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Note

Positive interferant in the chemiluminescent detection of nitrosamines

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Chemiluminescent detectors for the analysis of nitrogen oxides in the atmosphere have been available for a number of years. The interaction of nitric oxide and ozone results in a chemiluminescent emission which is detected with a photomultiplier tube. More recently this principle has been applied to the detection of N-nitrosamines after catalytic cleavage of the N–NO bond to produce nitric oxide[1]. These detectors incorporate an optical filter to eliminate emissions occurring below 600 nm, such as those between ethylenic compounds and ozone. Further selectivity is introduced by combining the detector with a gas chromatograph[2,3], so that an additional criterion for the presence of a nitrosamine is retention coincidence with an authentic nitrosamine. Finally, a cold trap situated between the catalyst and the nitric oxide–ozone reaction chamber will remove all but the most volatile compounds eluting from the gas chromatograph.

Chemiluminescent detectors thus provide substantially stronger evidence for the presence of an N-nitrosamine than detectors which are only nitrogen- rather than NO-selective. For the analysis of foodstuffs and other commodities in which nitrosamines may occur, the Coulson and alkali flame ionisation detector have been used for some years[4,5], but apart from their value in simple and model systems with relatively few components, they are only of value for screening prior to confirmation of the presence of nitrosamines by combined gas chromatography and mass spectrometry (GC–MS)[6]. The advent of chemiluminescent detectors has vastly improved the situation and, although it is recommended that results obtained by chemiluminescence should still be confirmed by MS, it was anticipated that the proportion of false positive results would be far fewer than those obtained using nitrogen-selective detectors. In a survey in which a large number of foods were examined the reliability of two chemiluminescent detectors, one commercially available and the other laboratory built, was assessed. Neither detector gave rise to false positive results at the retention time of the nitrosamines under study[3,7]. It has, however, been observed by Stephany and Schuller[8] that the chemiluminescent detector will give a response to some C-nitroso compounds. Strong responses were observed for some simple aliphatic C-nitroso compounds, although none were observed for some aromatic compounds listed. Even in those instances where a response was obtained, there was no coincidence of retention time with any known N-nitroso compound. We now report the occurrence of such an event.

EXPERIMENTAL

Samples for analysis were obtained from simple aqueous solutions in which strongly basic anion-exchange resins had been immersed. 250 ml of distilled water tap (containing less than 0.005 μg/l of N-nitrosocompounds) was added to 50 g of resin type Deacidite FF. After soaking for 24 h the water was decanted and the organic constituents which had been leached from the resin were extracted into dichloromethane, which was then dried over sodium sulphate. The dried extract was evaporated to 2.5 ml at 45°, 800 μl of hexane added and evaporation continued to 250 μl. 5-μl aliquots of these solutions were examined by GC with chemiluminescent and MS detection.

A Pye gas chromatograph was coupled to a chemiluminescent detector model TEA 502. The chromatograph was fitted either with a polar or a non-polar column. The polar column was 5% Carbowax 20M on Diatomite C AW DMCS and the non-polar column 5% Apiezon L on a similar support. Both columns were 4 m × 1.8 mm I.D. stainless-steel and used a carrier gas flow-rate of 11 ml/min. The column and transfer line between the chromatograph and the chemiluminescent detector were operated at 150°, the chemiluminescent detector furnace at 400°, and cold trap at −132° (n-pentane and liquid nitrogen) and the reaction chamber at a pressure of 1 torr. A modification to the chemiluminescent detector to prevent contamination of the reaction chamber has been described elsewhere[9].

A second Pye 104 chromatograph was interfaced to an AEI MS902 mass spectrometer. The column system was identical to that previously described[10] and consisted of a short 15% Carbowax 20M column followed by a Carbowax support coated open tubular column. Solvent venting facilities using micro volume switching valves were also fitted. The Apiezon L column used in conjunction with the chemiluminescent detector was also interfaced to the mass spectrometer for some of the work.

The extracts were examined for nitrosamines firstly using the chemiluminescent detector with the polar column, and identifying nitrosamine content on retention coincidence with known nitrosamines. Quantitation to a precision of ±3% was by peak area measurement after detector calibration with standard solutions of the appropriate nitrosamines. Each sample was re-examined by GC–MS to confirm the presence and amount of any nitrosamine detected by chemiluminescence. Nitrosamines were detected by parent ion monitoring using peak matching under high resolution[11], and the amounts were calculated from peak height measurements after calibration with standard solutions. The precision of these measurements is ±10%.

Unknown compounds also present in the extracts were identified on the basis of their low resolution spectra, which were generated at the appropriate retention times. Confirmation of identity was obtained by mass measurement of selected fragments at a resolution of 10,000 (10% valley).

RESULTS AND DISCUSSION

In a previous communication[12], in which nitrosamines were detected in de-ionised water, it was noted that in the extracts derived from one type of resin, the chemiluminescent detector gave a response at the retention time of N-nitrosodi-n-

propylamine (NDPA). An examination of these extracts by GC–MS established that NDPA was not present in the extracts. These observations of apparent NDPA only occurred in the case of a strongly basic resin and provide the first examples of discrepancies between the chemiluminescent detector and mass spectrometer. In order to establish the identity of the compound giving rise to the chemiluminescent response, further extracts of this type of resin have been prepared and examined by this detector and the mass spectrometer.

Using a Carbowax 20M GC column which is widely employed for the separation of nitrosamines, the presence of NDPA but no other nitrosamine was indicated by chemiluminescence. The samples were then examined by MS and found to be free from nitrosamines although the amount of NDPA indicated by chemiluminescence was an order of magnitude higher than the detection limit of the mass spectrometer. One such extract was divided into two and to one portion was added authentic N-nitrosodiethylamine (NDEA) and NDPA, each at the 5-mg/l level. This sample was examined by chemiluminescence, when NDEA and an enhanced value for NDPA were observed. MS confirmed the presence of both of these nitrosamines at the 5-mg/l level. It was therefore apparent that the original extract did not in fact contain NDPA despite the response of the chemiluminescence detector.

In order to resolve this interferant from authentic NDPA, an Apiezon L column was substituted for the Carbowax column. Under these conditions the chemiluminescent detector gave no response to the unfortified extract at the retention time of NDPA, confirming its absence. However, a response was obtained which matched exactly the retention time of NDEA on this column, and was equivalent to 4 mg/l. The sample was re-examined by MS using the Apiezon column and no response was obtained from either nitrosamine. The other half of the extract, with added NDEA and NDPA when examined by chemiluminescence, gave an erroneously high value for NDEA and the correct value for NDPA. MS gave the correct values for both nitrosamines. These observations are summarised in Table I. In order to establish that all the chemiluminescing species were generated in the chemiluminescent detector furnace, the furnace was cooled to 30° and the extracts re-examined. No response was obtained. Nitrosamines are decomposed by ultraviolet light, and the extracts were irradiated for 6 h after the method described by Doerr and Fiddler[13]. On re-

TABLE I

EFFECT OF CHEMILUMINESCING INTERFERANT ON RESULTS

NDEA = N-nitrosodiethylamine, NDPA = N-nitrosodipropylamine, N.D. = not detected. MS = mass spectrometer, CL = chemiluminescent detector.

Nitrosamine	Unspiked extract nitrosamine concn. (mg/l)		Spiked extract nitrosamine concn. (mg/l)	
	CL	MS	CL	MS
Carbowax 20M				
NDEA	N.D.	N.D.	5	5
NDPA	11	N.D.	16	5
Apiezon L				
NDEA	4	N.D.	9	5
NDPA	N.D.	N.D.	5	5

examining the extracts there was no trace of the authentic nitrosamines, but the interfering compound was still present at the same level, confirming that it was not a nitrosamine.

An investigation to establish the identity of the interferant was carried out using GC–MS. Low resolution spectra were run at the appropriate retention time, and the highest m/e values were 107 and 135. From the high resolution mass measurement of m/e 107, the empirical formula C_7H_9N was assigned. The low resolution spectrum was consistent with the fragmentation of the benzylamine moiety; m/e 135 corresponds to N-dimethylbenzylamine. This is consistent with the fact that the ion-exchange resin from which the extracts were derived was prepared from a benzyl-trialkyl ammonium salt, although the exact composition of the resin was unknown. Quaternary ammonium salts themselves will not pass through a gas chromatograph, and the chemiluminescent response is therefore the result of the pyrolysis in the chemiluminescent detector furnace, of a compound which, itself, is a breakdown product of the salt.

CONCLUSIONS

Chemiluminescence offers a highly selective method for detecting N-nitroso-compounds. However, it is possible, although unusual, to observe a response arising from other compounds at retention times corresponding to volatile aliphatic nitrosamines. In the present work this occurred using an extract from a relatively simple system. It is therefore desirable to confirm by MS the presence of nitrosamines detected by chemiluminescence, at least for materials which have not been extensively studied.

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REFERENCES

1 D. H. Fine, D. Lieb and F. Rufeh, *J. Chromatogr.*, 107 (1975) 351.
2 D. H. Fine and D. P. Rounbehler, *J. Chromatogr.*, 109 (1975) 271.
3 T. A. Gough, K. S. Webb and R. F. Eaton, *J. Chromatogr.*, 137 (1977) 293.
4 J. F. Palframan, J. MacNab and N. T. Crosby, *J. Chromatogr.*, 76 (1973) 307.
5 T. Fazio, J. N. Damico, J. W. Howard, R. H. White and J. O. Watts, *J. Agr. Food Chem.*, 19 (1971) 250.
6 K. Goodhead and T. A. Gough, *Food Cosmet. Toxicol.*, 13 (1975) 307.
7 T. A. Gough, K. S. Webb, M. A. Pringuer and B. J. Wood, *J. Agr. Food Chem.*, 25 (1977) 663.
8 R. W. Stephany and P. L. Schuller, in B. J. Tinbergen and B. Krol (Editors), *Proceedings of 2nd International Symposium on Nitrite in Meat Products, 7–10 September 1976, Zeist*, Pudoc, Wageningen, 1977, p. 249.
9 T. A. Gough, *Environmental Aspects of N-nitrosocompounds*, International Agency for Research on Cancer, Scientific Publications No. 19, 1978.
10 T. A. Gough and K. Sugden, *J. Chromatogr.*, 109 (1975) 265.
11 T. A. Gough and K. S. Webb, *J. Chromatogr.*, 64 (1972) 201.
12 T. A. Gough, K. S. Webb and M. F. McPhail, *Food Cosmet. Toxicol.*, 15 (1977) 437.
13 R. C. Doerr and W. Fiddler, *J. Chromatogr.*, 140 (1977) 284.