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DIRECT DETERMINATION OF ELEMENTAL PHOSPHORUS BY GAS–LIQUID CHROMATOGRAPHY

R. F. ADDISON AND R. G. ACKMAN
Fisheries Research Board of Canada, Halifax Laboratory, Halifax, Nova Scotia (Canada)
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SUMMARY

A rapid and sensitive method for the analysis of elemental phosphorus is described. Phosphorus is extracted into a suitable organic solvent, isolated by gas–liquid chromatography, and measured in a highly sensitive and specific flame photometric detector. Phosphorus levels as low as 10^{-12} g (corresponding to sensitivities of about 2 parts in 10^{12} in concentrated extracts) can be measured in a few minutes. Application of this method to the analysis of water, mud and biological samples is described.

INTRODUCTION

Methods currently in use for the determination of elemental phosphorus rely on isolation of phosphorus by wet or dry distillation, or by organic extraction, followed in either case by oxidation to phosphate which is determined by any standard method[1,2]. This approach has a number of drawbacks: it is not specific for elemental phosphorus, since it measures total distillable or organic-extractable phosphorus; it is relatively slow, and large samples are required for high sensitivity.

A recent problem of pollution of Newfoundland waters by yellow phosphorus led us to develop an alternative method. We chose analysis by gas–liquid chromatography, since (a) phosphorus is relatively low-boiling and volatile, and has been gas chromatographed[3], and (b) the development of various phosphorus-specific detectors[4,5] allows the detection of as little as 10^{-12} g of this element. As in the classical approach phosphorus is partially isolated by extraction into a suitable organic solvent; a sample of this extract is then subjected to GLC and the phosphorus response is compared to that of standards. This method offers considerable advantages in specificity, sensitivity and rapidity of analysis over methods currently in use.

EXPERIMENTAL

Extraction of phosphorus

All extractions were carried out with either benzene (pesticide quality: Matheson, Coleman and Bell) or isooctane (Fisher Scientific Co., or British Drug Houses).

Operations were carried out in an inert atmosphere (nitrogen or argon) wherever possible. Samples of three types were dealt with as follows:

(*a*) *Water.* Phosphorus was extracted from water samples by vigorous shaking with an organic extractant (2:1, v/v) for a few minutes. Samples in which large amounts of phosphorus were expected were re-extracted once.

(*b*) *Mud.* Up to 5 g mud was extracted with 50 ml extractant by swirling in a stoppered erlenmeyer-type flask with a few 5-mm glass beads for 10–15 min. The samples were then filtered (Whatman No. 1 paper) and the residue re-extracted once where necessary. The filtrates were allowed to separate and the organic layers were recovered for analysis.

(*c*) *Tissue.* Up to 10 g tissue was homogenized for 2 min with 50 ml extractant in a stainless steel blender (Sorvall "Omnimixer") cooled in ice. The blend was filtered and the residue re-extracted if necessary. The filtrate was allowed to separate and the organic layer was taken for analysis.

The efficiency of the extraction methods was determined as follows:

In the case of water, weighed amounts of yellow phosphorus (BDH) were suspended ultrasonically (particles at 5–50 μ) in distilled water. After a short period to allow some coarse unsuspended materials to settle out, extractions were performed as described above.

In the case of mud, aqueous suspensions containing known concentrations of phosphorus were pipetted on to mud samples, under argon, and mixed. Extractions were then carried out immediately as described above.

In the case of tissue, aqueous suspensions containing known concentrations of phosphorus were injected (hypodermic) into weighed amounts of trout muscle. These were then extracted as described above.

Gas–liquid chromatography

Standard solutions of phosphorus were prepared by weighing yellow phosphorus under water, drying it by a brief (10 sec) immersion in acetone, and dissolving it in benzene. The solutions were stored at 0° in the dark under argon. These solutions were stable over periods of several weeks when used daily or longer if used only occasionally to prepare fresh dilutions.

Chromatography was carried out on either a MicroTek 220 or a laboratory-built instrument. Both were fitted with the Melpar (Tracor, Inc., Austin, Texas) flame photometric detector (FPD) employing a 526-mμ filter. (This detector was also operated as a slightly inefficient flame ionisation detector (FID).) Signals were recorded on Honeywell ElektroniK 1-mV recorders fitted with disc integrators of Disc Instrument Co., Inc. Conditions for chromatography are shown below:

Instrument:	MicroTek	Lab. built
Column:	2 m × 3 mm U-glass	2 m × 3 mm coiled glass
Packing:	OV 1, 3% on Chromosorb W	SE-30, 3 % on Chromosorb W
Column temp.:	100°	120°
Injector temp.:	200°	200°
Detector temp.:	200°	200°
Carrier gas flow:	He, 80 ml/min	He, 80 ml/min

The H_2, O_2 and air flows were those specified in the FPD manufacturer's instructions.

All injections were made using Hamilton No. 701 syringes. Under these conditions phosphorus was eluted as a sharp peak less than 2 min after injection (Fig. 1).

Detector response (load of phosphorus *vs.* peak area) was found to vary slightly from day to day, possibly because of small variations in flame characteristics. A calibration curve was therefore plotted immediately before each series of analyses. Although the manufacturer claims[6] that FPD response is linear over a wide load range (10^3-fold), we found it much more practical to work within a narrower (50-fold) range of 1×10^{-10} to 5×10^{-9} g over which peak area response was consistently linear. The reasons for this were: (a) loads below 1×10^{-10} g were approaching the limit of sensitivity of the detector (around 10^{-12} g for a peak twice as high as mean noise level), and (b) loads of more than 10^{-8} g were eluted as multiple peaks (discussed further below). Samples were therefore diluted where necessary, or appropriate aliquots taken for injection so that loads fell within the calibration range. The phosphorus content of the injected samples was then easily read from the calibration curve.

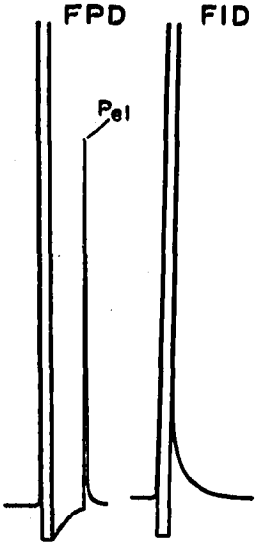


Fig. 1. FPD and FID responses to 5×10^{-10} g elemental phosphorus.

Determination of phosphorus by oxidation method

An organic extract of phosphorus from mud or water samples was brominated, hydrolysed[7], and the phosphate produced determined by the method of ALLEN[8].

RESULTS AND DISCUSSION

Efficiency of extraction

It is known that suspensions of elemental phosphorus in aqueous suspensions deteriorate rapidly[9], even after the initial settling of undispersed or clumped particles. The exact recovery of known weights of added phosphorus is therefore impossible in any experimental system containing water. Our experimental approach of preliminary isolation by extraction with organic solvent is necessarily deficient in absolute terms, but the recovery from water systems is independent of amount of phosphorus present,

TABLE I

RECOVERY OF ELEMENTAL PHOSPHORUS ADDED TO WATER, MUD AND TISSUE BY TWO SUCCESSIVE EXTRACTIONS

Medium	*Phosphorus originally added (μg)*	*Phosphorus recovered (μg)*		*% recovery*	
		Ext. No. 1	*Ext. No. 2*	*Ext. No. 1*	*Ext. No. 2*
Water	25.0	19.4	0.6	78	2
	100.0	78.0	2.0	78	2
	100.0	74.0	2.0	74	2
Mud	1.8	1.62	ND[a]	90	–
	1.8	1.62	ND[a]	90	–
	1.8	1.38	0.12	77	6
Tissue	23.5	18.2	2.9	77	12
	47.0	40.4	4.2	86	9

[a] ND = not detected.

and 95–98 % of recovered phosphorus is extracted in one step (Table I). A second extraction recovers only a small proportion, often below detection limits when the aqueous concentration is very low. For many practical purposes, therefore, one extraction would be adequate. The organic extract, although saturated with water, is remarkably stable in respect to phosphorus, and may be safely stored at ambient temperature for some time if in a closed container.

Recoveries from mud and tissue samples are also shown in Table I. Again, the apparent failure to recover all the phosphorus applied is probably attributable to deterioration of the phosphorus in the added aqueous suspensions. As with water samples, one extraction would probably be adequate for most practical purposes.

Comparison of GLC and classical methods of elemental phosphorus determination

There seemed little point in attempting to measure elemental phosphorus in tissue by the oxidation method, since organic-extractable phosphate would interfere with the classical approach, and comparisons were carried out on water and mud extracts only. Results of six analyses of benzene extracts are shown in Table II. It is clear that agreement between the two methods is satisfactory.

TABLE II

COMPARISON OF PHOSPHORUS ANALYSES BY GLC AND CONVENTIONAL METHODS

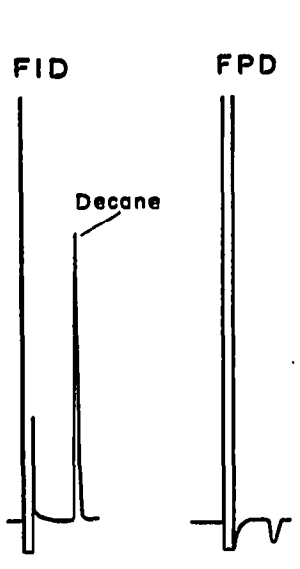
Sample	*Phosphorus concentration (p.p.m.) in extract*	
	GLC	*Conventional*
Benzene extract of water	25	24
	50	38
	50	50
	100	98
Benzene extract of mud	0.600	0.640
	0.850	0.784

Sensitivity and specificity to elemental phosphorus

At best, the FPD will detect around 10^{-12} g phosphorus, but measurement at this level is imprecise (± 100%). However, in a typical 5-μl injection from a 50-ml organic extract of water (2:1, v/v) this represents a minimum detectable concentration of phosphorus in water of around 0.1 parts per thousand million. While this is acceptable for most applications, recent (unpublished) work from this laboratory suggests that elemental phosphorus suspensions in water may be toxic to fish at well below the parts per thousand million level. We have therefore increased the sensitivity of the method by carefully concentrating the organic extracts, avoiding going to dryness, under a gentle stream of inert gas. In this way, it is possible to increase the sensitivity by a factor of 25 or 50; *i.e.*, to the order of two parts in 10^{12}.

The specificity for elemental phosphorus is based (a) on the characteristic retention time of phosphorus and (b) on the relative responses of FPD and FID to phosphorus. The FPD with 526-mμ filter is highly sensitive to P-containing compounds but is considerably less sensitive to purely organic materials. Thus, the minimum detectable amount of decane (which co-migrated with elemental phosphorus under the particular GLC conditions described above) was around 10^{-7} g in the FPD, and this was recorded as a negative peak in the absence of phosphorus. Elemental phosphorus, furthermore, gives no FID response (even in mg loads on an efficiently run detector) (Fig. 1). By using the FPD and FID simultaneously (dual channel electrometer and two pen recorder) it is therefore possible to distinguish between elemental phosphorus and any organic material emerging with the same retention time. Typical responses for FPD and FID detectors to hydrocarbons are shown in Fig. 2.

A further illustration of the specificity of the method is shown in Fig. 3. Industrial treatment of waste colloidal solutions of phosphorus involves flocculation at a high pH. During this process, some phosphine (PH_3) is formed, and as this is extractable with organic solvents it interferes with the determination of elemental phosphorus



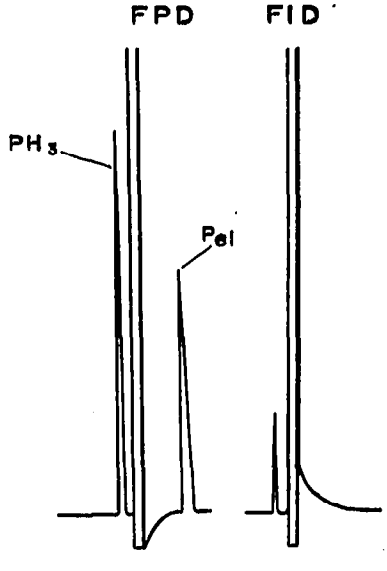


Fig. 2. FPD and FID responses to 1 × 10^{-6} g decane.

Fig. 3. FPD and FID responses to a mixture of phosphine and elemental phosphorus.

by the conventional oxidation method. The GLC approach, however, allows the isolation and independent determination of elemental phosphorus, as well as indicating the presence of phosphine.

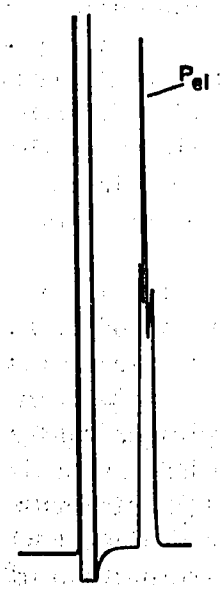


Fig. 4. FPD response to 10^{-8} g elemental phosphorus.

GLC behaviour of heavy loads of phosphorus

Loads of phosphorus exceeding 10^{-8} g were eluted as multiple peaks (Fig. 4). A possible explanation of this is that in very low concentrations (as would occur in injections of 10^{-12} to 10^{-9} g phosphorus) phosphorus vapour may exist monatomically whereas at higher concentrations there is some recombination. Under the GLC conditions used, it would not be surprising if species of (say) mol. wt. 31, 62 and 124 were partially separated. It is interesting to note that at high temperatures (above 800°) phosphorus vapour is also partially dissociated[10].

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