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APPLICATION OF HIGH-PERFORMANCE LIQUID CHROMATO-GRAPHY–FLOW INJECTION ANALYSIS FOR THE DETERMINATION OF POLYPHOSPHORIC ACIDS IN PHOSPHORUS SMOKES

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SUMMARY

A flow injection analysis system for the determination of inorganic phosphate has been coupled to a high-performance liquid chromatograph for use as a specific post-column detector for linear and cyclic condensed phosphoric acids. The condensed phosphates are separated by anion exchange (4 and 8% cross-linked resins), hydrolyzed to orthophosphoric acid, and condensed with molybdic acid to form a molybdenum blue complex which is detected in a flow through absorption cell. The system has been used to analyze the aerosol formed from the combustion of red and white phosphorus. Phosphates up to the P_{18} polymer have been detected. The phosphorus species formed depend upon specific combustion conditions. The age of the aerosol at which a sample is collected for analysis has been found to have a pronounced effect on composition.

INTRODUCTION

The separation of linear and cyclic condensed phosphoric acids by anion-exchange chromatography with detection by automatic air segmented phosphate analyzers has been previously described[1–4]. The disadvantages of this type of detection system which include relatively long analysis time and band broadening have been eliminated by substituting a continuous flow injection system as the post-column detector[5,6]. The flow injection analysis (FIA) system has been applied to the analysis of the lower oxo acids of phosphorus and to orthophosphate, pyrophosphate, and tripolyphosphate[5,7]. In the present study, an FIA system has been applied to the analysis of a complex mixture of condensed phosphates.

The combustion of phosphorus in air produces a dense white smoke which has been used by the military for screening and signaling purposes[8]. The smoke is composed of orthophosphoric acid and a mixture of polymeric phosphoric acids formed from the hydration of the initial combustion product, phosphorus pentoxide (P_4O_{10}), with the moisture present in air. The exact composition of the acids and the compositional variation as a function of combustion conditions has not, however, been determined. In a study conducted by Katz *et al.*[9], on the aerosol produced from burning white phosphorus, acids ranging in polymer number from 1 to 8 were iden-

tified. Higher polymeric species could not be determined with their method of analysis and no relationship between experimental combustion conditions, i.e., humidity and air flow across the burning fuel, and the resulting acids was determined.

In the present study, the aerosols formed from the combustion of two formulations, red phosphorus impregnated in butyl rubber (RPBR) and white phosphorus contained in a felt matrix (WP-F), were chemically characterized. The aerosols were analyzed by high-performance liquid chromatography (HPLC) coupled to a flow injection analyzer specific for phosphate. This method of analysis allowed determination of species ranging from orthophosphoric acid (H_3PO_4) up to the P_{18} polymer. Variations in composition as a function of air humidity, aerosol concentration, air flow-rate, and aerosol age are reported.

EXPERIMENTAL

Combustion

The RPBR smoke was produced at uniform concentrations with an extrusion combustion generator developed at ORNL[10]. In this system, RPBR is extruded through a 2-mm stainless-steel orifice and the emerging filament continuously burned in a 2-in. glass pipe tee. Humidified air enters one side of the tee and the aerosol exists the other. An air flow of 250 l/min carries the aerosol through flexible stainless-steel tubing to a 425-l volume Young and Bertke exposure chamber. The air velocity at the burn site is varied by restricting the air flow at the inlet of the burn chamber. Aerosol concentration is controlled by adjusting the extrusion rate and monitored on-line by a back-scattering particle sensor[11]. Moisture levels corresponding to a range of 20–100% relative humitity are produced by adding dry air or steam to the feed stream air.

In addition to the aerosol produced by the generator, RPBR and WP-F were also statically burned. In this case, fragments of the phosphorus formulations were placed in stainless-steel crystallization dishes filled with clean sand and placed under a small hood to collect the smoke. The hood was connected to the exposure chamber where the aerosol was sampled. Aerosol concentrations were controlled approximately by periodically adding fragments to the fire zone.

Sampling

Samples of 50–100 mg total weight were drawn directly from the exposure chamber with a vacuum pump onto 47-mm glass fiber filter pads. Following collection, the samples were immediately extracted by placing the pads in 100 ml of 0.22 M NaCl containing 5 mM Na_4EDTA. The pads remained in solution for a minimum of 2 h prior to analysis to assure maximum recovery. Samples placed in NaCl/EDTA preserved the polyphosphate species for a minimum of 72 h at room temperature.

Reagents

All chemicals were reagent grade and were used as received. Pyrophosphate, trimetaphosphate, tripolyphosphate, and tetrapolyphosphate were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Sodium phosphate glasses Types 5, 15, and 25 containing a mixture of polyphosphates of average chain length 5.5, 17, and 22, respectively, were also obtained from Sigma. Sodium tetrametaphosphate

was donated by E. J. Griffith (Monsanto, St. Louis, MO, U.S.A.). Orthophosphate standards were prepared from sodium hydrogen phosphate purchased from the Office of Standard Reference Materials, National Bureau of Standards (Washington, DC, U.S.A.). All standards were prepared in 0.22 M NaCl containing 5 mM Na$_4$EDTA.

The reagent used in the flow injection system for the determination of phosphates was a 5:1 mixture of 5.7 × 10^{-3} M ammonium molybdate, (NH$_4$)$_6$Mo$_7$O$_{24}$ · 4H$_2$O, in 1.8 M H$_2$SO$_4$ and 0.05 M L-ascorbic acid containing 5% (v/v) acetone. The ascorbic acid partially reduces the Mo(VI) to Mo(V). The Mo(V)–Mo(VI) complex combines with orthophosphate to form the heteropolyblue complex[12]. The individual molybdate and ascorbic acid solutions were stable for several months. The mixed reagent had limited stability and was prepared daily.

Flow injection analyzer

The flow injection system was adapted from Hirai *et al.*[5], with a few modifications. An Eldex Model E-120-S pump (Eldex, Menlo Park, CA, U.S.A.) delivers the molybdenum reagent at a flow of 0.5 ml/min. Samples for total phosphate analysis are introduced into the reagent stream with a Rheodyne Model 7125 six-port injection valve equipped with a 20-μl sampling loop. The sample and reagent react in a 10-m PTFE coil heated to 140°C. This coil is wrapped around a 6 × 2 in. O.D. glass pipe which is then wrapped with electrical heating tape. The temperature is controlled with a Barber Coleman Model 520 Solid State Controller. The reacted sample passes through a 1-m coil cooled by chilled water before flowing through an Altex Model 153 UV detector (volume 8 μl, path 10 mm). Adsorption of the phosphate complex is measured at 660 nm. A 10-m backpressure coil attached to the exit of the detector prevents bubble formation caused by heating the reaction coil preventing anomalous responses.

Chromatographic separations

A Spectra Physics 8700 solvent delivery system was used for programming and delivering the solvent at a constant flow of 1 ml/min through the analytical column. Samples were injected with a Rheodyne 7125 six-port injection valve with a 50-μl sample loop onto a Vydac SC anion guard column (4 cm × 4 mm I.D.; Varian, Palo Alto, CA, U.S.A.). The polyphosphates were separated on an Animex A-27 8% cross-linked quaternary ammonium anion-exchange column (30 cm × 4 mm I.D.; Bio-Rad, Richmond, CA, U.S.A.) using NaCl solutions (pH 10) containing 5 mM Na$_4$EDTA. A linear gradient from 0.28 M NaCl to 0.53 M NaCl over a 40-min time interval was used and the final eluent concentration was held for an additional 15 min. Some analyses were also conducted on an Animex A-14 column (4% cross-linked resin, 30 cm × 7.8 mm I.D., Bio-Rad). In this case, the solvent was programmed from 0.31 M NaCl to 0.53 M NaCl at a linear rate over a 260-min time interval. The effluent from the analytical columns was introduced directly into the stream of reagent used for FIA. A diagram of the entire system is shown in Fig. 1.

RESULTS AND DISCUSSION

HPLC–FIA system

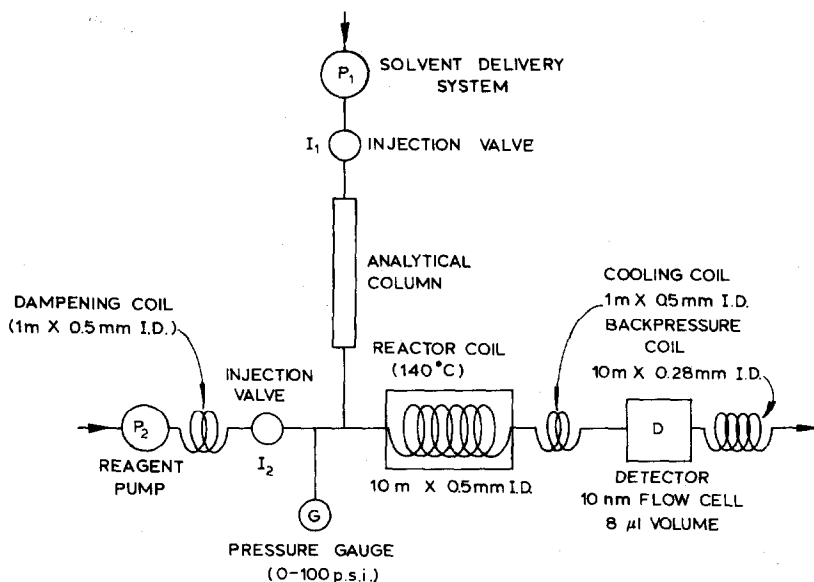The analytical system described could be used for either the rapid "batch"

Fig. 1. Schematic diagram of HPLC-FIA system.

analysis for total phosphate or as a detector for individual phosphate species follow-
ing their chromatographic separation. The polyphosphates are hydrolyzed in the
reaction coil by the acidic reagent and elevated temperature to orthophosphate which
then condenses with Mo(V)–Mo(VI) to form the colored complex. To obtain maxi-
mum response the hydrolysis must be complete. The system was optimized by ana-
lyzing various standards including the phosphate glasses at different reactor coil tem-
peratures. The results including the response obtained orthophosphate are shown in
Fig. 2. Color development for orthophosphate was essentially complete above 125°C
and maximum response for the other standards was obtained at 150°C. These results
confirm that the hydrolysis is the rate limiting reaction in the overall determination[5].
The responses for the pure standards also indicate that hydrolysis was 95% or greater
at 140°C. This lower temperature was chosen for the analyses because of the accom-
panying lower operating pressure for the FIA system. There were also only slight
differences in the responses of the glasses at 140 and 150°C. Since the amount of
phosphate in these glasses was calculated from the average chain length and was not
accurately known, their response was not equivalent to the other standards.

The maximum absorption of the complex using the conditions described was
at 820 nm. Rather than modifying the detector to allow measurements at this wave-
length, determinations were made at 660 nm. The sensitivity was satisfactory and the
linear range was from 1.5 to 105 ppm. The relative standard deviation for replicate
injections was less than 1% and determinations for total phosphate could be made
at a rate of 25 samples per hour.

Fig. 3 shows the results obtained for the analysis of the Type 5 glass on Aminex
A-27 using the FIA system as a post-column detector. The individual compounds are
well separated and the total analysis time is less than 1 h. The mobile phase contained
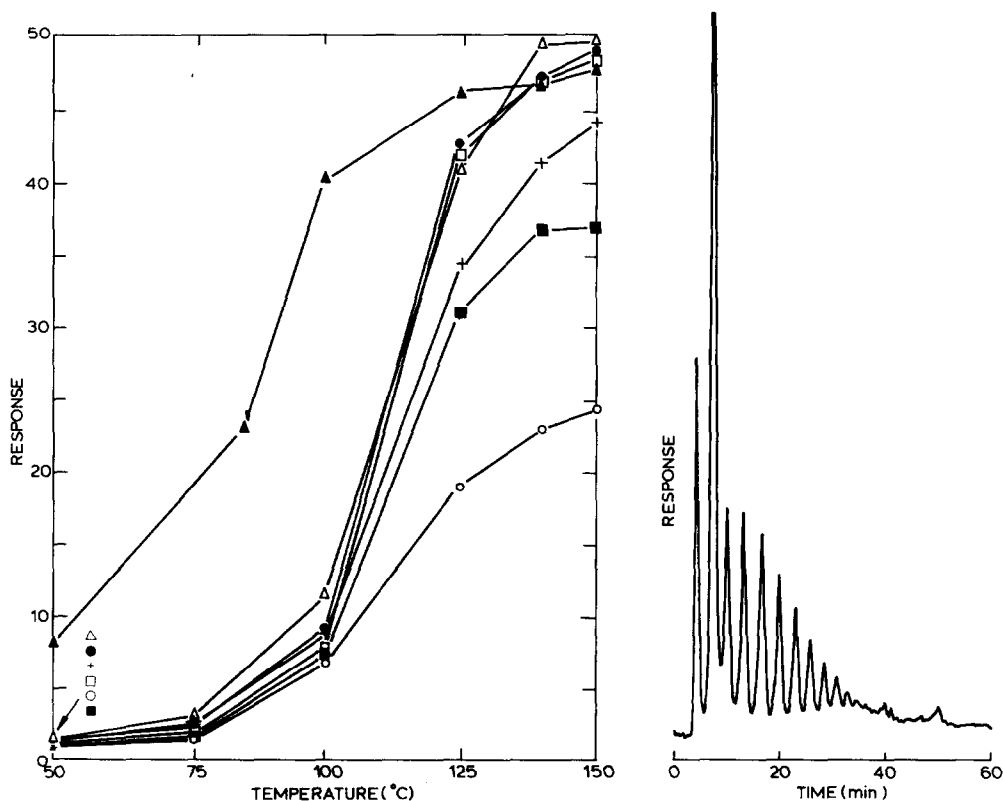Na_4EDTA which has been reported to improve the resolution and reproducibili-

Fig. 2. Response of orthophosphate (▲), tripolyphosphate (△), trimetaphosphate (●), tetrapolyphosphate (□), and Type 5 (+), Type 15 (■), and Type 25 (○) phosphate glass at different reactor coil temperatures. Concentration of each standard: 25 μg PO_4^{3-}/ml. Phosphate concentration of glasses calculated from average chain length. Response given in arbitrary units.

Fig. 3. HPLC profile of Type 5 phosphate glass.

ty[4,13]. The chelating agent inhibits the hydrolysis of polyphosphates which is accelerated by trace metal ions that may be present in the chromatographic system. The addition of EDTA to the extracting agent for the aerosol samples and to standard solutions was also found to preserve their integrity for extended periods.

Phosphate aerosols

A typical profile of a generator-produced RPBR aerosol sample on the Aminex A-27 column is shown in Fig. 4. The sample was collected at an aerosol concentration of 1.8 mg/l, relative humidity 58%, chamber temperature 22°C, and air velocity 252 m/min across the burn site. A series of polyphosphate species ranging from orthophosphate to the P_{13} polymer were determined. With the exception of ortho- and occasionally pyrophosphate, P_5 or P_6 is found in the highest concentration and as the polymer number increases beyond P_6, there is an approxinate linear decrease in concentration. The large "envelope" that elutes from the column is believed to contain an unresolved mixture of longer straight chain (> P_{14}), branched, and cyclic phosphates. Analysis of the samples on the Aminex A-14 column provided greater

TABLE I

COMPOSITION OF THE PHOSPHORIC ACIDS IN PHOSPHORUS SMOKES

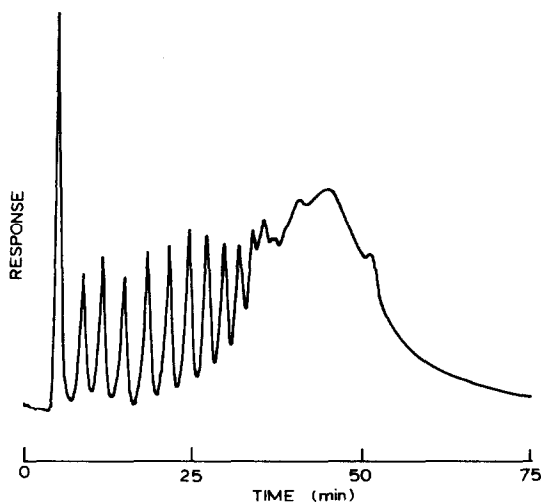| Parameter	Generation conditions			Composition (%)							
	Air velocity (m/min)	% Relative humidity	Aerosol concentration (mg PO_4^{3-}/l)	Ortho-phosphate	Pyro-phosphate	Tripoly-phosphate	Tetrapoly-phosphate	P_5-P_{13}	Higher polyphosphates	Tetrameta-phosphate	Trimeta-phosphate
Humidity	252	20	1.4	20.0	7.9	5.4	6.3	27.1	32.8		
		45		17.6	4.9	6.0	6.2	34.8	29.1		
		58		7.1	2.1	3.0	2.6	17.2	67.4		
		80		4.9	1.8	2.6	2.5	17.3	69.8		
		100		4.0	1.9	2.5	3.0	17.7	69.7		
Air velocity	123	45	1.3	15.0	3.9	4.7	4.8	30.5	40.7		
	252			17.6	4.9	6.0	6.2	34.8	29.1		
	493			22.2	5.0	6.3	7.4	43.5	13.4		
Aerosol concentration	252	51	0.8	17.6	7.7	5.7	5.7	26.3	36.9		
			2.0	12.4	4.1	4.1	4.5	26.9	48.0		
			4.6	16.8	7.7	5.8	6.2	30.3	33.2		
Aerosol age	252	30	1.5								
Unaged				9.5	3.6	3.9	4.5	24.3	53.3		
30 min				16.6	7.0	3.4	3.0	12.1	57.9		
60 min				31.6	14.0	5.5	4.7	15.2	29.0		
				49.0	22.1	7.2	5.4	13.9	2.4		
Hydrolysis (Sample analysis time)	252	30	1.5								
4 h				10.0	3.5	3.8	4.9	27.2	49.5	0.7	0.4
24 h				12.0	4.8	6.5	9.8	42.3	16.5	0.9	7.2
48 h				15.2	6.5	9.8	13.9	36.3	–	1.4	16.9
71 h				16.8	7.5	12.3	16.2	26.2	–	2.3	18.7
Free burning	252	38									
RPBR			1.7	22.8	19.6	13.3	11.5	32.8	–		
WP-F			2.1	23.8	26.6	16.3	11.3	22.0	–		

Fig. 4. HPLC profile of generator-produced RPBR aerosol sample.

resolution of this mixture and allowed determinations up to the P_{18} polymer. Because the concentration of these phosphates (P_{14}–P_{18}) was very low, *i.e.,* <1% of the total phosphate each, and the analysis time on this column was very long, *i.e.,* 260 min, the samples were routinely analyzed on Aminex A-27. The concentration of the higher polymers in the envelope was calculated as the difference between the total phosphate concentration and the other distinct peaks which could be quantitated. Small amounts of the cyclic phosphates, tetrameta- and trimetaphosphate were also detected in the generator-produced aerosols. They eluted at 42.5 and 53 min respectively, and contributed less than 1% of the total phosphate each.

Table I shows the variation in the composition of the aerosol as a function of generator conditions. The effects of humidity, air velocity, and concentration were investigated separately while other parameters which could influence composition were held constant, *i.e.,* as the air velocity was modulated, the concentration and humidity were maintained at a fixed level. All samples for analysis were collected at ambient chamber temperatures. The results for the concentration study show that there were only slight variations in the contributions of each species with no major compositional differences. As the chamber humidity was varied from 20% to 100%, however, some species differences were noted. The level of orthophosphate decreases and the concentrations of the higher polymers increase between 45 and 58% relative humidity. The concentrations of the various species above 58% were essentially the same and there was also little difference between the polymers at the 20 and 45% moisture levels. Since hydrolysis is accelerated by acidic conditions, there may be less depolymerization at high humidities where individual aerosol particles may be less acid. As the air velocity was increased across the burn site, lower quantities of higher polymeric species were formed. The concentration of orthophosphate was also slightly elevated at the fastest burn velocity.

A study was also conducted to partially simulate the aging process of the phosphorus smoke cloud in the field. In the environmental exposure chamber the aerosol concentration was allowed to reach a steady state as indicated by light scat-

tering monitors. Samples collected at this point were designated unaged. The chamber was then sealed at both the entrance and exit to prevent loss of the aerosol. Filter pad samples were collected periodically for 2 h. It was determined under these conditions with time that the concentrations of orthophosphate and straight chain polymers from P_2 through P_{13} increase while higher polymeric forms decrease (see Table I). At 120 min the unresolved envelope has almost completely disappeared. Although the exact mechanism for these changes is not presently understood, the transitions probably occur because of the rapid hydrolysis of the polymeric species in the acidic aerosol.
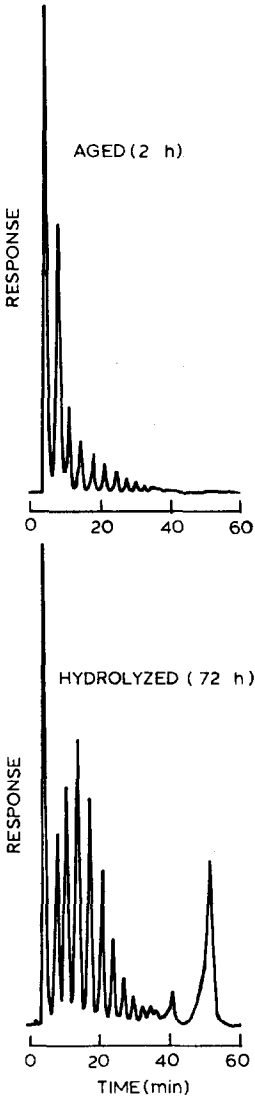

Fig. 5. HPLC profiles of an aerosol sample allowed to age in the exposure chamber and an aerosol sample extracted and hydrolyzed in water.

To examine the hydrolytic process in solution, aerosol pad samples were extracted in water rather than NaCl/EDTA. The aqueous solution was then analyzed approximately every 24 h up to 72 h. It was determined as the extracted sample hydrolyzes, the concentrations of long and branched chain polymers decrease but the concentrations of trimeta- and tetrametaphosphate increase. The level of orthophosphate also increases, but only slightly. These results are reported in Table I and profiles of an aged aerosol sample and hydrolyzed sample are shown in Fig. 5 for comparative purposes.

The aerosols produced from free-burning RPBR and WP-F may more closely simulate the smoke produced in the field and samples collected under free-burning conditions were analyzed to evaluate the similarities of the generator *vs.* free burn and RPBR *vs.* WP-F smokes. The free burns were produced under nearly identical conditions. Air flow through the burn chamber was ≈ 100 l/min and dilution air was added to adjust the total flow through the exposure chamber to 250 l/min. Aerosol concentrations fluctuated because the burn rate is not controlled in a static burn but an average concentration of 2.5 mg/l for the RPBR and 2.9 mg/l for the WP-F was obtained. The HPLC profiles for the two phosphorus formulations were very similar with regard to the number of compounds detected and their concentration levels. P_{11} was the highest polymeric phosphate found and no cyclic compounds were determined in either the WP-F or RPBR smokes. Contributions for individual phosphates are reported in Table I. The major difference between the generator and free-burn aerosols is the absence of higher polymeric forms in the free burns. Some differences are expected, however, since the burn rate and temperature (which are expected to influence the degree of polymerization) are not controlled in the latter. In addition, the samples from the free burns were collected at a low humidity which was shown to influence the concentration of the higher polymers.

CONCLUSIONS

An HPLC–FIA system was assembled and used to analyze the complex mixtures of polyphosphoric acids found to be present in various phosphorus smokes. As a specific post-column detector, the FIA system was both sensitive and accurate allowing determination to be made readily. Costly modifications to the absorbance detector were avoided by making measurements at a fixed wavelength which did not correspond to the absorbance maximum but still provided sufficient sensitivity. Polyphosphates up to P_{13} could be easily analyzed in less than 1 h.

The system facilitated the analysis of the phosphorus smokes as they were produced under various conditions. Differences in phosphate composition at different humidities were noted although the most pronounced changes in speciation occurred as the aerosol was allowed to age. In this case, hydrolysis occurs rapidly and depolymerizes the higher phosphates to lower forms. Hydrolysis of very dilute samples, *i.e.*, aerosol extracts in water, was much slower and was characterized by a marked increase in the concentration of some of the cyclic phosphates. Samples analyzed from free-burning red and white phosphorus were found to contain only straight chain species less than P_{11}.

REFERENCES

1 D. P. Lundgren and N. P. Loeb, *Anal. Chem.*, 33 (1961) 366.
2 F. H. Pollard, G. Nickless, D. E. Rogers and M. T. Rothwell, *J. Chromatogr.*, 17 (1965) 157.
3 N. Yoza, K. Ito, Y. Hirai and S. Ohashi, *J. Chromatogr.*, 196 (1980) 471.
4 H. Yamaguchi, T. Nakamura, Y. Hirai and G. Ohashi, *J. Chromatogr.*, 172 (1979) 131.
5 Y. Hirai, N. Yoza and S. Ohashi, *Anal. Chim. Acta*, 115 (1980) 269.
6 J. Ruzicka and E. H. Hansen, *Anal. Chim. Acta*, 114 (1980) 19.
7 Y. Hirai, N. Yoza and S. Ohashi, *J. Chromatogr.*, 206 (1981) 501.
8 K. Wasti, K. J. R. Abaedoo and J. E. Villaume, *A Literature Review — Problem Definition Studies on Selected Toxic Chemicals. Volume 2. Occupational Health and Safety Aspects of Phosphorous Smoke Compounds*, Franklin Institute Research Labs., Rockville, MD, April, 1978.
9 S. Katz, A. Snelson, R. Butler, W. Bock, N. Rajendron and S. Relwani, *Physical and Chemical Characterization of Military Smokes. Part III. White Phosphorous-Felt Smokes*, Final Report, IIT Research Institute, Chicago, IL, May 29, 1981.
10 R. W. Holmberg and J. H. Moneyhun, *Proc. Smoke/Obscurants Symposium VI, Adelphi, MD, April 27-29, 1982*, U.S. Army Report DRCPM-SMR-T-001-82, Aberdeen Proving Ground, MD, 1982, pp. 767-775.
11 T. M. Gayle, C. E. Higgins and J. R. Stokely, *A Continuous Smoke Monitoring Device for Continuous Animal Exposure Systems*, in *Tobacco Smoke Inhalation Bioassay Chemistry*, ORNL-5424, Oak Ridge National Laboratory, Oak Ridge, TN, 1979.
12 Y. Hirai, N. Yoza and S. Ohashi, *Chem. Lett.*, (1980) 499.
13 T. Nakamura, T. Yano, T. Nunokawa and S. Ohashi, *J. Chromatogr.*, 161 (1978) 421.