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Photochemical micro-digestion in a multi-pumping flow system for phosphorus fractionation in cereals

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ABSTRACT

Drawbacks of sample preparation can be overcome by mechanization, minimizing systematic errors and analysis time, as well as improving precision. Multi-pumping flow systems (MPFS) attain the requirements for mechanization in a versatile and robust way, minimizing reagent consumption and waste generation. A MPFS was proposed for the fractionation of water soluble phosphorus in cereals, which is important because the bioavailability of this nutrient depends on its chemical form. Besides, phytic acid yields stable complexes with some micronutrients (*e.g.* Fe^{3+}, Ca^{2+} and Mg^{2+}), thus also altering their bioavailability. The flow system incorporated on-line photochemical conversion of organic phosphorus to phosphate, which was quantified by the spectrophotometric molybdenum blue method. A linear response was observed between 5 and 40 mg L^{-1} for both inorganic (P_I) and organic (P_O) phosphorus, with detection limits of 0.5 and 1.2 mg L^{-1}, respectively. Coefficients of variation (n = 20) were estimated as 1.2 and 3.6% for P_I and P_O, respectively, with a sampling rate of 80 determinations per hour. Per determination, 380 μg of $(NH_4)_6Mo_7O_{24}$, 620 μg of ascorbic acid and 790 μg of $K_2S_2O_8$ were consumed, generating only 2.5 mL of waste. Interferences of organic matter were avoided by sample pretreatment with active charcoal (20 mg/mL). The results for cereal samples agreed with those obtained by the reference procedure based on nitro-percloric digestion. The proposed procedure is then a reliable, fast, inexpensive and more environmentally friendly alternative for phosphorus fractionation.

1. Introduction

Sample preparation is often the most critical step in analytical procedures [1]. The decomposition of organic matter is generally carried out by supplying energy to break chemical bonds and ultraviolet radiation can be employed to this end [2]. The process is enhanced by generation of free radicals for organic matter oxidation, which usually employs compounds such as $K_2S_2O_8$ (Eqs. (1) and (2)).

$$S_2O_8^{2-} + h\nu \rightarrow 2SO_4^{-\bullet} \quad (1)$$

$$SO_4^{-\bullet} + H_2O \rightarrow HSO_4^{-} + OH^{\bullet} \quad (2)$$

Despite the common use in view of simplicity, sample preparation in open vessels requires large amounts of reagents, being also time-consuming, susceptible to contamination and analyte loss, thus affecting accuracy and precision. Additionally, safety of the analyst may be hindered depending on the process and sample composition. Flow-based procedures, in which sample preparation is carried out in closed systems, show characteristics that overcome most of these difficulties.

On-line digestion in flow systems [2,3] is highly reproducible in view of the strict control of both sample processing conditions and residence time, therefore allowing analytical applications without quantitative conversion. High sampling rate, low reagent consumption and minimal waste generation are additional advantages [4]. Versatility of flow systems is increased by the multicommutation [5] and multi-pumping [6] approaches, in which computer-controlled devices (*e.g.* solenoid valves or micro-pumps) allow independent solution handling. These systems show better analytical performance and significantly lower reagent consumption because solutions can be sampled only when required in the analytical procedure. Solenoid micro-pumps actuate as both fluid propellers and injectors yielding a reproducible pulsed flow [6], which promotes better mixing conditions. In spite of the potentialities, the employment of flow-based procedures with solenoid micro-pumps aiming sample preparation is scarce [7].

Phosphorus is an essential element for animals and plants. In humans, the deficiency of this nutrient leads to organism disorders such as muscle and bone weakness, anemia and rickets [8]. In plants, phosphorus participates in important biological processes, such as growth [9], being found in seeds, fruits and leaves as free inorganic (orthophosphate) or organic bounded forms, including inositol phosphates, phospholipids and nucleic acids [9].

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Orthophosphate on the soluble fraction is readily available for plants and animals, while the organic forms need to be mineralized before absorption in the intestinal tract or plant roots. Phytic acid (inositol-6-phosphate) is the main form of organic phosphorus in plants. Other species show similar chemical structure, except for the partial substitution of phosphate groups by hydroxyls. In cereals and derivatives, the differentiation of inorganic and organic fractions is important for nutrition studies to evaluate phosphorus bioavailability, which depends on its chemical form [8]. Availability of other essential nutrients can also be affected because phytate forms stable complexes with metal ions (*e.g.* Fe^{3+}, $Ca^{2+}$and Mg^{2+}).

Several flow injection systems have been developed for determination of inorganic, total phosphorus or species fractionation, mainly in water samples [10]. The fractionation of soluble phosphorus species in plant materials is generally carried out by determining the total phosphorus and the inorganic fraction separately. Mineralization of organic phosphorus species (*e.g.* by nitro-percloric [11] or photo-oxidative [12] digestions) is required to yield orthophosphate, whose detection is more feasible (*e.g.* by spectrophometry). In another approach, phytic acid is separated on an anion exchange column and determined after precipitation with iron(III). This strategy, recommended by AOAC [13], was also adopted in a flow system for phytic acid determination [14]. However, this approach was questioned in a previous work [15] because other inositol phosphates yield an overestimated value for phytic acid. Phosphorus fractionation in cereals was also carried out in flow systems with immobilized phytase enzyme [16] or with potentiometric detection at different pH [17].

On-line photochemical conversion of organic phosphorus was employed for fractionation in waters [7,18–20]. The procedures usually employ long photo-reactors to enhance the residence time, but this also increases sample dispersion. The heating produced due to the lamp operation generates air bubbles and cooling or debubbling systems are often required.

In this work, on-line photochemical conversion was employed for the first time for fractionation of soluble phosphorus in vegetables (cereals and derivatives). A multi-pumping flow system was proposed for sample micro-digestion under mild conditions, aiming minimizing analysis time, reagent consumption, waste generation and risks of sample contamination.

2. Experimental

2.1. Apparatus

The flow system (Fig. 1) was constructed with five solenoid micro-pumps (Biochem Valve Inc., Boonton, NJ, USA), dispensing volumes of 8.0 μL (P_1), 10 μL (P_2 and P_5) and 15 μL (P_3 and P_4) per stroke, a three-way solenoid valve and PTFE tubes (0.8 mm *i.d.*). The micro-pumps were operated at 5 Hz, except for P_3 at the degradation step, which was actuated at 3 Hz (2.7 mL min^{-1}). The manifold was controlled by an Intel Pentium micro-computer through a parallel interface, employing a current drive based on the ULN2803 integrated circuit. Control software was developed in Visual Basic 6.0 (Microsoft).

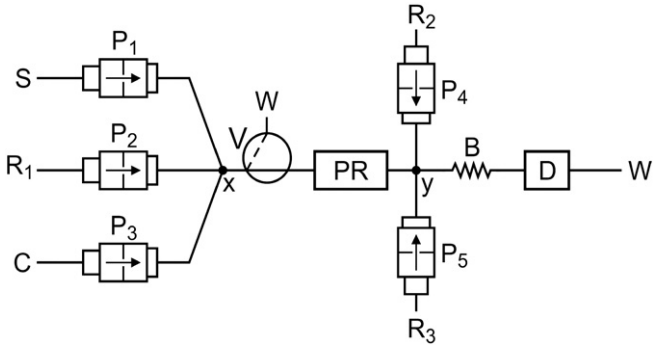

Fig. 1. Multi-pumping flow manifold for phosphorus fractionation. **S**: Sample; **R_1**: 0.15 mol L^{-1} $K_2S_2O_8$ or H_2O; **R_2**: 7.5 mmol L^{-1} $(NH_4)_6Mo_7O_{24}$ in 0.5 mol L^{-1} HNO_3; **R_3**: 60.0 mmol L^{-1} Ascorbic acid; **C**: H_2O; **W**: Waste vessel; **P_1–P_5**: Solenoid micro-pumps; **V**: Three-way solenoid valve; **x** and **y**: Confluence points; **PR**: Photo-reactor; **B**: 50-cm coiled reactor; **D**: Spectrophotometric flow cell. Valve V is activated only for sample replacement (Table 1, step 8).

Measurements were carried out with a multi-channel CCD spectrophotometer (Ocean Optics, Dunedin, FL, USA; model USB2000), with the software supplied by the manufacturer. Optical fibers transmitted the radiation from a tungsten-halogen lamp (Ocean Optics, model LS-1) to a 1.0 cm optical path flow cell (Hellma, Plainview, NY, USA; 80 μL internal volume) and from the cell to the spectrometer. The photo-reactor was constructed with a 125-W mercury vapor lamp (Osram, HQL E27), which presents high emission at 254 and 365 nm and was powered by a reactor (Helfont, 127 V, 2 A, 60 Hz). A 30-cm long thin-wall PTFE tube (0.8 mm i.d.) coiled in a 3-cm helix was placed *ca.* 2.0 cm under the lamp bulb and fixed on a wooded piece covered with aluminum paper. The photo-reactor was inserted in a lab-made dark box without a cooling device.

2.2. Reagents and solutions

All solutions were prepared with deionized water and analytical grade chemicals. R_1 was a 0.15 mol L^{-1} $K_2S_2O_8$ (Merck, Darmstadt, Germany) solution or deionized water and R_2 was a 7.5 mmol L^{-1} ammonium molybdate solution prepared from $(NH_4)_6Mo_7O_{24}.4H_2O$ (Merck) in 0.5 mol L^{-1} HNO_3. A 60 mmol L^{-1} ascorbic acid solution (R_3) was prepared daily by dissolving the reagent (Sigma, St. Louis, MO, USA) in water. Stock 1000 mg L^{-1} P solutions were prepared from sodium phytate (Sigma) or KH_2PO_4 (Merck) by dissolution in water.

2.3. Proposed procedure

The flow-based manifold employed for fractionation of phosphorus (Fig. 1) was operated according to the routine described in Table 1, based on the binary sampling approach [5]. Two sampling cycles (steps 1 and 2) were used for the insertion of sample and R_1 in the photo-reactor (PR). The sample zone was pumped through the photo-reactor (step 3) by the actuation of micro-pump P_3 at 3 Hz. Afterwards, the digested sample zone was inserted in reactor B (step 4), intercalated by aliquots of R_2 (step 5) and R_3 (step 6) reagents, employing four sampling cycles. The sample zone was carried towards the flow cell by the actuation of the micro-pump P_3 (step 7). Measurements at 700 nm were based on peak height and carried out in triplicate, except for the estimative of the coefficients of variation, based on twenty measurements.

For sample replacement, micro-pump P_1 and valve V were simultaneously actuated (step 8), changing the flow direction to waste. The

Table 1
Switching course of solenoid micro-pumps for fractionation of phosphorus in cereal extracts[a].

Step	Description	Pump	Stroke (μL)	Pulses
1[b]	Sample insertion	P_1	8	5
2[b]	$K_2S_2O_8$ or H_2O insertion	P_2	10	1
3	Photo-conversion	P_3	15	14
4[c]	Digest transport	P_3	15	2
5[c]	$(NH_4)_6Mo_7O_{24}$ insertion	P_4	10	1
6[c]	Ascorbic acid insertion	P_5	15	1
7	Transport and detection	P_3	15	120
8	Sample or R_1 replacement	P_1 or P_2	8 or 10	100
		P_3	15	30

[a] All pumps operated at 5 Hz except in step 3 (pulse frequency of 3 Hz).
[b] 2 sampling cycles.
[c] 4 sampling cycles.