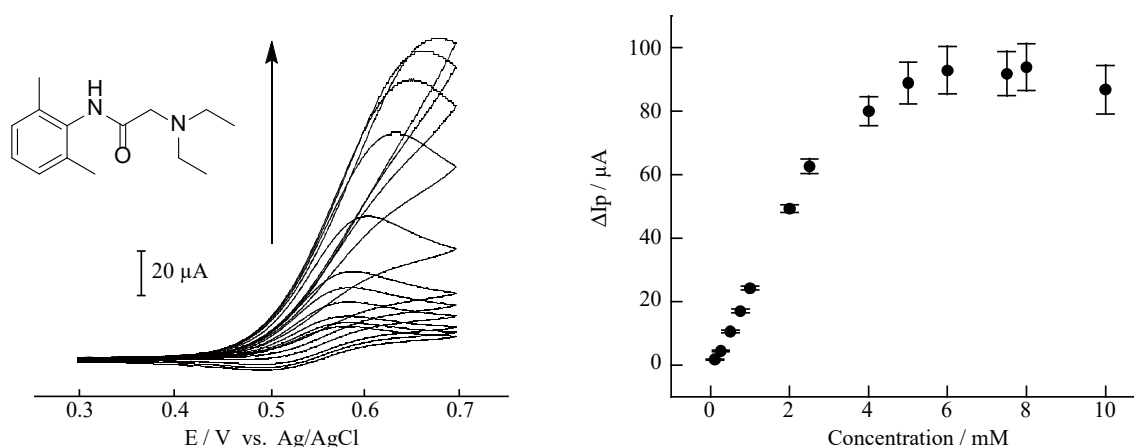
Figure 7. Cyclic voltammograms of NNO (1 mM) in the absence and presence of 0.1, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, and 10 mM lidocaine in 100 mM phosphate buffer solution (pH 7.4). Scan rate was 100 mV s^{-1} (left). Anodic peak current in cyclic voltammograms of NNO was a function of the concentration of lidocaine in 100 mM phosphate buffer (pH 7.4). Average values of three electrodes with standard deviation are plotted (right).

3. Materials and Methods

The NNO was synthesized from nortropine according to a previously reported single-step procedure, with slight modifications [27]. All amines (hydrochloride form) and the lidocaine were purchased from Tokyo Kasei Co. (Tokyo, Japan). All other reagents used in this study were commercially available and reagent grade.

Cyclic voltammetry was conducted using an electrochemical analyzer (ALS model 660B, BAS, Tokyo, Japan) in a conventional three-electrode cell consisting of a glassy carbon electrode (diameter: 3 mm) as the working electrode, a platinum wire as the counter electrode, and an Ag/AgCl (3.33 M KCl) reference electrode. The CV was measured in 100 mM phosphate buffer solution containing NNO

(1 mM) and amines (0–100 mM) at pH 7.4 with a scan rate of 100 mV s^{-1}. The third cycle of the cyclic voltammograms was recorded and the electrocatalytic responses were evaluated from the difference in oxidation peak current in the cyclic voltammograms in the absence and presence of substrate (ΔIp). All experiments were conducted at room temperature (*ca.* 20 °C).

4. Conclusions

The results demonstrated that NNO electrochemically oxidizes secondary and tertiary amines and isopropylamine under physiological conditions. In addition, the response current depends on the concentration of the amine in the solution, indicating that this system can be used as a probe for electrochemical analysis. Using NNO, lidocaine concentration was determined and a good calibration curve was prepared between 0.1 and 10 mM. Therefore, NNO is useful for the development of electrochemical sensors without enzymes or antibodies.

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