Journal of Chromatography, 112 (1975) 443–453

CHROM. 8492

SELECTIVE FLAME EMISSION DETECTION OF PHOSPHORUS AND SULFUR IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A selective detector for phosphorus and sulfur based on flame emission has been developed for use with high-performance liquid chromatography. Up to 5 ml/min of the total column effluent is nebulized and directed into a cool hydrogen–nitrogen flame. In this flame, phosphorus- and sulfur-containing compounds emit characteristic emissions at 526 and 383 nm, respectively, which are measured by a simple band-pass filter/photomultiplier system. In favorable situations, the apparatus permits the detection of about $2 \cdot 10^{-8}$ and $2 \cdot 10^{-7}$ g/ml of phosphorus and sulfur, respectively, in the column effluent. However, with optimization of the system, sensitivity may be increased by an order of magnitude.

The flame emission detector can be used for compounds containing phosphorus and sulfur in different forms. Excellent short- and long-term quantitation has been experienced in trace analysis. To date, application of the detector has been limited to systems with aqueous mobile phases since significant concentrations of organic materials affect detector sensitivity. Factors influencing the signal-to-noise response of the detector have been studied. These include: mobile phase nebulization rate, hydrogen, nitrogen and air flow-rates, and the effect of organic materials and salts.

INTRODUCTION

High-performance liquid chromatography (HPLC) has become a very important tool in analytical chemistry; however, the availability of suitable detectors generally has lagged behind developments in column packings and other equipment. There are a number of selective detectors for HPLC that are particularly useful for trace analysis (*e.g.*, fluorimetric, amperometric)[1]. However, there is further need for sensitive detection devices that permit the selective measurement of trace compo-

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nents separated in HPLC. We wish to describe the design, characteristics, and use of a flame emission detector for phosphorus- and sulfur-containing compounds in HPLC.

The principle of detection for sulfur or phosphorus is based on flame excitation in a hydrogen-rich flame. The detector itself is somewhat similar to the flame photometric detector widely used in gas chromatography[2], but uses a special burner assembly to handle the total liquid effluent from HPLC columns.

West and co-workers[3,4] have shown that a cool hydrogen–nitrogen flame can be used to measure phosphorus and sulfur in aqueous solutions by observing the molecular emission of HPO and S_2 species. Aqueous solutions were aspirated directly into a total consumption burner to obtain detection limits of 7 and 80 ppb* for phosphorus and sulfur, respectively. Following this study, other workers used flame emission to determine very low concentrations of phosphorus-containing compounds in natural waters and air[5–7]. Recently, the flame photometric principle has been used as a selective detector for measuring metallic ions in ion-exchange chromatography[8]. A general description of the potential for a flame emission detector in HPLC also has been given in the patent literature[9]. However, no practical application for the detection of low concentrations of phosphorus- and sulfur-containing compounds in HPLC has been described.

In this study the total aqueous effluent from the column is nebulized and directed into a cool, stabilized hydrogen–nitrogen flame. The characteristic emissions of phosphorus- and sulfur-containing compounds are sensed by a simple band-pass filter/photomultiplier system. Factors influencing the signal-to-noise response of the detector have been determined. Although no attempt has been made to optimize the detector, it has been applied with excellent results to several analytical systems.

EXPERIMENTAL

Burner design

To insure maximum sensitivity, the burner must be capable of handling the total effluent from a liquid chromatograph (*e.g.*, 0.5–2 ml/min). Burners for aspirating static liquid samples do not handle satisfactorily the volume flow-rates normally used in HPLC[10]. Therefore, a special burner was constructed to accept the total column effluent.

The detector is illustrated in Fig. 1, and a photograph of the actual burner assembly is shown in Fig. 2. The burner is a composite of the base and nebulizer of a Model 82-341 Hetco total consumption burner with an adapter from a Model 82-368 burner head assembly (Jarrell-Ash, Waltham, Mass., U.S.A.). The laboratory-constructed burner head is similar to the standard 1.8 × 7.5 cm air–acetylene emission head supplied with a Unicam SP-900A flame photometer, except that the end of the burner is perforated with a 1.5 cm diameter circular pattern containing thirty-eight 0.15 cm holes. Nitrogen and hydrogen enter a mixing chamber at the bottom of the burner to aspirate the column effluent into the flame. Using these gases, the total column effluent is aspirated into the nebulizer which converts much of the liquid into small droplets. Undesired larger droplets are collected and removed via a drain. With

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

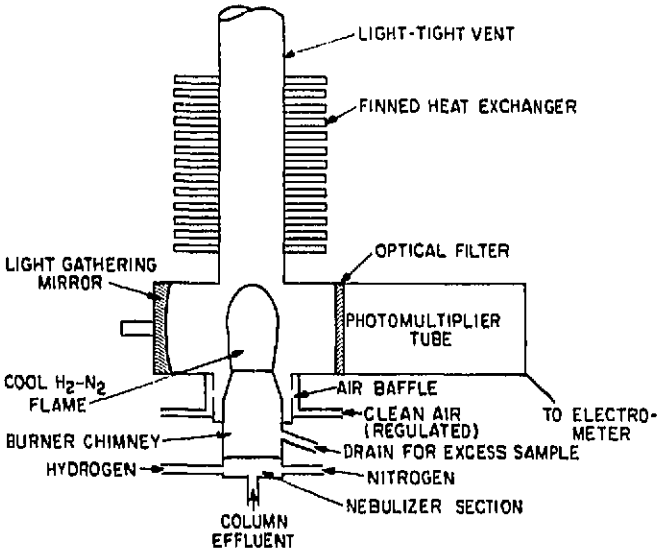

Fig. 1. Schematic of emission detector.

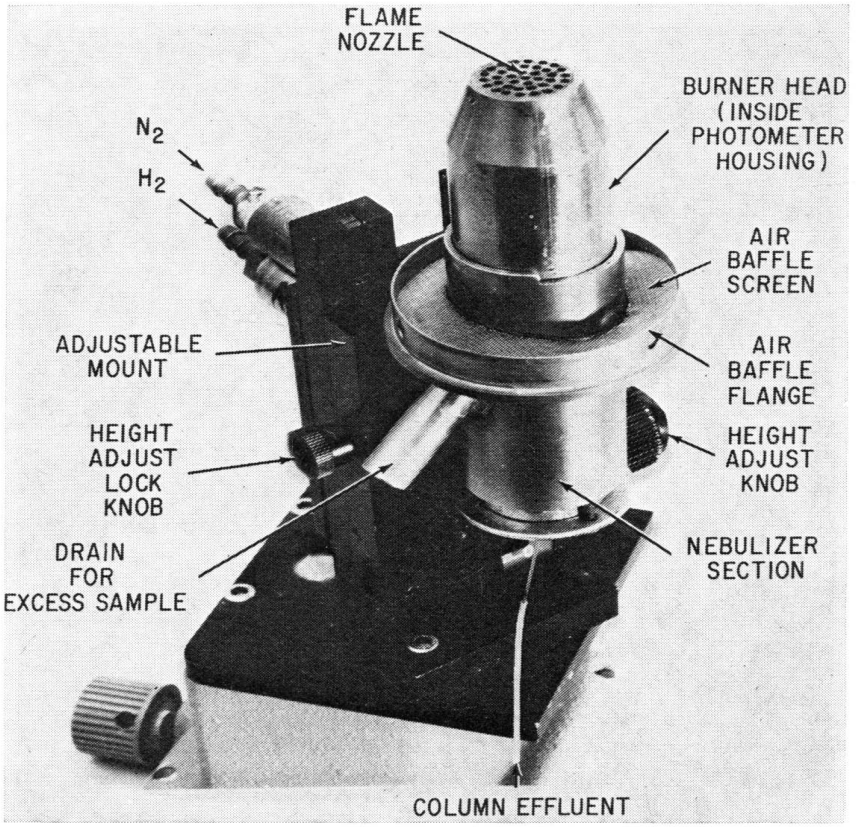

Fig. 2. Burner assembly.

the present burner design, about 25% of the nebulized column effluent passes through the burner head to the flame to produce emission.

Air for the diffusion-fed flame is obtained from an air cylinder and passed through Type 4A molecular sieves to remove trace contaminants. The purified air enters the flame region via three ports arranged circularly around the flame base. An air-baffle system reduces currents in the incoming air stream, so a constant, uniform pattern of air is fed to the flame envelope. To minimize possible flow noise, the gas supplies are regulated by Brooks flow-controllers (Brooks Instrument, Hatfield, Pa., U.S.A.). To facilitate vertical alignment of the flame in the photometric housing, the burner assembly is mounted on a Jarrell-Ash adjustable burner mount.

Baseline stability for the detector system depends on a stable, quiet flame, independent of the laboratory environment. Therefore, the flame is completely enclosed to prevent drafts from affecting flame stability. As indicated in Fig. 1, the gas and combustion products from the flame exit through a closed, finned aluminum heat exchanger, through a flexible light-tight metal pipe into a laboratory hood. The delivery rate of column effluent into the burner can be varied over relatively wide ranges by changing the internal diameter of the aspirator needle in the nebulizer. For example, 0.8–3.0 ml/min is introduced into the burner with a 0.0155-in. I.D. aspirator needle using appropriate nitrogen flow-rates. A 0.018-in. I.D. needle delivers 2.2–5.4 ml/min.

Photometer

The photometer is a wide band-pass filter system using an EMI Type 9524B photomultiplier tube operated at 840 V (EMI Electronics, Ruislip, Great Britain) and the amplifier section of a DuPont luminescence biometer (Instrument Products Division, E.I. du Pont de Nemours, Wilmington, Del., U.S.A.) having a maximum response of 10^{-9} A full-scale. The detector output was connected to a 10 mV potentiometric recorder. Phosphorus (526 nm) or sulfur (383 nm) emission is selected by placing the appropriate 15-nm band-pass filter (Corion Instrument, Waltham, Mass., U.S.A.) in front of the photomultiplier tube with a sliding mount. A fixed 0.25 × 1.5 cm slit admits the light from the central portion of the cool flame to the photomultiplier tube. A 5.0 cm diameter concave front-surface aluminized mirror collects the emitted light from the burner and focuses this energy on the entrance slit.

Liquid chromatograph

The liquid chromatograph was custom-built and uses a Model DST-22 Haskel constant pressure pump (Haskel, Burbank, Calif., U.S.A.). It was necessary to store distilled water in this pump overnight and rinse the pump carefully before proceeding with the first chromatographic run of the day. If the mobile phase was left in the pump overnight, emission from phosphorus-containing compounds was greatly diminished or eliminated. This problem was traced to low levels of iron that built up in the stagnant mobile phase in the stainless-steel pump.

The liquid chromatographic columns were made from 100 × 0.21 cm I.D. precision-bore stainless-steel tubing which was cleaned and packed using procedures previously described[11]. Zipax* superficially porous chromatographic support and

* DuPont registered trade-mark for chromatographic support.

Zipax* SAX strong anion-exchange packing were obtained from DuPont. An experimental superficially porous strong anion-exchanger (38–44 μm) was used in some of the work[12,13].

Sample injections were made with a microsyringe (Precision Sampling, Baton Rouge, La., U.S.A.) through a laboratory-built septum sampling inlet using the stopped-flow mode.

Other equipment

The desired emission bands for phosphorus and sulfur were identified with a Jarrell-Ash 0.5 meter Ebert scanning monochromator.

RESULTS AND DISCUSSION

Studies by other workers[3,4] have shown that the same emission spectra of phosphorus- and sulfur-containing compounds are observed for different structures, indicating that the origin of the emission is a common species in the flame. The species giving rise to the green phosphorus emission is the HPO moiety with a maximum at 526 nm; the blue sulfur emission is caused by the S_2 species with an emission maximum of 383 nm. Although not attempted, halogens probably can be determined by observing the emission of InX species (X = chlorine, bromine, iodine) formed by contact of the effluent with indium metal present inside the burner[14].

A number of factors significantly influence the emission intensity of phosphorus- and sulfur-containing compounds. These include the molecular structure of the compound, the flame temperature (as affected by the relative flow-rates of the gases, etc.), and the presence of certain organic materials and metal ions. These parameters were all studied to define those which limit detector performance.

Observation of the flame while aspirating solutions showed that the blue or green color emanates from the central portion of the flame, as previously suggested by Dagnall *et al.*[3]. Thermocouple measurements indicate that the center portion of the normally colorless flame has a temperature of 300–400°, while the outer mantle is about 800°. A conventional total-consumption burner was found to be unsatisfactory, presumably because this design produces a flame too hot for the production of the HPO and S_2 species. In hot flames (*e.g.*, pre-mixed air–hydrogen flames) temperatures are sufficient to cause breakdown of the desired emitting components.

The flow-rates of the gases producing the flame in the emission detector largely determine both the flame temperature and the resultant emission intensity. Since detector noise also might be influenced by the flow-rates of the individual gases, this effect on the signal-to-noise ratio (per cent relative emission/noise) was studied. As shown in Fig. 3, 11–28 l/min of air has little effect on the emission of a dilute organic phosphonate solution. A constant signal-to-noise level was obtained at hydrogen flow-rates of 4.6–6.8 l/min. However, lower hydrogen flow-rates produced less response, and no flame could be sustained below about 3.5 ml/min.

The signal-to-noise ratio of the detector for phosphorus emission is most sensitive to changes in nitrogen flow. At the particular hydrogen setting used to obtain the data in Fig. 3, the signal-to-noise ratio peaks out at about 12 l/min nitrogen. Lower nitrogen flow-rates result in decreased emission, higher flow-rates in increased noise. At different hydrogen flow-rates, the optimum flow-rate for nitrogen changes, but

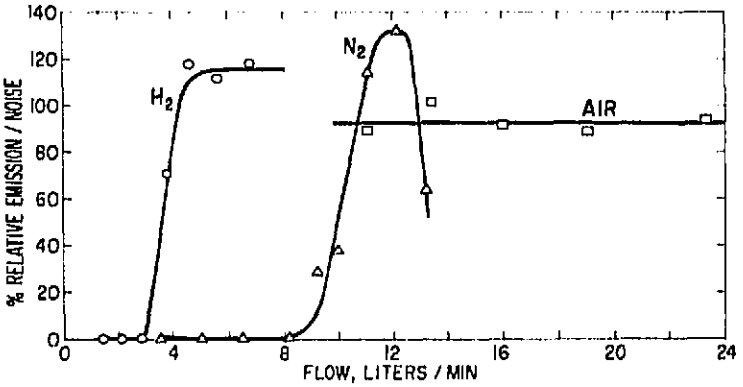

Fig. 3. Effect of gas flow-rates on detector signal-to-noise response. Conditions: 0.9 ppm P (from an organic phosphonate) directly aspirated into the flame at a rate of 2.2 ml/min; H_2 tests: 21 l/min air, 11.5 l/min N_2; N_2 tests: 3.8 l/min H_2, 21 l/min air; air tests: 3.8 l/min H_2, 11.5 l/min N_2.

the general shape of the signal-to-noise curve for nitrogen remains essentially the same. With optimum conditions the noise of the present flame emission system for phosphorus is $\pm 5 \cdot 10^{-6}$ A.

The temperature of the flame required to produce the desired emission apparently is not high enough to produce the emitting species at a rate independent of structure of the chromatographed compound. Consequently, as is common with most liquid chromatographic detectors, the response varies with the identity of the nebulized species, and calibration curves must be made for each compound to be quantitatively analyzed. The effect of structure on the emission of some compounds is indicated in Table I.

TABLE I

EFFECT OF STRUCTURE ON EMISSION

Structure	*Relative molar response*
$NH_4H_2PO_4$	181
Organic phosphonate	109
NH_4POF_2	32
$(NH_4)_2HPO_4$	28
Sulfide > sulfite > thiosulfate ≫ sulfate ≈ sulfuric acid ≫ alkali metal sulfate*	

* From ref. 3.

The presence of organic solvents in the column effluent can adversely affect the intensity of the emitting species. Adding 1% methanol to a mobile phase of water at an aspiration rate of 2.4 ml/min decreased phosphorus emission intensity by approximately one-half. At 50% methanol, the emission intensity for phosphorus is about one-fifth that in water. With pure organic solvents essentially no phosphorus emission is observed. However, the effect of organics on phosphorus emission can be unpredictable, depending on the phosphorus concentration and other variables. For

example, adding methanol to an aqueous mobile phase used to chromatograph an organic phosphonate resulted in increased phosphorus emission at concentrations just above the detection limit. Similar effects were demonstrated with the sulfur-containing compounds, but fewer studies were carried out.

The effect of organic solvent concentration in the mobile phase does not appear to be due to changes in flame temperature. No differences in flame temperature could be detected with a thermocouple when water, acetone–water (1 :1), or acetone was aspirated into the flame at about 1 ml/min. Dagnall *et al.*[3] have made similar observations and conjectured that the decreased emission in the presence of organics arises from the quenching of reactions leading to an emitting species by radicals formed from the organic materials. Presumably, the large volume flow-rate of gases in the flame tends to stabilize the temperature of the flame even though combustible organic materials are also present. These preliminary results suggest that reversed-phase liquid chromatography probably could be used with this detector, but with reduced sensitivity. At present it does not appear that this detector can be used with totally organic mobile phases.

Metal cations also have a significant effect on phosphorus emission intensity, usually causing a decrease. However, copper gives a positive interference, because of emission from the CuH and CuCl bands which overlap with the HPO emission. Small amounts of sodium decrease the emission intensity, but as the concentration increases, a positive interference occurs because of emission from sodium, as illustrated in Fig. 4. Potassium appears to have a much lower level of positive interference. Dagnall *et al.*[3] observed the following order in the decreased phosphorus emission caused by metal ions: Al $>$ Mg $>$ Ca $>$ Na $\gg$ Li $>$ Cd $>$ Fe $>$ Pb. There appears to be a better correlation of emission with ease of reduction of the metal phosphate than with thermal stability. This pattern suggests that the breakdown of the nebulized

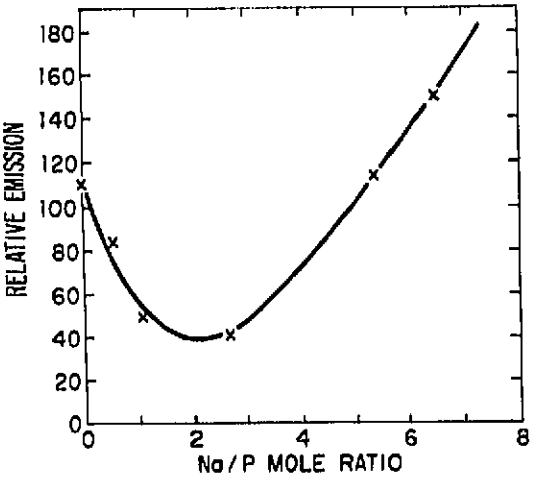

Fig. 4. Effect of sodium on phosphorus emission. Conditions: sample, 25-μl solutions of sodium acetate containing varying amounts of an organic phosphonate. Column, 100 × 0.21 cm I.D. of uncoated Zipax chromatographic support; mobile phase, distilled water; temperature, ambient; pressure, 500 p.s.i.; flow-rate, 1.7 ml/min; gas flow-rates: air, 23.5 l/min; H_2, 3.5 l/min; N_2, 14.6 l/min.

species to HPO is caused principally by reduction rather than thermal degradation. The effect is consistent with the cool temperature and highly reducing nature of the flame optimized for phosphorus emission.

The characteristics of the emission detector are particularly favorable for use in trace analysis, since the device has good sensitivity (particularly for phosphorus), relatively low noise, good linearity with concentration, and excellent short- and long-term reproducibility. No detailed study of the upper limit of linear dynamic working range has been attempted, but a linear response of >500 as a function of concentration has been obtained in application studies. Examples of typical calibrations obtained for phosphorus-containing compounds are shown in Fig. 5.

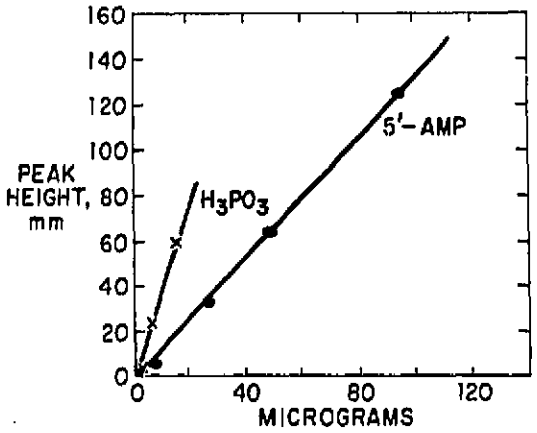

Fig. 5. Calibration plots for phosphorus-containing compounds. Conditions: H_3PO_3 plot: column, 100 × 0.21 cm I.D. experimental Zipax-SAX, chromatographic support; mobile phase, 0.04 *M* formic acid (pH 3.2); temperature, ambient; pressure, 500 p.s.i.; flow-rate, 1.6 ml/min; sample, 100 µl; amplifier, 10^{-4} A; gas flow-rates: air, 23.5 l/min, H_2, 3.5 l/min; N_2, 14.6 l/min. 5'-AMP plot: column as above; mobile phase, 0.1 *M* formic acid; temperature, 60°; pressure, 200 p.s.i.; flow-rate, 0.7 ml/min; sample, 100 µl; amplifier, 10^{-4} A; gas flow-rates, as above. 5'-AMP = adenosine 5'-monophosphate.

In a typical situation the emission detector can sense about 20 and 200 ng of phosphorus and sulfur, respectively, injected into a moderately efficient chromatographic column. It is anticipated that this detection limit could be lowered at least ten-fold, since the photometer or the photomultiplier–electrometer combination was not optimized. In addition, emission intensity could be enhanced at least three-fold by redesigning the nebulizer so 75% or more of the column effluent is aspirated into the flame (25% in the present detector). Ultrasonic energy for this nebulization process appears attractive.

Excellent short- and long-term reproducibility of the flame emission detector has been observed in routine trace analyses. Table II shows the response of the flame emission detector for quantitative calibration of a proprietary phosphorus-containing compound over about a six-week period. Variations in these data encompass all the usual chromatographic variations in flow-rate and sample volume (by syringe delivery) and changes in instrument and column characteristics. Peak heights show a relative standard deviation of <15% for 90 pg of phosphorus, which is about four

TABLE II

REPRODUCIBILITY OF CALIBRATION FOR PHOSPHORUS

Conditions: column, 200 × 0.21 cm I.D. Zipax SAX; mobile phase, 0.015 *M* formic acid (pH 5.0); temperature, ambient; pressure, 2000 p.s.i.; flow-rate, 1.0 ml/min; sample volume, 100 μl; emission detector amplifier, 10^{-8} A, gas flow-rates: air, 23.5 l/min; hydrogen, 3.5 l/min; nitrogen, 14.6 l/min.

Date	*Relative emission (%)*		
	90 pg P	*180 pg P*	*400 pg P*
11-29	8.5	16.0	41.0
12-3	9.0	17.0	44.0
12-4	8.8	15.5	41.1
12-11	7.7	15.7	34.5
12-12	8.0	14.5	39.0
12-14	8.0	16.5	41.1
12-17	8.3	18.7	40.5
12-18	8.3	17.2	38.8
12-19	11.0	17.6	40.6
12-21	8.0	14.5	37.0
1-2	—	14.7	41.3
1-3	7.0	17.2	37.3
1-4	—	10.7	34.5
1-7	—	15.5	36.9
1-10	6.3	12.0	38.1
1-11	—	11.2	38.5
1-15	6.5	16.0	38.0
1-16	8.4	17.5	41.0
Average	8.1	15.4	39.1
σ	1.18	2.23	2.49
Relative σ	14.5%	14.5%	6.4%

times the detection limit for phosphorus in this chromatographic system. With 460 pg of phosphorus the relative standard deviation of the peak heights is about 6%. This reproducibility is comparable to data obtained with the stable ultraviolet photometric detectors in similar HPLC trace analyses.

Extra-column band-broadening appears to have no significant effect with the emission detector system described. With columns of $\approx 40\,\mu$m superficially porous packings the variance (σ^2) of peaks monitored with the emission detector were no larger than those measured with a commercial UV photometric detector with an 8-μl cell. The "dead volume" of the emission detector apparently is insignificant since the sample is nebulized and immediately swept by a high flow-rate of gases into the flame. In addition, the connector between the column outlet and the inlet to the flame emission detector can be made with a short length of small inner diameter capillary tubing (0.010–0.015 in. I.D.), so unwanted re-mixing effects are insignificant. While no studies were made with very high-efficiency columns of small particles ($< 10\,\mu$m), it is believed that extra-column band-broadening effects associated with such systems also will be small.

Because of the limitations in the mobile phase, the emission detector appears best suited for ion-exchange and perhaps reversed-phase liquid chromatography. As indicated previously, some organics can be tolerated in the mobile phase but a lower response is obtained. In ion-exchange chromatography, possible interference by metal

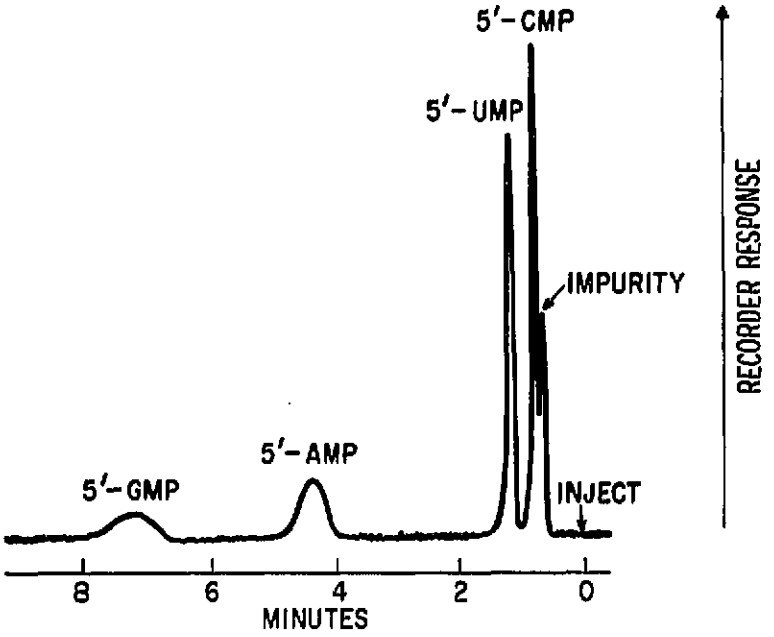

Fig. 6. Separation of 5'-monophosphate nucleotides using phosphorus detector. Conditions same as for 5'-AMP in Fig. 5 except: pressure, 500 p.s.i.; flow-rate, 1.8 ml/min; sample, 100 μl of 0.4 mg of each compound per ml. 5'-GMP = guanosine 5'-monophosphate; 5'-AMP = adenosine 5'-monophosphate; 5'-UMP = uridine 5'-monophosphate; 5'-CMP = cytidine 5'-monophosphate.

ions associated with buffers used as the mobile phase often can be avoided by using ammonium formate or ammonium borate systems. Fig. 6 shows a chromatogram of a test mixture of four 5'-monophosphate nucleotides, using the phosphorus-selective flame emission detector together with a formic acid mobile phase. Other studies have shown that the excellent selectivity of this detector permits measurement of trace amounts of organic phosphorus-containing compounds in naturally occurring systems with minimum interference. Similar selectivity for sulfur-containing compounds probably can be obtained, but at about a factor of ten reduced sensitivity. The flame emission detector can also detect certain inorganic species, as illustrated in Fig. 7.

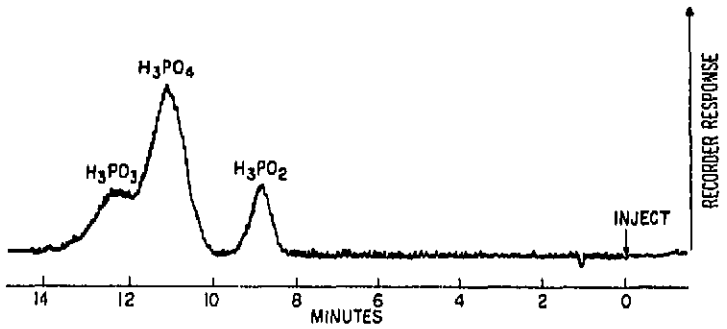

Fig. 7. Separation of oxides of phosphorus using flame emission detector. Conditions same as for H_3PO_3 in Fig. 5 except: pressure, 300 p.s.i.; flow-rate, 1.3 ml/min; sample, 100 μl of 0.3 mg of each compound per ml.

The good sensitivity and excellent selectivity of flame emission makes this detector an excellent candidate for certain trace analyses. For example, the emission detector probably can determine low levels of phosphorus oxides in surface water and other aqueous systems. Using known chromatographic techniques for optimizing the sensitivity and selectivity of trace analyses by HPLC will be helpful[1]. Further improvements could result in increased sensitivity, versatility and applicability of the flame emission detector in HPLC.

ACKNOWLEDGEMENTS

The assistance of Anthony J. Cecchini in the experimental work is gratefully acknowledged. We greatly appreciate the assistance of DuPont's Instrument Products Division in fabricating the photometer housing, and for the use of the amplifier.

REFERENCES

1 J. J. Kirkland, *Analyst (London)*, 99 (1974) 859.
2 S. S. Brody and J. E. Chaney, *J. Gas Chromatogr.*, 4 (1966) 42.
3 R. M. Dagnall, K. C. Thompson and T. S. West, *Analyst (London)*, 92 (1967) 506.
4 K. M. Aldous, R. M. Dagnall and T. S. West, *Analyst (London)*, 95 (1970) 417.
5 A. Syty and J. A. Dean, *Appl. Opt.*, 7 (1968) 1331.
6 W. N. Elliott and R. A. Mostyn, *Analyst (London)*, 96 (1971) 452.
7 M. J. Prager and W. R. Seitz, *Anal. Chem.*, 47 (1975) 148.
8 D. J. Freed, *Anal. Chem.*, 47 (1975) 186.
9 H. Oster, *Ger. Pat.*, 1,965,547, Sept. 2, 1971.
10 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley-Interscience, New York, 1974.
11 J. J. Kirkland, *J. Chromatogr. Sci.*, 10 (1972) 129.
12 J. J. Kirkland, *J. Chromatogr. Sci.*, 8 (1970) 72.
13 J. J. Kirkland, *U.S. Pat.*, 3,488,922, Jan. 13, 1970.
14 P. T. Gilbert, *Anal. Chem.*, 38 (1966) 1920.