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## NEW THERMIONIC DETECTOR UTILIZING ORTHOGONAL NEBULIZATION FOR CAPILLARY COLUMN LIQUID CHROMATOGRAPHY

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### SUMMARY

A new version of the previously studied phosphorus-sensitive, dual-flame thermionic detector has been developed for microcolumn liquid chromatography. The total column effluent is orthogonally nebulized and aspirated directly into a primary air-hydrogen diffusion flame. Phosphorus compounds with molecular weights extending beyond 500 a.m.u. are then selectively detected by measuring the conductivity of the secondary flame in the presence of a rubidium silicate bead. The sensitivity was found to be  $1.36 \cdot 10^{-11}$  g phosphorus/sec at the maximum of a Gaussian peak, and the signal increased linearly with concentration over 2 orders of magnitude for dilute samples. Possible mechanisms accounting for the negative orientation of the signal and for the limited dynamic range are discussed.

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### INTRODUCTION

The subject of flame-based detectors for high-performance liquid chromatography (HPLC) has received some attention in the past because of the need for element-specific liquid chromatographic analysis. Unfortunately, the continuous introduction of aqueous and organic mobile phases at the milliliter-per-minute flow-rates typical of HPLC has caused numerous technological problems, especially with regard to the selective nature of these detectors. Consequently, neither direct introduction of the column effluent<sup>1-5</sup>, nor the use of transport-based interfaces<sup>6,7</sup> has been entirely successful.

Capillary column liquid chromatography (LC) may, however, provide a solution to the above problems. The very low ( $\mu\text{l}/\text{min}$ ) flow-rates typical of microcolumns allow the total column effluent to be directly introduced and fairly efficiently aspirated into the flame<sup>8-12</sup>, resulting in an extremely low dead-volume, total consumption detection system. The utilization of the flame ionization<sup>5,11,12</sup>, flame emission<sup>8,9</sup>, and thermionic<sup>10</sup> detectors for microcolumn LC has been previously described. However, the nebulization of larger molecules proved to be extremely difficult. In this investigation, modifications have been made to a thermionic detector for microcolumn HPLC<sup>10</sup> such that phosphorus-containing species having molecular weights of over 500 a.m.u. can be readily detected.

Of the detectors available to liquid chromatography, few offer element specificity, a feature which is of particular importance in the detection of a given compound or class of compounds contained within a highly complex matrix. The thermionic detector (or nitrogen-phosphorus detector) provides just such capabilities. Although Karmen and Giuffrida<sup>13</sup> first described the thermionic detector for gas chromatography, Kolb and Bischoff<sup>14</sup> later modified its design to increase both the sensitivity and the stability of its response. This later configuration utilized a constant current source, rather than the flame itself, to heat the alkali bead, which was negatively polarized with respect to the collector. The proposed mechanism, therefore, involved the combustion of molecules entering the flame, thereby forming CN<sup>•</sup> or PO<sup>•</sup> and PO<sub>2</sub> radicals from nitrogen- and phosphorus-containing compounds, respectively. These radicals then abstracted an electron from an excited state alkali atom to form a stable anion, which, in turn, donated its extra electron at the collector electrode before leaving the detector as a neutral species. The alkali cation, on the other hand, was recaptured by the negatively charged bead. Further modifications to the detector are numerous and have been reviewed by Brazhnikov *et al.*<sup>15</sup>.

As described in this communication, a dual-flame thermionic detector designed in our laboratory<sup>10</sup> has been modified to incorporate orthogonal, rather than concentric, nebulization of the column effluent. This modification dramatically increased the nebulization efficiency for larger molecules without substantial alteration in the operating parameters of the detector. The detector did, however, yield an anomalous response under certain circumstances.

## EXPERIMENTAL

### *Modified thermionic detector*

The modified dual-flame thermionic detector, which appears in Fig. 1, is of the Kolb-Bischoff<sup>14</sup> configuration and follows the previously outlined design<sup>10</sup>. Several changes were, however, made in the original specifications, as detailed below. The detector (Part No. 332-5300, Perkin-Elmer, Norwalk, CT, U.S.A.). The stability of the thermionic detector is largely a function of the careful regulation of high-purity flame gases, and the gas flow-rates were more carefully controlled than in the previous detector. Single-stage regulators at the cylinder were followed by conventional pressure regulators (Veriflo, Richmond, CA, U.S.A.) for the air and nitrogen to the lower flame. The more critical air flow to the upper flame was controlled with a Dwyer rotameter (Dwyer Instruments, Michigan City, IN, U.S.A.), and the hydrogen flows, being most critical to the operation of the detector, were regulated with high precision rotameters (PR series FM042-07C, lower flame; PR series FM042-07G, upper flame, Aalborg Instruments and Controls, Monsey, NY, U.S.A.). Carrier grade hydrogen (Air Products, Tomaqua, PA, U.S.A.) and nitrogen (No. UN1066, Air Products) were premixed and nebulized the microcolumn effluent at the capillary orifice. Compressed air (No. 1002, Air Products) was uniformly supplied to both flames by diffusion. Type 4A molecular sieves (No. 987990, P. J. Cobert Assoc., St. Louis, MO, U.S.A.) were utilized to remove trace impurities from all three gases.

Perhaps the most important of the modifications was the orientation of the fused-silica capillary (50  $\mu\text{m}$  I.D., 5–10 cm length), which was connected at one end to the microcolumn with PTFE tubing and which entered the detector orthogonally

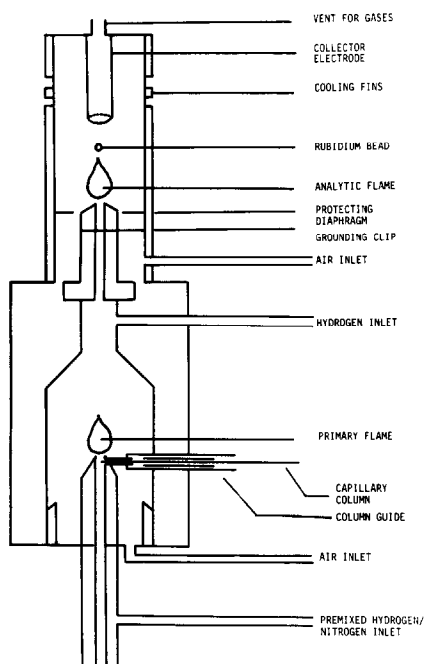


Fig. 1. Schematic diagram of the modified phosphorus-sensitive thermionic detector.

through the lower burner base and flame jet. It was carefully positioned approx. 1 mm below the tip and one-third of the way across the inner diameter of the jet and was held securely in place by a series of stainless-steel guide tubes and Swagelok (Crawford Fitting, Solon, OH, U.S.A.) fittings, as shown in Fig. 1. Nebulization, thus, occurred as the flame gases flowed past the capillary tip and lifted the entire column effluent into the flame.

The decomposition products from the lower flame were combined with additional fuel and were subsequently swept into the analytical flame. As previously reported<sup>8</sup>, excessive noise resulted if this flame was allowed to ignite and burn freely. A rubidium silicate bead, fabricated in-house according to the procedure of Lubkowitz *et al.*<sup>16</sup>, was negatively polarized to  $-200$  V with respect to the collector electrode and was electrically heated by a finely controlled constant-current source located in the detector's electrometer. The bead was positioned 1–2 mm above the analytical flame jet, which was also maintained at  $-200$  V. A cylindrical electrode, approx. 8 cm in length, was located 6 mm above the analytical jet to monitor the electrical signal from the negatively charged species produced in the flames and at the bead. The ion current was converted to a voltage and then amplified in the electrometer, and the final signal was filtered to remove high-frequency noise before appearing on a strip chart recorder (Model B-5000 Omniscrite, Houston Instruments, Austin, TX, U.S.A.). A block diagram of the system has been shown previously<sup>10</sup>.

### *Chromatographic system*

Slurry-packed capillary columns, prepared as previously described<sup>17</sup>, were utilized in this study. A high-pressure syringe pump (Model 8500, Varian Instrument Division, Palo Alto, CA, U.S.A.) was operated in the constant pressure mode to provide a steady, pulsation-free mobile phase flow through the column. Samples were injected in 0.25- $\mu$ l aliquots using a direct, stopped-flow injection method<sup>18</sup>.

### *Reagents*

Trimethyl phosphate (Reagent grade, Aldrich, Milwaukee, WI, U.S.A.) was employed as a model solute in the optimization and characterization of the detector.

The hydroxy steroids 1,3,5(10)-estratrien-3-ol-17-one, 1,3,5(10)-estratrien-3,16 $\alpha$ ,17 $\beta$ -diol, and 5-androsten-3 $\beta$ ,17 $\beta$ -diol (Sigma, St. Louis, MO, U.S.A.) were derivatized to incorporate phosphorus using dimethylthiophosphinic chloride (Riedel de Haen, Seelze, F.R.G.) according to the procedure of Jacob and Vogt<sup>19</sup> to yield the dimethylthiophosphinic esters. The structures of the derivatives were verified through both their NMR and their mass spectra. The dimethylthiophosphinic ester of 1,3,5(10)-estratrien-3 $\beta$ ,17 $\beta$ -diol was obtained in its pure form from Dr. Karl Jacob (Ludwig-Maximilians-Universität, München, F.R.G.).

## RESULTS AND DISCUSSION

### *Detector optimization*

Both the sensitivity and the selectivity of the thermionic detector are, in general, highly dependent on a wide variety of operating parameters<sup>15</sup>, many of which vary with the design specifics of the individual detector. For example, the exact shape and position of the flame housing, alkali bead, and collector electrode can all significantly affect the detector's performance. For this reason, a nominal optimization using an independent linear variation of the gas flow-rates and bead heating current was performed to obtain the maximum signal-to-noise ratio (S/N) of the modified phosphorus-sensitive thermionic detector. Trimethyl phosphate in a 100% methanol mobile phase served as the model solute.

The resulting optimization curves were very similar to those obtained for detection in the nitrogen sensitive mode using the same detector<sup>20</sup>, rather than to those previously obtained for a phosphorus-sensitive thermionic detector<sup>10</sup>. The general trends did, however, correspond closely between the two phosphorus-sensitive detectors. The exact flow-rate of the nebulizing gas, nitrogen, had little effect when it was kept between 50 and 120 ml/min. However, the fuel gas, hydrogen, showed a sharp optimum at 22 ml/min for the primary flame, with higher rates leading to a decreased signal level, and lower rates to increased noise. The air to the primary flame yielded optimal S/N ratios when it flowed at 700 ml/min, while higher flows served mainly to increase the background noise level. The gas flows to the analytical flame acted mainly to guide the solutes upward and to cool the alkali bead. Therefore, they had a negligible effect on the signal intensity once they were sufficient to carry the solute molecules. Instead, their main effect was on the background noise level: increasing the hydrogen flow beyond its optimum of 28 ml/min increased the baseline noise, while increasing the air flow appeared to have little effect across the range of values tested. As previously reported, the highest S/N ratio was found with a minimal

beadheating current<sup>10</sup>, and with both the bead and the analytical flame jet maintained at the same potential ( $-200$  V) with respect to the collector electrode.

### Detector characterization

The optimal response of the detector was determined for compounds of differing molecular weight. Trimethyl phosphate in methanol yielded detection limits in the order of 490 pg of injected phosphorus or 13.6 pg of phosphorus per sec at a signal to root mean square (rms) noise ratio of 8 (99.9% confidence level)<sup>21</sup>. The dimethylthiophosphinic ester of estradiol (MW = 456 a.m.u.) yielded a detection limit of 5 ng of injected phosphorus or 139 pg of phosphorus per second at a  $S/N_{\text{rms}}$  ratio of 10 (99.9% confidence level). These values compare well with the detection limit of 22 pg phosphorus per sec previously obtained for trimethyl phosphate<sup>10</sup>.

There are two important features of this detector which should be noted at this point. First, the absolute sensitivity was actually greater than is evident from the reported  $S/N$  ratios. Since more effluent was effectively swept into the combustion flame with right-angle, as compared to concentric, nebulization, a substantial increase occurred in the amount of liquid fuel (methanol) available to the flame, and, consequently, the flame temperature increased over that observed with concentric nebulization<sup>20</sup>. The net result was an increase in the background noise level, which resulted both from the increased heat of the zone surrounding the alkali bead and from the greater number of background ions created by the solvent in the hotter flame. Fortunately, the increase in nebulization efficiency also introduced a correspondingly larger number of solute molecules into the flame, so that the detection limits remained similar to those obtained with concentric nebulization.

The second feature worthy of note was that, in the quantitative mode, the peaks extended in the negative direction. Also, the linear dynamic range of this detector was reduced from that reported earlier<sup>10</sup>, since response linearity was obtained only for very small quantities of sample, as shown in Fig. 2.

The response was actually quite unconventional in that the peaks remained negative beyond the linear region, but became bipolar at approx. 123 ng of injected phosphorus, where a positive response was intermittently seen. For larger injections,

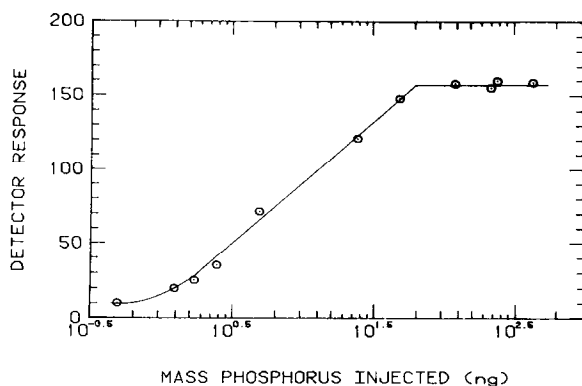


Fig. 2. Linearity of detector response to phosphorus as a function of injected sample mass. Column: slurry-packed capillary column ( $1.1 \text{ m} \times 250 \mu\text{m}$  I.D.) packed with  $5\text{-}\mu\text{m}$  Spherisorb ODS; mobile phase: methanol ( $1.3 \mu\text{l}/\text{min}$ ); solute: trimethyl phosphate.

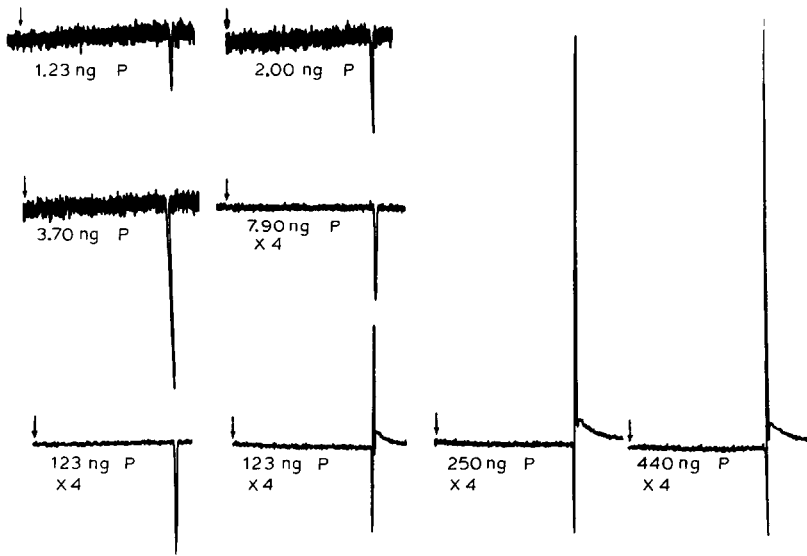
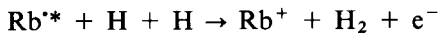
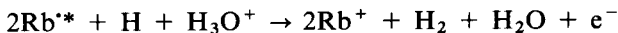


Fig. 3. Transition from negative to bipolar detector response as a function of increasing sample mass.

the negative peak became an initial downward spike of constant size, while the positive portion of the response exhibited a non-quantitative spike of increasing intensity followed by a very small peak of constant height, which was both broad and tailing. This transition is illustrated in Fig. 3. While flame chemistry is highly complex and poorly understood, a reasonable explanation of the detection phenomena may be derived as follows. With side-on nebulization, a relatively large amount of methanol reached the combustion flame, where it formed both cations, such as  $\text{CH}_3^+\text{O} \begin{smallmatrix} \text{H} \\ \text{H} \end{smallmatrix}$ ,  $\text{CH}_2^+\text{OH}$ ,  $\text{CH}_2 = ^+\text{OH}$  and  $\text{CH}_4^+$ , and radicals, such as  $\text{CH}_3\text{O}^\cdot$ ,  $^\cdot\text{CH}_2\text{OH}$ , and  $^\cdot\text{CH}_3$ . Indeed, cation formation was favored, since the flame was oxidizing and at relatively high temperature. Upon ignition, an initial positive shift was seen in the baseline as electrons were donated by rubidium radicals, which had been excited at the heated bead according to the following possible reactions:



or



When solvent flow began, the baseline fell as the cations present scavenged electrons from the excited rubidium to become radicals and, possibly, anions with lower ionization potentials, having, therefore, lower signal levels than the excited alkali metal. In addition, the radicals or anions may have combined with hydrogen atoms or ions to form neutral species which yielded no detector response. As a phosphorus-containing molecule entered the flame, the abundant solvent cations and radicals could combine with the  $^\cdot\text{PO}$  or  $^\cdot\text{PO}_2$  radicals to produce a variety of species within the flame. The phosphorus radicals may have combined with hydrogen to yield neutral

molecules, such as  $\text{HPO}$  or  $\text{HPO}_2$  or, alternatively, with solvent derived species to form many neutral compounds, cations, and complex anions. The combination to form neutral or cationic molecules obviously lowered the signal level, but even anionic species may have had this effect, if they lost electrons less willingly than the excited rubidium. At higher concentrations, however, the percentage of solute molecules dissociating in the primary flame decreased, and excess solute molecules traveled straight to the bead without undergoing many of the complex reactions of the lower region. These molecules reached the bead primarily as radicals and, hence, produced the characteristically positive response. Originating mainly from excess molecules, this response remained non-quantitative, although it may have predominated at very high solute concentrations. The presence of two positive peaks can be accounted for if one assumes that both the rapid gas-phase and the kinetically much slower surface-catalyzed electron-transfer reactions occurred at the bead.

### Applications

Many molecules best suited to analysis by liquid chromatography are those which are either thermally labile or too large or polar to be conveniently separated using gas chromatographic (GC) methods. Many such compounds are biological in origin and are contained within complex sample matrices, for which the high resolving power of the capillary LC column is ideally suited. Although phosphorus is frequently not an inherent part of these compounds, derivatization methods exist by which it may be added to various compound types<sup>19,21-25</sup>. Jacob and co-workers have derivatized hydroxy and amine compounds to increase their volatility for GC, while simultaneously adding a phosphorus moiety to allow thermionic detection. Since LC is frequently the method of choice in the analysis of biological samples, it is important that the thiophosphinic esters formed in this derivatization are stable in aqueous and

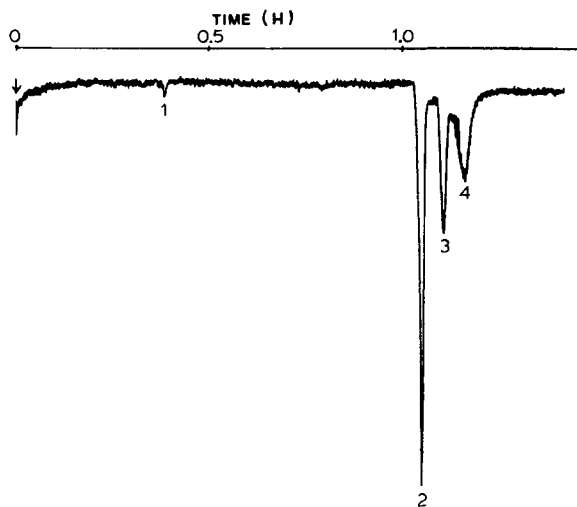


Fig. 4. Chromatogram of derivatized hydroxy steroids. Column: slurry-packed capillary column (3 m  $\times$  250  $\mu\text{m}$  I.D.) packed with 5- $\mu\text{m}$  Spherisorb ODS; mobile phase: acetone (1.3  $\mu\text{l}/\text{min}$ ); solutes: 1 = solvent peak, 2 = estrone derivative (20 ng injected phosphorus), 3 = estratriol derivative (10 ng injected phosphorus), 4 = androstendiol derivative (10 ng injected phosphorus).

organic solutions. Until recently these molecules were too large to be effectively nebulized for detection with the thermionic detector designed for microcolumn liquid chromatography. The modified detector is, however, capable of quantitatively nebulizing and monitoring these compounds in the low concentrations at which they typically appear. Such a separation appears in the chromatogram of Fig. 4, wherein the dimethylthiophosphinic esters of three steroid hormones were separated on a reversed-phase microcolumn and detected at the nanogram level.

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