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ON-LINE FLAME PHOTOMETRIC DETECTION IN MICRO-COLUMN LIQUID CHROMATOGRAPHY

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SUMMARY

An improved interface coupled to a commercially available flame photometric detector designed for gas chromatography has been investigated for its applicability in micro-column (0.32 mm I.D.) liquid chromatography. The column effluent is directly introduced into the flame via a 0.1 mm I.D. fused-silica capillary. The influence on the detector performance of parameters such as the position of the effluent introduction into the flame, the composition and flow-rates of the gases and the quenching by the eluent is discussed. With a series of organophosphorus acids as model compounds, plots of peak area *vs.* amount injected are linear ($r > 0.998$) in the range 0.5–200 ng investigated. The repeatability is better than 6% ($n = 148$). The system shows a detection limit of 20 pg of phosphorus per second (0.5–2 ng of analyte) when using an aqueous ammonium acetate or nitric acid solution as the eluent. The addition of methanol or acetonitrile to the eluent quenches the detector response. A mass flux of 1.2 mg/s of oxidizable carbon causes a 20% loss in detector signal.

INTRODUCTION

In recent years, the use of miniaturized liquid chromatography (LC) with 0.2–1 mm I.D. columns has increased considerably. One major advantage is the possibility to couple LC directly to several types of flame-based gas chromatographic (GC) detectors, such as, *e.g.*, phosphorus-selective detectors. Initial research in this field was carried out by Novotny and co-workers[1–4] who used packed micro-columns (0.2–0.3 mm I.D.) and a nebulization interface to introduce the effluent into a dual-flame thermionic detector. A different approach was used by Brinkman and co-workers[5–7] who applied narrow-bore columns (0.7–1 mm I.D.) and an interface designed to vaporize the LC effluent before it enters the thermionic detector. For a series of

[a] Author deceased.
[b] Publication delayed at the authors' request.

organophosphorus pesticides the authors reported detection limits varying from 0.2 to 0.5 pg/s of phosphorus[7]. The use of a flame photometric detector in micro-column LC has been described by McGuffin and Novotny[8], who were able to detect 2 ng of phosphorus by means of the direct introduction of the column effluent into the flame. The mass flux at the peak maximum was 71 pg/s of phosphorus when using the relatively volatile trimethyl phosphate as a test compound. Karnicky *et al.*[9] have reported on an ultrasonic micro nebulizer-flame photometric detector as an LC detector using both dual-flame and dual-wavelength operation to improve signal-to-noise ratios and to eliminate baseline shifts. With this relatively sophisticated interface, the authors were able to detect sugar phosphates and phospholipids in water down to 50 pg/s of phosphorus. Unfortunately, no further work using this approach has been published.

Our group is interested in the trace-level determination of polar, acidic and other non-volatile phosphorus-containing compounds. Generally, LC with UV detection and GC analysis are unsuitable for such compounds without prior derivatization. Therefore, the direct coupling of packed capillary fused-silica LC columns to a commercially available flame photometric detector is being investigated in our laboratories. Preliminary experiments[10] showed that such a system allows the detection of volatile organophosphorus compounds. However, non-volatile analytes such as organophosphorus acids or high-molecular-weight compounds were not detected. In the present study the interface has been further improved to obtain a suitable introduction of non-volatile organophosphorus compounds into the flame.

EXPERIMENTAL

Materials

All solvents were of HPLC-grade quality (Merck, Darmstadt, F.R.G.). LiChrosorb RP-18, particle size 10 μm (Merck), and PRP-1 styrene–divinylbenzene copolymer, particle size 10 μm, from Hamilton (Reno, NE, U.S.A.) were used as column packing materials. Phosphoric acid (PA) was supplied by Merck, methylphosphonic acid (MPA), ethyl methylphosphonic acid (EMPA), isopropyl methylphosphonic acid (IMPA), dimethyl phosphoric acid (DMP) and diethyl phosphoric acid (DEP) were synthesized at the Prins Maurits Laboratory TNO.

Apparatus

The system was assembled from an LC-5A pump (Shimadzu, Kyoto, Japan) a Valco sample injection valve (VICI, Schenkon, Switzerland) with a 60-nl internal volume and a flame photometric detector Model 380 (Carlo Erba, Milan, Italy). The different fused-silica connective tubings (0.02–0.3 mm I.D.) were supplied by Chrompack (Middelburg, The Netherlands). The fused-silica capillaries (0.3 mm I.D.) were packed with LiChrosorb RP-18 or PRP-1 according to the procedure of Gluckman *et al.*[11].The column performance was tested using a laboratory-made 40-nl micro-flow UV cell[12]. In the experimental set-up a second Valco valve was inserted between the micro-column and the detector interface. The micro-column was connected to both Valco valves using finger-tightened PTFE ferrules and nuts (Hibar, Merck), its packing being held by porous PTFE frits (Alltech, Eke, Belgium) inserted into both valves. This experimental set-up allows easy inspection or replacement of the fused-silica capillary outlet (see Fig. 1) without the need to disconnect or depressurize the micro-LC column.

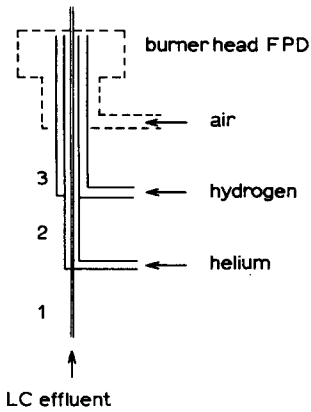

Fig. 1. Schematic interface design. 1 = Fused-silica capillary (0.10 mm I.D.); 2 = fused-silica capillary (0.32 mm I.D.); 3 = stainless-steel tube (0.50 mm I.D., 1.6 mm O.D.); 4 = burner head. For further details, see text.

Data acquisition was performed on a personal computer (Type PC 350; Digital Equipment Corporation, Maynard, MA, U.S.A.) via a Minichrom interface and the appropriate software (VG Laboratory Systems, Altrincham, U.K.). Additional calculations were carried out on a VAX 8200 computer (Digital Equipment Corporation). To evaluate the external peak broadening of the system a computer program was written to calculate the second moment, M_2, of the chromatographic peaks with the statistical method, using the algorithm described by Yau[13] and with the Foley and Dorsey[14] equations. The program was validated using the exponentially modified gaussian (EMG) peak generation method[15]. The M_2 value was obtained with an accuracy of 0.6% as derived from generated M_2 values in the range of 0.05–1.8 μl^2 at a peak asymmetry ratio of 0.1–3 τ/σ as defined according to ref. 15.

RESULTS AND DISCUSSION

Liquid chromatography–flame photometric detection (FPD)

The interface previously constructed and used for micro-column LC–FPD studies[10], was based on the principle of evaporation of the liquid eluent before its introduction into the flame. When using this interface it was found that organophosphorus acids were not detected. These experiences were similar to those published in another paper on the utilization of an evaporation interface for LC with thermionic detection (TID)[6]. In that study a variety of phosphorus-containing compounds including polar pesticides was measured. All non-volatile or high-molecular-weigth compounds were found to be difficult to detect. This may well indicate the limitation of interfaces based on the principle of simple evaporation. In order to determine non-volatile organophosphorus acids with LC–FPD, the effect of several modifications to the interface mentioned before was examined.

In the final set-up (see Fig. 1) the LC effluent is introduced into the detector by means of a fused-silica capillary (no. 1), while an helium purge flows through the wider coaxial fused-silica capillary (no. 2). In the previous design a nitrogen flow was

added. The stability of the system was found to improve upon replacing nitrogen by helium. The hydrogen flows through a stainless-steel tube (no. 3) into the burner just below the tips of the two fused-silica capillaries. The hydrogen flow-rate, the air flow-rate and the helium purge flow-rate are set at 650, 350 and 40 ml/min, respectively, as deduced from experiments discussed below.

The position of the tip of the fused-silica capillary which introduces the column effluent into the flame (Fig. 1, no. 1) was varied as well as the internal diameter of this capillary. The best results were obtained by introducing the liquid effluent into the relatively cold centre of the hydrogen flame. Finally, the position of the tip of the fused-silica capillary was fixed 1.4 mm above the outlet of the hydrogen flow (Fig. 1, no. 3) to obtain maximum stability of the detector response. Comparing the performance of fused-silica outlet capillaries with internal diameters of 25, 50 and 100 μm, the best stability and reproducibility of the detector response were found using the 100-μm capillary.

The flame photometric detector used contains a burner head configuration as originally presented by Brody and Chaney[16]. The flame burns in a hollow metal tip of the burner head which shields the blue flame envelope from direct view by the photomultiplier. In this way interferences from flame emission are eliminated whereas, if phosphorus or sulphur atoms are present, the emission of the excited POH an S_2 fragments appears above the shield[16]. In order to maintain this situation the hydrogen outlet and, thus, the position of the stainless-steel tube (Fig. 1, no. 3) should be at its original position, *i.e.*, at the bottom of the burner head. However, considerable heat transfer will then take place towards the lower part of the burner, causing clogging of the 100-μm fused-silica capillary when non-volatile solutes are present. From these experiments it was concluded that a cooler burner base must be used. This was obtained by lifting the entire interface (Fig. 1, nos. 1–3) to the same level as the top of the burner head.

The helium flow was added close below the LC column outlet (Fig. 1, no. 2) which probably provides an additional cooling effect.

Detector gas flows

In the present system, hydrogen may have a two-fold function, *viz.*, as a fuel and as an LC effluent transporting gas. Consequently, the flow-rate affects the shape and, more importantly, the position of the diffusion flame. In all experiments, the flow was set at 650 ml/min which is relatively high in comparison with conditions in GC, where a flow of 50–200 ml/min is conventionally used[17]. A decrease of the present flow-rate causes a lower flame position, that is, too high a position of the fused-silica tip via which the LC effluent is introduced into the flame. On removing the photomultiplier tube, bad positioning of this fused-silica capillary in the flame was seen: when decreasing the hydrogen flow-rate the tip of the capillary started to glow. Under such conditions non-volatile compounds will block the fused-silica capillary.

The dependence of the response or, rather, the signal-to-noise ratio, for the test compounds on the air flow-rate is shown in Fig. 2. As is seen, there is a distinct optimum air flow-rate at about 375 ml/min for all three compounds. At this optimum the hydrogen-to-oxygen ratio amounts to approximately 5, which corresponds nicely with the ratio of 4 to 7 generally reported in the literature for GC–FPD applications

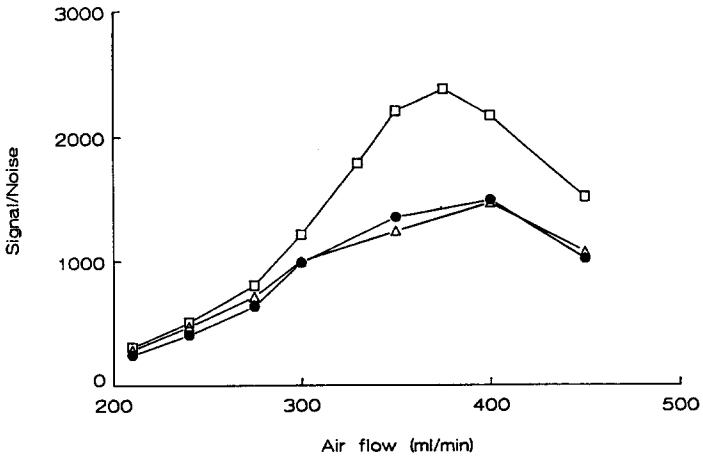

Fig. 2. Influence of air flow-rate on signal-to-noise ratio. □, Methylphosphonic acid; ●, ethyl methylphosphonic acid; △, isopropyl methylphosphonic acid. Hydrogen flow-rate 650 ml/min and helium flow-rate 40 ml/min.

with similar, inverted air-hyperventilated flames as introduced by Burgett and Green[18]. The flow-rate of helium was varied between 0 and 100 ml/min. Flow-rates up to 50 ml/min did not affect either the detector response or the noise level. However, higher flow-rates resulted in an irregular and spiking baseline, whereas the absence of a helium flow caused system instability. Therefore, the helium flow was set at 40 ml/min.

Performance of the micro-column LC–FPD system

Chromatography. The performance of the micro-column LC–FPD system was studied by investigating the separation and detection of a number of organophosphorus acids. The evaluation of the chromatographic conditions has been previously reported[19]. Fig. 3 presents a characteristic chromatogram for the separation of meth-

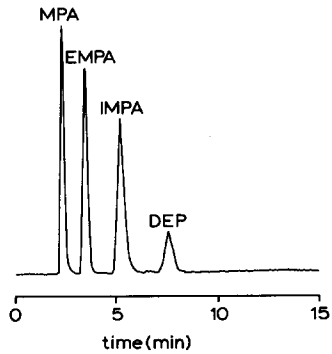

Fig. 3. Chromatogram of a number of organophosphorus acids, using FPD. Eluent: 0.05 *M* ammonium acetate, pH 5; flow-rate 8 μl/min. Column: 300 mm × 0.32 mm I.D. fused-silica capillary packed with 10-μm LiChrosorb RP-18. Injection volume: 60 nl. MPA = Methylphosphonic acid; EMPA = ethyl methylphosphonic acid; IMPA = isopropyl methylphosphonic acid; DEP = diethyl phosphoric acid.

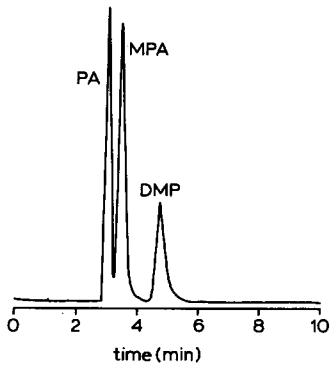

Fig. 4. Chromatogram of three phosphorus-containing acids, using FPD. Eluent: 0.1 *M* nitric acid; flow-rate, 6 μl/min. Column: 300 mm × 0.32 mm I.D. fused-silica capillary packed with 10-μm PRP-1. Injection volume: 60 nl. PA = Phosphoric acid; MPA = methylphosphonic acid; DMP = dimethyl phosphoric acid.

ylphosphonic acid, ethyl methylphosphonic acid, isopropyl methylphosphonic acid and diethyl phosphoric acid under ion-pair LC conditions. Closely related compounds such as phosphoric acid, methylphosphonic acid and dimethyl phosphoric acid are difficult to separate under these conditions. For these analytes, separation was achieved using a micro-LC column packed with the styrene–divinylbenzene copolymer PRP-1, and working under ion-suppression conditions at pH 1 (Fig. 4).

The repeatability of the system was determined by injecting 60 nl of a solution containing 3–250 ng of each compound. Peak height determination gave a relative standard deviation (S.D.) of 1.7–6.1%, derived from 31 series of measurements with a total of 148 injections. In the range 0.5–200 ng a good linear correlation of over 0.998 was obtained.

External peak broadening. The total peak broadening of the system was calculated as the initial M_2 values [k' (lim 0)] derived from the statistical moment method, the method of Yau and that of Foley and Dorsey using analytical data such as are shown in Fig. 3. The results obtained for 50-μm and 100-μm fused-silica capillary outlets of the interface are given in Table I; the average values are 0.28 and 0.50 μl^2, respectively.

From separate experiments with a micro-column LC–UV system[12], the peak broadening caused by the injector, column and connection tubes is known to be 0.22 μl^2. This indicates that the contribution of the flame photometric detector and the

TABLE I

TOTAL PEAK BROADENING, M_2, OF THE MICRO-COLUMN LC–FPD SYSTEM

Capillary I.D. (μm)	*M_2 (μl^2) according to* [a]		
	1	*2*	*3*
100	0.48	0.48	0.53
50	0.25	0.28	0.31

[a] 1 = Statistical moment method; 2 = method of Yau[13]; 3 = method of Foley and Dorsey[14].

TABLE II

DETECTION LIMITS OF ORGANOPHOSPHORUS ACIDS IN MICRO-COLUMN LC–FPD

Compound	*Detection limit of phosphorus*[a] *(pg/s)*
Phosphoric acid	34
Methylphosphonic acid	18
Ethyl methylphosphonic acid	22
Isopropyl methylphosphonic acid	20
Dimethyl phosphoric acid	20
Diethyl phosphoric acid	22

[a] Signal-to-noise ratio, 2:1; average mass flux.

interface amounts to 0.06 and 0.28 μl^2 for the 50- and 100-μm capillary outlets, respectively, at an effluent flow-rate of 8 μl/min. Although the 100-μm capillary is seen, contrary to the 50-μm capillary, to contribute significantly to the total peak broadening, it is still preferred because of the higher stability and reproducibility of the detector response.

Detection limits. Table II shows the detection limits calculated for the six organophosphorus acids measured in the present system, using various aqueous mobile phases. The average detection limit is about 20 pg/s of phosphorus as against about 1 pg/s of phosphorus in GC. This result, which corresponds with an injected amount of about 1 ng of analyte (injection volume 60 nl), is rather promising when one realizes that due to the necessity of (i) the position of the flame above the shield, and (ii) the relatively high hydrogen flow-rate, the photomultiplier probably is not in its optimum position relative to the flame and the POH emission.

Quenching effects. From the literature, the phenomenon of quenching of the POH emission intensity is well known[20] for a flame photometric detector used as a GC detector. The degree of quenching depends on various factors such as the type and concentration of the organic analyte, the oxygen-to-hydrogen ratio in the gas stream passing through the detector, the detector construction[21] and, finally, the temperature of the detector[22].

In the present study aqueous LC effluents were used containing 0.01–0.5 *M* ammonium formate or ammonium acetate and, occasionally, 0.05 *M* tetraethylammonium hydroxide (in order to achieve ion-pair chromatography), or 0.01–0.1 *M* nitric acid (in order to achieve ion suppression). Neither type of eluent affects the baseline stability or detector limit. The absence of quenching is in good agreement with results obtained in molecular emission spectroscopy experiments by Dagnall *et al.*[23] and Aldous *et al.*[24], who found no interference upon the addition of several acidic and ionic compounds which included nitric acid and acetates.

Table III shows the influence of the addition of methanol on the detection limit. As found previously, the quenching strongly depends on the flow-rate[10]. With increasing flow-rate, the increase of the detection limit in the absence of methanol is caused by an increased noise level, while the response remains constant. In the presence of methanol, the increase of the detection limit is chiefly due to a decrease of the

TABLE III

INFLUENCE OF THE METHANOL PERCENTAGE AND THE ELUENT FLOW-RATE ON THE DETECTION LIMIT OF METHYLPHOSPHONIC ACID (M) AND DIMETHYL PHOSPHORIC ACID (D) IN MICRO-COLUMN LC–FPD

–, Not detected due to early evaporation of the effluent.

Methanol in eluent (%)	*Detection limit (pg/s of P) at flow-rate (μl/min) of:*							
	2		*5*		*10*		*15*	
	M	*D*	*M*	*D*	*M*	*D*	*M*	*D*
0	18	25	24	30	44	60	61	78
5	19	27	30	40	97	67	125	158
10	24	30	29	43	83	120	200	290
20	–	–	80	110	288	440	1260	2160
30	–	–	100	160	850	1150	2520	4940

signal intensity. In order to limit the loss in detection performance in the case of water–methanol eluents, flow-rates of 5 μl/min or below are recommended. This is also advantageous, because the negative effect of methanol on the detection limit is less at lower flow-rates.

When using 10% acetonitrile in water as the eluent, at a flow-rate of 8 μl/min, detection limits of 44 and 77 pg/s of phosphorus were found for methylphosphonic acid and dimethyl phosphoric acid, respectively. This indicates that the quenching is of the same order of magnitude as when using 10% methanol in water as an eluent. The magnitude of the quenching effect is nicely illustrated in Fig. 5, where the dependence of the intensity ratio, Φ, as defined according to Sugiyama *et al.*[20]

$$\Phi = \frac{\text{FPD response (eluent with modifier)}}{\text{FPD response (eluent without modifier)}} \quad (1)$$

is plotted as a function of the mass flux of methanol. The data obtained with dimethyl phosphoric acid as a test compound indicate that a mass flux of 0.1 μmol of methanol

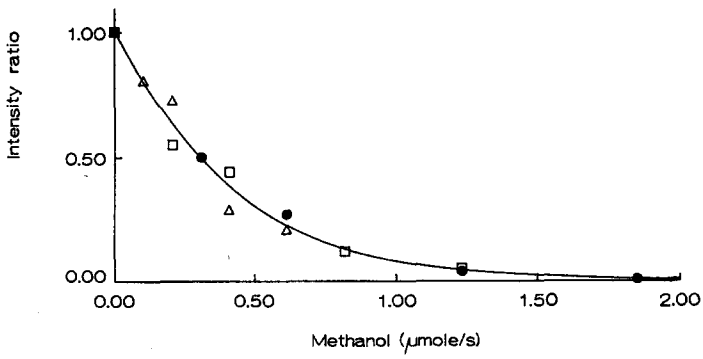

Fig. 5. Influence of mass flux (μmol/s) of methanol on intensity ratio (eqn. 1). Eluent flow-rate: ●, 15; □, 10; △, 5 μl/min. Test compound: dimethyl phosphoric acid.

or 1.2 μg of oxidizable carbon per second is permitted if one accepts a 20% loss in intensity ratio.

In GC using FPD in the sulphur mode, Dressler[22] found a 22% quenching effect at 4 ng/s of oxidizable carbon. However, using the same detector mode and quenching compound (cyclohexane), Fredriksson and Cedrgren[21] reported a quenching value of about 20% for as high a carbon flow-rate as 4 mg/s. They applied a hydrogen hyperventilated burner type as designed by Brody and Chaney[16], their results being obtained after optimization of the hydrogen-to-oxygen ratio as a function of the amount of carbon per second. Optimization of the hydrogen-to-oxygen ratio in the presence of a modifier and exchanging the positions of the hydrogen and air entrance to create an hydrogen hyperventilated flame, may therefore also be interesting when trying to extend the use of organic modifiers in microcolumn-LC–FPD.

CONCLUSIONS

The on-line coupling of microcolumn-LC to a commercially available flame photometric detector has been improved to permit the detection of non-volatile organophosphorus acids. As yet, these analytes cannot be handled by LC with on-line thermionic detection. The system has been succesfully applied to the separation of a series of these acids with a detection limit of 20 pg/s of phosphorus, a relative S.D. of less than 6% and a linear range of more than two orders of magnitude. The interface used is of simple design and may be suitable for other flame-based GC detectors as well. Preliminary experiments on coupling the microcolumn-LC interface to a thermionic detector are rather promising. Future research will deal with the utilization of the present microcolumn-LC–FPD system in the analysis of various types of samples. Besides, the potential and limitations of the use of several organic modifiers will be studied.

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