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AN EVALUATION OF THE ALKALI FLAME IONISATION DETECTOR AND THE COULSON ELECTROLYTIC CONDUCTIVITY DETECTOR IN THE ANALYSIS OF N-NITROSAMINES IN FOODS

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SUMMARY

A comparison is made of the performance characteristics of two dissimilar nitrogen detectors. The various parameters influencing the specificity and sensitivity of these detectors are discussed and experience gained in their use for the analysis of foods for traces of nitrosamines is described. Only slight differences in the sensitivities of the two detectors were found but the Coulson electrolytic conductivity detector was more selective and less disturbed by small changes in operating parameters, and hence is considered the more suitable for routine use in this field of analysis.

A flow diverting system is described which enables the thermionic detector to be used both with chlorinated solvents and injection volumes of hydrocarbon solvents greater than 2 μ l.

INTRODUCTION

The carcinogenic properties of many of the N-nitrosamines are well documented¹⁻³. Foods are known to contain secondary amines⁴ and with the widespread use of nitrite as a preservative⁵, the possibility of these precursors reacting to form nitroso compounds has been discussed.

The analysis of food extracts for nitrosamines has been reported in the literature, and diverse instrumental techniques have been applied⁶⁻¹⁰. However, not all of these are equally suitable and such unspecific methods as polarography^{11,12} and thin-layer chromatography¹³⁻¹⁵ have been shown to produce false positives^{7,10,17}. Even gas-liquid chromatography (GLC) when using a flame ionisation detector (FID) is generally unsuitable for the majority of sample concentrates, despite elaborate clean-up procedures (Fig. 1). This is due to the very low levels of nitroso compounds present in the extract in comparison with co-extractives. It has therefore been necessary to use gas-liquid chromatography/mass spectrometry (GLC-MS) with a

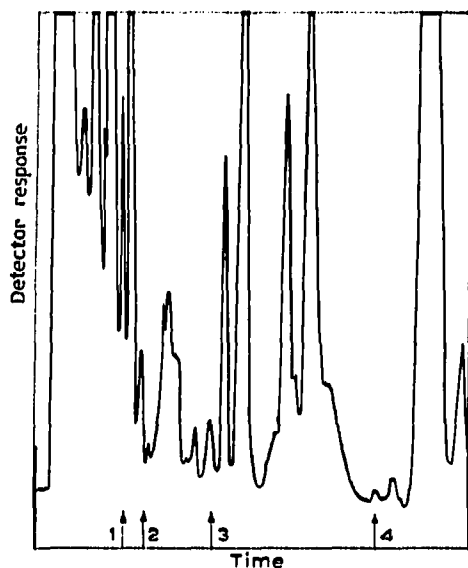


Fig. 1. Chromatogram of a concentrated extract of Gouda cheese using a flame ionisation detector. The retention times of the nitrosamines are indicated by arrows. 1 = DMN; 2 = DIEN; 3 = DPN; 4 = DBN.

high resolution instrument as the most selective technique available for the analysis of extracts for traces of nitrosamines¹⁸⁻²⁰. As the use of this procedure is expensive and requires highly trained operators, the approach in these laboratories has been to develop methods based upon steam distillation and solvent extraction, followed by GLC using nitrogen-selective detectors. Only when apparent positives are recorded are the samples subjected to confirmation by GLC-MS^{10,21,22}.

This paper compares the results we have obtained when using two dissimilar nitrogen detectors, *viz.* the alkali flame ionisation (thermionic) detector and the Coulson electrolytic conductivity detector, in conjunction with commercial gas chromatographs.

ALKALI FLAME IONISATION DETECTOR

The alkali flame ionisation detector (AFID) is a modification of the FID, in which alkali metal ions are introduced into the flame to vary the ionisation processes and to enhance selectively the response to compounds containing certain heteroatoms. This phenomenon was first reported by GIUFFRIDA²³, and KARMEN AND GIUFFRIDA²⁴, who showed that the response to phosphorus was increased by a factor of 100. The close similarity in electronic configuration between phosphorus and nitrogen prompted AUE *et al.*²⁵ to experiment with a variety of alkali salts in order to improve the selective determination of nitrogen, and their results indicated that rubidium sulphate gave the best overall performance. In a comprehensive review of thermionic detectors, BRAZHNIKOV *et al.*²⁶ compared and contrasted the performance of the detector with tips formed from a number of salts, and concluded that rubidium chloride was preferable for nitrogen compounds. Caesium bromide has also been

considered suitable in this respect²⁷. No less conflicting are the reports of the significance of detector geometry²⁸, electrode spacing^{29,30}, nature of carrier gas³¹ and hydrogen flow²⁸⁻³². The importance of operating under carefully controlled conditions is exemplified by the use of slight adjustment of these parameters to vary the selectivity of the detector and conditions for the following elements have been described: chlorine, iodine and bromine^{31,33,34}; silicon, tin and lead³⁵; and sulphur³⁶. It seems clear, therefore, that the choice of parameters for thermionic detectors is still empirical, and that further development and understanding is essential before they can be considered suitable for anything except specialist or research applications.

COULSON ELECTROLYTIC CONDUCTIVITY DETECTOR

The Coulson electrolytic conductivity detector (CECD), as described by COULSON in 1965³⁷, is claimed to be a specific and sensitive detector of compounds containing nitrogen and, by varying the mode of operation, also of compounds containing sulphur or halogens. For the estimation of nitrogen-containing compounds the effluent from a gas chromatographic column is mixed with hydrogen and passed through a reduction furnace at 800° over a nickel catalyst. Nitrogen-containing compounds are reduced to ammonia, which is subsequently dissolved in deionised water and passed through a micro cell in which changes in the electrolytic conductivity are measured. Halogens, which, if present, would be reduced to the corresponding acid and hence interfere, are removed by a strontium hydroxide coated plug of glass fibre placed between the furnace and the detector cell. Other organic species form pyrolysis hydrogenation products which give no response in the conductivity cell. PATCHETT³⁸ has recorded several refinements of the detector which enabled him to detect nitrogen at about 100 pg. COCHRANE AND WILSON³⁹ compared the response of the CECD with an electron capture detector for the detection of nitrogen-containing herbicides. The response of the former was found to be lower, possibly due to inherent peak tailing when using the CECD.

EXPERIMENTAL

Alkali flame ionisation detector

No attempt has been made to evaluate the various critical parameters exhaustively and attention has been concentrated upon obtaining reliable and repeatable results from a standard detector, based on manufacturers' instructions. The experiments were carried out with a Varian 1700 Series gas chromatograph fitted with a rubidium sulphate thermionic detector. Optimum background current is first achieved by careful stepwise adjustment of hydrogen and air flows. These require careful control to obtain reproducible results and, even when using mass flow controllers, a drift in background current is often experienced at maximum sensitivities. The hydrogen flow is especially critical and has to be controlled to 0.01 ml/min if stable conditions are to be maintained. Rotameter type flow indicators were removed from the system as they cause instability in the gas flow, possibly due to small leaks or oscillations of the float.

Operating conditions chosen are: carrier gas (helium) flow-rate, 25 ml/min;

hydrogen flow-rate, 40 ml/min*; air flow-rate, 240 ml/min*; sample size, 2–10 μ l; injection temperature, 200°; detector temperature, 230°.

The chromatographic column is prepared from 6.5 m \times 2 mm I.D. stainless-steel tube packed with 15% FFAP (15 g FFAP on 100 g Chromosorb W, acid-washed, DMCS treated, 80–100 mesh), and conditioned for 48 h at 250° with carrier gas flowing, prior to use. The column is normally held isothermally at 130° but occasionally it has been temperature programmed at 1°/min in the range 110°–160°. The response of thermionic detectors is disturbed by chlorinated solvents and the flame is extinguished by injection of more than 2 μ l of hydrocarbon solvent. These difficulties have been overcome by inserting a VALCO flow diverting valve between the column and the detector (Fig. 2) so that solvent can be vented to atmosphere.

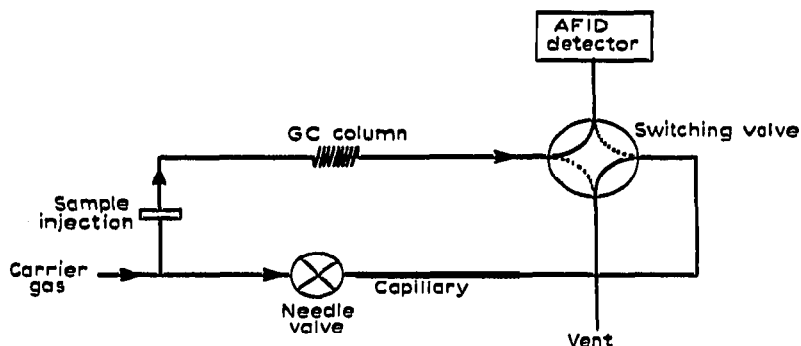


Fig. 2. Diagram of the insertion of a flow switching valve between a GC column and an alkali flame ionisation detector.

If the outlet flow from the valve is carefully balanced by means of capillary tubing and a needle valve, there is no disturbance of baseline stability on switching, despite the sensitivity of this type of detector to changes in gas flows.

Coulson electrolytic conductivity detector

The Coulson electrolytic conductivity detector was operated in conjunction with a Varian 1200 Series gas chromatograph housing a column prepared as described above. In a typical analysis the following conditions were found to be satisfactory: carrier gas (helium) flow-rate, 25 ml/min; hydrogen flow-rate, 60 ml/min; sweep gas (helium) flow-rate, 25 ml/min; cell flow (ion-exchange water), 1 ml/min.

The transfer line from the gas chromatograph comprises a stainless-steel capillary tube 180 mm \times 0.2 mm I.D. housed in a copper tube of 20 mm O.D. surrounded with glass fibre packing. The tube is heated by electrical tape and the temperature maintained at about 220° by a variable voltage transformer. A VALCO flow diverter valve is incorporated before the reduction furnace and by use of this we have found it possible to inject samples of up to 50 μ l, although 10 μ l is sufficient for most extracts under test. Unlike the AFID, the response of the CECD appears to be independent of small changes in the various gas flows and hence conditions of

* Initial flow-rates which are then adjusted during optimisation.

operation are much less critical. Injector and column oven temperatures were similar to those used with the AFID.

Standard solutions

The nitrosamines included in the present survey have been limited to the steam volatile lower dialkyl compounds—dimethyl (DMN)-, diethyl (DEN)-, dipropyl (DPN)- and dibutyl (DBN)-nitrosamines—and the heterocyclics N-nitrosopiperidine (PIP) and N-nitrosopyrrolidine (PYRN). Primary standard solutions were prepared by weighing appropriate quantities of 'pure' grade nitrosamines into calibrated flasks and dissolving in demineralised water. The reagents were used as received from the manufacturers without prior treatment, other than checking that only one peak was obtained by gas chromatography using a FID. As nitrosamine solutions are light sensitive⁴⁰ the standards are stored in glass containers wrapped in aluminium foil and kept in a constant temperature room at 10°. No deterioration of the standards was observed under these conditions over a period of six months.

RESULTS AND DISCUSSION

Linearity

In comparing the linearity of the AFID and CECD detectors, calibration curves were prepared in the range of interest *i.e.* 0-200 ng of nitrogen. The response of the

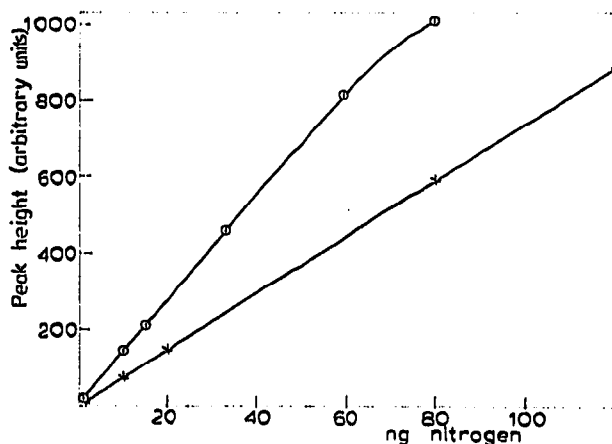


Fig. 3. Response of the alkali flame ionisation detector to DMN and DEN. ×, DMN; O, DEN.

AFID to increasing amounts of DMN and DEN is illustrated in Fig. 3. In a similar curve shown in Fig. 4, the linearity of the response of the CECD to DMN and DEN is demonstrated. In both cases the curves were prepared from data obtained from peak height measurements, hence one would not expect the lines to be coincident.

Sensitivity

For practical purposes the sensitivity of both detectors was calculated from the minimum detectable amount at twice the noise level, under normal operating

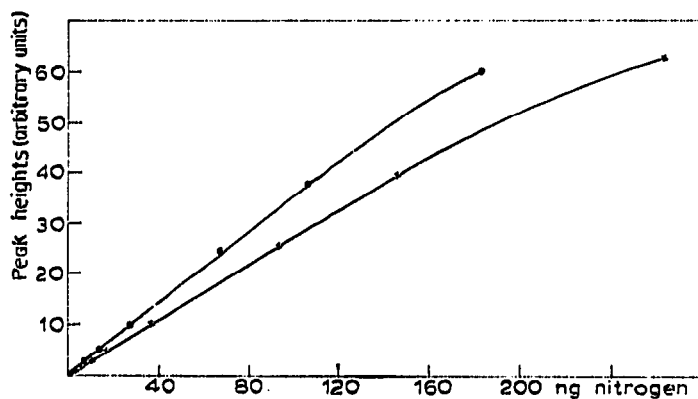


Fig. 4. Response of the Coulson electrolytic conductivity detector to DMN and DEN. \times , DMN; \circ , DEN.

conditions. For DMN, both detectors have a limit of about 100 μg calculated as nitrogen. This figure is of the same order as data previously reported by PATCHETT²⁸ for the CECD, but HARTMANN²⁹ has claimed a detection level of 10 μg nitrogenous material on a modified Varian AFID. Sample size is an important factor when chromatographing dilute solutions, and in the case of the AFID a maximum loading of 2 μl is normally recommended, but by using the vent valve referred to previously, it has been possible to use samples of up to 20 μl . The only limit to the amount of sample that can be injected under these conditions would be the capacity of the gas chromatographic column. A chromatogram of 50 μl of a dilute aqueous nitrosamine solution obtained using the CECD is illustrated in Fig. 5. The minimum detectable amount is dependent on the background signal, and with the CECD this is maintained

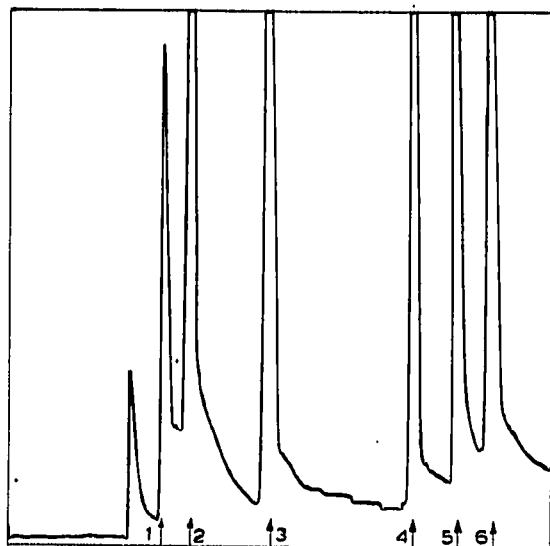


Fig. 5. Chromatogram of 50 μl of an aqueous standard of six nitrosamines at a concentration of 1 mg/kg . 1 = DMN; 2 = DEN; 3 = DPN; 4 = DBN; 5 = PIP; 6 = PYRN.

at a low level by continuously recirculating the water through an anion-exchange resin.

Selectivity

The CECD is nitrogen-specific in the reductive mode when a strontium hydroxide scrubber is used in line to remove acid produced by the reduction of halogenated species. RHOADES AND JOHNSON⁴¹ and ISSENBERG AND TANNENBAUM⁴² have described further refinements in which the detector is operated in the pyrolytic rather than reductive mode, and by this method they claim a response only to nitrosamines and amines. However, this selectivity is gained only at the expense of sensitivity, and in our present work, in which detection levels of $1 \mu\text{g}/\text{kg}$ of nitrosamines in the original samples are required, this approach has not proved satisfactory.

When using the AFID the operating parameters are adjusted to achieve a compromise between maximum sensitivity and selectivity, and in practice the

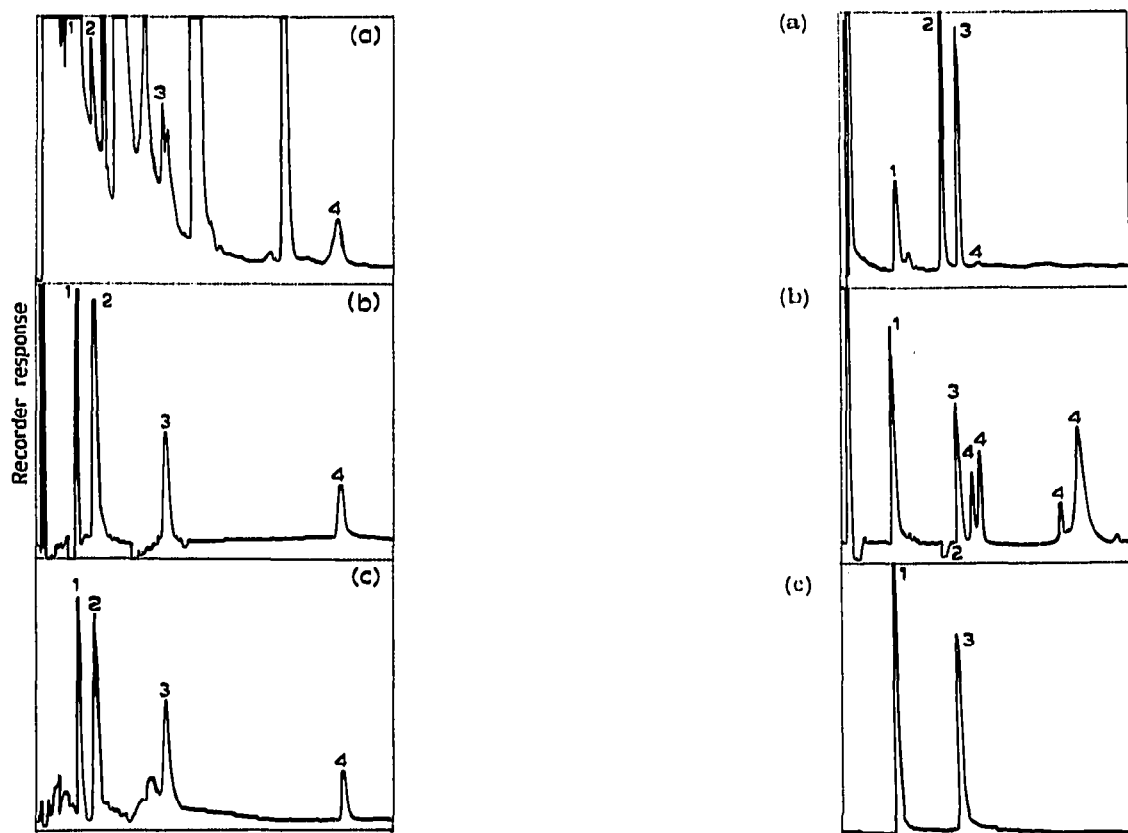


Fig. 6. Chromatograms of a mixture of equal parts of toluene, dodecane, pentadecane, hexan-1-ol and $10 \text{ mg}/\text{kg}$ each of (1) DMN, (2) DEN, (3) DPN, and (4) DBN. (a) FID; $10^{-12} \text{ A}/\text{mV}$; attenuation $\times 8$; sample $3 \mu\text{l}$. (b) AFID; $10^{-12} \text{ A}/\text{mV}$; attenuation $\times 32$; sample $4 \mu\text{l}$. (c) CECD; attenuation $\times 8$; sample $10 \mu\text{l}$.

Fig. 7. Chromatograms of a mixture of (1) DMN, (2) dichlorobenzene, (3) DEN, and (4) phorate ($10 \text{ mg}/\text{kg}$ of each in acetone). (a) FID; $10^{-12} \text{ A}/\text{mV}$; attenuation $\times 32$; sample $3 \mu\text{l}$. (b) AFID; $10^{-12} \text{ A}/\text{mV}$; attenuation $\times 128$; sample $2 \mu\text{l}$. (c) CECD; attenuation $\times 64$; sample $10 \mu\text{l}$.

detector response to phosphorus is normally greater than that to nitrogen. Although the AFID is claimed to be about 10 000 times less sensitive to hydrocarbons than the FID⁴³, a negative peak is commonly observed when large quantities of hydrocarbons are present. This phenomenon is particularly troublesome in nitrosamine analysis as hydrocarbons are commonly used as solvent for injection. Thus far, investigations have shown that the production of the negative peak is dependent upon hydrogen flow-rate and is generally most apparent when the latter is adjusted for maximum sensitivity. The different response profiles of the two specific detectors and the standard FID, under similar gas chromatographic conditions, can be seen in the chromatograms shown in Fig. 6. These were derived from a mixture of three hydrocarbons and an alcohol spiked with four nitrosamines in the ratio of 1000:1. It can be inferred from these chromatograms that interference from the major components would prohibit the use of the FID for this analysis. The lack of response of the CECD to phosphorus and halogen compared with the AFID and FID is illustrated in Fig. 7. For this a mixture of 10 mg/kg DMN, DEN, dichlorobenzene, and an organophosphorus pesticide (phorate) in acetone was chromatographed using the three detectors. The CECD responds only to the nitrogen-containing compounds whereas the FID

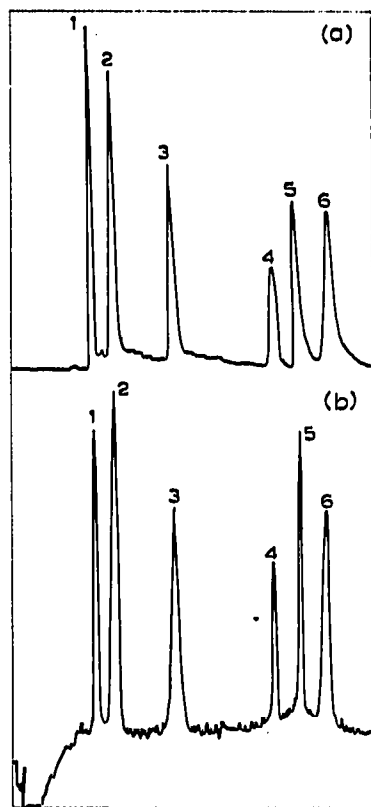


Fig. 8. Temperature-programmed chromatograms of a mixture of (1) DMN, (2) DEN, (3) DPN, (4) DBN, (5) PIP, and (6) PYRN (10 mg/kg of each in aqueous solution). (a) CECD; programmed at 1°/min, 110–150°. (b) AFID; programmed at 1°/min, 110–150°.

trace has peaks corresponding to all the components, but with a much reduced phosphorus response. By comparison, the AFID produced a negative peak for solvent and dichlorobenzene, and a greatly enhanced response to the phosphorus compound. The additional peaks may be due to either decomposition on the GC column or to the presence of impurities in the sample. Some adjustment of the hydrogen flow to the AFID would improve the selectivity, but only at the expense of sensitivity.

Temperature programming

One of the advantages of selective nitrogen detectors is their insensitivity to increased column bleed produced during a temperature-programmed analysis. The absence of baseline drift is a noteworthy feature, especially when operating at high sensitivities and using columns of high liquid phase loading. Typical chromatograms of a temperature-programmed analysis are shown in Fig. 8 indicating that both detectors are suitable for this type of operation, even when as in this case the liquid phase (FFAP) contains nitrogen. Under similar conditions the trace from a FID would rise rapidly off scale. When temperature programming the AFID we recommend that the background current be checked and, if necessary, readjusted before the next injection.

Response factors

Experiments were designed to test the response of both nitrogen-selective detectors to nitrosamines containing different proportions of nitrogen and carbon in the molecule. In this study the response of the detector was calculated by measurement of peak areas using a Disc integrator. The response of each detector relative to DPN was then computed and the results are presented in Table I, together with the nitrogen and carbon contents of each nitrosamine, again calculated relative to DPN. It can be seen that the response of the CECD follows the nitrogen content, within experimental error. On the other hand, ISSENBERG AND TANNENBAUM⁴² have reported that when using the CECD in the pyrolytic manner alone, the detector response is dependent on the structure of the nitrosamine. Presumably in this mode of operation the molecule is incompletely fragmented.

In the case of the AFID there is a more complicated relationship amongst the

TABLE I

RELATIVE RESPONSE FACTORS AND RELATIVE NITROGEN AND CARBON CONTENTS OF N-NITROSAMINES

<i>Nitrosamine</i> ^a	<i>Relative response factor</i>			<i>Nitrogen content</i>	<i>Carbon content</i>
	<i>AFID</i>	<i>CECD</i>	<i>FID</i>		
DMN	0.96	1.64	0.29	1.76	0.59
MEN	1.02	1.41	—	1.48	0.74
DEN	1.12	1.35	0.87	1.28	0.85
DPN	1.00	1.00	1.00	1.00	1.00
DBN	0.83	0.81	1.06	0.82	1.10
PIP	1.12	1.07	0.68	1.14	0.95
PYRN	1.04	1.20	0.41	1.31	0.87

^a For abbreviations, see text.

various parameters affecting its response to nitrogen. Table I shows that the correlation between the AFID response factors and nitrogen content is less good, but it is still more closely related to nitrogen than to carbon content. A survey of the literature shows apparently conflicting opinions on the correlation between nitrogen content and response characteristics of alkali flame detectors to heteroatoms. AVE *et al.*³³ found a non-proportional response from certain nitro compounds. On the other hand, Hewlett-Packard claim⁴³ that their detector responds linearly to nitrogen in any organic molecule independent of the molecular structure.

Quantitative analysis

In a recent international collaborative study organised by the IARC (International Agency for Research on Cancer, W.H.O., Lyon) two aqueous samples

TABLE II

RESULTS OF ANALYSIS OF SOLUTION B FOR DEN AND PYRN

Detector	Composition of solution B (calculated)			
	DEN (mg/l)	A/B	PYRN (mg/l)	A/B
CECD	9.6		10.1	
	9.7		10.1	
	9.6		10.4	
	9.6		10.5	
	9.7		10.1	
	9.3		10.5	
	9.4		10.0	
	9.9		10.3	
	9.4		10.2	
	9.1		10.2	
	9.5		10.5	
	9.9			
	Average calculated	9.6	1.03	10.3
Actual composition	8.8	1.12	10.6	0.94
AFID	9.1		9.8	
	9.4		11.0	
	9.1		10.2	
	9.1		9.5 ^u	
	10.2 ^u		11.8 ^u	
	8.8 ^u		10.2 ^u	
	9.8 ^u		10.2 ^u	
Average calculated	9.4	1.05	10.4	0.96
Actual composition	8.8	1.12	10.6	0.94
FID	9.2		9.4	
	9.1		9.8	
	9.2		10.2	
	9.1		9.6	
	9.4		10.2	
	9.6		10.1	
Average calculated	9.3	1.06	9.9	1.01
Actual composition	8.8	1.12	10.6	0.94

^u Solvent vented for 6 min.

(A, B) each containing known amounts of two nitrosamines (DEN, PYRN) were circulated to participants for analysis. Solution A was to be used as a reference standard; the composition of solution B was not disclosed to the analyst. The result was expressed as a ratio A/B by means of peak height measurements. From Table II it can be seen that the average compositions as calculated for each detector are in good agreement with each other and with the actual composition disclosed at the conclusion of the study.

Sample analysis

Satisfactory results have been obtained from the analysis of samples of meat, fish and other food products. Both detectors have been used in this work although the greater specificity of the CECD generally produces a cleaner chromatogram. A characteristic trace from a CECD is illustrated in Fig. 9, this was obtained from a hexane extract of a sample of Gouda cheese. A 250-g sample was steam distilled and the aqueous distillate extracted with methylene chloride. The solvent was then reduced to small volume in a Kuderna-Danish evaporator and 1 ml *n*-hexane added to the concentrate. The evaporation was continued to a final volume of 250 μ l. This represents a concentration factor of $\times 1000$ on the original weight of cheese as is required to achieve a detection limit of 1 μ g/kg. Each sample is spiked with 10 μ g/kg of DPN as internal standard and as a check on the recovery process. Overall recoveries fall within the range 60 to 80%. DPN was chosen since we have not yet identified it in any of the samples tested, and it has a suitable GLC retention time.

The majority of foods contain nitrogenous compounds, many of which are co-

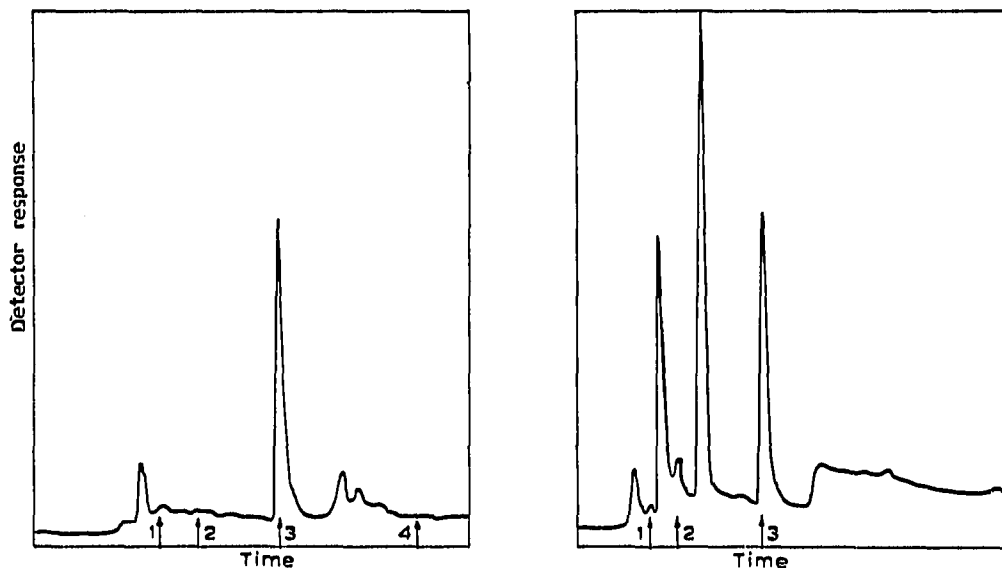


Fig. 9. Chromatogram of an extract of Gouda cheese spiked with 10 μ g/kg DPN using the Coulson electrolytic conductivity detector. 1 = DMN; 2 = DEN; 3 = DPN; 4 = DBN.

Fig. 10. Chromatogram of an extract of eel spiked with 10 μ g/kg DPN using the Coulson electrolytic conductivity detector. 1 = DMN; 2 = DEN; 3 = DPN.

extracted with nitroso compounds, and hence produce interference even when using selective detectors. The chromatogram of a fish extract spiked with DPN illustrated in Fig. 10 shows peaks which do not correspond to the nitrosamines sought. This interference is especially noticeable when analysing cooked foods, or foods which have been kept for extended periods in storage tests, due to chemical or bacteriological decomposition. Our present objectives are to improve clean-up techniques and to devise a more sophisticated GLC approach, for example "heart cutting" using two dissimilar columns in a manner described by DEANS⁴⁴. In the meantime samples which are found to contain components of similar retention times to nitrosamines are submitted for analysis by the combined GLC-MS techniques reported by GOUGH AND WEBB¹⁹.

CONCLUSION

In our experience of the examination of a variety of food samples for very low levels of nitrosamines^{10,32} both the AFID and CECD have given satisfactory results. However, the most important criteria of a specific nitrogen detector are that it should respond to nitrogen-containing materials without interference from other molecules present, also the detector should be capable of a high degree of sensitivity and reproducibility and be relatively free from high noise level and baseline fluctuations. Considering these factors, the experience of operators in this laboratory indicates that the electrolytic conductivity detector is more convenient and reliable than the ionisation detector for the analysis of foodstuffs for traces of nitrosamines.

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