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Note

Effect of ethanol on the determination of N-nitrosodimethylamine using chemiluminescent detection

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It is widely accepted that the only unambiguous method for the estimation and identification of N-nitrosamines in complex mixtures involves confirmation by combined gas chromatography (GC) and high-resolution mass spectrometry (MS). However, much work has been devoted to the development of reliable and reasonably specific screening techniques to be used prior to GC–MS confirmation[1]. The most satisfactory technique is that based on the catalytic denitrosation of N-nitrosamines and subsequent measurement of the chemiluminescence of the reaction between nitric oxide and ozone[2,3]. This technique shows high specificity and results are in excellent agreement with those obtained by MS[4]. There have, however, been occasional reports of false positive results[5,6]. The enhanced and erratic response of a chemiluminescent detector to solutions of N-nitrosodimethylamine (NDMA) in hexane–ethanol mixtures is described here.

EXPERIMENTAL

Standard solutions of NDMA (1 and 10 mg/l) in *n*-hexane and ethanol mixtures covering the range 0–50% ethanol were prepared. The *n*-hexane was at least 99% pure and the ethanol was PBS grade. All glassware was scrupulously cleaned prior to use, including treatment with hydrobromic acid and glacial acetic acid. The solvents were free from nitrosamines above the detection limit of 1 μg/l, and gave rise to no chemiluminescent responses other than the normally observed response immediately on injection. The standard solutions were examined immediately on preparation, by GC with flame ionisation, chemiluminescent and MS detection.

All chromatography was carried out on Pye 104 and Varian 1200 instruments with Carbowax 20M columns. For flame-ionisation and chemiluminescent detection these were 4 m × 1.8 mm I.D. stainless steel with 5% Carbowax 20M on Diatomite CLQ. The column temperature was 150° and carrier gas flow-rate 11 ml/min of argon. For MS the column system consisted of a 15% Carbowax packed column in series with a support-coated open tubular column containing the same stationary phase[7]. The flame-ionisation detector (Varian) and one chemiluminescent detector (Thermo Electron Corp.) were standard commercial instruments, although the latter was modified as previously described[8]. A second chemiluminescent detector was built in

the laboratory[3], and the mass spectrometer was an AEI high-resolution instrument type MS 902, used in the parent ion peak matching mode[9].

The chemiluminescent detectors were calibrated using dilute gas samples of nitric oxide in argon, from which the anticipated response toward NDMA was calculated. Previous work[3] was repeated to demonstrate that quantitative cleavage of nitric oxide from aliphatic nitrosamines was taking place and that there had been no deterioration of the catalyst. A cold trap at −150° was placed between the catalyst chamber and the detector chamber of each detector to remove the many volatile constituents eluting from the gas chromatograph. It is possible that some of these, or their pyrolysis products, may induce chemiluminescence. For example, ethylenic compounds interact with ozone and emit over the range 400–700 nm. Further elimination of extraneous chemiluminescence was afforded by an optical filter which only passes wavelengths over 610 nm. In practice the detectable range of emissions is between 610 and 900 nm, the upper limit being governed by the photomultiplier.

RESULTS AND DISCUSSION

An examination of the NDMA–hexane solutions using both chemiluminescent detectors gave rise to the expected response. However, the detector responses to the various NDMA solutions also containing ethanol were unexpectedly high by as much as one order of magnitude. An example of this effect is shown on Fig. 1 for the 1-mg/l NDMA solutions. For solutions containing less than 10% ethanol results were so erratic that they have been excluded from the figure. Aliquots of the same samples were concurrently examined by MS and gave the expected value, irrespective of the ethanol content. The possibility that a chemiluminescent species coeluting with NDMA and causing the enhanced response is unlikely, as on re-examining the samples using the flame-ionisation detector the correct quantitative values for NDMA were obtained. It was, however, noted that although the solvents were both eluted prior to NDMA, excessive tailing of the ethanol resulted in partial overlap with the NDMA. It is well known that during partition of polar compounds within a column some loss by adsorption on to active sites of the stationary phase can occur. This can result in the displacement of a previously adsorbed but less polar compound. The possibility that ethanol might be displacing NDMA which had been adsorbed from a previous injection was ruled out since Carbowax 20M was also used for the MS determinations and no enhancement was observed. Similarly the possibility of nitric oxide displacement from the catalysts of the chemiluminescent detectors is unlikely. Both detectors have been in use for several months and have been subjected to a large number of widely different samples. It is unlikely that they will therefore exhibit exactly the same erroneous response to the alcoholic standards. Regeneration of the catalysts by the passage of oxygen at 500° overnight and subsequent re-examination of the samples gave the same results.

A study was made of the effects of catalyst temperature on the NDMA results using the commercial detector. Fresh 1- and 10-mg/l solutions of NDMA were prepared, one pair with only *n*-hexane and the other with *n*-hexane and 10% ethanol. The catalyst temperature was varied from 280° to 475°, and the results are shown on Fig. 2. As expected, at the low temperature the values observed for the concentration of NDMA in hexane were less than the true value, as a result of incomplete

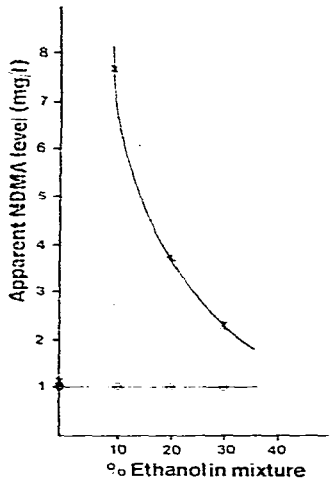

Fig. 1. Effect of the proportion of ethanol on the apparent N-nitrosodimethylamine level in 1-mg/l solutions in hexane–ethanol mixtures. X = chemiluminescent data, O = MS data.

conversion to nitric oxide. Above 430° a constant and correct value for the NDMA concentration was observed. In the case of the standard containing 10% ethanol, the apparent concentration of NDMA rose rapidly with increasing temperature to a maximum in the region of 420°, representing up to nine times the true level. The observed level rapidly fell thereafter, but results over the whole range were erratic.

In summary, chemiluminescent detectors give an enhanced response to NDMA in hexane–ethanol mixtures, which decreases as the proportion of alcohol is increased.

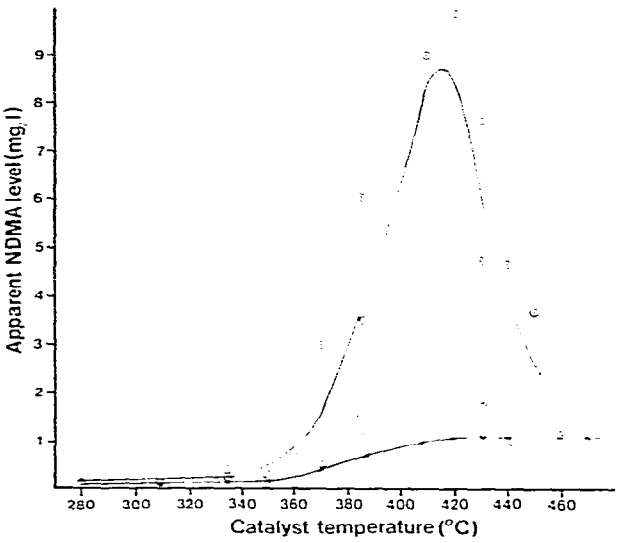

Fig. 2. Effect of catalyst temperature on apparent N-nitrosodimethylamine level in 1-mg/l standard solutions, using a chemiluminescent detector. X = 1 mg/l NDMA in hexane, O = 1 mg/l NDMA in hexane–ethanol (90:10) mixture.

Such an observation cannot be explained on the basis of impurities in either solvent unless further chemical reactions are involved. Hexane and hexane–ethanol mixtures in the absence of NDMA do not give a response, and the presence of all three constituents is essential for the erroneous response to be observed. It must be concluded that the alcohol (or an impurity therein) acts as a catalyst to the interaction of NDMA (or one of its pyrolysis products) with hexane (or an impurity). Increasing the proportion of alcohol in the mixture decreases the amount of material available to interact with NDMA, and hence a lower erroneous response is obtained. The change in erroneous response with catalyst temperature is a function of the amount of reactant generated in the chamber. Further pyrolysis at higher temperatures decreases the amount of reactant available. The reaction product is sufficiently volatile to survive the cold trap and either gives rise to chemiluminescence itself, or improves the efficiency of the $NO–O_3$ reaction, larger quantities causing quenching. This reaction product may effect the energetics of reaction so that the excited NO_2^* is in a different (higher) energy state and on decomposition to the ground state emits light of a lower wavelength. Sensitivity of the photomultiplier tube changes greatly even over a small wavelength shift[10] which would thus result in an apparent increase in the amount of NDMA detected.

CONCLUSIONS

Whilst the above observations do not enable us to establish conclusively the cause of enhancement of response, they do illustrate the need for caution when interpreting results derived from chemiluminescent measurements. Claims that preliminary clean-up of samples for nitrosamine analysis may be dispensed with must be regarded with caution, particularly when dealing with alcohol-based commodities. If the enhanced effect could be controlled, these observations may be employed as a means of improving the sensitivity of chemiluminescent detectors.

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