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NITROGEN-SENSITIVE THERMIONIC DETECTION IN MICROCOLUMN LIQUID CHROMATOGRAPHY

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SUMMARY

The dual-flame thermionic detector for microcolumn liquid chromatography has been improved and optimized for nitrogen sensitivity. The total column effluent is concentrically nebulized and aspirated directly into an air–hydrogen diffusion flame, while detection limits of $1.4 \cdot 10^{-11}$ g nitrogen/sec at the maximum of a Gaussian peak are achieved. Detection linearity spans three orders of magnitude. An example of the analysis of underivatized barbiturate standards is provided.

INTRODUCTION

The ability to achieve detection selectivity without sacrifice to sensitivity has become a major asset of certain detectors in gas chromatography (GC). The micro-wave-induced plasma and the flame-based thermionic, photometric, and electron capture detectors have allowed compounds containing specific heteroatoms or functional groups to be quantitatively detected in complex sample mixtures with little interference from coeluting species[1]. While the selectivity and sensitivity of these devices should be of equal value to modern high-performance liquid chromatography (HPLC), numerous technological problems have hindered their successful implementation. In particular, the relatively large (ml/min) volumetric flow-rates typical of conventional-scale HPLC introduce quantities of solvent which severely disrupt the spectral emission or selective ionization of eluting compounds. Both direct nebulization of the column effluent and introduction via transport based devices have been employed to interface HPLC systems with such detectors, but these attempts have met with little success[2–4].

The development of miniaturized HPLC has, however, allowed the total column effluent to be successfully nebulized without excessive solvent interference[5–10]. The characteristically low (μl/min) mobile phase flow-rates permit direct introduction and fairly efficient aspiration of the entire eluent and have led to the successful implementation of flame ionization[8], flame emission[5,6], and phosphorus-sensitive thermionic[7,10] detection in microcolumn HPLC. However, the detection of nitrogen-containing compounds, potentially important in the analysis of many biologically and pharmaceutically important mixtures, presents a more difficult challenge. While

the thermionic detector appears best suited to selectively monitor such substances, it is intrinsically an order of magnitude less sensitive to nitrogen than to phosphorus and care must be taken to maintain a sufficiently cool flame for maximum response[11]. These difficulties, while already significant in GC, present an additional challenge when interfacing the detector to a liquid chromatograph, where efficient aspiration of the mobile phase becomes a major concern. The lower gas flows required for a lower flame temperature tend to increase the difficulty of mobile phase nebulization, while the hotter flames produced by burning mobile phase necessitate lowering the gas flows even below those used in GC. In this investigation, modifications have been made to a previously described thermionic detector for microcolumn HPLC[5-7,9] and detection parameters have been optimized to allow the sensitive and selective detection of nitrogen-containing compounds.

The GC thermionic detector was first developed in 1964 by Karmen and Giuffrida[12], and later modified by Kolb and Bischoff[11] to increase both the sensitivity and stability of its response. The improved design, which is widely used in GC today, incorporated a constant current source, rather than the flame itself, to heat the alkali bead, which was negatively polarized with respect to the collector electrode. Nitrogen- and phosphorus-containing molecules, combusted in the flame to $CN^{\cdot}$ or $PO^{\cdot}$ and $PO^{\cdot}_2$ radicals, were detected as their radical by-products traveled to the excited alkali bead, abstracted an electron to become negatively charged, and then migrated to the positive collector, where the extra electron was donated before the neutral species were swept from the detector[11]. The alkali cation was recaptured by the negative bead, increasing the lifetime of the detector.

Thermionic detection has also been described for conventional LC using a transport interface for solvent evaporation[13,14] and for microcolumn HPLC with direct introduction of the entire column effluent into the flame[7,9,10]. This later dual-flame system, previously designed and described in its phosphorus-sensitive mode[7,10], has now been modified and optimized for nitrogen sensitivity. Through more careful control of the flame gas flows and by increasing the size of the collector electrode over 5-fold as compared to the original phosphorus-sensitive thermionic detector[7,10], among other improvements, many nitrogen-containing substances, including under-ivatized barbiturate sedatives, are now sensitively and selectively detected.

EXPERIMENTAL

Thermionic detector

The nitrogen-sensitive thermionic detector, illustrated in Fig. 1, is of the Kolb-Bischoff[11] configuration and is similar in design to the modified phosphorus-sensitive detector described previously[10]. The present detector, however, employs concentric nebulization via the Bernoulli principle to introduce the column effluent into the flame. A fused-silica capillary (10 cm × 50 μm I.D.) was inserted through the lower burner base and flame jet to approximately 1 mm above the tip of the jet and was held securely in the center of the flame by a stainless-steel guide tube, as shown in Fig. 1. The other end of this capillary was connected to the microcolumn with a PTFE frit as previously described[15]. In this manner, the total column effluent was aspirated directly into the primary diffusion flame by the concentric flow of the flame and nebulizing gases over the capillary orifice.
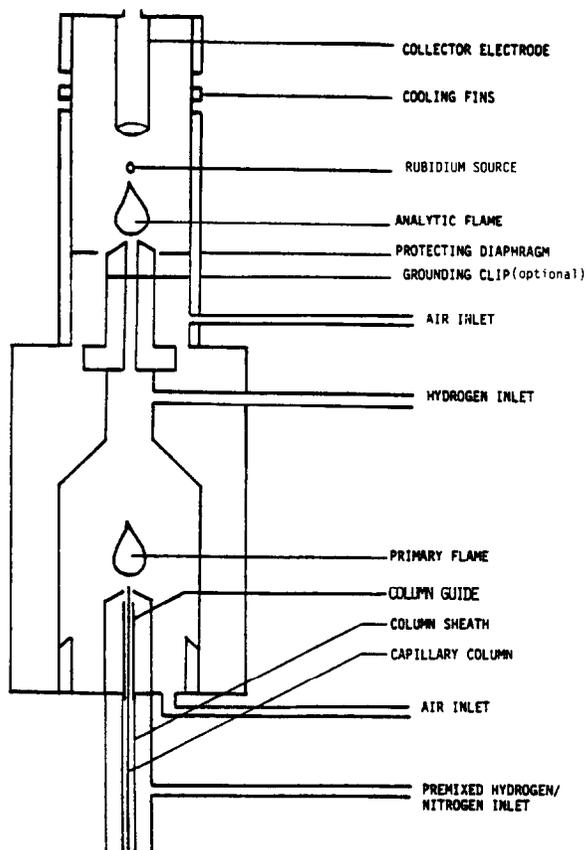
Fig. 1. Schematic diagram of the nitrogen-sensitive thermionic detector for microcolumn HPLC.

Chromatographic system

Slurry-packed capillary columns prepared as previously described[16] and terminated with a PTFE end-frit[15] were utilized in this study. A high-pressure syringe pump (Model 8500, Varian, Palo Alto, CA, U.S.A.) was operated in the constant pressure mode to provide steady mobile phase flow through the column. Samples were injected in 0.25-μl aliquots using direct, stopped-flow injection[17].

Reagents

Both tetramethylthiourea (reagent grade, Aldrich, Milwaukee, WI, U.S.A.) and secobarbital in its free acid form were employed as model solutes in the optimization and characterization of the detector. The aminophyline, caffeine, diethylamine, N,N-diethylaniline, pyrazine, pyridine, pyrrole-2-carboxaldehyde, and quinoline utilized in measuring the detector's response to compounds of varying structure were all reagent grade (Aldrich). The barbiturate sedatives pento-, pheno-, and secobarbital, as well as barbital itself, were obtained as their respective sodium salts. They were converted to the free acid form with 0.15 M sulfuric acid and subsequently extracted into chloroform. All samples were injected onto the chromatographic column without prior derivatization.

All solvents with the exception of acetone, which was spectroquality, were of HPLC grade (Fisher Scientific, Fair Lawn, NJ, U.S.A.), and were passed through a 0.2-μm filter (Rainin, Woburn, MA, U.S.A.) to remove particulates.

RESULTS AND DISCUSSION

Detector optimization

In general, the sensitivity and selectivity of the thermionic detector rely heavily on a great many variables[11,12], most of which are a function of the specific design of the individual detector. The shape and location of the flame housing, alkali bead, and collector electrode are only three examples of parameters which may influence the performance of the detector. The exact composition of the alkali salt from which the bead is made can also profoundly influence the detection response. These structural considerations were held constant insofar as possible throughout the investigation. The capillary and flame jet dimensions were similar to those previously described[7]. The detector was nominally optimized by independently varying the gas flow-rates and bead heating current in a linear fashion. In addition, the signal was also monitored with the analytical flame jet held at both ground and -200 V to determine the effect of this parameter. The optimization was performed using tetramethylthiourea in 100% methanol as a model solute.

As in the earlier described[7,10] phosphorus-sensitive detector, the addition of a non-combustible gas was found to be necessary for proper nebulization efficiency. Both nitrogen and helium were tested in this capacity, and it was found that the nitrogen gave slightly lower flame temperatures. It is evident from Fig. 2 that the exact rate of the nitrogen flow did not significantly effect the signal-to-noise (S/N) ratios obtained, provided the flow was within 50–120 ml/min. A value of 70 ml/min was chosen for routine detector operation. In reality, the organic solvents of the mobile phase were probably combusted, rather than nebulized, at the capillary orifice so that the nitrogen served mainly to lift the column effluent into the flame and not
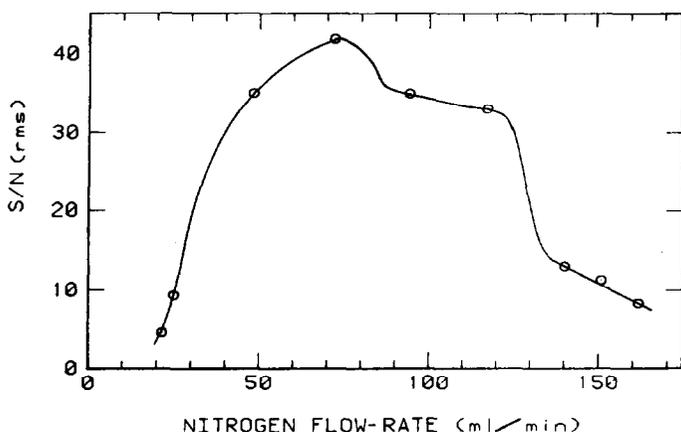
Fig. 2. Thermionic detector response as a function of nitrogen gas flow-rate in the primary flame. Column: capillary column, 1 m × 240 μm I.D., packed with Spherisorb 5 μm ODS; mobile phase: 100% methanol (1.2 μl/min); solute: tetramethylthiourea (16 ng nitrogen injected).
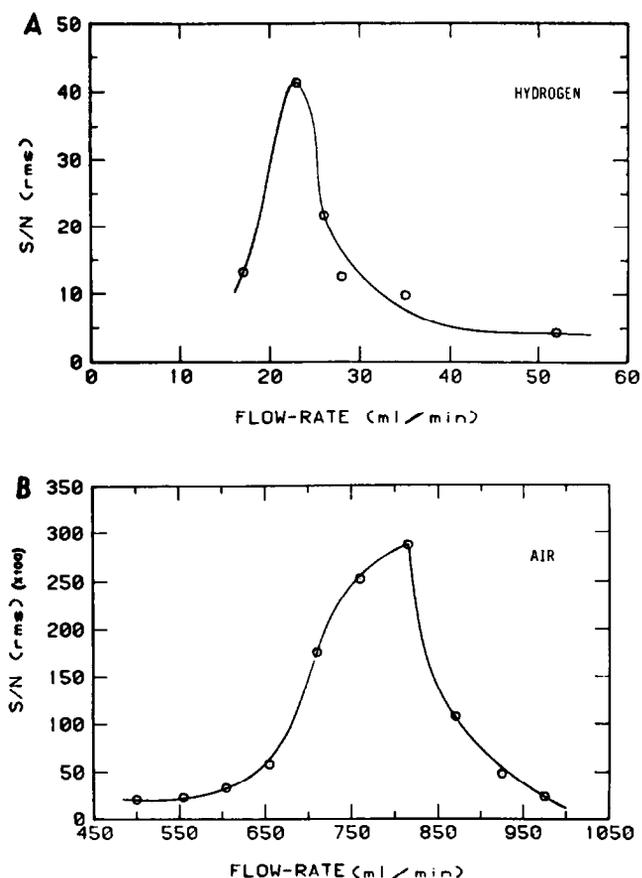
Fig. 3. Thermionic detector response as a function of the flame gas flow-rates in the primary flame. A, Hydrogen; B, air. Chromatographic conditions as in Fig. 2.

to truly nebulize it. Indeed, with the flame housing removed, the mobile phase could be seen to burn over a distance of 1–2 mm above the tip of the capillary.

The spatial separation of the primary combustion and secondary analytical flames allowed the independent optimization of the gas flows to each flame individually. In the primary flame, varying the hydrogen flow from 15 to 55 ml/min yielded a rather sharp optimum at 22 ml/min, as illustrated in Fig. 3A. Below approximately 15 ml/min a stable flame could no longer be maintained while solvent was flowing, causing flame noise to predominate and S/N values to drop sharply. At high hydrogen flows, the increased flame temperature inhibited the formation of the requisite CN· radicals[11], and the signal dropped, while the noise remained relatively constant, again leading to a decrease in the corresponding S/N measurements. The air flow to the primary flame was varied between 475 and 975 ml/min, as presented in Fig. 3B. At flows below 820 ml/min, increasing the oxidizing nature of the flame significantly increased the signal level. Higher air flows, however, served only to facilitate solute aspiration, and rapid increases in the background noise level frequently negated this benefit. The optimum air flow was significantly higher than that reported earlier for

either gas[11] or liquid[7,9] chromatographic detectors. With a fuel-to-oxidant ratio of only 0.1, the combustion flame was highly oxidizing, and the combustible organic solvents of the mobile phase undoubtedly served as an auxiliary fuel source.

The gas flows to the analytical flame served mainly to direct the flow of solutes upward and to cool the alkali bead. Therefore, they played a negligible role in altering the signal intensity once the flows were sufficient to carry the solutes. Rather, their main effect was on the background noise level, which increased with increasing amounts of fuel, but remained remarkably constant across the range of air flows examined, as shown in Fig. 4A and B, respectively.

The importance of a relatively cool bead temperature was further demonstrated by the effect that varying the bead-heating current had on the S/N levels obtained. The temperature of the bead was systematically raised by increasing the current passing through it until the bead glowed a bright red. As the bead temperature increased, the signal intensity remained relatively constant; however, the background noise level increased exponentially with a linear increase in bead-heating current. As a result, the S/N level decreased dramatically. These results, while in direct conflict with those
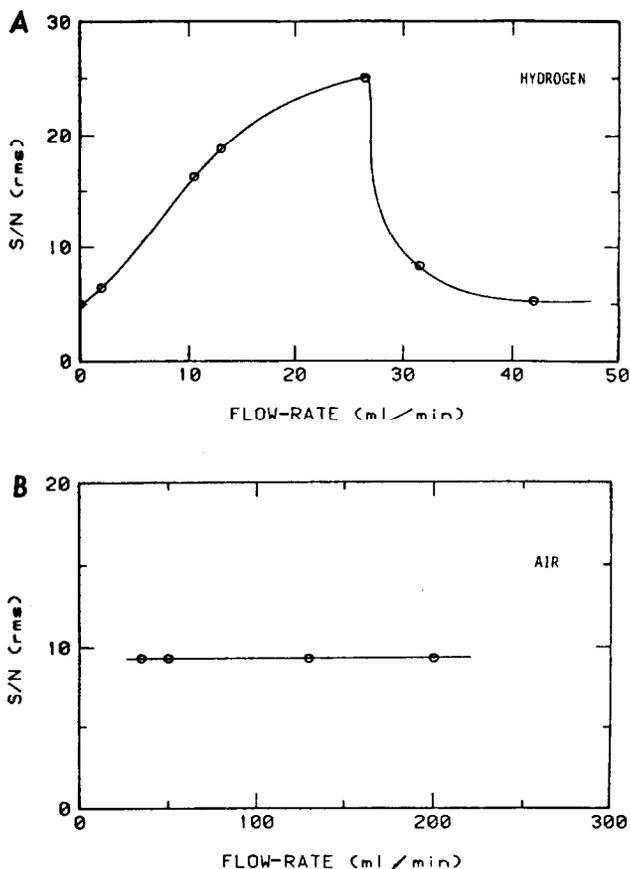

Fig. 4. Thermionic detector response as a function of the gas flow-rates in the analytical flame. A, Hydrogen; B, air. Chromatographic conditions as in Fig. 2.
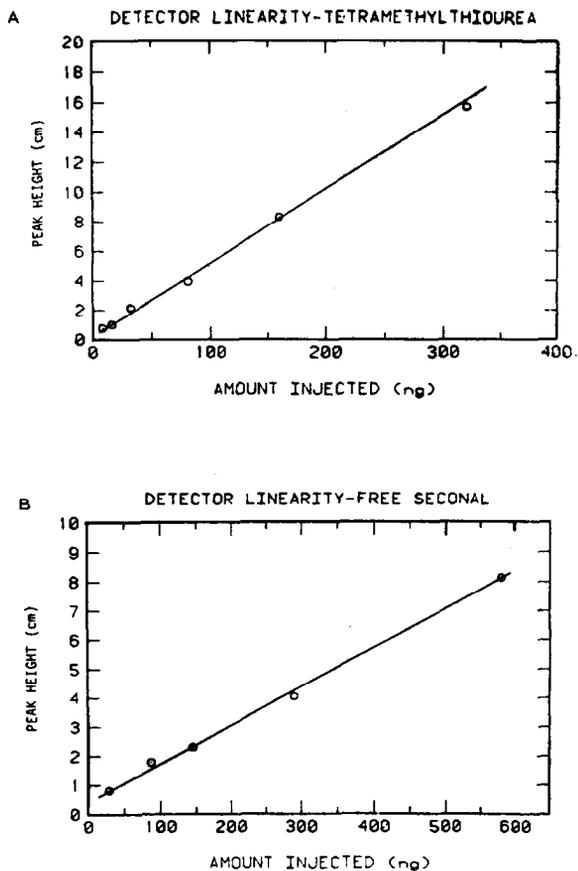
A   DETECTOR LINEARITY-TETRAMETHYLTHIOUREA

B   DETECTOR LINEARITY-FREE SECONAL

Fig. 5. Linearity of the thermionic detector response to nitrogen-containing species as a function of injected mass. Chromatographic condititions as in Fig. 2, except for solute. A, Tetramethylthiourea; B, secobarbital (free acid).

reported for GC thermionic detectors[11], correspond well with the trends described for phosphorus-sensitive thermionic detection in LC[7,9] and are, no doubt, due to the increased operating temperature which arises from the combustion of organic solvents in the mobile phase.

The potential of the analytical flame jet was also varied by holding it both at ground and at the same potential as the alkali bead, -200 V with respect to the collector electrode. The greatest signals were obtained at the later potential, although grounding the jet slightly reduced the baseline noise level by minimizing the background ion current, since many ions created from combustion in the primary flame were conducted to ground before reaching the collector. Because the detection of nitrogen-containing compounds was most often signal, rather than noise, limited, the jet was maintained at -200 V. Placing a Faraday cage around the detector body and electrically isolating the gas inlet lines also improved the background noise level by minimizing environmental interferences.

Detector characterization

The optimal response of the nitrogen-sensitive thermionic detector was determined using tetramethylthiourea in 100% methanol as a minimally retained model solute. Detection limits were established using a previously reported method[6]. The minimum detectable quantity of nitrogen was found to be an injected amount of 500 pg, corresponding to a mass flux of 14 pg nitrogen per sec at a S/N ratio (root mean square) of 5 (99.9% confidence level).

The linear dynamic range of the detector was investigated for two individual model solutes, as shown in Fig. 5. The response for tetramethylthiourea was linear over three orders of magnitude from the detection limit to more than 300 ng of injected nitrogen (8.3 ng/sec), at which point the solute began to precipitate in the injection capillary. The barbiturate secobarbital, on the other hand, yielded a linear response for up to 600 ng of injected nitrogen (16.7 ng/sec). The study was terminated at this solute concentration to avoid contamination of the detector's interior, although the response linearity was maintained.

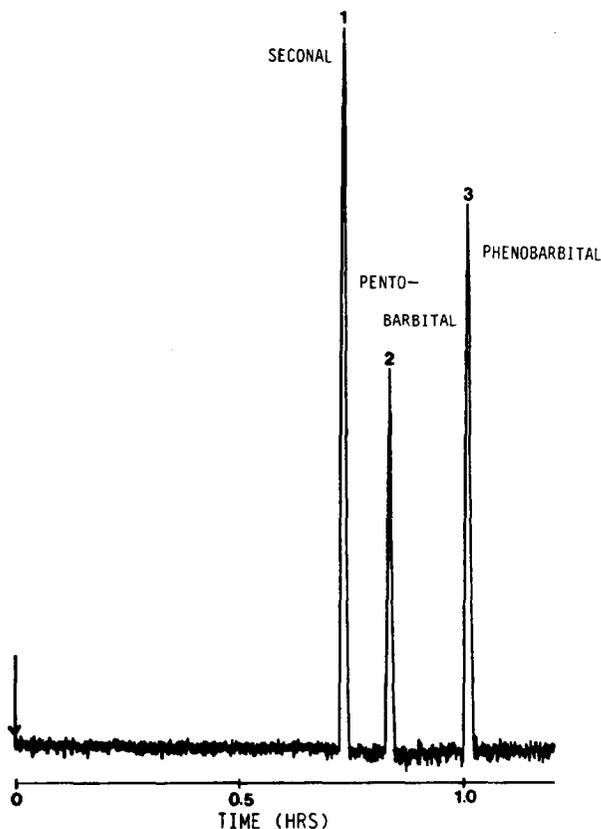The role played by the structure and volatility of the solute molecules was

Fig. 6. Chromatogram of three barbiturate standards in their free acid form. Column, 1.8 m × 200 μm I.D., packed with Spherisorb 5 μm C$_8$ packing; mobile phase: 100% methanol (1.3 μl/min); injected amount: 20 ng nitrogen per compound.

briefly explored using several compounds of varying structures and molecular weights. The compounds included in this investigation were diethylamine, pyridine, pyrazine, pyrrole-2-carboxaldehyde, quinoline, N,N-diethylaniline, caffeine, aminophylline, and the barbiturates amo-, pento-, pheno-, and secobarbital, as well as barbital itself. Injected at the 20 ng level, equally prominent signals were obtained for all compounds of similar volatility, indicating equal detector sensitivity both for compounds incorporating nitrogen into an aromatic structure and for aliphatic nitrogen-containing molecules, provided CN· radicals could be formed on combustion. Some discrimination in response sensitivity did occur on the basis of compound volatility. This presents a difficult, general problem in the detection of nitrogen-containing species, since increasing the flame temperature to increase nebulization efficiency, simultaneously reduces the probability of CN· radical formation.

Applications

The ability to selectively detect nitrogen-containing compounds in complex sample matrices is potentially important in the analysis of many polynuclear heteroatomic species in fossil-fuel related mixtures, alkaloids in plant materials, nitrogen-containing drugs and their metabolites in physiological fluids, etc. Since a variety of pharmaceutically important compounds contain nitrogen, and there is a great need to monitor certain potentially toxic ones among them at the low concentrations typically found in the human blood stream[18,19], the barbiturate sedatives were chosen to demonstrate the applicability of the thermionic detector to such analyses. The sedatives barbital, pentobarbital, phenobarbital, and seconal were converted to their free-acid forms, separated by capillary column LC, and readily detected at the nanogram level, as shown in Fig. 6.

Uses of nitrogen-sensitive detection, in various instances where elaborate sample prefractionation schemes are normally needed, should be quite attractive. Future applications can greatly benefit from this and similar element-specific detectors.

ACKNOWLEDGEMENT

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