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MICRO-COLUMN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND FLAME-BASED DETECTION PRINCIPLES

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SUMMARY

Novel detectors are described for micro-column high-performance liquid chromatography; they are based on the well-known principles of flame photometric and thermionic detection. These devices exhibit good selectivity and sensitivity for organophosphorus compounds in both aqueous and selected organic solvents. The extremely small volume of these flame-based detectors and their enhanced response at low flow-rates make them particularly attractive for capillary-column high-performance liquid chromatography.

INTRODUCTION

Miniaturized high-performance liquid chromatography (HPLC) systems are rapidly gaining recognition in modern separation science because of their very high efficiency and reduced consumption of sample and solvent. In recent communications, Guiochon[1] and Knox and Gilbert[2] discussed the theoretical and practical potential of such systems with optimistic conclusions, yet both papers emphasized the necessity for low-volume, sensitive detectors in order to realize the full potential of capillary-column technology.

Conventional concentration-sensitive detectors, such as UV absorbance[3], fluorescence[4], and electrochemical[5] devices, have been modified to incorporate low-volume (*ca.* 0.1 μl) flow-cells. Although such devices are adequate at the current stage of column development, the consequences of further cell-volume reduction become immediately apparent. It will be necessary in the future to develop certain detection and ancillary techniques that are inherently well suited to the unique conditions of micro-column HPLC. Among such techniques, direct-interface micro-liquid chromatography–mass spectrometry (LC–MS) has recently been demonstrated[6,7] and shows promise in its application to complex samples of limited volatility. Similarly, direct-interface Fourier-transform IR spectroscopy should be feasible, and will greatly facilitate solute identification. In addition, detectors based on a transport system or flame should also benefit greatly from the reduced flow-rates characteristic of micro-columns.

Flame-based detection has been largely unsuccessful in conventional HPLC because the large volume of solvent disrupts the delicate balance of chemical and

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physical processes that occur in the flame. Chemical interference severely limits the range of compatible mobile phases. In addition, the introduction of combustible organic solvents changes the temperature and fuel-to-oxidant ratio of the flame, thus influencing detector response and restricting the use of gradient elution. Despite the inherent incompatibility of these methods, there have been several attempts to use flame-based detection in conventional HPLC.

Julin *et al.*[8] described a flame photometric detector (FPD) in which the HPLC effluent was pneumatically nebulized, and a fraction (25%) was aspirated into a cool, hydrogen-rich diffusion flame. Phosphorus and sulfur compounds were selectively detected by monitoring the optical emission of the HPO (526 nm) and S_2 (384 nm) species. Under favorable conditions, this device was capable of sensing $2 \cdot 10^{-5}$ g/l of phosphorus, and $2 \cdot 10^{-4}$ g/l of sulfur. Organic solvents severely quenched emission from the chemiluminescent species, so that applications of this detector were limited to purely aqueous systems. Chester[9,10] later developed an inverted-flame FPD for conventional HPLC in which interference by many organic solvents, metal ions and buffers was minimized. However, the detection limits obtained with this modified FPD were not significantly improved; $1 \cdot 10^{-5}$ g/l of phosphorus could be detected with continuous sample introduction.

Flame ionization[11] and thermionic[12,13] detectors have been utilized in HPLC with a transport system for solvent evaporation before analysis. Such devices permit the use of a wide range of solvent systems and of gradient elution, but the sensitivity and stability are frequently inadequate for routine applications. The transport system introduces a significant amount of band dispersion and limits the linear dynamic range of the detector. The volatility of lower-molecular-weight solutes may further limit the accuracy of quantitative measurements.

In contrast to conventional HPLC columns, micro-columns (and capillary columns in particular) are very well suited to selective flame-based detection. The reduced flow-rate of solvent, typically 1 μl/min for open-tubular or packed capillary columns, may be directly introduced with minimal disruption of the flame.

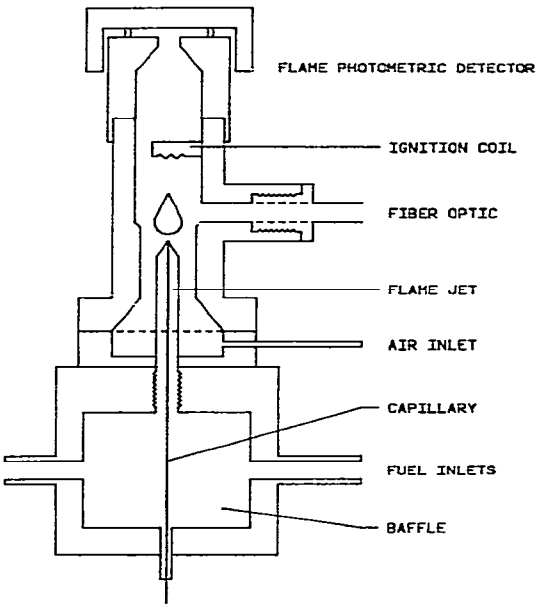
A novel flame-based detector has been developed and characterized, in which the total capillary-column effluent is concentrically nebulized and aspirated directly into a hydrogen–air diffusion flame. Solutes are then selectively detected using either optical emission or secondary ionization phenomena. This preliminary report illustrates the performance and potential of these versatile new detectors, which are believed to be the predecessors of other useful flame-based devices for micro-column liquid chromatography.

EXPERIMENTAL

Flame photometric detector

The flame base and housing are shown schematically in Fig. 1 and have been described previously[14].

Hydrogen (55 ml/min) and nitrogen (90 ml/min), a non-combustible nebulizing gas, are pre-mixed in a baffle and flow through a stainless-steel flame jet (0.76 mm I.D.). Purified air (75 ml/min) is supplied to the fuel-rich flame by diffusion through a fritted metal disk. A glass capillary (50 μm I.D., 0.6 mm O.D., and 10 cm in length) is inserted through the baffle, burner base and flame jet, and extends approximately 1



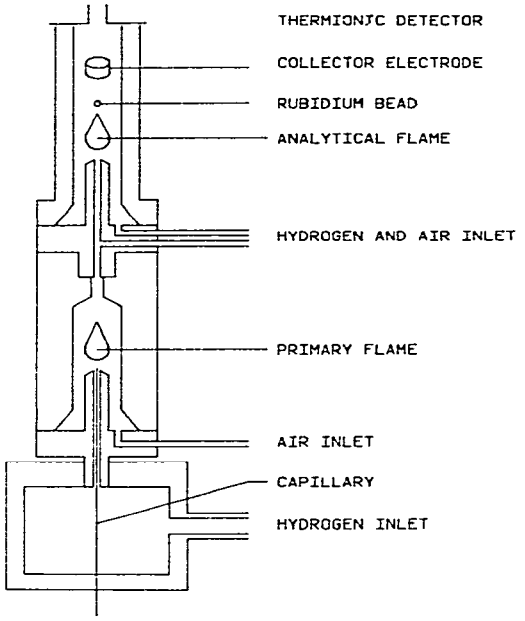


Fig. 1. Schematic diagram of the flame photometric detector for capillary-column HPLC.

Fig. 2. Schematic diagram of the dual-flame thermionic detector for capillary-column HPLC.

mm above the top of the flame jet. The other end of this capillary is connected to the HPLC micro-column with shrinkable PTFE tubing. The total column effluent is nebulized by the concentric flow of gases over the orifice of the capillary.

A fiber-optics probe is fixed in the wall of the housing, and views the cooler central region of the diffusion flame. The emission from the chemiluminescent species is then measured with an interference filter (530 nm, FWHM 14 nm) and photomultiplier tube (Hamamatsu Corp., Model R-372). The electrical signal from the photomultiplier is amplified by a picoammeter (RCA, Model WV-511A) and filtered to remove high-frequency noise; the output is then displayed on a strip-chart recorder (Shimadzu, Model R-101).

Thermionic detector

A schematic diagram of the dual-flame thermionic detector for micro-column HPLC is shown in Fig. 2. The baffle and lower burner base are similar to those described for the FPD. The lower flame housing is constructed of aluminum and includes an ignition coil and a thermocouple to monitor flame conditions.

Hydrogen (60–70 ml/min) and nitrogen (90 ml/min) are pre-mixed in the baffle, and nebulize the micro-column effluent at the glass-capillary orifice; purified air (140 ml/min) is supplied to the flame by diffusion through a fritted metal disk. Organic solutes and solvents are decomposed in the lower primary flame; the combustion products are combined with additional fuel (50–65 ml/min of hydrogen) and are swept into the analytical flame, which is also supplied with air by diffusion (280 ml/min). The analytical flame is not allowed to ignite and burn freely; the best response is obtained when combustion is carefully controlled in the region near the

alkali bead. If the upper flame should accidentally ignite, a large increase in background ion current is observed.

The alkali bead (rubidium nitrate), fabricated according to the procedure of Lubkowitz *et al.*[15], is positioned 1–2 mm directly above the analytical flame jet. This bead is electrically heated by a controlled-current source (Perkin-Elmer). The cylindrical collector electrode is polarized to 200 V by a high-voltage power supply (Keithley, Model 244), and the upper flame jet is grounded to minimize solvent background. The ion current is amplified by using an electrometer (Perkin-Elmer), filtered to remove high-frequency noise and then displayed on a strip-chart recorder (Linear, Model 355).

Liquid chromatograph

A syringe pump (Varian, Model 8500) was utilized in this investigation to minimize fluctuations in pressure and flow-rate, which were expected to significantly affect the mass-sensitive FPD and the thermionic detector.

Packed micro-capillary HPLC columns were prepared as described previously[14]; octylsilane and octadecylsilane bonded phases were utilized in this investigation. Samples (0.2 μl) were injected directly on to the column as described by Hirata and Novotný[4].

A variable-wavelength UV detector (Jasco Uvidec 100-II), with modified flow-cell (*ca.* 0.1 μl), was used for comparison of response and dead volume with those of the flame-based detectors.

Reagents

Trimethyl phosphate, a model solute used to characterize the flame-based detectors, was obtained from Aldrich.

Organophosphorus pesticide standards were of "qualitative grade", and were purchased from Anspec. Cygon [O,O-dimethyl-S-(N-methylcarbamoylmethyl) phosphorothioate; 98% purity], DDVP (2,2-dichlorovinyl phosphate; 93% purity), ethion [O,O,O′,O′-tetraethyl-S,S′-methylenebis(phosphorothioate); 95% purity], guthion [O,O-dimethyl-S-(4-oxo-1,2,3-benzotriazin-3(4H)-ylmethyl) phosphorothioate; 99% purity], malathion [O,O-dimethyl-S-(dicarbethoxyethyl)dithiophosphate, 95% purity] and zolone [O,O-diethyl-S-(6-chloro-2-oxybenzoxazolin-3-ylmethyl)phosphorodithioate; 98% purity] were used as standards in this investigation.

"Nanograde" methanol was purchased from Mallinckrodt; water was deionized, distilled, and finally redistilled from alkaline permanganate.

RESULTS AND DISCUSSION

Flame photometric detector

The FPD was nominally optimized in the phosphorus mode by independently varying the hydrogen, nitrogen and air flow-rates to obtain the maximum signal-to-noise ratio. The temperature of the central portion of the flame under optimum conditions was 400–450°C (measured with a thermocouple), when water was aspirated at 1 μl/min. The optimum fuel-to-oxidant ratio was 3.7, which is comparable with that of the gas chromatographic detector of this type[16]. These results confirmed the work of Dagnall *et al.*[17]: the HPO species is formed most efficiently in a cool, highly reducing flame.

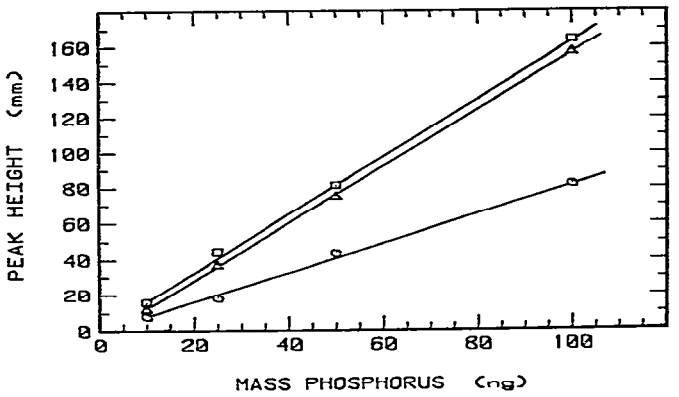


Fig. 3. Linearity of the flame photometric detector as a function of mobile phase composition. Column: 10-m octylsilane micro-column. Mobile phase: ○, pure water; □, 10% aqueous methanol; △, 25% aqueous methanol. Solute: trimethyl phosphate.

The effect of selected organic solvents on phosphorus emission was studied using trimethyl phosphate as a model solute. Methanol, ethanol, acetone and acetonitrile were added to the aqueous mobile phase in concentrations ranging from 0 to 50%. For moderate concentrations of alcohol or acetone, a slight enhancement in emission intensity was observed. As shown in Fig. 3, a twofold enhancement was achieved when 10% aqueous methanol was used as mobile phase. Methanol could be tolerated in concentrations up to 50% with no loss in signal intensity; however, the flame background was substantially increased. Similarly, ethanol and acetone provided small increases in analytical sensitivity, and could be tolerated in concentrations up to 40%. In contrast, acetonitrile severely quenched phosphorus emission and increased background noise even at very low concentrations (1–5%). Similar results were reported by Chester[9,10] in an investigation of solvent effects on the inverted-flame FPD in conventional HPLC.

The increase in emission intensity in the presence of alcohols and acetone does not indicate that quenching of the type described by Julin *et al.*[8] and Dagnall *et al.*[17] does not occur in this detector. It merely suggests that vast improvement in other areas may overshadow any slight decrease in emission due to chemical quenching in the flame. The signal enhancement is most probably due to changes in the physical properties of the HPLC effluent, such as surface tension, viscosity, volatility and combustibility, which result in improved nebulization and desolvation.

The flame-based detector was able to accept in excess of 20 μl/min of 10–25% aqueous methanol without extinction of the flame or production of luminous soot. The best response was obtained at flow-rates less than 5 μl/min, which is typical of open-tubular and packed capillary HPLC columns.

The detection limits were determined according to the method of McGuffin and Novotny[14], with trimethyl phosphate as a model solute under non-retained conditions. The minimum detectable quantity was 2.0 ± 0.1 ng of phosphorus at a signal-to-noise (RMS) ratio of 5 (99.5% confidence level). This corresponds to a mass flux of 71 ± 3 pg/sec at the maximum of the Gaussian peak.

The linear range of this detector was determined for mobile phases containing

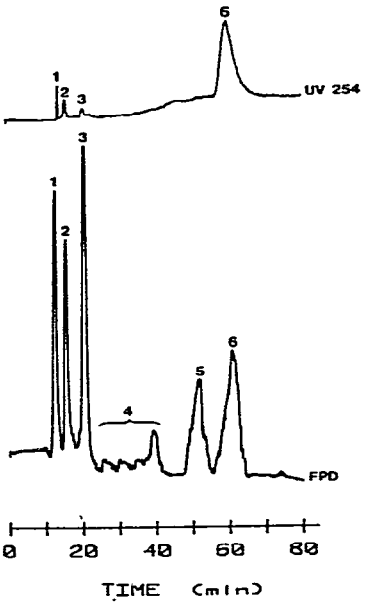


Fig. 4. Chromatogram of organophosphorus pesticides using the FPD. Column: 10-m octylsilane micro-column. Mobile phase: 42% aqueous methanol (50 atm). Peaks: 1 = solvent front; 2 = cygon (80 ng of phosphorus); 3 = DDVP (280 ng of phosphorus); 4 = phosphorus-containing impurities; 5 = malathion (230 ng of phosphorus); 6 = guthion (200 ng of phosphorus). UV detector range: 0.02.

pure water, 10% aqueous methanol and 25% aqueous methanol. The results summarized in Fig. 3 indicate that response is linear for all three solvent systems from the detection limit to at least 100 ng of phosphorus.

To illustrate the potential of the FPD, a mixture of four structurally diverse organophosphorus pesticides was separated on a short reversed-phase micro-column. The response of the FPD was compared with UV detection at 254 nm; the sensitivity of the UV detector was adjusted to give approximately the same signal-to-noise ratio as the FPD for the last peak (guthion). As shown in Fig. 4, the FPD exhibited good sensitivity and selectivity for all pesticides, and additionally revealed the presence of three phosphorus-containing impurities in the pesticide standards. In general, flame photometric detection was superior to UV detection whenever the molar absorptivity (ε) of the phosphorus-containing solute was less than 500 to 800 l/mol·cm at the wavelength of interest.

Although this investigation has been limited to the determination of organophosphorus compounds, flame-emission detection may also be utilized for sulfur, nitrogen, boron, and some organometallic species.

Thermionic detector

The dual-flame thermionic detector spatially separates the analytical measurement process from the fundamental flame processes, such as nebulization and desolvation. For this reason, higher concentrations of organic modifiers may be added to the mobile phase with less matrix interference. The thermionic detector has been

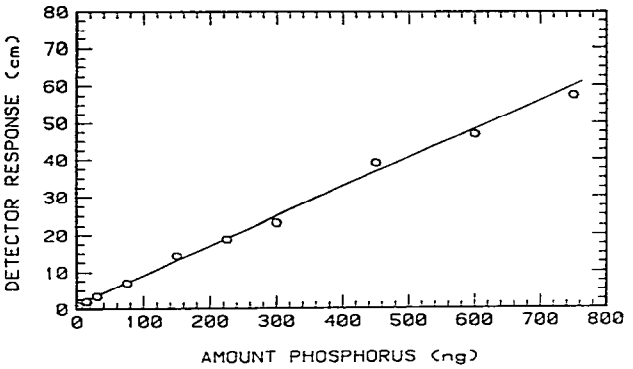


Fig. 5. Linearity of thermionic detector. Column: 10-m octylsilane micro-column. Mobile phase: methanol. Solute: trimethyl phosphate.

utilized with mobile phases containing 75 to 100% aqueous methanol; gradient elution is also feasible with minimal baseline drift. In addition, pump pulsations seem to have little, if any, effect on the detector response.

Although the device has not as yet been optimized, the sensitivity seems quite good. From preliminary experiments, the detection limit appears to be at least one, and possibly two, orders of magnitude less than that of the FPD. An injected quantity of 2 ng of phosphorus was clearly visible above the background noise [signal-to-noise (RMS) ratio >30].

The linear dynamic range of the thermionic detector was investigated using

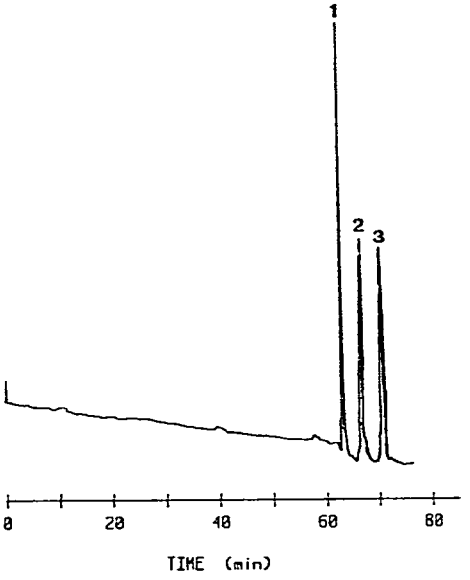


Fig. 6. Chromatogram of organophosphorus pesticides using thermionic detection. Column: 27-m octadecylsilane micro-column. Mobile phase: 85% aqueous methanol (120 atm). Peaks: 1 = guthion (97 ng of phosphorus); 2 = zolone (87 ng of phosphorus); 3 = ethion (130 ng of phosphorus). Detector range: 16 × 1.

trimethyl phosphate as a model solute and methanol as mobile phase. As shown in Fig. 5, the device was linear from 2 ng up to at least 750 ng of phosphorus injected.

To demonstrate the potential of the thermionic detector, several of the more strongly retained organophosphorus pesticides were chromatographed on an octadecylsilane capillary column, with 85% aqueous methanol as mobile phase. The high sensitivity and low background noise characteristic of the dual-flame thermionic detector are illustrated in Fig. 6; guthion, zolone and ethion were easily detected in amounts ranging from 87 to 130 ng of phosphorus.

In these preliminary investigations, the thermionic detector has been utilized primarily for the determination of organophosphorus compounds; however, selectivity has also been demonstrated for some nitrogen-containing compounds, such as caffeine and pyrazine.

CONCLUSION

A novel flame-based detector has been developed, which benefits highly from the reduced flow-rates of capillary HPLC columns. The total micro-column effluent is nebulized and aspirated directly into a hydrogen–air diffusion flame. Solutes may then be selectively detected by their characteristic optical emission or secondary-ionization phenomena.

The total-consumption burner utilized in this investigation provides efficient use of the small samples and low flow-rates associated with capillary column HPLC. In addition, it ensures that a representative fraction of the effluent reaches the flame; thus, no pre-concentration of volatile solutes or solvents occurs, as it might with separate, heated nebulizer chambers. Another advantage is the very small dead volume of this design; the end of the capillary column may be inserted directly into the flame. Although this investigation has been limited to packed capillaries, the detectors should also be compatible with open-tubular columns, provided that chemically bonded stationary phases are utilized.

The FPD and the thermionic detector exhibit high selectivity and good sensitivity for organophosphorus compounds. Moreover, the detectors permit the use of a wide range of organic mobile-phase concentrations; thus, sufficient variety is available to design chromatographic systems that will accomplish useful separations.

The selective detection of nitrogen-containing compounds will be the desirable extension of this research. Potential applications include the determination of nitrogen-containing drugs and their metabolites in physiological fluids, the detection of polynuclear heteroaromatic compounds in energy-related samples, and the determination of alkaloids in plant material. A more thorough characterization and optimization of the thermionic detector will be necessary before routine application is possible.

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REFERENCES

1 G. Guiochon, *J. Chromatogr.*, 185 (1979) 3.
2 J. H. Knox and M. T. Gilbert, *J. Chromatogr.*, 186 (1979) 405.
3 Y. Hirata, M. Novotny, T. Tsuda and D. Ishii, *Anal. Chem.*, 51 (1979) 1807.
4 Y. Hirata and M. Novotný, *J. Chromatogr.*, 186 (1979) 521.
5 Y. Hirata, P. T. Lin, M. Novotný and R. M. Wightman, *J. Chromatogr.*, 181 (1980) 287.
6 J. D. Henion and G. A. Maylin, *Biomed. Mass Spectrom.*, 7 (1980) 115.
7 J. D. Henion, *J. Chromatogr. Sci.*, 19 (1981) 57.
8 B. G. Julin, H. W. Vandenborn and J. J. Kirkland, *J. Chromatogr.*, 112 (1975) 443.
9 T. L. Chester, *Anal. Chem.*, 52 (1980) 638.
10 T. L. Chester, *Anal. Chem.*, 52 (1980) 1621.
11 R. P. W. Scott, *Liquid Chromatography Detectors*, Elsevier Scientific Publishing Co., New York, 1977.
12 K. Šlais and M. Krejčí, *J. Chromatogr.*, 91 (1974) 181.
13 B. J. Compton and W. C. Purdy, *J. Chromatogr.*, 169 (1979) 39.
14 V. L. McGuffin and M. Novotny, *Anal. Chem.*, 53 (1981) 947.
15 J. A. Lubkowitz, B. P. Semonian, J. Galobardes and L. B. Rogers, *Anal. Chem.*, 50 (1978) 673.
16 S. S. Brody and J. E. Chaney, *J. Gas Chromatogr.*, 4 (1966) 42.
17 R. M. Dagnall, K. C. Thompson and T. S. West, *Analyst (London)*, 93 (1968) 72.