Determination of Ammonia in Exhaled Breath by Flow Injection Analysis with Electrochemical Detection

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ABSTRACT

Flow injection analysis with electrochemical detection (FIA-ECD) using a-tocopherol (a-TOH) as a reagent was developed for determining ammonia in exhaled breath. An ethanol-water (4:1, v/v) mixture containing 3 mM a-TOH and 50 mM NaCl was used as the carrier solution. The FIA response at +0.70 V vs. Ag/AgCl in the flow cell was linear over a range of the ammonia concentration from 0.11 to 1.1 ppmv (r = 0.999, n = 7). The lower limit of detection for ammonia was 130 pg (S/N = 3), and the relative standard deviation (RSD) was 2.1% (17 ng, n = 10). The collected exhaled breath in a Tedlar® bag was mixed with water containing NaCl to dissolve ammonia in exhaled breath, and then it was diluted with ethanol containing a-TOH to the same composition of the carrier solution to be injected into the FIA-ECD. The present FIA-ECD method required simple sample preparation and was applied to the determination of ammonia in exhaled breath from a healthy human and gerbils.

Keywords: Ammonia, Exhaled Breath, Flow Injection Analysis, a-Tocopherol

1. Introduction

Ammonia is the major basic gas in human exhaled breath, as well as ambient atmosphere and indoor air. In human exhaled breath, the ammonia concentration of healthy human subjects is about sub part per billion by volume (ppbv) levels,1 and that in patients with renal failure is about part per million by volume (ppmv) levels.2-3 Moreover, ammonia levels in the exhaled breath of patients who have impaired liver or kidney functions have also been reported to be elevated.3,4 Helicobacter pylori infection can significantly elevate ammonia levels in exhaled breath by breaking down urea.5,6 Ammonia can also be generated in cavities from infections, and exhaled breath ammonia is increased in halitosis.7 Although the determination of ammonia in exhaled breath is useful and significant to examine and diagnose the aforementioned diseases, a complex mixture of volatile organics in exhaled breath makes measurement of ammonia difficult and unreliable with conventional ammonia sensors. Therefore, a simple, reliable, and rapid assay for the determination of ammonia is desirable in the clinical fields.

To determine ammonia in exhaled breath, several methods were proposed. Selected ion flow tube mass spectrometry (SIFT-MS) is particularly popular in measuring a variety of breath gases.8-12 SIFT-MS can obtain simultaneous data for various breath gases, including ammonia, and thus constitute good research tools. The complexity and cost, however, deter its routine use for the analysis of ammonia in exhaled breath. Laser photoacoustic spectrometry (LPAS) is very sensitive and can easily be used for the measurement of ammonia in both exhaled breath and ambient air.13-17 However, both SIFT-MS and LPAS are unfamiliar methods to clinical and practical analysis, because they require expert experimental techniques. An electroanalytical method is simple, facile, and sensitive, and the apparatuses are low-cost compared with optical and mass spectrometric detection. Using a conductivity sensor, the determination of ammonia in exhaled breath has been previously reported,18 but other electroanalytical methods such as amperometric and/or coulometric detection have never been applied to the analysis of exhaled breath. Our preliminary study showed that the flow injection analysis with electrochemical detection (FIA-ECD) by means of the voltammetric oxidation of a-tocopherol (5,7,8-trimethyltocol, a-TOH)19 was applicable for determining ammonia in pharmaceutical preparations.20 However, the reported FIA-ECD was inapplicable for the determination of exhaled breath ammonia, because the sensitivity was poor and there was no method provided for sample preparation to the analysis of exhaled breath. In the present study, an FIA-ECD system with high sensitivity, selectivity, and good precision is provided for determining ammonia in exhaled breath, subsequent to optimize FIA system and conditions. Moreover, a method for the collection of exhaled breath and preparation of a test solution with a simple and rapid procedure has also been developed in consideration of practical and clinical uses. To show the present method is effective for real samples, the determination of ammonia in exhaled breath from healthy humans and gerbils was carried out by the present FIA-ECD system.

2. Experimental

2.1 Reagents

a-Tocopherol (a-TOH, >98%), NaCl (Japanese Industrial Standards (JIS) grade, >99.5%), ethanol (JIS grade, 99.5%), ammonia water (25.0%), urea (≥99%), and urease (from jack bean, 143 U mg⁻¹) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The water used was distilled and purified with a NANO Pure II filtering system (Barnstead Co., Ltd., Boston, MA, USA).

2.2 Voltammetry

A linear potential sweep voltammogram was obtained using a voltammetric system composed of a potentiostat (HZ-3000, Hokuto Denko Co., Tokyo, Japan) with a personal computer as a recorder and 50 mL capacity commercially available conventional beaker-type electrochemical cell with a silicone rubber cell top. A working electrode of plastic formed carbon (PFC, a disk of 3 mm diameter, Mitsubishi Pencil Co. Ltd., Tokyo, Japan), an Ag/AgCl (3.3 M KCl) reference electrode, and a platinum counter electrode were inserted...
through the lid into the cell. Linear potential sweep voltammetry was performed with a potential sweep rate of 20 mV s⁻¹.

An ethanol-water (4:1, v/v) mixture solution containing 50 mM NaCl was used as a supporting electrolyte. α-TOH for determining ammonia was dissolved in the supporting electrolyte to a concentration of 3.0 mM and served as a working solution. Ammonia water was dissolved in the working solution and served as a test solution.

2.3 FIA-ECD

As shown in Fig. 1, the equipment for the FIA-ECD system was composed of a pump (DP-8020, Tosoh, Tokyo, Japan), a sample injector with a 20 µL sample loop (model 7125, Rheodyne, Cotati, CA, USA), a damper (HPD-1, GL Science, Tokyo, Japan), a electrochemical detector, electrochemical flow cell and potentiostat; R, recorder. Between the outlet of sample injector and the inlet of electrochemical flow cell was connected by a PEEK tube (0.5 mm i.d. × 300 mm length).

2.4 Collection of exhaled breath and preparation of test solution

2.4.1 Human

A healthy adult subject (subject W, male, 23-years-old, non-smoker) participated in our study upon receipt of his informed consent. Before commencement of the trial, the protocol and the subject’s informed consent were submitted to and approved by the institutional human research committees of Tokyo University of Pharmacy and Life Sciences.

The collection of human exhaled breath and preparation of the test solution is explained, based on the following EC pathway involves a two-electron transfer coupled with a one-proton transfer giving a phenoxonium ion:

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\text{α-Tocopherol (α-TOH)} \quad + \quad H^+ \quad + \quad 2e^- \quad (1)
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than ammonia was made by measuring the current peak heights of the 3.2 FIA-ECD system for determining ammonia thus it is useful for determining ammonia.

The voltammogram gave a maximum at +0.75 V vs. Ag/AgCl in the flow cell. The potential of prepeak on the hydrodynamic voltammogram by the present FIA-ECD was more positive than that on the linear potential sweep voltammogram. This is due to the solution resistance between working and reference electrodes in the thin-layer flow cell in the present FIA-ECD. Thus, the original α-TOH oxidation in the present FIA-ECD would be occurred at more than 0.8 V vs. Ag/AgCl in the flow cell. A correlation coefficient $(r, n = 7)$ between the current peak height of the flow signal and the ammonia concentration ranging from 0.11 to 1.1 ppmv at +0.75 V and +0.70 V vs. Ag/AgCl in the flow cell was 0.985 and 0.999, respectively. Thus, because the linearity of the peak height at +0.70 V vs. Ag/AgCl in the flow cell is superior to that at +0.75 V vs. Ag/AgCl in the flow cell, the applied potential for determining ammonia was fixed at +0.70 V vs. Ag/AgCl in the flow cell.

Figure 5 shows the flow signal of a peak caused by ammonia of 0.55 ppmv and 1.1 ppmv. The peak signal current was recovered until the baseline level within 1 min, thus the FIA operation conditions allowed 60 samples h$^{-1}$ to be processed. The FIA response was linear over a range of the ammonia concentration in a test solution from 0.11 to 1.1 ppmv $(r = 0.999, n = 7)$, i.e., the ammonia amount from 2.2 to 22 ng $(r = 0.999, n = 7)$. The regression expression for the calibration curve of the ammonia concentration $(ppmv)$ and the peak signal current (nA), and thus a base with small $pK_a$ means greater electron donation of nucleophiles, and thus a base with small $pK_a$ value would form a stable complex with α-TOH and consequently accelerate the first electron transfer reaction. Nucleophiles serve to decrease the energy for the transfer reaction.

Based on the findings described above, the FIA-ECD of ammonia was made by measuring the current peak heights of the flow signals at the fixed potential. The hydrodynamic voltammogram for ammonia was measured by the present FIA-ECD (Fig. 4). The voltammogram gave a maximum at +0.75 V vs. Ag/AgCl in the flow cell. The potential of prepeak on the hydrodynamic voltammogram by the present FIA-ECD was more positive than that on the linear potential sweep voltammogram. This is due to the solution resistance between working and reference electrodes in the thin-layer flow cell in the present FIA-ECD. Thus, the original α-TOH oxidation in the present FIA-ECD would be occurred at more than +0.8 V vs. Ag/AgCl in the flow cell. A correlation coefficient $(r, n = 7)$ between the current peak height of the flow signal and the ammonia concentration ranging from 0.11 to 1.1 ppmv at +0.75 V and +0.70 V vs. Ag/AgCl in the flow cell was 0.985 and 0.999, respectively. Thus, because the linearity of the peak height at +0.70 V vs. Ag/AgCl in the flow cell is superior to that at +0.75 V vs. Ag/AgCl in the flow cell, the applied potential for determining ammonia was fixed at +0.70 V vs. Ag/AgCl in the flow cell.

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To determine ammonia in environmental water samples and/or atmospheric gases, some methods have been reported using gas chromatography with flame photometric detection (GC-FPD). The FIA-ECD system for determining ammonia

3.2 FIA-ECD system for determining ammonia

where ROH is a nucleophile such as a solvent molecule. The protons produced by oxidation of α-TOH at the electrode surface are likely to be neutralized by the weak base, a nucleophile stronger than the solvent molecule. Nucleophiles serve to decrease the energy for the oxidation in Eq. (1). Weak base addition to the α-TOH solution thus produces a new two-electron oxidation wave in the voltammogram at a potential less positive than that of the original α-TOH wave without base. Moreover, a smaller $pK_a$ means greater electron donation of nucleophiles, and thus a base with small $pK_a$ value would form a stable complex with α-TOH and consequently accelerate the first electron transfer reaction. And, the half-peak potential ($E_{1/2}$) of prepeak shifted to a more negative value accompanied by a decrease in the $pK_a$. Thus, the occurrence of the prepeak is considered in terms of the acceleration of the first electron transfer reaction of α-TOH. Presumably, ammonia, being a nucleophile, would form an intermediate complex with a phenoxonium ion, and would consequently accelerate the first electron transfer reaction.

The prepeak height was found to be proportional to the ammonia concentration from 0.082 to 2.14 mM $(r = 0.998, n = 10)$. By the present voltammetry, ammonia is detected without derivatization, thus it is useful for determining ammonia.

![Figure 3. Linear potential sweep voltammograms of 3.0 mM α-TOH (a) without and with (b) 0.507, (c) 1.01, (d) 1.51, and (e) 2.00 mM ammonia in ethanol-water (4:1, v/v) mixture containing 50 mM NaCl. Scan rate was set at 20 mV s$^{-1}$.](image)

Figure 4. Hydrodynamic voltammogram of ammonia. FIA conditions: carrier solution, ethanol-water (4:1, v/v) mixture containing 3.0 mM α-TOH and 50 mM NaCl; flow rate, 1.0 mL min$^{-1}$.

![Figure 5. FIA signals caused by ammonia. FIA conditions used were the same as in Fig. 4 except for the applied potential. Applied potential was set at +0.70 V vs. Ag/AgCl in the flow cell. The injected concentrations of ammonia into the FIA are shown in the figure.](image)
with flame thermionic detector (GC-FTD),23 high-performance liquid chromatography with UV detection (HPLC-UV),24 HPLC with fluorescence detection (HPLC-FL),25 FIA-UV,26 and spectrometry.27 Although GC-FTD is the most sensitive ($L_3 = 30 \text{ pg}$ for ammonia in test solution), it requires complicated and particular experimental tools to collect ammonia from air samples. HPLC-UV, HPLC-FL, and FIA-UV are necessary for the derivatization of ammonia with chromophore to perform highly sensitive determination. Thus, the flow system is complex compared with the present FIA-ECD, and the controls of the accurate reaction conditions for the derivatization are essential for quantitative analysis with the sufficient accuracy. Furthermore, various chromatographic methods are generally difficult to finish one measurement of ammonia within 1 min. Thus, the present FIA-ECD for determining ammonia is a rapid and simple method in comparison with the above analytical methods, and it would be useful for the analysis of exhaled breath from humans.

3.3 Determination of ammonia in human exhaled breath

A suitable reservoir to collect exhaled breath was selected by additional recovery tests prior to the determination of ammonia in human exhaled breath. In this study, three reservoirs were compared as follow: a polyvinyl fluoride (PVF) film gas sampling bag, an aluminum film gas sampling bag, and an airtight glass bottle, respectively. The collected room air in each vessel, which was spiked with the standard ammonia solution to become an ammonia concentration of 0.45 ppmv, was analyzed by the present FIA-ECD. By using the PVF film gas sampling bag, aluminum film gas sampling bag, and airtight glass bottle, the recovery of the ammonia for the spiked test solutions were 99.8%, 84.9%, and 97.4%, respectively, and the RSD ($n = 3$) was 1.8%, 7.4%, and 3.4%, respectively. Thus, the PVF film gas sampling bag was selected to collect the exhaled breath in the present study.

The determination of ammonia in human exhaled breath was carried out by the present FIA-ECD. For three straight days, the healthy human subject W (male, 23 years old, non-smoker) exhaled breath and it was collected after 15 h fasting and analyzed by the present method. The results of ammonia concentration in the healthy human exhaled breath from the first day of sampling were 0.031, 0.11, and 0.044 ppmv, respectively. The results for the determination of ammonia concentration by the present FIA-ECD would be reasonable because ammonia is normally present in the exhaled breath of healthy humans at 0.01–1 ppmv.5,18

3.4 Determination of ammonia in gerbils exhaled breath after administration of urea

The concentration of ammonia in the exhaled breath of patients who have impaired liver or kidney function has been reported to be elevated.3,4 If the elevation of ammonia concentration in exhaled breath can be measured by the present FIA-ECD, it could be useful in the biological and clinical fields.

By the high urease activity of the Helicobacter pylori organism, orally administrated urea was degraded to carbon dioxide and ammonia in stomach.28–31 Thus, concentration of ammonia in exhaled breath of human who is infected by Helicobacter pylori is elevated after administration of urea compared with before.5,31 In this study, the determination of ammonia in the exhaled breath of urease administrated gerbils before and after ingesting urea was carried out to examine whether the elevation of ammonia in exhaled breath was monitored by the present FIA-ECD system. As shown in Fig. 6A, the ammonia concentration in the exhaled breath of urease administrated gerbils after urea administration was elevated in comparison with that in the exhaled breath of the negative control gerbils and urease administrated gerbils before urea administration. The mean difference ($\pm$SE) of ammonia concentration in the exhaled breath of each gerbil before and after urea administration are shown in Fig. 6B. Via the urea administration, the ammonia concentration in the exhaled breath of urease administrated gerbils was elevated, but that of the negative control gerbils was reduced. By the present FIA-ECD system, the elevation of ammonia in the exhaled breath of gerbils, which was derived from urea degradation in the stomach by urease, was observed after the administration of urea.

4. Conclusions

To determine ammonia in exhaled breath, SIFT-MS and LPAS are normally used in lab setting, but electroanalytical methods are not. In this study, a novel FIA-ECD utilizing $\alpha$-TOH oxidation was developed for determining ammonia in exhaled breath. The present method with good accuracy, high sensitivity, and simple operation was performed to determine ammonia in exhaled breath from a human and that from gerbils after administration of urea. Moreover, it was found that the present method was easily and rapidly carried out to determine ammonia compared with other reported methods such as GC-FPD, GC-FTD, HPLC-UV, HPLC-FL, FIA-UV, and spectrometry.22–27 Thus, the present method would be suitable to measure ammonia in the exhaled breath of patients that have impaired liver or kidney functions, and it would be useful in the clinical fields.

References