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ANALYTICAL PERFORMANCE OF A NOVEL NITROGEN-SENSITIVE DETECTOR AND ITS APPLICATIONS WITH GLASS OPEN TUBULAR COLUMNS

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SUMMARY

Sensitivity, selectivity, linear range, long-term stability and operating conditions of a new nitrogen-sensitive thermionic detector were investigated. The detector was combined with high-efficiency glass open tubular columns in order to detect trace nitrogen-containing constituents in the complex mixtures of volatiles from human urine and tobacco aroma. A complex nitrogen profile was also obtained from a nitro-methane extract of airborne particulate matter.

INTRODUCTION

Since its first description by Karmen and Giuffrida^{1–3} in 1964, the alkali flame detector (also known as the thermionic detector) has generated and maintained considerable interest among scientists and instrument manufacturers. Although much still remains unexplained about the detection principles involved⁴, the alkali flame detector has been used in different operational modes for the detection of organic compounds containing phosphorus, halogens, arsenic, nitrogen, sulphur, tin and some other less common elements. These detectors have usually been constructed by modification of conventional flame ionization detectors in which the alkali source is located above the flame and heated either by the flame or electrically. As reported by Aue and co-workers^{5,6}, small changes in design and operating conditions of these detectors may significantly influence their response for different elements. While this fact would seem beneficial for convenient adjustment of detector sensitivity, many instrumental parameters must be very carefully controlled.

In spite of obvious competition with electron capture and flame photometric detectors, the alkali flame detector has met with moderate success in the practical detection of phosphorus and halogen compounds. Although routine determination of nitrogen compounds at low concentration levels is needed in many analytical problems, application of the nitrogen-specific thermionic detector has been less common. The nitrogen-specific detector has been described and studied by numerous workers^{7–10},

but its limited stability and reliability have been serious drawbacks for wider use.

A new thermionic detector has recently been developed¹¹ which uses a glass bead with rubidium silicate as an alkali source. The bead is electrically heated, and thus freed from dependence upon the flame as a source of thermal energy. As previously reported^{5,6}, shape and temperature of the flame and the area of its contact with the alkali surface are very critical factors for detector performance. Thus, background variations due to inconsistent flame conditions are eliminated with the new detector. This detector is specific to nitrogen and phosphorus compounds, or, in an alternative mode of operation, to phosphorus compounds only. More advanced technology of the new alkaline source appears to be a further advantage over the earlier described rubidium sulphate pellets.

This paper describes the analytical performance of the novel detector and demonstrates its high sensitivity and selectivity to organic nitrogen compounds. Important operating parameters, including linear range and stability of this detector, are discussed.

In addition to the utilization of this detector in common "nitrogen problems", using conventional packed columns, important applications have also been found in conjunction with glass open tubular columns. The combination of this nitrogen-specific detector with such inert columns is capable of enhancing greatly the qualitative information obtained from extremely complex mixtures. Applications to the high-resolution analysis of complex volatile profiles of human urine, tobacco aroma and extracts of airborne particulate matter are demonstrated.

EXPERIMENTAL

Detector and operating parameters

A schematic diagram of the nitrogen-phosphorus detector is shown in Fig. 1. The novel alkali source consists of a glass bead containing primarily non-volatile rubidium silicate ($\text{Rb}_2\text{O}\cdot\text{SiO}_2$), in contrast to the previously described detectors

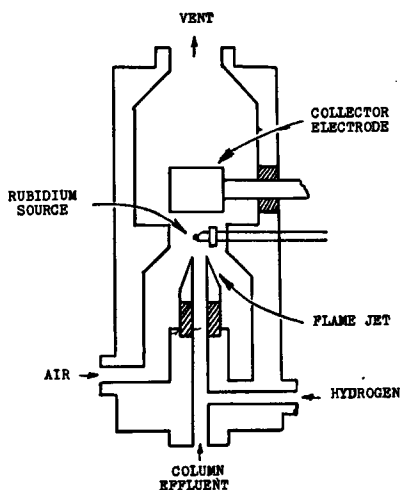


Fig. 1. Schematic diagram of the nitrogen-phosphorus detector.

which generally employ volatile or water-soluble salts. The bead is heated electrically by a variable power supply and the detection sensitivity is a function of the controlled bead temperature.

In the nitrogen-phosphorus mode, both the jet and bead are held at a negative potential (-180 V) relative to the collector electrode. A very small, cool flame is generated by using hydrogen flows between 1 and 5 ml/min and an air flow of 100 ml/min. Hydrogen is burned around the hot bead, and there is no need for critical adjustment of the bead and collector electrode with this detector design. The flame adjusts itself around the bead and the alkali source is situated automatically in the reducing center of the flame.

In the phosphorus-only mode, the polarity of the jet is changed by an external polarity switch and the air and hydrogen flows used are the same as in the standard flame ionization detector. The flame heats the bead, and no electrical heating is required. In this arrangement, the detector functions in a manner similar to the other thermionic detectors which have been described in the past⁴.

Performance studies

The nitrogen detector was mounted on a Perkin-Elmer 3920 gas chromatograph in place of one of the standard flame ionization detectors. The effects of different carrier gases (helium and nitrogen) and their flow-rates on the detector background current were first evaluated. The values of background current were also studied under different flow conditions of the combustion gases.

Standard solutions of different amounts of nitrogen-containing compounds (azobenzene, methamphetamine and caffeine) in *n*-hexane and cyclohexane were used to obtain data on linearity, selectivity and sensitivity of the nitrogen detector. *n*-Octadecane was chosen as a representative hydrocarbon for use in the selectivity studies. Conventional packed glass columns were used in all performance studies.

In order to test the stability and reproducibility of the nitrogen detector, replicate injections of methamphetamine at two different concentration levels were made over a period of 1 month.

Applications of open tubular glass columns

A Perkin-Elmer 990 gas chromatograph with a modified injector and detector base was used throughout these experiments. The standard flame ionization detector was interchanged with the nitrogen detector when needed, and comparative runs were recorded under identical sampling and chromatographic conditions. A 55 m \times 0.22 mm I.D. glass open tubular column coated with GE SF-96 silicone oil was used to obtain the chromatograms of urinary volatiles and tobacco headspace samples. Both types of samples were pre-concentrated by a modified sampling procedure^{12,13} of Zlatkis *et al.*¹⁴ using a thermostable porous polymer (Tenax GC). The details of sampling have been described elsewhere^{12,13}.

An air-pollution glass fiber filter through which Indianapolis city air had been drawn under standard conditions was extracted, and the extract treated according to the procedure established in our laboratory¹⁵ for screening sources of air pollution. A 35 m \times 0.25 mm I.D. glass open tubular column coated with SE-52 silicone gum was used to chromatograph the nitromethane fraction of airborne particulates. A solvent-free sampling technique¹⁶ was employed for this sample.

RESULTS AND DISCUSSION

Although the factors that affect the response of other thermionic detectors have been extensively studied^{4-6,9}, all detection phenomena have not been conclusively explained. Lakota and Aue⁵ reported that the shape and size of collector electrode and its distance from the flame, the shape of the bead, the size of the flame and the flow-rates of gases may all significantly influence the response for different types of molecules. Even negative peaks could be produced under certain conditions. In a later study, Gerhardt and Aue⁶ observed that the response for nitrogen compounds is affected by the type of carrier gas.

The detection mechanism for the nitrogen-sensitive detector studied in this work has recently been proposed by Kolb and Bischoff¹¹. In the nitrogen-phosphorus detection mode, the pyrolytic formation of cyano radicals ($\cdot\text{C}\equiv\text{N}$) in the cold flame zone is essential for detecting nitrogen compounds. The radicals produced gain one electron from neutral alkali atoms in the vicinity of the bead, thus resulting in a symmetrical cyanide ion and a positive alkali ion, which is recaptured by the negative bead. The cyanide ion migrates to the collector electrode, where it liberates one electron either by oxidation to yield neutral products or by reaction with hydrogen atoms to produce hydrogen cyanide. The collected electron is responsible for the specific nitrogen response.

The nitrogen detector has been used with either helium or nitrogen as the carrier gas. A change in the carrier gas flow will affect the temperature of the bead owing to thermal conductivity phenomena. As sensitivity is a function of background current and the bead temperature, the potentiometer setting on the power supply must be adjusted so as to maintain a desired background current and sensitivity level if the carrier gas flow-rate is changed. For instance, with a change in helium flow-rate from 20 to 35 ml/min (at the same power supply setting), both the detector background current and the relative sensitivity for azobenzene will be decreased by a factor of two. A change in the type of carrier gas will also have a large effect on the background current owing to a difference in thermal conductivity of the two gases. The bead temperature can again be electrically re-set to provide a standard background current.

The dependence of the background current on the applied air flow (pressure) is shown in Fig. 2 for both nitrogen and helium carrier gas. The maximum with helium occurs at 22 p.s.i.g. (corresponding to 85 ml/min), while its position for nitrogen is at a much lower value. Again, thermal conductivity effects are likely to be involved. The increasing air flow dilutes the highly efficient cooling effect of the helium carrier gas and, consequently, the bead tends to increase its temperature. However, too much air provides a cooling effect of its own, as evidenced by the descending part of the curve. It has been observed that the bead will not glow with helium as the carrier gas if no air is applied. No dilution is needed with nitrogen carrier gas and the bead will glow in the absence of air flow. When nitrogen is the carrier gas of choice, air appears to act mainly as a cooling agent and the background maximum occurs at very low flow-rates.

As demonstrated in Fig. 3, the plot of the detector background current against the hydrogen flow-rate also produces a bell-shaped curve with a maximum at about 28 p.s.i.g. (5 ml/min). Although drift and non-specific response occur to a lesser extent at lower flow-rates, the sensitivity to nitrogen compounds is significantly better at the hydrogen flow corresponding to the maximum background current.

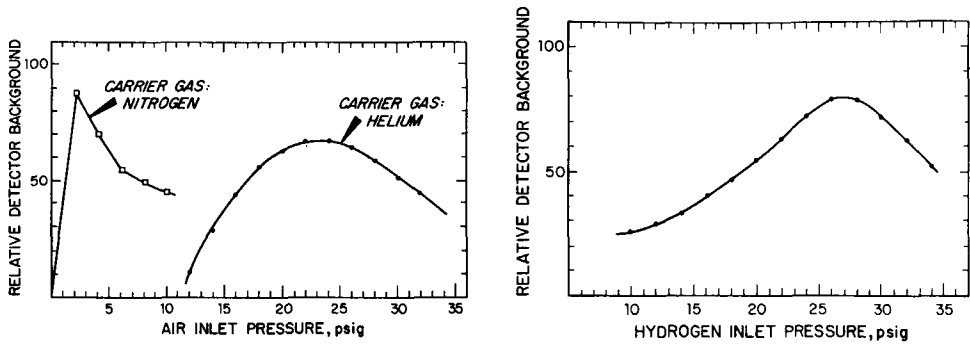


Fig. 2. Dependence of the relative detector background on air inlet pressure (flow) for two different carrier gases. Constant settings: carrier gas flow-rate, 16 ml/min; hydrogen flow-rate, 5 ml/min.

Fig. 3. Dependence of the relative detector background on hydrogen inlet pressure (flow). Constant settings: carrier gas (helium) flow-rate, 16 ml/min; air flow-rate, 85 ml/min.

The sensitivity of the detector can be conveniently expressed in coulombs per gram of nitrogen, and the sensitivity calculated for caffeine from Fig. 4 is 1.7 C/g. For most nitrogen-containing compounds, this detector is about 50 times more sensitive than the flame ionization detector. The minimum detectable amount may often be of the order of 10^{-13} g of nitrogen/sec under favorable analytical conditions.

There are some nitrogen-containing compounds for which cyano radicals cannot be readily formed and for which the detector shows decreased sensitivity. Barbiturates with both nitrogen atoms adjacent to carbonyl groups on either side, nitrate esters, and amides belong into this group. The sensitivity of the barbiturate determinations can be significantly improved through the formation of methyl derivatives¹⁷ and the consequent possibility of the formation of cyano radicals.

The selectivity was determined by analyzing a solution containing standard amounts of azobenzene and *n*-octadecane, and a response discrimination of 1:7,500 was observed. The selectivity may, of course, vary when different experimental conditions and samples are used, but no lower ratio than 1:5,000 was consistently observed for a variety of nitrogen compounds.

The detector linearity is demonstrated for azobenzene and caffeine in Fig. 5. Excellent linear response is obtained within a 10^4 range of concentration.

In order to test the stability and reproducibility of the detector, replicate injections of methamphetamine at two different concentration levels were made over a period of 1 month. The results listed in Table I show the excellent reproducibility and attest to the stability of this detection system.

Owing to its sensitivity, selectivity and, in particular, long-term stability and reliability, this new nitrogen-selective detector appears to have great potential use for routine biomedical analyses, drug research, toxicology, environmental studies, etc. The detector has been applied in our laboratories to diverse analytical problems in connection with packed, support-coated open tubular and glass open tubular columns. Examples include the determination of caffeine in the headspace over commercial coffee samples, nitrosamines, various nitrogen-containing drugs and methoxime-trimethylsilyl derivatives of steroids. The detector selectivity is a great advantage in

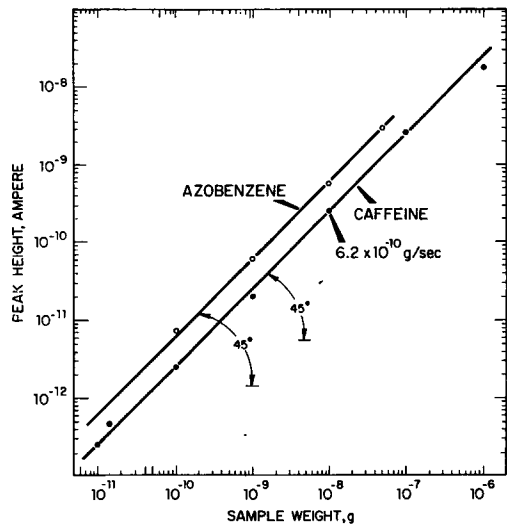
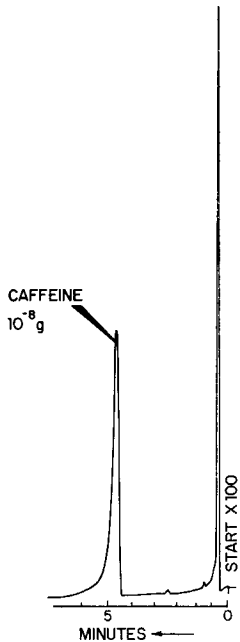


Fig. 4. Analysis of caffeine in *n*-hexane solution. The caffeine peak corresponds to $1 \cdot 10^{-8}$ g of substance. Conditions: 6 ft. \times 0.08 in. I.D. glass column packed with 3% OV-1 on Gas-Chrom Q (100–120 mesh); carrier gas (helium) flow-rate, 20 ml/min; hydrogen flow-rate, 5 ml/min; air flow-rate, 85 ml/min. Injector, 250°; column, 160°; detector, 300°.

Fig. 5. Linearity plot of the nitrogen detector for two nitrogen-containing substances.

TABLE I

STABILITY DATA

Replicate injections of methamphetamine.

Day	Peak area (arbitrary units)	
	50 ng injected	500 ng injected
1	48.0	489
2	49.5	487
3	48.7	492
4	47.9	494
5	49.2	472
10	48.4	491
30	47.9	484
	Mean 48.5	487
	Standard deviation	0.75 7.4
	Relative standard deviation (%)	1.5 1.5

many such cases, as many otherwise interfering sample constituents remain undetected.

Volatile nitrogen-containing compounds are present in abundance in biological materials of both animal and plant origin. Their appearance may be traced to many important metabolic pathways involving amino acids, purine compounds, vitamins, etc. Certain metabolic changes associated with these substrates may then be reflected in the composition of volatile nitrogen compounds. For example, Zlatkis *et al.*¹⁸ have reported that several pyrazine derivatives are found in much higher concentrations in the urine of diabetic patients compared with normal subjects. As the volatile mixtures of biologic origin are extremely complex and many nitrogen compounds present in small amounts may be relatively unstable, the combination of high-efficiency glass open tubular columns with the described detector appears to be important. Fig. 6 shows the comparison of metabolic profiles of a healthy male obtained with the flame ionization detector and the nitrogen detector, respectively.

The detector selectivity is perhaps even more dramatically shown in Figs. 7 and 8. While some peaks appear in both chromatograms A and B, the presence of nitrogen-containing compounds is strongly suggested by enhanced response.

Fig. 7 shows the chromatograms obtained by the two detectors from pre-concentrated headspace of tobacco aroma. It is known that numerous nitrogen

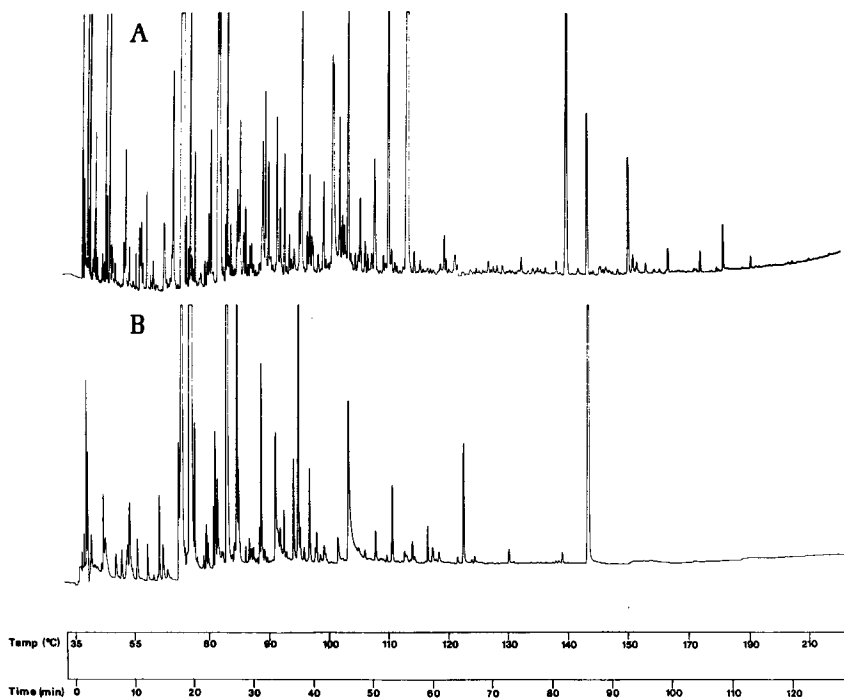


Fig. 6. Chromatograms of volatiles from a 24-h urine of a normal male. A, Flame ionization detector; B, nitrogen-phosphorus detector. Sample, 100 ml urine with 30 g $(\text{NH}_4)_2\text{SO}_4$; helium purge rate, 120 ml/min; time, 30 min; temperature, 100°. Column, 55 m \times 0.22 mm I.D. glass open tubular column coated with SF-96 silicone oil; injector, 250°; detector, 270°.

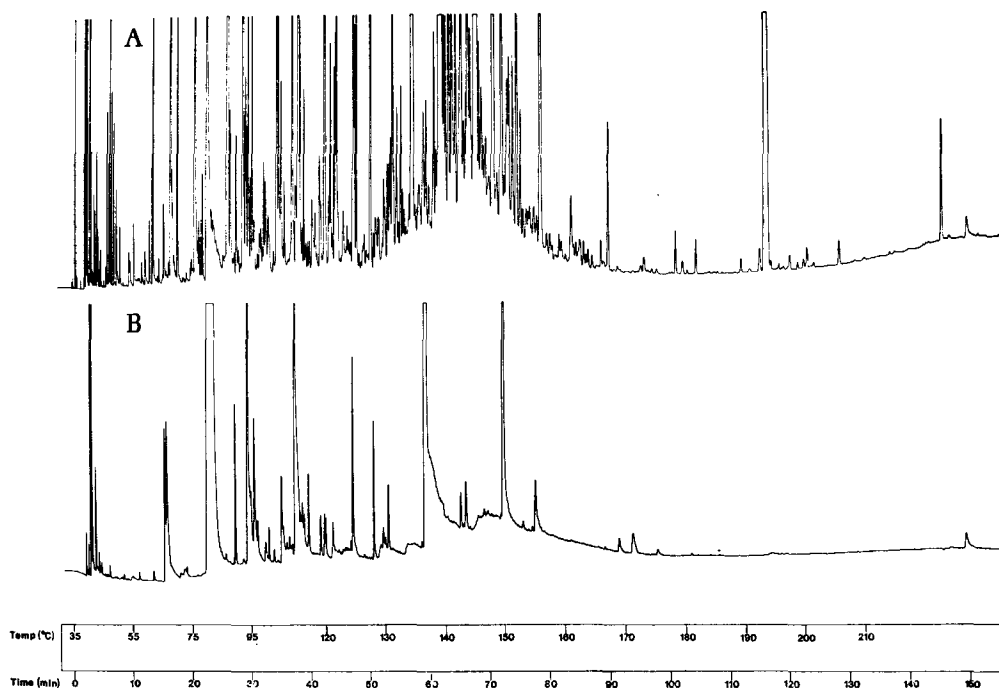


Fig. 7. Chromatograms of headspace volatiles of tobacco from standard cigarettes obtained from Tobacco-Health Research Institute, University of Kentucky, Lexington, Ky, U.S.A. A, Flame ionization detector; B, nitrogen-phosphorus detector. All conditions as in Fig. 6.

volatiles occur in this and other flavor samples; the presence of phosphorus compounds is unlikely.

With the recent emphasis on ecological problems, the development of rapid screening methods for identification of pollution sources has generated considerable interest during recent years. Bartle *et al.*¹⁵ have recently reported that the high-resolution gas chromatographic screening of airborne particulate extracts (containing mainly polynuclear aromatic hydrocarbons) is indicative of the area of sampling. Fig. 8 suggests that the potential of such chromatographic profile methods may be conveniently expanded with the use of the nitrogen-sensitive detector. In a similar fashion, the sources of oil spills were identified from typical sulphur profiles obtained with the flame photometric detector¹⁹. In addition, many trace nitrogen-containing polycyclic compounds, which are known to be potent carcinogens, may now easily be detected in complex mixtures.

CONCLUSIONS

The nitrogen-sensitive detector studied in this work has significant advantages in terms of sensitivity, selectivity, linearity and ease of operation over both previously described thermionic detectors and other nitrogen-selective detection systems^{20,21}. Its long-term stability and reliability are comparable with those of the flame ionization

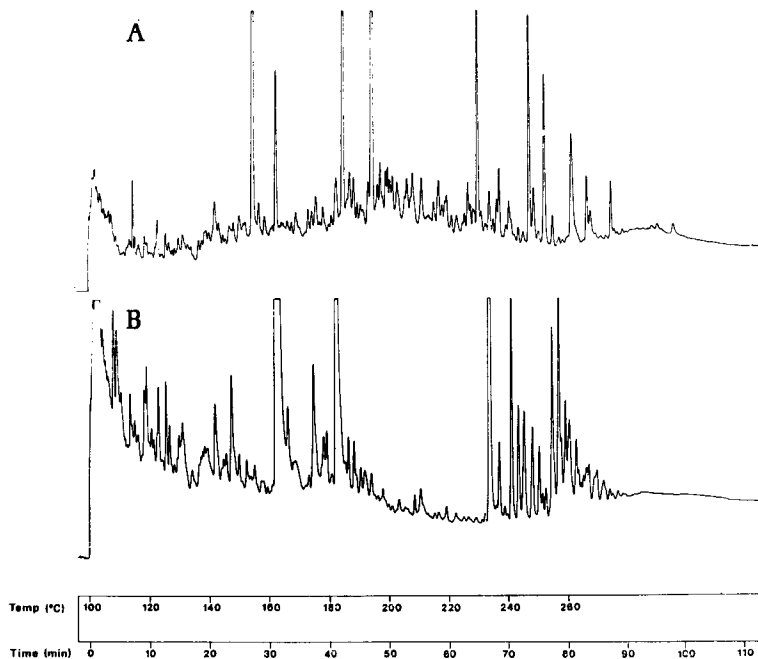


Fig. 8. Chromatograms of the nitromethane extract of air particulate matter extracted from glass fiber filters through which Indianapolis city air had been passed under standard conditions. A, Flame ionization detector; B, nitrogen-phosphorus detector. Column, 35 m \times 0.25 mm I.D. glass open tubular columns coated with SE-52 methyl phenyl silicone gum; injector, 260°; detector, 260°.

detector. While only selected applications have been considered in this paper, this detector is likely to be useful in many other analytical problems.

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