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Note

Suppression of chemiluminescent detector response toward nitrosamines

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INTRODUCTION

Chemiluminescent detectors for nitrosamine analysis have been available for about three years[1,2] and during this time ample evidence for their reliability and specificity has accumulated[2-4]. There have, however, been a few reports of response of chemiluminescent detectors to compounds other than N-nitrosamines. Whilst not detracting from the value of these detectors, these examples[5-7] do serve as a reminder that confirmation of the presence of nitrosamines by other methods, usually mass spectrometry, should also be made. We now report the occurrence of a potentially more serious form of interference in chemiluminescent detection, which can result not in an enhancement of response but a suppression. This could give rise in an extreme case to a false negative result.

Discussion

Since nitrosamines are formed by the interaction of amines and nitrite, it is frequently necessary to analyse samples for nitrosamines which are rich in amines. A consideration of the various stages involved in chemiluminescent detection reveals that the presence of an amine could interfere with the detection of any nitrosamine present. Chemiluminescent detectors are normally used in conjunction with a gas chromatograph (Fig. 1) or, less frequently, with a high-performance liquid chromatograph. Material eluted from the chromatographic column (A) passes into a furnace containing a catalyst (B), typically at 400–600°, in which the –NO moiety is cleaved from the nitrosamine. This passes, as nitric oxide, via a cold trap to remove most extraneous compounds, into a reaction chamber through which an oxygen–ozone mixture is passing. Interaction of nitric oxide and ozone results ultimately in chemiluminescence which is detected with a photomultiplier tube. Cleavage of the nitrosamine can be quantitative. The detector response is a function of the proportion of NO in the nitrosamine and is linearly related to nitrosamine concentration over a wide range. Consider the effect of an amine and nitrosamine simultaneously passing through the catalyst chamber. It is possible that nitric oxide generated from the nitrosamine in the chamber could itself interact with the amine on leaving the chamber to form another nitrosamine. There is then no further opportunity for this nitrosamine to react with ozone to give rise to nitric oxide, and hence no chemiluminescence. If the recombination is complete no response at all will be
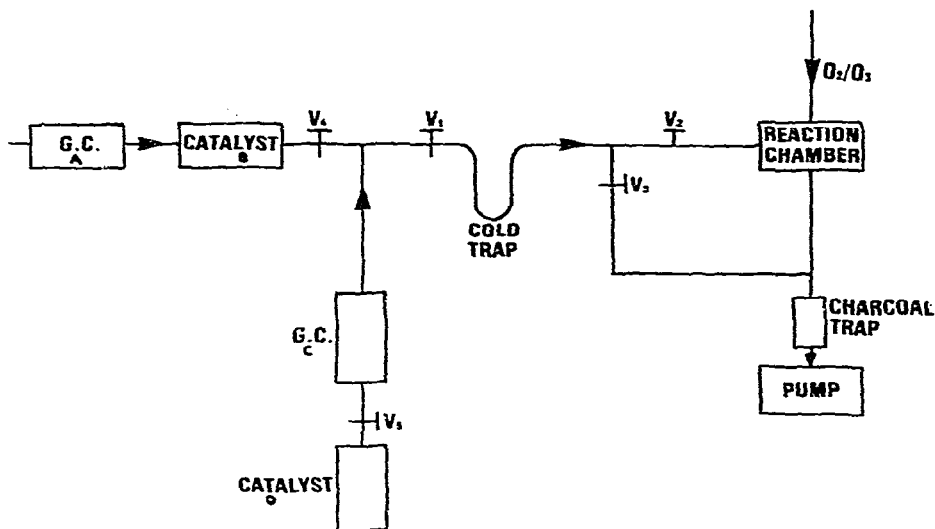
Fig. 1. Modified TEA system.

observed: if only a partial reaction occurs a diminished response will be seen. Experiments have been carried out to test the validity of this argument, using simple nitrosamine and amine mixtures.

EXPERIMENTAL

The chemiluminescent detector was a Thermal Energy Analyser (TEA) Model 502 (Thermo Electron Corporation), with facilities for injection of samples via a chromatograph (A) or directly into a catalyst (D) which was at 450°. The gas chromatograph (C) is not part of the commercial detector, and neither are the valves V_1 to V_4 whose function has been described elsewhere[8]. The gas chromatograph was operated under the conditions previously prescribed for nitrosamine analysis[5]. It was also fitted with a flame ionisation detector to determine the retention times of the nitrosamine and amine.

In the commercial instrument, direct sample introduction into the catalyst suffers from a number of disadvantages, making quantitative detection of nitrosamines unreliable. The injection of any solution into the chamber causes an immediate detector response as a result of pressure changes in the system and, for example, trace ethylenic compounds in solvents. The procedure affords no means of distinguishing these effects from a response to a nitrosamine which is itself dependent on the rate of sample injection and the residence time in the catalyst. Direct injection is thus of little value in this form. However, by incorporating a gas chromatograph (C) between the catalyst (D) exit port and the reaction chamber separation of these effects from nitric oxide response is achieved. This also forms the basis of a procedure for determining the total nitroso content of a sample (to be published elsewhere) as well as illustrating the effect of amine suppression. The transfer line from the catalyst to the reaction chamber was disconnected and a 1.5 m × 4 mm I.D. glass column containing Porapak Q connected to the catalyst chamber exit port. The column was

operated at 20° with an argon flow-rate of 15 ml/min and the effluent taken to the reaction chamber. In order to determine the retention time of nitric oxide derived from the nitrosamine and to calibrate the detector with respect to nitric oxide, dilute mixtures of the latter in argon (1–10 μl/l) were prepared. Under these conditions, nitric oxide had a retention time of 9 min. Trowell[9] has reported the interaction of nitrogen dioxide with porous polymer bead columns, but comparison of calculated and observed responses in the present work gave no evidence for such interactions involving nitric oxide.

To demonstrate changes in response of the TEA to a given amount of a nitrosamine when an amine is present, two solutions were prepared. One contained 10 mg/l of N-nitrosodimethylamine (NDMA) in hexane, and the other 10 mg/l NDMA and 100 mg/l of dimethylamine in hexane. 5-μl aliquots of these solutions were injected into the chromatograph (A) and directly into the catalyst (D) connected to the second chromatograph (C). In the former case repetitive injections at fixed time intervals were made to ensure the co-incident presence of NDMA and dimethylamine in the TEA.

RESULTS

Polar gas chromatographic columns are the most satisfactory for the separation of most volatile nitrosamines, but they give rise to excessive tailing of the corresponding amines. Under the operating conditions described, NDMA had a retention time of 10 min and dimethylamine of 8 min. Tailing of the amine peak extended over several minutes, resulting in the simultaneous elution of some dimethylamine and the NDMA. They were therefore both present in the chemiluminescent apparatus at the same time.
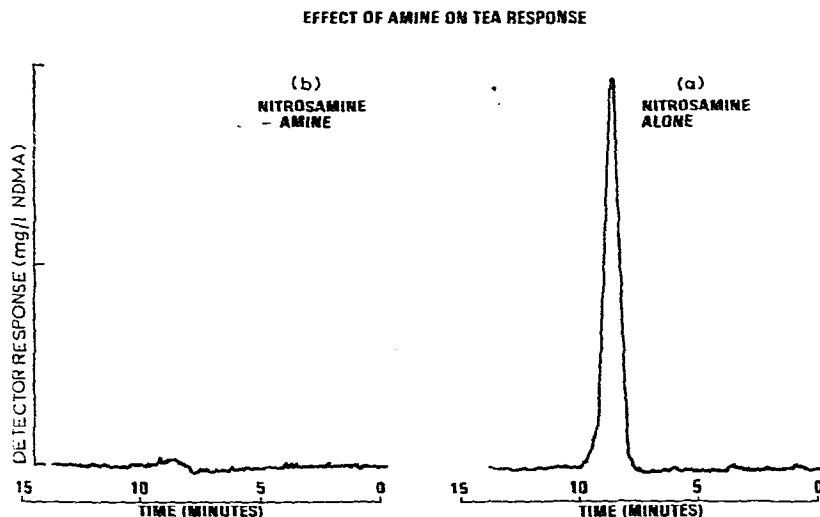

Fig. 2. Effect of amine on TEA response. Samples introduced directly into TEA fitted with post-catalyst column. (a) Response to a 5-μl aliquot of a 10 mg/l solution of NDMA in hexane; (b) response to a 5-μl aliquot of a solution containing 10 mg/l NDMA and 100 mg/l dimethylamine in hexane.

The solution containing NDMA alone gave rise to the same response whether introduced via the chromatograph or directly. Injection of nitric oxide gas confirmed that cleavage was quantitative. On repeated injection of the nitrosamine and amine mixture into the chromatograph, results were variable and it was not possible to obtain the true value for the NDMA concentration in the mixture. Suppression occurred even with the first injection and variable results were still being obtained after 15 replicate injections. Regeneration of the catalyst in oxygen overnight did not give any improvement. The direct injection offers more favourable conditions for recombination of nitric oxide with the amine, in that they are present simultaneously in the catalyst chamber. On injecting the nitrosamine and amine mixture directly, very small and variable responses were obtained and in some instances no nitrosamine at all was detected (Fig. 2).

Consideration was given to trapping the effluent from the catalyst chamber and re-injecting the contents of the trap into the catalyst chamber. This would not, however, provide any further evidence to support the explanation of response suppression. The excess unchanged amine and the reformed nitrosamine would both be present in the trap and re-injecting such a mixture would only repeat the effect.

CONCLUSIONS

It is concluded that the presence of amines in solutions under analysis for nitrosamines can cause partial or complete suppression of detector response toward nitrosamines. It is therefore desirable to destroy potentially interfering amines in mixtures prior to analysis by chemiluminescence.

ACKNOWLEDGEMENT

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