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TRACE ANALYSIS OF VOLATILE N-NITROSO COMPOUNDS BY COMBINED GAS CHROMATOGRAPHY AND THERMAL ENERGY ANALYSIS

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SUMMARY

Thermal energy analysis (TEA) has been combined with gas chromatography (GC). The new GC–TEA technique is highly specific to compounds which contain heat labile nitrosyl groups. Because of the specificity of the technique, full use may be made of the TEA sensitivity. Analysis by direct injection of solutions containing less than 1 ng/ml N-nitroso compound is demonstrated.

INTRODUCTION

Quantitative and selective gas chromatographic (GC) analysis procedures for volatile nitrosamines at the sub-μg/ml level, using either the alkali flame ionization detector or the Coulson electrolytic conductivity detector have been described[1–3]. When combined with adequate clean-up techniques, including concentration by factors of over 1000, these procedures can be used for μg/kg analysis of certain nitrosamines in foodstuffs. Elaborate clean-up and concentration procedures, apart from being tedious and costly, greatly limit the number of compounds which may be screened. In addition, it is never certain whether the nitrosamine concentrations which are found are due to artifacts of the clean-up procedures themselves. For these reasons, an N-nitroso compound specific, ultra sensitive GC detector would be of major value in evaluating the importance to human cancer of N-nitroso compounds in the environment.

Thermal energy analysis (TEA) has been shown[4–6] to be selective to N-nitroso compounds at the sub-ng/ml level. This paper describes the interfacing of a thermal energy analyzer with a gas chromatograph, the combined GC–TEA system retaining all the sensitivity and selectivity characteristics inherent in both GC and TEA.

APPARATUS

The principle of operation[6] and the detailed design parameters[7,8] of the TEA detector are described elsewhere.

The GC–TEA interface is shown schematically in Fig. 1. The GC effluent is introduced directly into the TEA catalytic pyrolyzer. In order to prevent condensa-

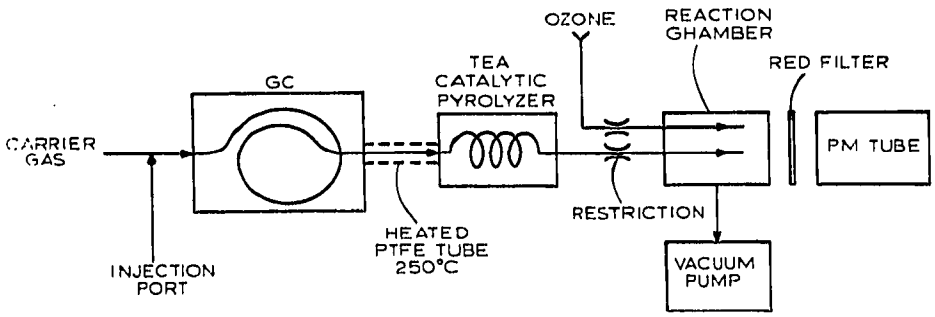
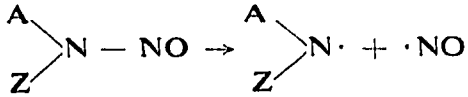


Fig. 1. Scheme of GC–TEA interface.

i on or adsorption of non-volatile components, it is preferable to have the TEA pyrolyzer joined directly to the GC column. If this is not possible, a heated PTFE line maintained above 200° may be used. Stainless-steel interconnecting tubing is not suitable unless the inside walls have been chemically polished so as to minimize sites for radical adsorption. Using a selective catalyst, the TEA pyrolyzer selectively pyrolyzes N-nitroso compounds, splitting off the nitrosyl radical:



where A and Z may be any organic radical. After the TEA pyrolyzer, the effluent is expanded through a narrow constriction into an evacuated reaction chamber, where the nitrosyl radical is allowed to react with ozone, giving electronically excited nitrogen dioxide:

$$\cdot NO + O_3 \rightarrow NO_2^* + O_2$$

The excited nitrogen dioxide rapidly relaxes to its ground state with the emission of light in the near infrared region of the spectrum:

$$NO_2^* \rightarrow NO_2 + h\nu$$

The emitted light is monitored by means of an infrared sensitive photomultiplier tube, the intensity of the emission being proportional to the number of nitrosyl radicals present.

Following Palframan[2] *et al.*, a chromatographic column was prepared from 6.5 m × 2 mm I.D. stainless-steel tube packed with 15% free fatty acid phase (FFAP) (15 g FFAP on 100 g Chromosorb W AW DMCS, 80–100 mesh) and conditioned for 36 h at 250° with carrier gas flowing prior to use. The column was operated isothermally at 185°, with a carrier gas flow-rate in the range of 10–30 ml/min. Commercial grade argon or helium was used as carrier gas with equal success. Although the gas bottles do contain traces of nitric oxide (at ppb* levels), the TEA response due to the

* Throughout this article the American billion (10^9) is meant.

nitric oxide in the carrier gas is readily accounted for by adjusting the instrument zero. Purification of the carrier gas was not required, even for direct sensitivity at the sub-ng/ml level.

EXPERIMENTAL

Mixtures containing seven N-nitroso compounds, *viz.*, dimethyl nitrosamine (DMN), diethyl nitrosamine (DEN), dipropyl nitrosamine (DPN), dibutyl nitrosamine (DBN), N-nitroso piperidine (PIP), N-nitrosopyrrolidine (PYRN), and N-nitroso sarcosinate (SARCOSN), in dichloromethane (DCM) solvent were made up gravimetrically. Confirmation of the structure of all the N-nitroso compounds used was carried out by GC–mass spectrometry in the laboratories which supplied the chemicals. The solvents were used as received without further purification. In addition, for ease of comparing the data presented here with other techniques used in other laboratories, dilute solutions were made up from a standard mixture distributed for collaborative purposes by the International Agency for Research in Cancer (IARC) of the World Health Organization in Lyon, France. The IARC mixture contained 23.9 μg DMN/ml, 25.3 μg DEN/ml, 29.0 μg DBN/ml and 30.0 μg PYRN/ml in DCM.

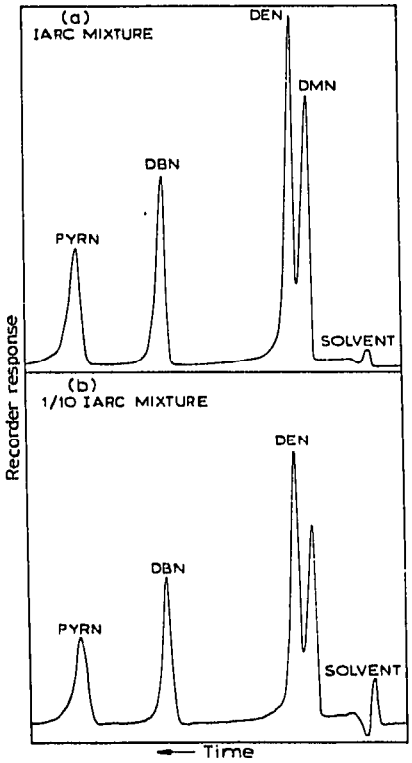
RESULTS AND DISCUSSION

Linearity

A chromatogram of 10 μl of the IARC standard solution is shown in Fig. 2a. Figs. 2b and 3a are chromatograms of 10 μl of DCM solutions obtained by 10-fold and 100-fold dilution of the IARC standard solutions. The chromatogram of 100 μl of a 1000-fold dilution of the IARC standard is shown in Fig. 3b, and a chromatogram of 500 μl of a 10,000-fold dilution is shown in Fig. 3c. Except for DMN and DEN in the 500 μl injection of the 10,000-fold dilution, all the chromatograms are clearly discernible. Even for the 10,000-fold dilution, in which each N-nitroso compound is present at less than the 3-ng/ml concentration level, the DBN and PYRN GC peaks are clearly visible above the noise level. A calibration plot for DMN, DEN, DBN and PYRN, obtained from the peak heights in Figs. 2 and 3 is shown in Fig. 4. The calibration is linear over five orders of magnitude, indicating that with the GC–TEA technique, quantitative analysis at the sub-3-ng/ml N-nitroso compound concentration level (30×10^{-12} moles/ml N-nitroso compound) is clearly feasible.

Sensivity

A chromatogram of 5 μl of the DCM standard solution containing each of the seven N-nitroso compounds at the 1 μg/ml concentration level (approximately, 1 ppm) is shown in Fig. 5. The chromatogram of 100 μl of the standard DCM solution diluted 200-fold, with each N-nitroso compound at the 5 ng/ml level (approximately 5 ppb) is shown in Fig. 6a. All seven GC peaks are seen to be clearly discernible. Fig. 6b is the chromatogram of 200 μl of the standard solution diluted 2000-fold, with each N-nitroso compound at the 500-pg/ml level (approximately 500 parts per trillion). Due to the extraordinary large amount of material injected onto the column, the baseline is seen to tail badly; nevertheless, the GC peaks of DMN, DEN, DPN



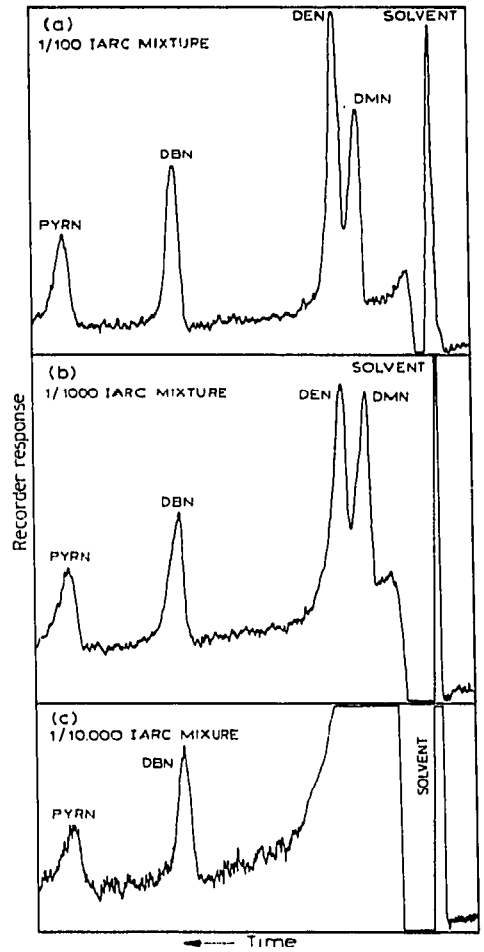


Fig. 2. (a) Chromatogram of 10 µl of IARC mixture containing 23.9 µg DMN/ml, 25.3 µg DEN/ml, 29.0 µg DBN/ml and 30.0 µg PYRN/ml in DCM. (b) Chromatogram of 10 µl of IARC mixture diluted 10-fold.

Fig. 3. Chromatograms of (a) 10 µl of IARC mixture diluted 100-fold; (b) 100 µl of IARC mixture diluted 1000-fold; (c) 500 µl of IARC mixture diluted 10,000-fold.

and SARCOSN are clearly identified above the noise level. The GC peaks of DBN, NIP and PYRN are not ambiguously displayed. A concentration level of 500 pg DMN/ml, corresponding to less than 1×10^{-12} moles N-nitroso compound introduced into the gas chromatograph, must therefore represent the practical detection limit of the GC–TEA technique as described here. Detection at levels below 1×10^{-12} moles N-nitroso compound is possible if steps are taken to remove the solvent, either by conventional temperature programming techniques or by freezing out the solvent in a cold trap placed between the TEA catalytic furnace and the TEA reaction chamber.

Because N-nitroso compound concentrations determined by direct injection at the 500-pg/ml level are so small as to be probably irrelevant (below the so-called

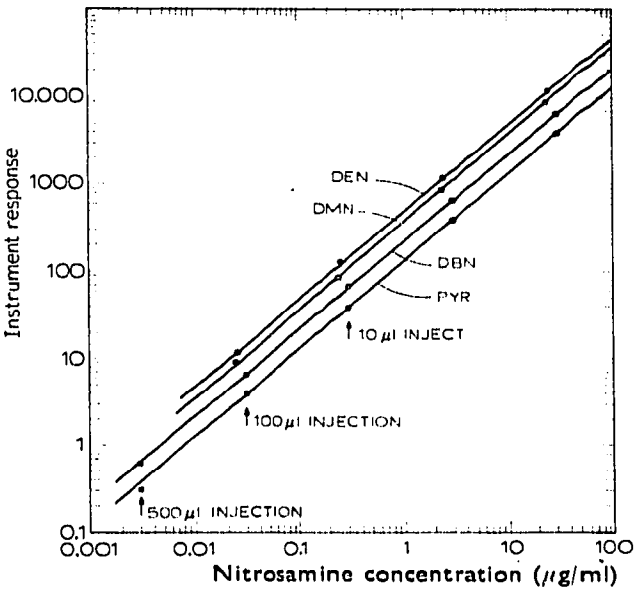


Fig. 4. GC–TEA calibration for DMN, DEN, DBN and PYRN calibration data taken from Figs. 2 and 3.

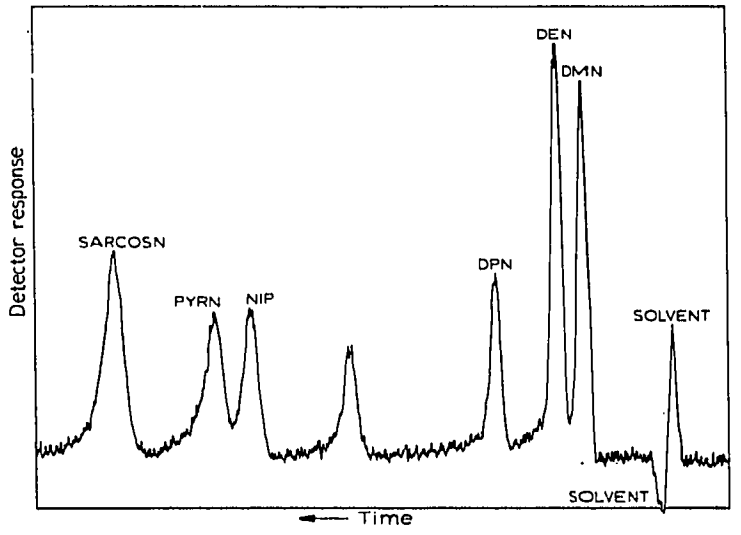


Fig. 5. Chromatogram of 5 µl of DCM solution containing 1 µg/ml of each of the following N-nitroso compounds: DMN, DEN, DPN, DBN, NIP, PYRN and SARCOSN.

"no effect" level) in terms of potential carcinogenic activity[9], we believe that efforts to enhance the direct sensitivity beyond what is reported here would be of limited practical value. Furthermore, if existing clean-up and concentrating procedures were used in conjunction with the GC–TEA system as reported here, detection by direct injection at the 500 fg/ml (5 parts per 10^{13}) concentration level would be possible.

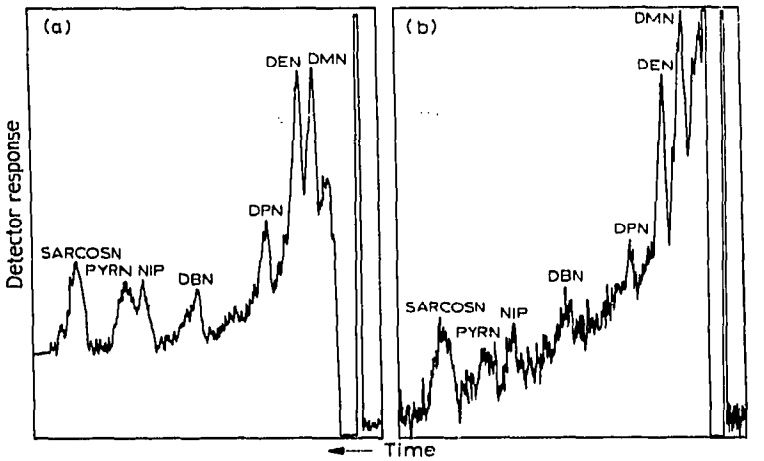


Fig. 6. Chromatograms of (a) 100 μl of DCM solution containing 5 μg/ml of DMN, DEN, DPN, DBN, NIP, PYRN and SARCOSN; (b) 200 μl of DCM solution containing 500 pg/ml of DMN, DEN, DPN, DBN, NIP, PYRN and SARCOSN.

Selectivity

The TEA detector is selective because it requires that the compounds be catalytically pyrolyzed at a low temperature to give a nitrosyl radical. The nitrosyl radical then reacts with ozone to produce light in the near infrared region of the spectrum Many other compounds also react with ozone to produce a luminescence, but the wavelength of the luminescence is in the blue or visible region of the spectrum. Thus, although a compound like carbon monoxide or ethylene produces an intense blue glow with ozone, the "blue" photons are screened out by the red optical filter. Many organic compounds with different functional groups have been evaluated for possible positive and negative interference[8], but to date none has been found.

At temperatures below −150°, the vapor pressure[10] of all but the lowest molecular weight species are substantially less than 1 torr. The nitrosyl radical, on the other hand, has a vapor pressure in excess of 760 torr at −150°. Thus, if compounds which are not N-nitroso are found which react with ozone to produce a luminescence in the near infrared, they may be removed by freezing them out in a cold trap placed between the TEA catalytic pyrolyzer and the TEA luminescent reaction chamber. Organic nitrites are extremely heat labile, and are readily distinguished on the GC–TEA because they decompose in the hot GC injection port (200°) or in the hot (185°) GC column, resulting in a large initial peak coincident with the solvent front. A similar behavior is observed with compounds such as N-nitrosodiphenylamine, N-nitroso urethane, and all the N-nitroso ureas.

Solvent front

As may be seen from Figs. 2, 3, 5 and 6, the GC–TEA solvent response becomes increasingly important as the injection volume is increased. Figs. 7a, b, c and d show the GC–TEA response for the injection of 25 μl, 15 μl, 10 μl and 5 μl of pure DCM, respectively. Three distinct effects are observed: There is an initial sharp positive peak, followed by a broader negative peak, and lastly, the negative peak is seen to overshoot

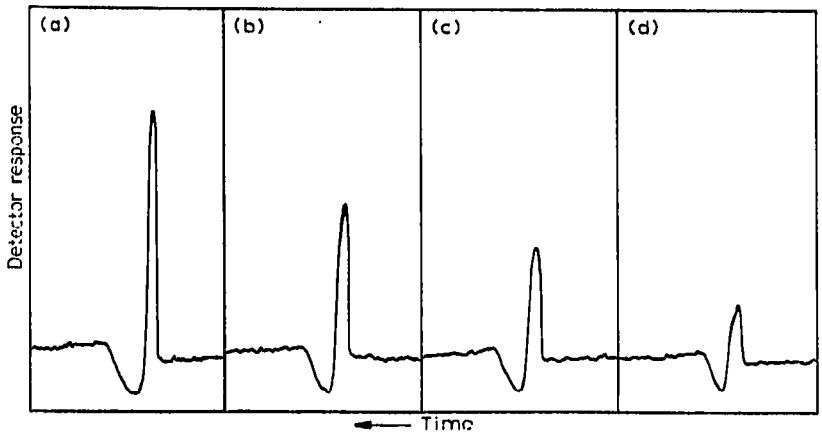


Fig. 7. Chromatograms of (a) 25 μl, (b) 15 μl, (c) 10 μl, and (d) 5 μl of pure DCM.

the baseline before slowly decaying back to the baseline. The behavior for other solvents is similar. All show a positive and then a negative peak, but the recovery of the negative peak may or may not rise above the baseline. The height of the initial positive peak is proportional to the volume of solvent injected. The proportionality is demonstrated in Fig. 8, which is a plot of the solvent peak height in Fig. 7 *versus* the injection volume. Because of the close relationship of peak height to the volume injected, and because the positive peak is sharp and well defined, even for a 500-μl injection (Fig. 3c), the peak must correspond either to a highly volatile impurity, such as dissolved nitric oxide, or more likely to an impurity such as an organic nitrite, which decomposes in the hot injection port to give nitric oxide.

As may be seen from Fig. 7, the negative peak falls to the same level whatever the volume of solvent which is injected. The lowest point corresponds to a zero level of nitric oxide impurity in the carrier gas. We believe that there is a negative peak

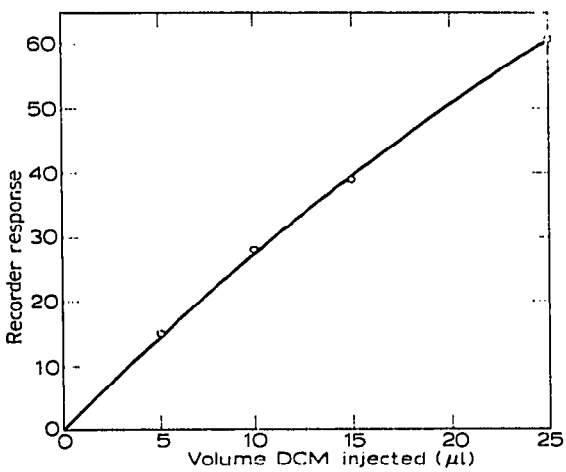


Fig. 8. Plot of solvent peak height versus volume injected for pure DCM.

because the solvent has displaced the carrier gas, with its nitric oxide impurity, from the reaction chamber. As the solvent vapor is in turn swept out of the chamber, the carrier gas with its nitric oxide impurity returns, returning the instrument response to the baseline. The relatively slow decay of the negative peak back to the baseline which may overshoot the baseline, as in the case of DCM, may be explained by a variation in the collisional deactivation of the excited nitrogen dioxide by solvent molecules.

The excited nitrogen dioxide can relax by one of two paths:

$$NO_2^* \rightarrow NO_2 + h\nu \quad (1)$$

$$NO_2^* \rightarrow NO_2 + M \quad (2)$$

The collisional deactivation by diluent species (eqn. 2) is always present but usually not important, unless the nature of M changes drastically, as may happen when the carrier gas is displaced by the solvent vapor. Further evidence for this explanation was obtained by observing the simultaneous thermal conductivity and TEA detector response. The solvent front, as seen by the thermal conductivity detector, extended far beyond the time that the TEA detector takes to return to its baseline.

In order to test these hypotheses further, a cold trap (20 × 3/16 in. I.D. stainless-steel tubing filled with steel wool and bent into a U-shape) was placed between the TEA catalytic pyrolyzer and the TEA luminescent reaction chamber. With the trap at a temperature of −150°, all the DCM is removed and prevented from entering the reaction chamber. A chromatogram of 10 μl of a DCM solution containing 1 μg DMN/ml is shown in Fig. 9, with and without the cold trap. The cold trap is seen to have eliminated the negative peak.

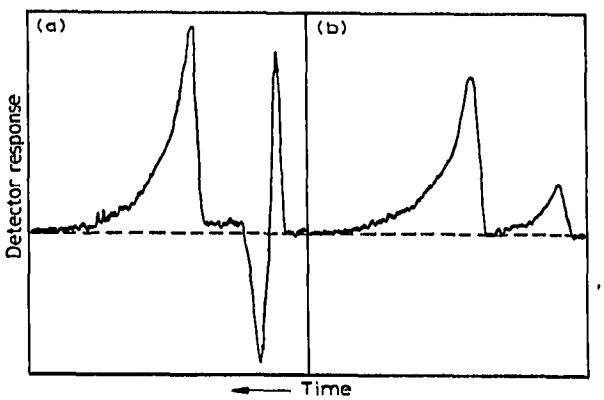


Fig. 9. Chromatograms of 1 μg DMN/ml in DCM: (a) without cold trap; (b) with cold trap at −150°.

CONCLUSION

The advantage of the GC–TEA system for the analysis of N-nitroso compounds over the existing GC–Coulson electrolytic conductivity detector and the GC alkali flame ionization detector techniques is that the GC–TEA is sensitive to sub-ng/ml

concentration levels and is at the same time selective to only N-nitroso compounds. Because of the selectivity, there is little need for extravagant clean-up or concentration procedures. Indeed, concentration is usually unnecessary and clean-up is only required so as to ensure that the extract is compatible with the GC column itself. No clean-up whatsoever is required for the TEA detector.

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