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Analysis of N-nitrosodiethylamine by ion chromatography coupled with UV photolysis pretreatment

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ABSTRACT

Nitrosamines such as *N*-nitrosodiethylamine (NDEA) are commonly detected by spectrophotometry after photolysis and Griess reaction (PG) in food industries for lower cost. Results of this research indicate that NDEA decays rapidly under UV irradiation, and concentrations of the generated NO_2^- and NO_3^- ions vary with photolysis conditions. Thus, the measurement of the PG method may be inconsistent because it is based on the amount of photoproduced NO_2^-. In addition, more errors may be present in the PG method since NO_3^- cannot be measured colorimetrically using Griess reagent. In this work, the sum of the concentrations of photoproduced NO_2^- and NO_3^- was found to be equivalent to the initial NDEA before photolysis, and a photolysis–ion chromatography method was validated, which may serve as a feasible and accurate method to determine nitrosamines.

1. Introduction

N-nitrosamines, such as *N*-nitrosodiethylamine (NDEA), have received considerable attention due to their highly carcinogenic nature and potentially harmful impacts on human health [1,2]. These compounds can be present in wastewater, as well as in ground and drinking water [3]. In addition, nitrosamines can be formed by the reaction of secondary amines with nitrosating agents in food processing, so they may appear in a wide variety of foods like cured ham, bacon, and sausages [4]. Therefore, much interest is focused on the quantification of nitrosamines that occur in the environment and diet.

Several methods are now available for the determination of nitrosamines, and among them the more frequently used are high performance liquid chromatography (HPLC)- and gas chromatography (GC)- mass spectrometry (MS) methods [5,6]. However, these methods are limited by expensive equipment and the requirement for a high level of expertise. Based on the photolability of nitrosamines, a cost-effective spectrophotometry method was developed. The photolysis of nitrosamines yields corresponding amine and nitrite ions [7]. The liberated NO_2^- can be measured colorimetrically by Griess

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reagent [8]. This photolysis and Griess reaction (PG) method can be conveniently implemented, so it has been widely used for the determination of nitrosamines in food industries. In addition, the PG method was also combined with other technologies like HPLC to improve the response and sensitivity of detection [9].

The PG method was based on the amount of photo-produced nitrite, but the latter may vary with photolysis conditions [10], which introduce errors in the determination of nitrosamines. Furthermore, the produced nitrite can convert to nitrate through a photo-oxidation process [11]. Thus, more errors may be included if the converted nitrate cannot be detected by the Griess reaction. In that case, a combination of photolysis with ion chromatography (IC) may be an alternative with a small error for nitrosamine determination. Although the analysis of nitrosamines by PG-related methods has been extensively studied, the possible error resulting from the coloring nature of photoproducts with Griess reagent is not well documented. In particular, there have been few studies on applying IC to nitrosamine analysis [5–7]. Therefore, in the present work, we attempted to study the ionic photoproducts and their Griess reaction by using N-nitrosodiethylamine (NDEA). A photolysis–IC method was investigated for NDEA determination.

2. Materials and methods

2.1. Reagents

A stock solution of NDEA (0.5mM) was prepared and stored in the dark, and the working solutions were prepared by dilution. Griess reagent consisted of 1% (w/w, solution A) 4-aminobenzenesulfonic acid and 0.1% (w/w, solution B) N-(1-naphthyl) ethylenediamine dihydrochloride in 30% acetic acid. HCl and NaOH (0.1M) were used for pH adjustment when necessary. Methanol was of chromatographic grade and other chemicals used were of analytical grade. All chemicals were purchased from Changzheng Chemical Co. (Chengdu, China), and all solutions were prepared in deionized water.

2.2. UV photolysis of NDEA

NDEA solution was exposed to UV irradiation by using a low-pressure Hg lamp (30 W, emission at 253.7 nm, Changzheng Chemical Co.). After irradiation, the solution was used for HPLC or IC analysis. Effects of pH, irradiation duration, and solution concentration on the photolysis of NDEA were investigated. For quantification analysis, the UV irradiation lasted 20 minutes unless otherwise specified.

2.3. HPLC analysis of NDEA

Samples were filtered through 0.45 μm filters before HPLC injection (30 μL). A reverse-phase HPLC system (Agilent 1100, Agilent Technologies Inc., Santa Clara, CA, USA) was employed, which was equipped with an ArchromBond-AQ C18 column (150×4.6 mm; GL-Science, Tokyo, Japan) and a G1315B diode array detector. A methanol–water mixture (35/65, v/v) was used as mobile phase at 1.0 mL/min. The column temperature was 30°C, and the detection was performed at 230 nm.

2.4. IC analysis of NDEA photoproducts

A Dionex ICS-90 ion chromatograph (Sunnyvale, CA, USA), equipped with a Dionex AS14 anion exchange column (250×4 mm) and a conductivity detector, was used to analyze the photoproducts of NDEA. The mobile phase was composed of 10mM Na_2CO_3 and 30mM $NaHCO_3$ at a flow rate of 1.0 mL/min. Samples were filtered through 0.45 μm filters before injection (30 μL) and the column temperature was set at 30°C.

2.5. Griess reaction of nitrite and nitrate

The colorimetric reaction of nitrite and nitrate with Griess reagent was comparatively investigated. The Griess reactions were investigated according to the methods of Wang et al [12] and Liao et al [13], with a slight modification. One milliliter of sodium nitrite solution (or sodium nitrate, 0.05mM) was mixed with Griess solution A (1.5 mL) in a tube. Five minutes later, 1.5 mL of Griess solution B was added. The tube was vortexed and kept still for another 5 minutes. The mixture was then scanned from 400 nm to 700 nm by using a UV/VIS spectrophotometer (UV-1800PC, Shanghai Mapada Co., Shanghai, China).

2.6. Statistical analysis

All experiments were conducted in triplicate and the data were expressed as mean value ± standard deviation (SD). Statistical analysis was performed with the software Origin 8.0. (Origin lab, Northampton, Mass, USA) Student's *t*-test was applied to determine the significance of differences between initial NDEA values with calculated NDEA values at a confidence level of 95%.

3. Results and discussion

3.1. Photolysis of NDEA under UV irradiation

N-nitrosamines are thermally stable and also resistant to biodegradation [10], but UV treatment is known to be an efficient method to degrade nitrosamines. This photolabile characteristic was used for the removal of nitrosamines from contaminated waters [14] and also for their determination. The photolysis of nitrosamine under UV irradiation generates equivalent moles of nitrite that can be measured colorimetrically by Griess reagent. UV irradiation is a key step for the PG method, and a partial photolysis of nitrosamine should be avoided in order to reach an accurate determination.

The photolysis of NDEA was studied by HPLC analysis, showing a rapid decay of NDEA under UV irradiation. As shown in Fig. 1, NDEA (0.05mM) was almost totally photolyzed when exposed to UV irradiation (253.7 nm, 30 W) for 10 minutes, and there was no reformation of NDEA during further irradiation. In fact, NDEA was no longer observed by HPLC after 20 minutes of irradiation even though it initially appeared at a much higher concentration (0.5mM), suggesting

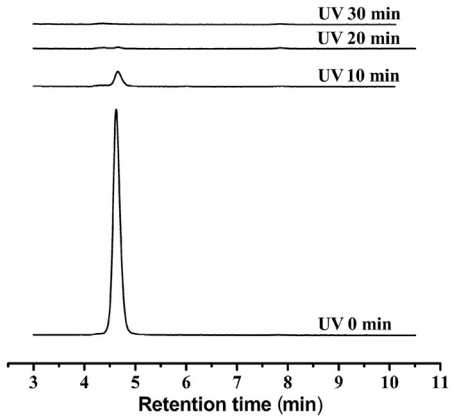

Fig. 1 – High performance liquid chromatography HPLC chromatograms of N-nitrosodiethylamine under different UV irradiation periods (0.05mM, pH 7.0).

a complete photolysis. To verify the possible errors of the PG method, the ionic photoproducts of NDEA were further analyzed by IC and their Griess reaction was also investigated.

3.2. *IC analysis of NDEA photoproducts*

Two photolysis processes of *N*-nitrosamines were suggested, including homolytic and heterolytic cleavage pathways of *N–N* bonds. The homolytic cleavage of *N–N* bonds generates an aminium radical and nitric oxide radical (NO) that may subsequently convert to nitrite, while nitrosamines may be degraded to form corresponding secondary amines and nitrite in the heterolytic cleavage pathway [15,16]. Based on Griess colorimetric reaction of liberated nitrite, the PG method was developed to determine nitrosamines. However, as shown in Fig. 2, NO_2^- was not the only photoproduced ion from NDEA. NO_3^- ions were generated along with NO_2^- ions when NDEA was exposed to UV irradiation for 5 minutes. Specifically, an increase of NO_3^- concentration was observed after 20 minutes of UV irradiation. Similar presence of NO_3^- was also observed

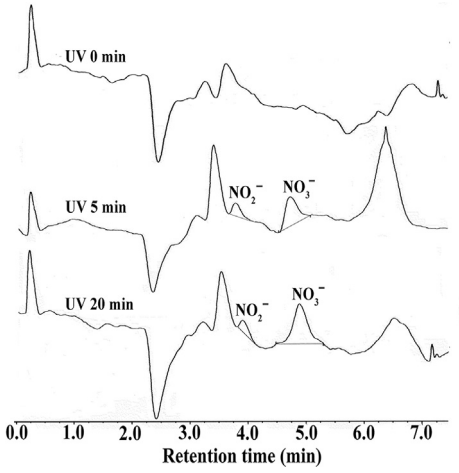

Fig. 2 – Ion chromatograms of N-nitrosodiethylamine under different UV irradiation periods (0.05mM, pH 7.0).

during the photolysis of nitrosamines, which was attributed to the oxidation of photoproduced NO_2^- [17].

The UV degradation of nitrosamines and the oxidation conversion of NO_2^- to NO_3^- depend on photolysis conditions [18]. This suggests that varying amount of NO_2^- may appear in the photoproducts of a nitrosamine solution. In that case, the determination of nitrosamine by the PG method may give a different result due to changes in photolysis conditions. As shown in Fig. 3, solution pH exhibited considerable effect on the generation of NO_2^- during the photolysis of NDEA. The NO_2^- level was remarkably low at pH 3, which was consistent with a prior study on NDEA photolysis [16]. A maximum NO_2^- level was observed at pH 7, but it was nearly half of the initial NDEA concentration, indicating that the complete degradation of NDEA (Fig. 1) did not generate equivalent moles of NO_2^-. Reports [17,19] demonstrated that the loss of NO_2^- during nitrosamine photolysis mainly resulted in the appearance of NO_3^- in the photoproducts because of oxidation, and the two ions might reach an equilibrium that varies with conditions. Therefore, the conversion of NO_2^- to NO_3^- may inevitably bring errors to the determination of NDEA by the PG method, as NO_3^- cannot be detected by the Griess reaction.

3.3. *Reaction of nitrite and nitrate with Griess reagent*

The UV–visible spectrum of nitrite–Griess product had an absorption maximum at 550 nm (Fig. 4), suggesting that the

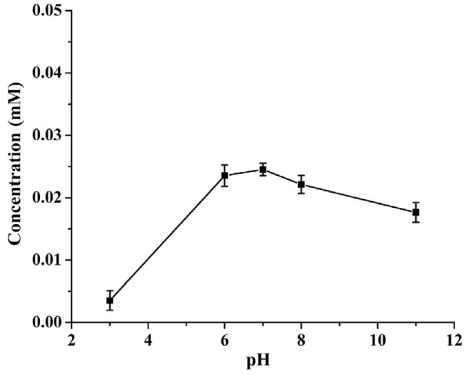

Fig. 3 – Concentrations of NO_2^- generated from the photolysis of N-nitrosodiethylamine at different pH values (0.05mM, 20 min).

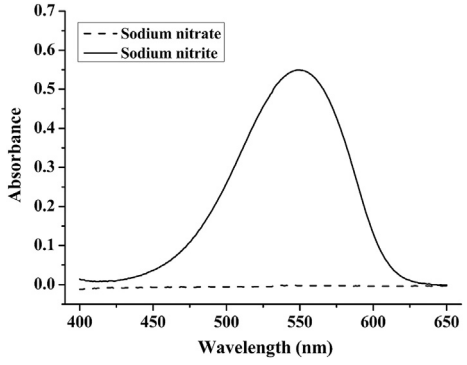

Fig. 4 – UV-visible spectra of nitrite- and nitrate-Griess products (nitrite, nitrate, 0.05mM).

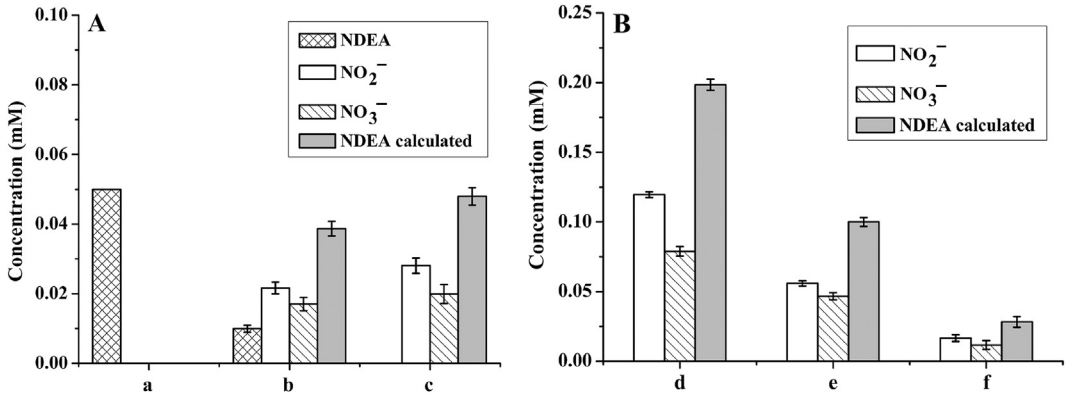

Fig. 5 – Quantification of *N*-nitrosodiethylamine (NDEA) by photolysis–ion chromatography under different UV irradiation periods or at different initial NDEA levels. (A) NDEA 0.05mM, pH 7.0, UV duration: a, 0 minutes; b, 5 minutes; c, 20 minutes. (B) pH 7.0, UV duration 20 minutes, NDEA: d, 0.2mM; e, 0.1mM, f, 0.03mM. NDEA = *N*-nitrosodiethylamine.

liberated nitrite ions from nitrosamine photolysis are detectable by the spectroscopic technique. However, unlike nitrite, nitrate gave no response after reaction with Griess reagent (Fig. 4), which indicates that NO_3^- cannot be detected by the colorimetrical Griess reaction. These observations illustrate that the conversion of NO_2^- to NO_3^-, occurring in the photolysis of nitrosamines, may result in quantification loss with the PG method. More errors may be present in the determination of nitrosamines when NO_3^- appears at higher concentration in the photoproducts. Results above suggest that a quantification method of nitrosamines, developed on the basis of their photolabile property, should take both NO_2^- and NO_3^- into account in order to reduce errors. Therefore, a combination of photolysis with IC may be an alternative for nitrosamine determination, which was further validated by using NDEA.

3.4. *Quantification of NDEA by photolysis and IC*

The method is based on the photolysis of nitrosamines to yield corresponding amine, nitrite and nitrate ions. The two types of ions are then detected by IC and the sum of both molar concentrations is calculated as that of nitrosamine before photolysis. This photolysis–IC method was validated by using NDEA, as affected by UV irradiation duration and initial NDEA concentration.

As shown in Fig. 5A, a 0.05mM NDEA solution was used for UV irradiation, which generated 0.022mM NO_2^- and 0.017mM NO_3^- in 5 minutes. The two ions amounted to a lower value (0.039mM) than the initial NDEA concentration due to a partial photolysis that had 0.011mM of NDEA residue. The NDEA solution was completely photolyzed after 20 minutes of UV irradiation (Fig. 1), and the sum (0.048 ± 0.002mM) of NO_2^- and NO_3^- concentrations was nearly equal ($p > 0.05$) to 0.05mM. HPLC analysis (data not shown) indicated that, when NDEA appeared at 0.2mM, 0.1mM and 0.03mM, it was also fully photolyzed in 20 minutes of UV irradiation. Meanwhile, the generated NO_2^- and NO_3^- amounted to 0.198 ± 0.002mM, 0.099 ± 0.001mM, and 0.028 ± 0.001mM (Fig. 5B), which are equivalent ($p > 0.05$) to the initial NDEA values, respectively. Results indicated that, on the basis of nitrosamine photolability, the developed photolysis–IC method is feasible and accurate for the determination of nitrosamines. More information about the method, including photolysis optimization and precision assay, will be discussed in a further study.

4. Conclusion

NDEA is photolabile and it showed a rapid decay under UV irradiation. Both NO_2^- and NO_3^- ions were observed in the photoproducts by using IC and their concentrations may vary with photolysis conditions such as solution pH. Unlike NO_2^-, NO_3^- was unable to be measured colorimetrically by Griess reagent. These observations demonstrated that the spectrophotometry determination of nitrosamines after PG has noticeable errors, though it is commonly adopted by food enterprises. At a few NDEA levels, the sums of concentrations of photoproduced NO_2^- and NO_3^- were found to be equivalent to those of NDEA before photolysis. Then, based on the photolability, a photolysis–IC method was suggested as a feasible and accurate method for the determination of nitrosamines.

Conflicts of interest

All authors declare no conflicts of interest.

Acknowledgments

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