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Hybrid flow system integrating a miniaturized optoelectronic detector for on-line dynamic fractionation and fluorometric determination of bioaccessible orthophosphate in soils

Marta Fiedoruk [a], David J. Cocovi-Solberg [b], Łukasz Tymecki [a], Robert Koncki [a], Manuel Miró [b,*]

[a] Department of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland
[b] FI-TRACE group, Department of Chemistry, University of the Balearic Islands, Carretera de Valldemossa km 7.5, E-07122 Palma de Mallorca, Spain

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ABSTRACT

An integrated Sequential Injection (SI)/Flow Injection (FI) system furnished with a miniaturized LED-based fluorometric detector is presented in this work for expedient bioaccessibility tests of orthophosphate in soils. Equipped with a microcolumn of conical shape containing 50 mg of soil, the hybrid flow system was resorted to on-line dynamic leaching and real-time quantification of pools of mobilizable orthophosphate using a bi-directional syringe pump and multiposition valve. The flexibility of the flow manifold was harnessed to explore both bi-directional and uni-directional flow extraction modes with the added degree of freedom of on-line dilution of extracts whenever needed. Bioaccessible orthophosphate was split in three fractions, the so-called NH_4Cl fraction containing labile exchangeable phosphates, the alkaline fraction with Fe and Al-bound phosphates and the acidic fraction containing Ca-bound phosphates.

The prevailing molybdenum blue photometric detection method is replaced by spectrofluorometric detection based on the ion pair formation between the phosphomolybdate heteropolyacid and rhodamine B with the subsequent quenching of the dye fluorescence. The dedicated optoelectronic detector was integrated in a secondary FI manifold and operated according to the fluorometric paired emitter–detector diode (FPEDD) principle involving two light emitting diodes as fluorescence inductors and one as detector of LED-induced fluorescence.

Demonstrated with the analysis of a standard reference material (SRM 2711) and a real agricultural soil, the developed FI/SI fractionation system with FPEDD detection is proven reliable against the standard molybdenum blue method ($p > 0.05$), and useful for investigation of the leaching kinetics of orthophosphate in bioaccessibility tests through in-line recording of the extraction profiles.

1. Introduction

Phosphorus plays an important role in the environment and food web as is contained in fertilizers for supporting the plant growth and in food additives as well. On the other hand, phosphorus is a factor of eutrophication in water reservoirs, such as lakes or rivers, and might pose severe risks to aerobic living organisms [1]. Phosphorus occurs in different forms – both inorganic, namely orthophosphates, metaphosphates and polyphosphates, and organic species [2–4], which include e.g. phospholipids, sugar phosphates, nucleic acids and phosphoproteins [5]. The labile organic forms of phosphorus might hydrolize on a short notice [6]. Immediate detection is thus needed after sampling or leaching for discrimination between inorganic and organic phosphorus species. This requirement is most likely met with flow analysis methodology [7]. In case of soil analysis in-line microcolumn/chamber extraction coupled to downstream detection allows the simplification of the analytical leaching procedures for orthophosphate and the minimization of the hydrolysis of the organic forms [8,9].

Free inorganic phosphorus forms can be measured using different analytical methods including potentiometry [10], voltammetry [11] and amperometry [12] but the most commonly used are optical methods in combination with the molybdenum blue chemistry. Flow methods have been frequently used in view of their green chemical credentials as a result of miniaturization, automation, high sample throughput with the extra degree of on-line sample handling at hand [13,14]. A large number of FI and SI methods focused on detection of

* Corresponding author.
E-mail address: manuel.miro@uib.es (M. Miró).

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the yellow heteropolyacid complex (phosphomolybdate acid) generated by reaction of orthophosphate with molybdate in acidic medium [13–15], yet this method is deemed not sensitive enough in multifarious environmental analysis. Sensitivity amelioration is gained by reducing the heteropolyacid to the molybdenum blue dye using ascorbic acid, tin (II) chloride, hydrazine or hydroquinone [14–16]. The molybdenum blue method was also used in combination with enzymatic reactions based on alkaline phosphatase to determine orthophosphate [17] and flow-based preconcentration setups using anion-exchange reactors [18]. Another existing photometric method for determination of orthophosphate is based on the ion-pair association between the yellow heteropolyacid and Malachite Green, yet it affords poorer repeatability and narrower linear range as compared to the molybdenum blue chemistry [13]. Besides photometric methods fluorometric detection is also feasible on the basis of the ion-pair formation between the heteropolyacid and fluorophores, such as rhodamine B [13,19] and rhodamine 6G [20,21] for indirect analysis relying upon fluorescence quenching. This procedure has been adapted to a flow-based format as well [13,14,19–21].

In this work, the concept of light-emitting diodes (LEDs) as both emitters and detectors of light (so-called paired emitter–detector diode (PEDD) device) is used. This idea has been recently launched but deemed prospective. New LED-based miniature detectors have thus been constructed and dedicated to particular analytical applications with no need of optical filters. LEDs are extremely affordable what makes the cost of PEDDs really low. They are also stable, sturdy, highly efficient and feature long-term operation. Furthermore, LEDs need not that much power to elicit light. Two additional merits are the narrow-band light emission (width at half maximum averages 20 nm) and their versatility with commercially available LEDs from UV to NIR spectral range.

The LED working as detector should operate in reversed mode. The current produced on this LED however is too small to be measured. Diamond's team was the first in building an optical detector based on paired LEDs, measuring the time of discharge of the LED detector [22,23]. Further research was done to improve the way of measurements of the analytical signal generated by PEDDs [24,25]. The voltage has also been used as analytical readout, which is proportional to the light intensity, and correlates with the concentration of analyte as described by the Shockley's diode equation and the Lambert–Beer–Bouguer law [24]. Critical comparison of LEDs against photodiodes as detectors has been recently reported by Hauser's team [26].

PEDDs are predominantly developed for photometric measurements and several research teams utilized this concept to build miniaturized detection platforms for analytical purposes [27–31]. LEDs can also be used as fluorescence inductors in dedicated cell geometries. Only recently, prototypes of fluorometric PEDD (FPEDD) have been reported [32–37]. FPEDD detectors are greatly cost-effective and miniaturized in comparison to conventional fluorometric spectrometers. These detectors are dedicated to a given analysis by careful selection of the optical properties of the LEDs. Enhancement of sensitivity of measurements is provided by using various diodes (with the same emitting wavelength) as fluorescence inductors and one LED working as detector of induced fluorescence [33]. Such optoelectronic detectors have been successfully used in fluorometric assays of calcium [33], phosphate [34], oxygen [35], riboflavin [36] and proteins [37].

A novel hybrid SI/FI system integrating in-line soil leaching (using the Hieltjes-Lijklema sequential extraction procedure) with flow-through FPEDD detection (using two LED emitters) is herein proposed for expedient bioaccessibility tests of orthophosphate in soils. To the best of our knowledge PEDD detection has not been resorted to the analysis of soil extracts as of yet. As compared to previous dynamic leaching methods for orthophosphate using advanced flow methodology [9,38] the proposed setup is more simple (merely needs one syringe pump instead of five liquid drivers [9]) and uses portable and affordable detection systems that are easily constructed in the lab. Combining an SI manifold for automatic leaching and a secondary FI system for on-line detection the phosphorus laden extracts are analyzed at real-time, with the subsequent minimization of the hydrolysis of organic phosphorus in the alkaline or acid fractions that was not avoided in previous studies with off-line detection of leachates [38]. The hybrid flow system also affords in-line dilution upon demand by using automatic flow programming.

2. Experimental

2.1. Reagents, solutions, samples

All chemicals were of analytical reagent grade. Solutions were prepared using double distilled water. The stock standard solution (100 mg PO_4^{3-}/L in water) was prepared from KH_2PO_4 (Merck). Working standard solutions were prepared separately in each extractant milieu (NH_4Cl, NaOH, HCl). In this work, the three-step Hieltjes-Lijklema (HL) sequential extraction procedure [39] was selected. In the first step, 1.0 mol/L NH_4Cl (Probus) adjusted to pH=7 with $NH_3 \cdot H_2O$ (25%, Scharlau) was used to extract the labile phosphate (water soluble and exchangeable fraction) from the soil sample. In the second step, 0.1 mol/L NaOH (Panreac) was pumped through the column to extract the Fe- and Al-bound phosphate. The last step, using 0.5 mol/L HCl (37%, Scharlau) as extractant, released Ca-bound phosphate. The derivatization reagent consisted of 12 g/L ammonium molybdate tetrahydrate (Scharlau) in 0.8 mol/L H_2SO_4 (Sigma-Aldrich). In some instances oxalic acid (Panreac) was added at the level of 0.25% (w/v). The solution of fluorophore was prepared by dissolving 70 mg of rhodamine B (Merck) in 1000 mL of distilled water. This solution contained 0.05% (w/v) of polyvinyl alcohol (30–70 kDa, Sigma). A standard reference material from the National Institute of Standards and Technology (NIST) – SRM 2711 (Montana Soil) and a surface agricultural soil in Mallorca (Spain) were selected to study the reliability of the hybrid microcolumn-based flow system and validate the FPEDD detection method. Prior to chemical analysis, the soil was oven-dried at 105 °C until constant weight and 2-mm sieved. Soil pH was measured in 0.01 mol/L $CaCl_2$ at a soil to solution ratio of 1:5 (w:v) after 2 h of equilibration using a combined pH electrode as specified by ISO 10390 [40]. The pH value was 7.52 ± 0.03. The total organic carbon (TOC) contents of 8.55% were determined by dry combustion at 900 °C after removal of carbonates with a few drops of a 20% (v/v) HCl solution. Particle size distribution of the fraction <2 mm for determination of soil texture was performed with the Bouyoucos hydrometer method (ASTM type 152H) [41]. The agricultural soil consisting of 51.1% sand (0.05–2.0 mm), 34.5% silt (2–50 μm), and 14.4% clay (<2 μm) was classified as loam soil.

2.2. Flow system

The hybrid flow system for in-line sequential extraction (fractionation) and automatic determination of orthophosphate in soil samples is depicted in Fig. 1. This integrated Sequential Injection (SI)/Flow Injection (FI) system is a combination of a microSIA setup (FIAlab Instruments, Seattle, US) furnished with a syringe pump and a 6-port multiposition selection valve (SV), controlled by its own software (FIAlab Instruments), with a dedicated secondary FI system incorporating a peristaltic pump (Minipuls 3, Gilson, Middleton, Wisconsin), a rotary injection valve (IDEX V-1451-DC, Upchurch scientific, Oak Harbor, Washington) and a solenoid valve (Parker Hannifin, Cleveland, Ohio). All components of the FI system are controlled by contact

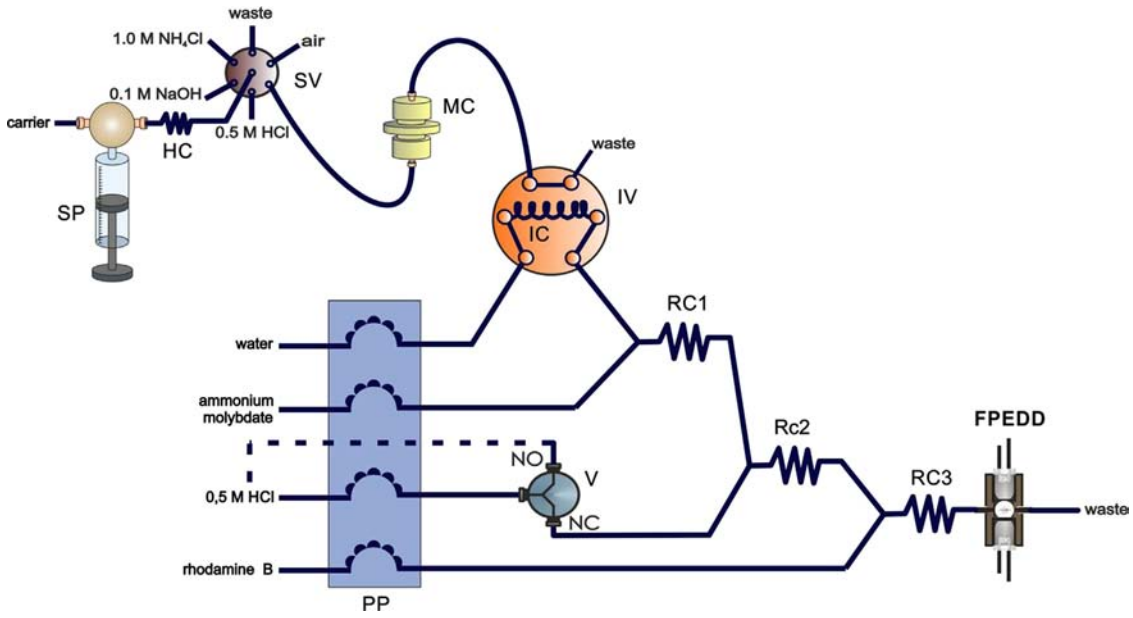

Fig. 1. Scheme of the hybrid flow system for in-line extraction and determination of bioaccessible orthophosphate in soils using fluorometric LED-based detection. SP: Syringe pump; PP: Peristaltic pump; SV: Selection valve, IV: Injection valve, MC: Microcolumn (containing soil); IC: Injection coil; RC: Reaction coil; V: Solenoid valve; FPEDD: Fluorometric paired emitter–detector diode; NO: Normally open port; NC: Normally closed port.

closure from a USB relay plate (ADU200, Ontrack Control Systems, Sudbury, Ontario, Canada), with the FIAlab software akin the SI extraction system. The syringe pump is furnished with a 5-mL glass syringe and a three-way valve at its head. It is connected by a 2.25 mL-holding coil (HC, 1.5 mm ID) with the multiposition valve. This valve allows also selecting the appropriate extractant which via the syringe pump is delivered to the soil containing microcolumn nested to one of the external ports of multiposition valve (see Fig. 1). The injection valve (IV) furnished with 1.0-mL PTFE loop (1 mm ID) enables the connection between the SI and FI systems. The peristaltic pump uses four channels to pump concurrently the derivatization reagents downstream for fluorometric detection of the heteropolyacid-fluorophore ion pair. An additional solenoid valve (V) is used for dilution of HCl extracts. The volumes of the PTFE reaction coils (0.8 mm ID, RC1, RC2 and RC3) are ca. 400 μL, 600 μL and 400 μL, respectively. Peristaltic pump Tygon tubing was of 0.89 mm ID for molybdate, water/sample and rhodamine B and of 1.85 mm ID for the HCl stream as diluent.

2.3. Flow-through microcolumn assembly

The PEEK extraction microcolumn has been described elsewhere [9,38]. It includes a central dual-conical shaped sample container, filters and caps at its both ends. Two different membrane filters (13 mm, FHLP01300, Fluoropore, Millipore) are used – 0.45 μm on the top of the column and 1.0 μm at the bottom to not allow the solid particles to flow freely to the detection part of the system. The free column volume was ca. 250 μL. The column was placed in upright configuration unlike previous dynamic microcolumn-based sequential extraction procedures for orthophosphate in soils [9,38].

2.4. Flow-through PEDD detector

The dedicated flow-through FPEDD detector has been constructed for fluorometric measurements of orthophosphate (see Fig. 2). The design has been described elsewhere [33,36]. Briefly, a 25 mm long and 15.5 mm diameter PEEK cylinder is used. In the equatorial plane, a 5 mm through hole is drilled so as to accommodate two identical green LEDs as inductors of

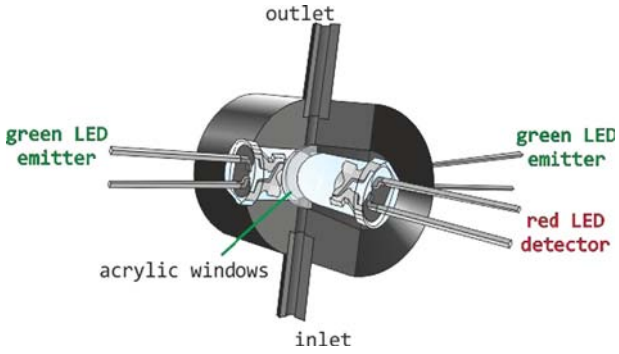

Fig. 2. Schematic illustration of the dedicated flow-through detector based upon fluorometric PEDD detection. The flow-cell is confined within the two acrylic windows.

fluorescence (525 nm Optosupply, China) facing each other. In the same equatorial plane but perpendicular to the LEDs, a 2 mm through hole is drilled for inserting the inlet and outlet tubes (PEEK, 2 mm OD, 1 mm ID, 8 mm protruding) which are glued to the cylinder walls. A 5 mm hole is drilled through the cylinder axis, but the entries are enlarged to 7 mm. The ridge so formed serves to retain the acrylic windows (7 mm diameter), which are blocked by a short tube adapter (7 mm OD, 5 mm ID). The red LED detector (650 nm, Optosupply, China) is press-fitted and held by the tube adapter. The volume of the as-obtained flow cell is ca. 60 μL. Both LED emitters operated at a current of 30 mA. An ordinary low-budget multimeter with RS232 serial communication (model UT70B from UNI-T, China) is used for reading the voltage signal [mV] generated by the FPEDD whereupon it is processed by a PC.

2.5. Analytical procedure

The automatic sequential extraction procedure using the SI manifold starts by drawing 100 μL of air into HC to avoid mixing of individual extractants with carrier and filling the free column volume with the first extractant. Then, 1100 μL of 1.0 mol/L NH_4Cl were aspirated into the HC whereupon the flow was reversed and

1000 μL (first subfraction) were dispensed through the soil laden column. The extractant was aspirated back into the HC and then again dispensed through the column into the injection coil of the FI manifold in a backward–forward mode. All these operations were performed while the injection valve position was set to "load". Afterward, the injection valve was activated to the "inject" position so as to introduce the first subfraction into the secondary FI system (described below). The subfraction was injected into a water carrier stream to maintain the same flow rate across the detector throughout. This procedure was repeated several times until the orthophosphate signal was negligible or when the sum of the last 5 consecutive measures is below 10% of the overall extracted orthophosphate so as to obtain the full extraction profile of the first NH_4Cl fraction. The FPEDD detector and FI tubes were then cleaned with ca. 4 mL denatured ethanol containing 0.1% (w/v) benzalkonium chloride to remove the adsorbed ion pair while the HC of the SI manifold was cleaned with double-distilled water (ca. 6 mL). A virtually identical dynamic extraction procedure was repeated for the second fraction (0.1 mol/L NaOH) for a given number of subfractions until no increase in phosphate leaching was detected. After cleaning the detection cell again, the analytical procedure was repeated with 0.5 mol/L HCl but this time using unidirectional flow, that is, the extractant is only pushed once through the column. All solutions of extractants were aspirated and dispensed at 1.5 mL/min.

In the FI manifold each leachate subfraction from the SI-microcolumn extraction method merges in the first and second reaction coils (RC1 and RC2) with ammonium molybdate to generate the phosphomolybdic acid. In case of HCl-fractions, the dilution module is turned on (NC on the microsolenoid valve) so as to dilute the subfraction by ca. 3 times in the second reaction coil. The heteropolyacid then reaches the third reaction coil (RC3) where the ion pair is formed with rhodamine B. The solution of the rhodamine B exhibits fluorescence and therefore the blank signal was taken as the baseline. As a result of ion pair generation the fluorescence was quenched and negative peak signals were recorded [15,19]. The flow rate of each FI channel was 3 mL/min, except of the dilution stream which was set to 9.4 mL/min.

In-line extraction and analysis of every single subfraction takes ca. 6 min for the NH_4Cl and NaOH fractions and ca. 5 min for the HCl fraction. For calculation of bioaccessible phosphate pools external matrix match calibration was used.

3. Results and discussion

3.1. Fluorometric detection of orthophosphate

Before analysis of the agricultural soil and the SRM 2711 (Montana Soil) different parameters affecting FPEDD detection were investigated in details. For low fluorophore concentrations, the fluorescence yield increases when the exciting intensity does, thus, the exciting LEDs were powered with 30 mA, that is, the maximal current recommended by the manufacturer.

Rhodamine B concentration was investigated so as to obtain the highest full FPEDD scale with appropriate sensitivity for detection of bioaccessible orthophosphate in soils. To this end, a phosphate standard concentration of 1 mg/L was analyzed with rhodamine concentrations ranging from 20 to 200 mg/L. The highest sensitivity was obtained with a fluorophore concentration at the 70 mg/L level.

To prevent fouling of the flow system by sorption/precipitation of the ion-pair onto PTFE tubing, polyvinylic alcohol (PVA) was added to the fluorophore reagent in the concentration spanning from 0.01% to 0.05% (w/v) (higher concentration is not recommended [19]). With a concentration of 0.05% (w/v) PVA the baseline drift was significantly refrained while improving simultaneously the analytical signal repeatability.

3.2. Analytical performance of the in-line dynamic leaching method with FPEDD detection

Different physical and analytical parameters of the hybrid flow system influencing orthophosphate leachability and the reliable analysis of leachates were thoroughly examined. The SI-microcolum system was proven to endure flow rates of extractants up to 3.0 mL/min, yet significant backpressure was observed from 1.5 mL/min onwards. As a compromise between system reliability and length of subfraction analysis, the flow rate was fixed to 1.5 mL/min for the three extractants.

A bidirectional (forward–backward–forward) extraction mode was used for both the exchangeable and NaOH fractions for amelioration of extraction profile repeatability while hindering filter clogging by soil particles. Unidirectional flow was however selected instead for the HCl fraction as a consequence of the large pools of P associated to calcite, which are released in the first subfractions. Otherwise undue dilution will be needed for reliable FPEDD measurements.

Triplicate fractionation analysis of either 50 mg of SRM 2711 or the agricultural soil with repeatabilities in all instances $\leq 8.8\%$ demonstrated that the test portion assayed was representative of the bulk soil medium.

The effect of the soil leachate matrix in the three extractants upon accurate FPEDD orthophosphate quantitation was investigated by off-line collection of subfractions and application of the method of the standard additions using two spikes. Deviations below 12% between the recovered and the expected value revealed the inexistence of matrix interfering effects.

The potential interfering effect of silicate onto the FPEDD phosphate signal was ascertained by spike recoveries of a phosphate standard at the 1 mg/L level containing increasing concentrations of silicate in the last two extraction milieus (0.1 mol/L NaOH and 0.5 mol/L HCl), as silicate extraction by NH_4Cl is expected to be negligible [9]. Silicate was tolerated up to 400 mg/L in 1.0 mol/L HCl with recoveries above 90%. Without masking agents the hybrid flow system however showed severe interfering effects from silicate in the NaOH medium at the same concentration level than that of orthophosphate. Addition of 0.25% (w/v) of oxalic acid to the molybdate derivatization reagent made the detection system immune to silicate at a Si/P ratio of 400 with recoveries above 87%.

The detection (LOD) and quantification (LOQ) limits of orthophosphate at the 3 and 10 s_{blank} level, respectively, using the FI automatic method with FPEDD detection were 0.02 and 0.07 mg P/L for NH_4Cl, 0.04 and 0.13 mg P/L for NaOH and 0.013 and 0.043 mg P/L for HCl, respectively. We have proven that the LOQ of the photometric PEDD counterpart using 650 nm red LEDs as emitter and detector, respectively, for determination of orthophosphate in water was 1.9 mg/L. The expected concentrations of bioaccessible orthophosphate in soils are lower than the LOQ thereby making the miniaturized photometric detection inappropriate for analysis of soil leachates.

Dynamic linear ranges (mg P/L) in the three extractant media were 0.019–0.32 mg/L for NH_4Cl ($R=0.9967$); 0.042–0.32 mg/L and 0.32–1.63 mg/L for NaOH ($R=0.9999$ and 0.9958, respectively) and 0.045–2.0 mg/L and 2.0–10 mg/L for HCl ($R=0.9949$ and 0.9946, respectively).

3.3. Applicability of the SI/FI-FPEDD setup for in-line sequential extraction and determination of orthophosphate in soil leachates

A reference material (SRM 2711-Montana soil) and a real soil (agricultural soil) were analyzed in this work to assess the reliability of the hybrid flow setup. The in-line extractrograms (kinetic extraction profiles) in individual extractants are shown in

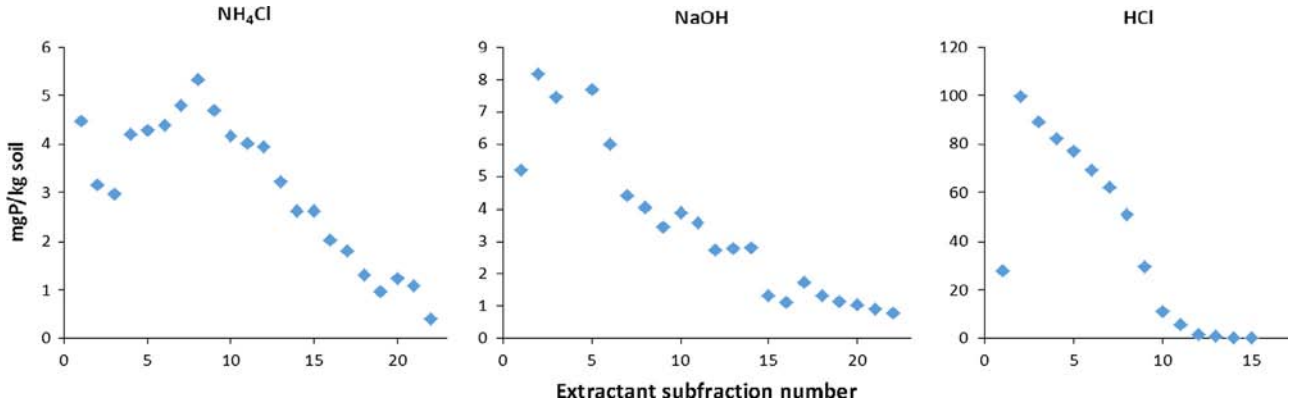

Fig. 3. Profiles obtained for in-line extraction and fluorometric FPEDD detection of bioaccessible orthophosphate in the agricultural soil using the Hieltjes-Lijklema sequential extraction scheme. The subfraction volume is 1 mL.

Table 1
Statistical comparison between on-line/off-line detection of orthophosphate in leachates obtained from the SI-microcolumn setup using FPEDD and a reference method based on the molybdenum blue chemistry for the SRM 2711 reference material and an agricultural soil.

	On-line FPEDD (mg P/kg)	Off-line FPEDD (mg P/kg)	Off-line molybdenum blue photometric method (mg P/kg)
SRM 2711 (Montana soil)			
NH_4Cl	45 ± 1	58 ± 6	69 ± 5
NaOH	102 ± 18	68 ± 10	58 ± 5
HCl	671 ± 60	581 ± 43	574 ± 86
Agricultural soil (Mallorca)			
NH_4Cl	100 ± 24	153 ± 49	123 ± 65
NaOH	115 ± 39	107 ± 21	107 ± 33
HCl	668 ± 58	731 ± 59	817 ± 14

Results are expressed as the mean ± standard deviation.
Results of paired *t*-tests: $p=0.901$ ($n=17$) for on-line FPEDD against standard photometric method; $p=0.946$ ($n=18$) for off-line FPEDD against standard photometric method; $p=0.917$ ($n=17$) for on-line FPEDD against off-line FPEDD.

Fig. 3 for the agricultural soil. The magnitudes of bioaccessible orthophosphate pools in both samples determined by the SI/FI setup with on-line FPEDD detection are listed in Table 1. The proposed hybrid system allows accurate measurements of inorganic phosphorus, mostly orthophosphate but fast hydrolyzing condensed inorganic phosphates, whenever available, while minimizing the undesirable hydrolysis of organic phosphorus forms because of immediate in-line analysis of the extracts. In the two first extractants the concentrations of bioaccessible orthophosphate are low (below the mg/L level in every subfraction) but the sensitivity of the fluorometric PEDD detector suffices for reliable measurements.

As shown in Fig. 3, the leaching kinetics of orthophosphate in the three extractant solutions are rather different in the agricultural soil. The labile orthophosphate (first fraction) is mostly leached within the first twelve sub-fractions and decayed in the ensuing sub-fractions. This is actually the most relevant inorganic phosphorus pool for plant uptake, and a sustained labile orthophosphate release is found in the agricultural soil assayed. Conversely, the leaching of the pools of phosphorus associated to hydrous oxides of Al and Fe using a more aggressive extractant (second fraction) takes place in a shorter time period. Similar profile is recorded for Ca-bound orthophosphate (third fraction) as a result of fast acid dissolution of Ca-laden mineralogical phases.

The SI/FI-FPEDD assembly merely detects bioaccessible orthophosphate but organic phosphorus species are also leached under alkaline and acidic extraction conditions [4,42,43]. In addition, organic phosphorus forms are hydrolyzed to inorganic phosphate in (microwave) digestion protocols aimed at measurement of the total phosphorus (or the residual phosphorus fraction after fractionation) in soils [9]. Hence, a mass balance validation usually employed in sequential extraction schemes as a quality control tool [44–46] is here inapplicable for orthophosphate. Taking this into account, a flow-through spectrophotometric method based on the molybdenum blue chemistry, reported elsewhere [9], was selected as a reference method using the SRM 2711 and the agriculture soil as model samples to investigate the reliability of the FPEDD detection system. To this end, leachates from the SI microcolumn extraction system were collected off-line and analyzed on a short notice (to circumvent the hydrolysis of organic phosphorus compounds) using both detection techniques. The results of the paired *t*-tests (see footnote in Table 1) indicated no significant differences between both methods at the 0.05 significance level ($p > 0.05$), thereby demonstrating the lack of biased results.

To investigate the potential hydrolysis of organic phosphorus in the SRM 2711 and the real soil in the different Hieltjes-Lijklema leaching media, the on-line leaching data obtained with the proposed FPEDD method were statistically compared against the results obtained by off-line FPEDD detection of 4 h-aged leachates. No statistically significant differences were found at the 0.05 significance level ($p > 0.05$) between on-line FPEDD against off-line FPEDD (see footnote in Table 1) using a paired t-test. Therefore, there was no hydrolysable organic phosphorus detectable in the analyzed soils, at least after a reaction time of ca. 90 s, which equals to the residence time of each extractant volume in the SI microcolumn extraction system and the FI detection part. The pools of bioaccessible orthophosphate in SRM 2711 as obtained by the hybrid SI/FI microextraction setup using the Hieltjes-Lijklema scheme were compared against previous flow-through dynamic sequential extraction methods [8,9,38] (see Table 2). A good agreement is found in both NH_4Cl and NaOH fractions for bioaccessible orthophosphate between the proposed SI/FI manifold and that of a previous SI-microcolumn extraction method [38]. Despite the fast FI detection, the residence time of the leachates in our SI manifold in the bi-directional extraction mode most likely suffices for hydrolysis of some condensed inorganic phosphates (pyrophosphate and polyphosphates), even in mild leaching conditions [4]. This might explain the increased amount of most readily available orthophosphate (first step in the Hieltjes-Lijklema procedure) with regard to a multisyringe flow injection microcolumn extraction scheme reported earlier [9]. As compared to continuous-flow extraction chamber devices [8], flow-based microcolumn procedures (see Table 2) afford decreased leachability of readily available orthophosphate because of the lack of mechanical agitation and the lower residence times of the

Table 2
Bioaccessible orthophosphate in SRM 2711 using dynamic flow-through Hieltjes-Lijklema-based fractionation assays.

	NH_4Cl (mg P/kg)	NaOH (mg P/kg)	HCl (mg P/kg)
Stirred-flow cell extraction [8]	189 ± 6	77 ± 4	413 ± 6
SI-microcolumn extraction [38]	45 ± 5	93 ± 10	373 ± 18
Multisyringe-based microcolumn extraction [9]	7 ± 1	13.1 ± 0.4	324 ± 45
This work	45 ± 1	102 ± 18	671 ± 60

extractant. With regard to the HCl fraction, our SI/FI hybrid assembly leaches significantly higher amounts of orthophosphate associated to calcite compared to previous flow-through microcolumn systems [9,38]. The main difference between our configuration and previous horizontal-type microcolumn arrangements [9,38] is the upright position of the column for up-flow extraction mode that fosters fluidized bed-like leaching with the consequent increase in leachability.

4. Conclusion

A fully software-controlled hybrid FI/SI assembly furnished with a miniaturized flow-through FPEDD detector has been proposed for expedient in-line dynamic fractionation and detection of bioaccessible orthophosphate in soils. The main advantage of combining and synchronizing two flow manifolds in parallel is that on-line analysis of extracts is immediately performed without delay after dynamic extraction. Further, the SI and FI manifolds allow for ease on-line manipulation of sample/extracts as herein demonstrated by dilution of the HCl subfractions. Using bi-directional flow the negative influence of flow backpressure is alleviated in the fractionation scheme and allowed faster extraction of orthophosphate from soils in the NH_4Cl and NaOH fractions. The actual applicability of the flow setup was demonstrated by analysis of a certified reference material and a real soil sample. It should be noted that previous works dealing with dynamic phosphorus fractionation did not analyze real samples [9,38]. Another advantage is the simplicity of the system as compared to previous flow setups furnished with up to 5 syringe pumps [9]. Our hybrid configuration merely uses one syringe pump, a multiposition valve and a peristaltic pump. Moreover, the upright configuration of the soil laden microcolumn for up-flow extraction has been demonstrated to perform in a much more efficient way than previously reported horizontal column setups [9,38].

As opposed to conventional bench-type spectrofluorometers, the portable fluorometric detection system herein presented is constructed from cost-effective LEDs with no need of special optical filters or monochromators and uses inexpensive fluorophores (i.e., rhodamine B). The detection limit and linear ranges characterizing the fluorometric method are low and proved adequate to the expected levels of extracted orthophosphate in soils.

Current work is underway to expand the scope of the hybrid flow system to automate other sequential extraction schemes; e.g., the endorsed by the Standards, Measurement and Testing (SM&T) Program (formerly BCR) of the European Commission, and adapt the flow-through FPEDD detector to measure other forms of phosphorus (inorganic and/or organic) in soil leachates.

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References

[1] O.F. Schoumans, W.J. Chardon, M.E. Bechmann, C. Gascuel-Odoux, G. Hofman, B. Kronvang, G.H. Rubæk, B. Ulén, J.-M. Dorioz, Sci. Total Environ. 468–469 (2014) 1255–1266.
[2] C. Forsberg, Water Sci. Technol. 37 (3) (1998) 193–200.
[3] I.D. McKelvie, A. Lyddy-Meaney, Phosphorus, in: A. Townshend, P.J. Worsfold, C. Poole (Eds.), Encyclopedia of Analytical Science, 2nd ed.,Academic Press, Elsevier, 2005, pp. 167–173.
[4] B.L. Turner, B.J. Cade-Menun, L.M Condron, S. Newman, Talanta 66 (2005) 294–306.
[5] D.S. Baldwin, Environ. Chem. 10 (2013) 439–454.
[6] A. Barbanti, M.C. Bergamini, F. Frascari, S. Miserocchi, G. Rosso, J. Environ. Qual. 23 (1994) 1093–1102.
[7] E.H. Hansen, M. Miró, Trends Anal. Chem. 26 (1) (2007) 18–26.
[8] W. Tiyapongpattana, P. Pongsakul, J. Shiowatana, D. Nacapricha, Talanta 62 (2004) 765–771.
[9] J. Buanuam, M. Miró, E.H. Hansen, J. Shiowatana, J.M. Estela, V. Cerdà, Talanta 71 (2007) 1710–1719.
[10] I. Novozamsky, W.H. Van Riemsdijk, Anal. Chim. Acta 85 (1976) 41–46.
[11] F. Szemes, D. Hesek, Z. Chen, S.W. Dent, M.G. Drew, A.J. Goulden, A.R. Graydon, A. Grieve, R.J. Mortimer, T. Wear, J.S. Weightman, P.D. Beer, Inorg. Chem. 35 (1996) 5868–5879.
[12] W.L. Cheng, J.W. Sue, W.C. Chen, J.L. Chang, J.M. Zen, Anal. Chem. 82 (2010) 1157–1161.
[13] M. Miró, J.M. Estela, V. Cerda, Talanta 60 (2003) 867–886.
[14] P.J. Worsfold, R. Clough, M.C. Lohan, P. Monbet, P.S. Ellis, Ch.R. Quétel, G.H. Floor, I.D. McKelvie, Anal. Chim. Acta 803 (2013) 15–40.
[15] S. Motomizu, Z.-H. Li, Talanta 66 (2005) 332–340.
[16] S. Karthikeyan, S. Hashigaya, T. Kajiya, S. Hirata, Anal. Bioanal. Chem. 378 (2004) 1842–1846.
[17] P.D. Tzanavaras, D.G. Themelis, Anal. Chim. Acta 467 (2002) 83–89.
[18] B.F. Reis, P.B. Martelli, F.G. Krug, C.A. Tumang, C.A. Flow, J. Braz. Chem. Soc. 3 (1992) 38–42.
[19] Z.-H. Li, M. Oshima, A. Sabarudin, S. Motomizu, Anal. Sci. 21 (2005) 263–268.
[20] L. Kröckel, H. Lehmann, T. Wieduwilt, M.A. Schmidt, Talanta 125 (2014) 107–113.
[21] C. Frank, F. Schroeder, R. Ebinghaus, W. Ruck, Talanta 70 (2006) 513–517.
[22] M. O'Toole, K.T. Lau, R. Shepherd, C. Slater, D. Diamond, Anal. Chim. Acta 597 (2007) 290–294.
[23] K.T. Lau, S. Baldwin, M. O'Toole, R.I. Shepherd, W.S. Yerazunis, S. Izuo, S. Ueyama, D. Diamond, Anal. Chim. Acta 557 (2006) 111–116.
[24] Ł. Tymecki, M. Pokrzywnicka, R. Koncki, Analyst 133 (2008) 1501–1504.
[25] Ł. Tymecki, R. Koncki, Anal. Chim. Acta 639 (2009) 73–77.
[26] D.A. Bui, P.C. Hauser, Talanta 116 (2013) 1073–1078.
[27] M. Pokrzywnicka, D.J. Cocovi-Solberg, M. Miró, V. Cerdà, R. Koncki, L. Tymecki, Anal. Bioanal. Chem. 399 (2011) 1381–1387.
[28] D.J. Cocovi-Solberg, M. Miró, V. Cerdà, M. Pokrzywnicka, L. Tymecki, R. Koncki, Talanta 96 (2012) 113–120.
[29] K. Strzelak, R. Koncki, Ł. Tymecki, Talanta 96 (2012) 127–131.
[30] M. Czugala, C. Fay, N.E. O'Connor, B. Corcoran, F. Benito-Lopez, D. Diamond, Talanta 116 (2013) 997–1004.
[31] Ł. Tymecki, J. Korszun, K. Strzelak, R. Koncki, Anal. Chim. Acta 787 (2013) 118–125.
[32] Ł. Tymecki, M. Pokrzywnicka, R. Koncki, Analyst 136 (2011) 73–76.
[33] M. Pokrzywnicka, M. Fiedoruk, R. Koncki, Talanta 93 (2012) 106–110.
[34] M. Fiedoruk, E. Mieczkowska, R. Koncki, Ł. Tymecki, Talanta 128 (2014) 211–214.
[35] I.M. Perez De Vargas-Sansalvador, C. Fay, M.D. Fernandez-Ramos, D. Diamond, F. Benito-Lopez, L.F. Capitan-Vallvey, Anal. Bioanal. Chem. 404 (2012) 2851–2858.
[36] Ł. Tymecki, M. Rejnis, M. Pokrzywnicka, K. Strzelak, R. Koncki, Anal. Chim. Acta 721 (2012) 92–96.
[37] M. Pokrzywnicka, Ł. Tymecki, R. Koncki, Talanta 96 (2012) 121–126.

[38] J. Buanuam, M. Miró, E.H. Hansen, J. Shiowatana, Anal. Chim. Acta 570 (2006) 224–231.
[39] A.H.M. Hieltjes, L. Lijklema, J. Environ. Qual. 9 (1980) 405–407.
[40] International Organization for Standardization, ISO 10390: Soil Quality – Determination of pH. Geneva, Switzerland, 1994.
[41] H. Dietrich, SOP METH004.00: Standard operating procedure of procedure for determining soil particle size using the hydrometer method, Environmental Monitoring Branch, California department of pesticide regulation, Sacramento: CA, 2005.
[42] G. Anderson, in: A.D. McLaren, G.H. Peterson (Eds.), Soil Biochemistry, vol. 1, Marcel Dekker, New York, 1967, pp. 67–90.
[43] E.W. Russell, Soil Conditions and Plant Growth, 11th ed., Longman Scientific & Technical, Harlow, UK, 1988.
[44] J.R. Bacon, C.M. Davidson, Analyst 133 (2008) 25–46.
[45] W. Boonjob, M. Miró, V. Cerdà, Anal. Chem. 80 (2008) 7319–7326.
[46] W. Boonjob, M. Zevenhoven, P. Ek, M. Hupa, A. Ivaska, M. Miró, J. Anal. At. Spectrom. 27 (2012) 841–849.