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Photolysis-ion chromatographic determination of *N*-nitrosodiethylamine

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RESEARCH ARTICLE

Abstract

Photolysis-Griess reaction (PG) method, as a cost-effective spectrophotometry, is widely used in food enterprises for determination of nitrosamines such as *N*-nitrosodiethylamine (NDEA). The PG method was conducted based on the amount of photo-produced nitrite ions. Results of this research showed the possible errors of the PG method, since photolysis of NDEA not only generated nitrite, but also nitrate ions. The sum of the concentrations of the two photo-produced ions was found equivalent to the initial NDEA concentration before photolysis. Based on this finding, a photolysis-ion chromatography method was established with good precision and accuracy.

Keywords: nitrosamine, *N*-nitrosodiethylamine, photolysis, ion chromatography

1. Introduction

N-nitrosamines are known as carcinogens (De Rainho *et al.*, 2010; Hebels *et al.*, 2009); for example, *N*-nitrosodiethylamine (NDEA) is able to induce hepatic and gastrointestinal tumours (Xu *et al.*, 2010). These compounds have been of great concern due to their highly carcinogenic characteristic and widespread occurrence. *N*-nitrosamines can be produced by the reaction of nitrosating agents with secondary amines in food processing, so they may occur in a wide variety of food like cured ham, sausages, and beer (Yang *et al.*, 2013). Moreover, nitrosamines can be found in drinking water, soil and the human stomach (Stefan and Bolton, 2002). Therefore, much interest is directed toward the determination of nitrosamines that appear in the environment and diet.

High performance liquid chromatography (HPLC) and gas chromatography coupled with mass spectrometry (GC-MS) methods are frequently used for quantification of nitrosamines (Krauss and Hollender, 2008; Sannino and Bolzoni, 2013), but these methods are limited to high level of expertise and expensive equipment. *N*-nitrosamines can be rapidly degraded by UV irradiation (Xu *et al.*, 2010). Thus, this photolabile property was applied to remove nitrosamines from contaminated water (Xu *et al.*, 2008) and also for quantification. Based on the photolability of nitrosamines, a cost-effective spectrophotometry method was established. The photolysis of nitrosamines generates corresponding amine and NO_2^- (Luque-Pérez *et al.*, 2001). The liberated nitrite ion can be detected colourimetrically by the Griess reagent (Rocha *et al.*, 2009). This Photolysis-Griess (PG) method can be conveniently implemented, and has been commonly adopted for the quantification of nitrosamines by food enterprises. Additionally, the PG method was also coupled with other technologies, such as HPLC, to improve the response and sensitivity of detection (Bellec *et al.*, 1996).

The PG method is based on the concentration of photo-produced nitrite, but the latter may be photo-oxidised to nitrate, which can lead to possible errors in the determination. In this work, the possible error of the method was shown, and a photolysis-ion chromatography (IC) method for nitrosamine quantification was established to reduce these errors. This method was subsequently validated by using NDEA under various photolysis conditions. The combination of photolysis with IC, developed on the basis of the ionic photoproducts from nitrosamine, could be an accurate alternative to measure nitrosamines.

2. Materials and methods

Reagents

Working standard solutions of NDEA were prepared in deionised water and stored in dark. Solution pH was adjusted by HCl and NaOH (0.1 M) when necessary. All reagents were obtained from Changzheng Chemical Co. (Chengdu, China P.R.) and of analytical grade or better. All solutions were prepared in deionised water.

UV photolysis of *N*-nitrosodiethylamine

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

Ion chromatography of *N*-nitrosodiethylamine photoproducts

Photoproducts of NDEA after UV irradiation were analysed by a Dionex ICS-90 ion chromatograph system (Sunnyvale, CA, USA) equipped with a Dionex AS14 anion exchange column (250×4 mm). Samples were filtered through 0.45 µm filters before injection (30 µl) and the column temperature was set at 30 °C. A mixture consisting of 10 mM Na_2CO_3 and 30 mM $NaHCO_3$ was used as mobile phase at a flow rate of 1.0 ml/min.

Validation analysis of photolysis-ion chromatography

The recovery and precision tests were performed using drinking water fortified with appropriate concentrations of the NDEA standard. The recovery (Rec) value was calculated from the concentration in the fortified sample (C_1), concentration in the unfortified sample (C_2), and concentration of the fortification (C_3) as follows:

$$\text{Rec}\ (\%) = [(C_1 - C_2) / C_3] \times 100$$

The concentration of NDEA residue was measured by the method of Xu *et al.* (2010).

Statistical analysis

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

3. Results and discussion

Quantification loss of Photolysis-Griess reaction method

NDEA can be totally degraded when exposed to UV irradiation, and no reformation was observed during further irradiation (Lee *et al.*, 2013). According to the PG method, the complete photolysis of nitrosamine forms equivalent mole of nitrite that can be quantified by the colourimetrical Griess reaction. However, Figure 1 reveals that nitrate ions were generated from the photolysis of NDEA, which suggests nitrite ions were not the only photoproduct of nitrosamine. The occurrence of nitrate ions might result from oxidation of nitrite, and the two ions could reach an equilibrium (Kwon *et al.*, 2012; Lee *et al.*, 2005a). The conversion of NO_2^- to NO_3^- may inevitably bring errors to the quantification of NDEA by PG, because NO_3^- cannot be measured by the Griess reagent. Results indicated that a determination method of nitrosamines, founded on their photolabile characteristic, should take both nitrite and nitrate into account in order to reduce errors. Hence, a combination of photolysis with IC may be an alternative for nitrosamine quantification, which was further studied by using NDEA.

Quantification of *N*-nitrosodiethylamine by photolysis-ion chromatography

The photolysis of nitrosamine generated nitrite and nitrate ions, and photolysis-IC was based on the two photoproducts. The sum of NO_2^- and NO_3^- concentrations

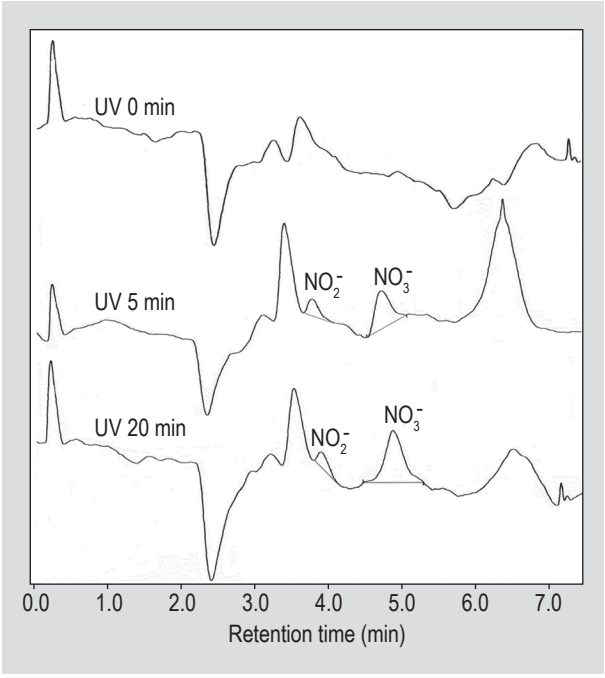

Figure 1. Ion chromatogram of *N*-nitrosodiethylamine under UV irradiation (0.05 mM, pH 7.0).

is calculated as that of nitrosamine before photolysis, which was investigated by employing NDEA under various photolysis conditions.

In Figure 2, 0.05 mM of NDEA solution was subjected to UV irradiation. Nitrate (0.022 mM) and nitrite ions (0.017 mM) were liberated from the partial photolysis of NDEA with 5 min of irradiation. Concentrations of NDEA residue (0.011 mM) and the two ions added up to 0.05 mM. The NDEA solution was completely photolysed after 20 min of UV irradiation, and the sum (0.048±0.002 mM) of NO_2^- and NO_3^- concentrations was nearly equivalent (P>0.05) to the initial NDEA value. Figure 3 illustrates concentrations of NO_2^- and NO_3^- from complete photolysis of NDEA at 0.2, 0.1 and 0.03 mM. The two ions amounted to equivalent values of the initial NDEA levels as 0.198±0.002, 0.099±0.001 and 0.028±0.001 mM, respectively.

Although yields of the two ions may vary with photolysis conditions, especially solution pH (Xu *et al.*, 2010), the sum of NO_2^- and NO_3^- concentrations was still equivalent to initial NDEA level. As shown in Figure 4, nitrite level from photolysis of NDEA at pH 7 reached the maximum, while the level was considerably low at pH 3, which was exactly opposite to the trend of NO_3^- concentrations. Solution pH could change yields of the two ions by influencing conversion of NO_2^- to NO_3^- (Lee *et al.*, 2005b). This suggests that PG method founded on the liberated NO_2^-, may give different results for nitrosamine determination at various photolysis pH. The developed photolysis-IC may be a feasible alternative, because the quantification equation that the photo-produced ions concentrations added up to the equivalent concentration of NDEA before photolysis was also available (Figure 4). Therefore, when the photolysis-IC is applied to food with pH ranging from acidity to alkaline, it can be precise for nitrosamine quantification.

Validation of photolysis-ion chromatography

The accuracy of the photolysis-IC was assessed by recovery tests. Tested samples were spiked with 0.02, 0.5 and 0.7 mM of NDEA standard. Table 1 lists that the recoveries were 89.5, 97.2 and 95.6%, respectively. The SD and relative standard deviation (RSD) values were low; not more than 0.017 and 2.79%, respectively. Compared with PG-HPLC and GC-mass selective detector methods for determination of NDEA (Lee *et al.*, 2013; Yurchenko and Mölder, 2007), the photolysis-IC showed higher accuracy.

For the precision experiment, the intra-day and inter-day RSD values for peak areas of nitrite and nitrate ions were tested. As shown in Table 2, the intra-day RSD value for nitrite ion was 1.41%, and the value for nitrate ions was 2.15%, indicating high repeatability for the photolysis-IC method. The method also exhibited acceptable

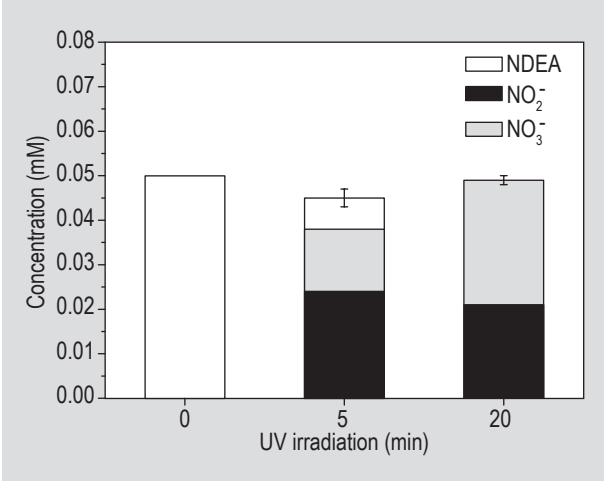

Figure 2. Quantification of *N*-nitrosodiethylamine (NDEA) by photolysis-ion chromatography under different UV irradiation periods (NDEA 0.05 mM, pH 7.0).

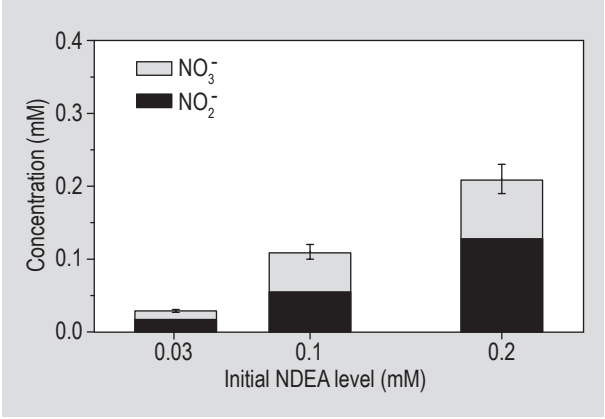

Figure 3. Quantification of *N*-nitrosodiethylamine (NDEA) by photolysis-ion chromatography at different initial NDEA levels (pH 7.0, UV duration = 20 min).

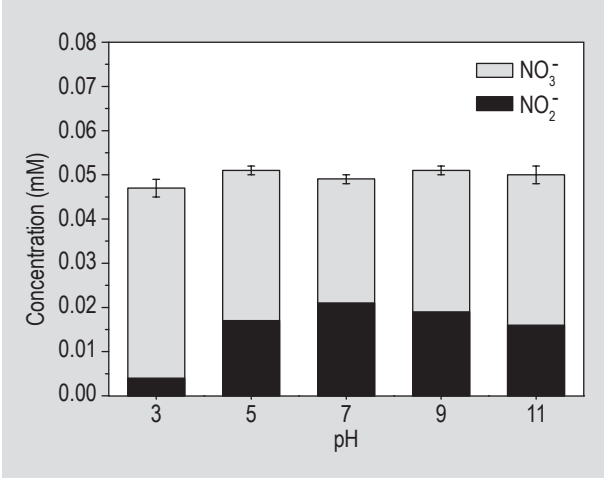

Figure 4. Quantification of *N*-nitrosodiethylamine (NDEA) by photolysis-ion chromatography at different pH (NDEA 0.05 mM, UV duration = 20 min).

Table 1. Validation data of *N*-nitrosodiethylamine spiked at three levels (n=5).[1]

	Rec (%)	SD	RSD (%)
Level I (0.02 mM)	89.5	0.005	2.79
Level II (0.5 mM)	97.2	0.011	2.26
Level III (0.7 mM)	95.6	0.017	2.54

[1] Rec = recovery; SD = standard deviation; RSD = relative standard deviation

Table 2. Precision assay (n=3) for peak areas of nitrite and nitrate ions.[1]

	Intra-day RSD (%)	Inter-day RSD (%)
Nitrite	1.41	2.68
Nitrate	2.15	3.02

1 RSD = relative standard deviation

reproducibility, as the inter-day RSD values were 2.68 and 3.02% for nitrite and nitrate ions, respectively. The photolysis-IC may serve as an accurate method for the determination of nitrosamines in food industry.

4. Conclusions

In this study, both nitrite and nitrate ions were observed in the photoproducts of NDEA by using ion chromatography. But the generation of nitrate ion may result in noticeable errors for the determination by the PG method that is commonly adopted in food industries. A photolysis-IC method based on the photolability of nitrosamines was established, and the sum of concentrations of photo-produced nitrite and nitrate ions was found equivalent to those of NDEA before photolysis. In addition, the method was validated to be an alternative with a small error for nitrosamine determination.

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