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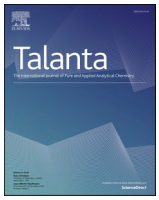

Single flow-based system for the automatic multiparametric nutrients (NPK & Fe) assessment in soil leachates

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ABSTRACT

A multiparametric sequential injection system for the determination of phosphate, nitrite, nitrate, potassium, and iron(III) in a single manifold was developed. The main goal of the proposed method was to develop an efficient tool to assess a number of essential chemical compounds in soils, providing the corresponding information on soil fertility and, additionally, information on possible groundwater contamination. The method was applied for the quantification of the aforementioned parameters in simulated leachates produced in laboratory-scale core columns. The relative standard deviations of ten replicate analyses of a standard were: 6% for phosphate; 2% for nitrite; 2% for nitrate; 5% for potassium; and 6% for iron(III). The limits of detection and quantification were: 2.15 and 7.18 μmol/L for phosphate determination; 0.22 and 0.73 μmol/L for nitrite determination; 3.42 and 8.00 μmol/L for nitrate determination; 39 μmol/L (limit of detection) for potassium determination; and 0.46 and 1.85 μmol/L for iron(III) determination. The sequential injection system was successfully applied for the quantification of multiple soil chemical components (PO_4^{3-}, NO_2^-, NO_3^-, K^+, and Fe^{3+}) in soil leachates. The analysis of a sample, involving all the analytes, has a duration of 28 min.

1. Introduction

Soil analysis is a worldwide concern involving different areas, it is crucial to monitor/characterize soil properties for agriculture purposes but also to evaluate groundwater quality/contamination due to the natural soil leaching. Nutrients content in soil have a great impact on agriculture since the soil is the main source of nutrients for plant growth and crop productivity, which leads to the great importance of the soil monitoring [1–5]. Nitrogen, phosphorous, and potassium are considered to be macronutrients of major importance in agriculture, as they are involved in plant growth and, for that purpose, they are needed in large quantities [6]. This is why they are usually present in commercially available soil fertilizers, to contribute to the improvement of soil fertility. In the past decades, the large increase in the world population led to a huge increase in agricultural activity and consequently to the massive use of fertilizers. The use of fertilizers led to another worldwide problem, groundwater contamination as a result of the natural soil leaching [5–7].

Nitrogen plays a crucial role in plant development, as it is part of the structure of a variety of plant molecules (amino acids, nucleic acids, chlorophyll, phytohormones, and ATP). Therefore, it is involved in various plant biological processes: photosynthetic process, phytohormonal, proteomic changes, and growth development, among others [8,9]. Nitrogen plant requirement can be considered one of the most limiting conditions for plant production [10]. The most frequently used method for the quantification of nitrite and nitrate is based on the modified colorimetric Griess reaction. The basic principle of the method is the reaction of nitrite with sulfanilamide and then with N-(1-naphthyl)ethylenediamine, forming a pink compound [11,12]. The nitrate analysis is based on the same reaction, but it requires the nitrate's previous reduction to nitrite, usually through the use of a chemical reducing agent like cadmium [13,14].

Phosphorous is an essential nutrient for plants, as it is involved in plant growth, energy transportation, plant structure, and genetic transfer. Additionally, it has a major role in biochemical reactions, especially in converting the sun's energy into plant compounds [15,16]. Phosphorous determination can be performed spectrophotometrically, using the molybdenum blue chemical reaction. This determination is based on the quantification of soluble phosphates, through the formation of a reduced form of the phosphomolybdate, a blue compound [17].

Potassium is a macronutrient that plays an important role in the water levels balance in plants and as part of the activation process of

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plant enzymes [6]. Potassium is present in the soil, usually adsorbed on clay surfaces, and the water-soluble species can be easily leached from surface soils [18]. The determination of potassium is commonly performed by flame emission (FES) and atomic absorption spectrometry (AAS) [19]; however, as a less expensive and portable method, it can also be determined by potentiometry [20].

Iron is an essential micronutrient for plants, being the principal component of electron chains, and a co-factor of several enzymes, participating in photosynthesis and chlorophyll synthesis. Iron content in soil receives great attention, as it determines the yield and nutritional quality of crops [21]. Iron determination is mainly performed by atomic absorption, inductively coupled plasma (ICP), or molecular absorption spectrophotometry [22]. The spectrophotometric methods present some advantages over the other methods as they are usually less expensive (equipment and maintenance), no need for a skilled operator, and a wide variety of selective colorimetric ligands are available for iron determination [23]. Among these colorimetric ligands, 3-hydroxy-4-pyridinones have been recently reported as chromogenic reagents with high affinity and specificity for iron(III) with associated low toxicity [24]. Some of these chelators, like the MRB12, have been used in the spectrophotometric determination of iron in a variety of matrices, including soil leachates [25,26].

The main goal of this work was to devise a single analytical system for the multi-parametric determination of several nutrients in soil, enabling the evaluation of soil fertility and foreseeing eventual groundwater contamination. The methods herein described were already developed in a flow-based approach. Nevertheless, these determinations were only described on an individual basis, namely for nitrite/nitrate in natural waters [14], phosphate in soil leachates [27], potassium in soils [28], and iron in soil leachates [25]. Because of the high importance of these parameters to predict soil fertility and soil nutrient requirements, it would be of great importance to perform all the determinations in a single analytical system. Flow-based methods, like sequential injection analysis (SIA), are appealing to implement this kind of multiparametric determinations in an automated and miniaturized strategy. This is due to some of the associated characteristics such as low reagents and sample consumption, high throughput, and the apparatus versatility [29]. In this scenario, in this paper, we propose a sequential injection system for the determination of NPK & Fe in the same manifold.

2. Material and methods

2.1. Reagents and solutions

All solutions were prepared with analytical-grade chemicals and boiled deionized water (conductivity< 0.01 mS/cm).

For the phosphate determination, a 2.00 mmol/L phosphate stock solution was prepared by dissolving the corresponding quantity of the solid (KH_2PO_4, Panreac) in deionized water. A 200 μmol/L phosphate solution was prepared by dilution of the 2.00 mmol/L stock solution. Working standards in the range of 10–80 μmol/L were prepared by dilution of the 200 μmol/L phosphate solution. A 30 g/L ascorbic acid solution was weekly prepared by dissolution of the corresponding quantity of the solid ($C_6H_8O_6$, Normapur) in deionized water. The molybdate reagent was prepared weekly, by dissolving 2.5 mg of potassium antimony(III) oxide tartrate hemihydrate extra pure ($K(SbO)C_4H_4O_6{\cdot}0.5H_2O$, Merck), 0.4 g of ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, Fluka) and 0.18 g of tartaric acid ($C_4H_6O_6$, Merck) in 10 mL of 2 M sulfuric acid. After dissolution, deionized water was added to a final volume of 25 mL. The final concentrations of the molybdate solution were: 0.1 g/L $C_4H_4KO_7Sb$, 16 g/L $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 7.2 g/L $C_4H_6O_6$ and 0.8 mol/L H_2SO_4. All the solutions used for phosphate determination were stored in the refrigerator.

For the nitrite and nitrate determination, the solutions were prepared as described below. A 100 mmol/L nitrite stock solution was prepared by dissolution of the corresponding quantity of sodium nitrite (Merck) in deionized water. This stock solution was monthly diluted to a 1 mmol/L stock solution, that was used to prepare, weekly, a 20 μmol/L standard solution. A 100 mmol/L nitrate stock solution was prepared by dissolution of the corresponding quantity of sodium nitrate (Merck) in deionized water. This stock solution was monthly diluted to a 1 mmol/L stock solution. From the 20 μmol/L nitrite standard solution and the 1 mmol/L nitrate standard solution, mixed working standard solutions in the range of 0.50–8.00 μmol/L for nitrite and 12.5–300 μmol/L for nitrate, were prepared weekly. The Griess reagent preparation involved the monthly preparation of two solutions, a sulfanilamide solution and an N-(1-naphthyl)-ethylenediamine dihydrochloride (N1NED) solution. The sulfanilamide solution was prepared by dissolving 4 g of the solid (Sigma) in 20 mL of a 5 mol/L *ortho*-phosphoric acid and then, the volume was made up to 100 mL with deionized water. The N1NED solution was prepared by dissolving 0.4 g of the solid (Merck) in 100 mL of deionized water. The Griess reagent was prepared weekly by mixing 1:1 of the sulfanilamide and N1NED solutions, to a final concentration of 20 g/L and 2 g/L, respectively in 0.5 M of *ortho*-phosphoric acid.

A stock solution of *ortho*-phosphoric acid 5 mol/L was obtained by proper dilution of the concentrated acid (Merck, d = 1.71, 85%).

The cadmium column conditioner was a solution with 0.4 g/L of disodium ethylenediamine tetraacetic acid (EDTA, Merck) and 20 g/L of ammonium chloride (Merck). This solution was monthly prepared by dissolution of the corresponding quantity of both solids in deionized water. In the end, the pH of the solution was adjusted to 9–9.5 with a commercial ammonia solution (Merck, d = 0.91, 25%).

For the potassium determination, a 0.108 mol/L potassium stock solution was prepared by dissolving the corresponding quantity of potassium chloride (KCl, Merck) in deionized water. The working standards were prepared in a range of 0.54–108 mmol/L by dilution of the stock solution and were stored in the refrigerator. A 0.5 mol/L sodium chloride (Merck) solution, used as an ionic strength adjuster solution (ISA), was prepared by dissolving the corresponding quantity of the solid in deionized water.

For the iron(III) determinations, a 5.00 mg/L iron(III) stock solution was prepared by diluting the 1000 mg/L atomic absorption standard of iron(III) (Fluka). The working standards were prepared from the stock solution within the range of 0.25–1.00 mg/L in 0.01 M nitric acid (Merck). A 4.9 g/L (15.2 mmol/L) stock solution of the MRB12 ligand [25] was prepared by dissolution of the corresponding quantity of the solid in water. The working solution of 0.6 mmol/L was weekly prepared by dilution of the stock solution. A 0.5 mol/L carbonate buffer solution was prepared by dissolving the correspondent quantity of sodium hydrogen carbonate (Panreac) in deionized water, the pH was adjusted to 10.5 with sodium hydroxide.

2.2. Cadmium column preparation

The cadmium granules were prepared according to the reference procedure for nitrate determination set in the Standard Methods for the Examination of Water and Wastewater [30]. The cadmium column was prepared according to Mesquita et al. [14], where the tube made of PTFE (3.2 mm i.d. and 90 mm long) was filled with the cadmium granules. An ordinary domestic sponge was placed at both ends of the column to entrap the solid material. After preparation, the column was firstly washed with the conditioner solution (NH_4Cl-EDTA) and then activated with a solution composed of 25% of the 20 μmol/L nitrate and 75% of the conditioner solution.

2.3. Apparatus

A syringe pump (Crison, Barcelona, Spain) equipped with a 10 mL syringe was connected to the central channel of a ten-port electrically actuated selection valve (Valco VICI Cheminert C25-381OD 06B–0699C,

USA). The connections between the different flow system components were made with 0.8 mm i.d PTFE tubing (Omnifit, UK). A computer (HP DC7600SFF) equipped with AutoAnalysis software (version 5.0.15.2, Sciware, Spain) was used to control the selection valve (SV) and the syringe pump.

For the spectrophotometric detection, an Ocean Optics (USA) USB 4000 charged coupled device detector (CCD), equipped with a pair of 200 μm optic cables and an HL-2000 halogen light source was used. A Hellma (Germany) 178.711-QS flow cell with a 10 mm light path and 30 μL inner volume was used. Data acquisition was performed at 710 nm (for phosphate determination), at 543 nm (for nitrite and nitrate determination), and at 460 nm (for iron(III) determination), using the OceanOptics - Spectrasuite software, running in an HP DC7600SFF computer. For the potentiometric determination of potassium, a Crison pH meter GLP 22 potentiometer equipped with a Crison 9661 N potassium ion-selective electrode (ISE) and a Crison 5241 reference electrode (Spain) was used.

2.4. Sequential injection system manifold and sequence protocol

The sequential injection manifold for the determination of phosphate, nitrite, nitrate, potassium, and iron(III) in soil is presented in Fig. 1.

As a multiparametric system, it was divided in four different determination protocols for the determination of phosphate, nitrite and nitrate, potassium, and iron(III). The phosphate, nitrite, nitrate, and iron (III) determination were based on spectrophotometric detection and the potassium determination was based on potentiometric detection. Both detectors, the CCD detector and the potentiometer equipped with a potassium ISE and reference electrode, were coupled to the selection valve of the sequential injection system. The protocol sequences are summarized in Table 1.

2.4.1. Phosphate determination

The sequence for the phosphate determination started with the aspiration of the ascorbic acid solution, molybdate reagent, and the sample/standard (steps 1A - 1C) into the holding coil. Then, the mixture between the different plugs is promoted by flow reversal (step 1D) and additionally by pausing the flow for 45 s when the mixture reaches the reaction coil (step 1E) before sending to the detector (step 1F, $\lambda = 710$ nm). The protocol was based on a published paper by Ferreira et al. for the determination of phosphate in soil leachates [27].

2.4.2. Nitrite and nitrate determination

To prepare the column for nitrate reduction, the conditioner solution was aspirated through the cadmium column (step 2A). Then, the sample/standard is aspirated to the holding coil and sent to the column for nitrate reduction (steps 2B and 2C). While the reduction of nitrate is taking place, the determination of nitrite was performed. For that, the Griess reagent and sample/standard are aspirated into the holding coil and, after flow reversal, the two plugs were propelled to the detector for nitrite determination (steps 2E - 2G, $\lambda = 543$ nm). Finally, for nitrate determination, the Griess reagent and the reduced solution were aspirated to the holding coil and sent to the detector (steps 2J - 2L, $\lambda = 543$ nm). The last two steps (steps 2 M and 2 N) of the protocol sequence aimed to prepare the column for a new cycle. The flow procedure was based on the method set by Mesquita et al. [14] applied to water analysis.

2.4.3. Potassium determination

For the potassium determination, the sample was aspirated to the holding coil (step 3A) and, after flow reversal, sent to the detector (step 2B); the detection system comprehends a pair of electrodes, a potassium selective electrode in a wall-jet arrangement, and a reference electrode. During step 2B, the sample/standard was mixed, in a confluence, with the ISA solution (NaCl) that was continuously flowing through the detector.

2.4.4. Iron(III) determination

For the iron(III) determination, MRB12, carbonate buffer, and the sample/standard were consecutively aspirated to the holding coil (steps 4A-4C). Afterward, the plugs were sent to the detector (step 4D) for the complex measurement ($\lambda = 460$ nm). The system protocol for iron(III) determination was based on the paper published by Mesquita et al. [25].

2.5. Laboratory-scale soil column

2.5.1. Laboratory-scale soil column setup

To simulate the leaching process, two laboratory-scale soil columns (LSSC) of 30 and 60 cm in height were assembled with different soil layers from different depths. Soil samples were collected from two different locations in Northwestern Portugal: Place A and Place B. The process for sampling the soil consisted of separately collecting three visually distinct layers while digging a hole with approximately 60 cm depth. For each layer of soil (about 20 cm depth each), around 1.5 kg was collected. Then, at the laboratory, some physical and chemical soil characterization was performed for both soils (Table 2). The soil layers

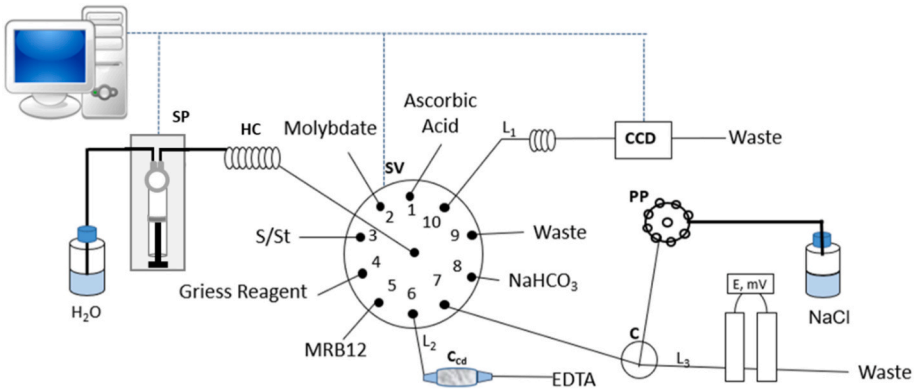

Fig. 1. Sequential injection manifold for the determination of phosphate, nitrite, nitrate, potassium, and iron. SP, syringe pump; SV, selection valve; PP, peristaltic pump; HC, holding coil (300 cm); C, confluence; ascorbic acid 30 g/L; molybdate reagent (0.1 g/L $C_4H_4KO_7Sb$, 16 g/L $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 7.2 g/L $C_4H_6O_6$ and 0.8 mol/L H_2SO_4); S/St, sample/phosphate, nitrite, nitrate, iron standard; $NaHCO_3$ 0.5 mol/L solution; Griess reagent (20 g/L of sulfanilamide, 2 g/L of N1NED, and 0.5 M of *ortho*-phosphoric acid); MRB12 0.6 mmol/L; EDTA (0.4 g/L of EDTA and 20 g/L of ammonium chloride); NaCl 0.5 mol/L, ionic strength adjuster; E, mV, potentiometer with K^+ ion-selective electrode and reference electrode; L_1, 140 cm reaction coil; CCD, charged coupled device detector ($\lambda = 460$, 543, 710 nm); L_2, 3 cm tube length connection; L_3, 10 cm tube length connection; C_{Cd}, cadmium column; W, waste.

Table 1
Sequential injection protocols for the determination of phosphate, nitrite, nitrate, potassium, and iron(III). At the beginning of each cycle the syringe was filled with an adequate volume of carrier (water) to proceed with the respective determination.

Determination	Step	SV position	Flow-rate (mL/min)	Volume (μL)	Description
Phosphate (1)	–	–	–	–	Fill the syringe with carrier to a volume of 9600 μL
	1A	1	2.6	186	Aspirate ascorbic acid solution
	1B	2	0.9	48	Aspirate molybdate reagent solution
	1C	3	0.9	79	Aspirate of sample/phosphate standard solution
	1D	10	1.8	425	Propel to the detector
	1E	–	–	–	Pause the solutions in the reactor
	1F	10	3.5	1758	Propel to the detector for phosphate determination (λ = 710 nm)
Nitrite and nitrate (2)	–	–	–	–	Fill the syringe with carrier to a volume of 8800 μL
	2A	6	2.6	214	Aspirate EDTA conditioner solution
	2B	3	2.6	119	Aspirate sample/standard solution
	2C	6	0.9	148	Propel through the cadmium column for nitrate reduction
	2D	9	3.5	243	Propel to waste to wash holding coil
	2E	4	3.5	548	Aspirate Griess reagent solution
	2F	3	3.5	560	Aspirate sample/standard solution
	2G	10	3.5	3348	Propel to the detector for nitrite determination (λ = 543 nm)
	2H	6	0.9	44	Aspirate solution from the cadmium column
	2I	9	3.5	122	Propel to waste to wash holding coil
	2J	4	3.5	303	Aspirate Griess reagent solution
	2K	6	1.8	102	Aspirate reduced sample/standard solution
	2L	10	3.5	2738	Propel to the detector for nitrate determination (λ = 543 nm)
	2 M	6	2.6	238	Aspiration of EDTA conditioner solution to wash the column
	2 N	9	2.6	304	Propel to waste to wash holding coil
Potassium (3)	–	–	–	–	Fill the syringe with carrier to a volume of 9600 μL
	3A	3	3.5	225	Aspirate sample/standard solution
	3B	7	3.5	2200	Propel to the detector for potassium determination
Iron(III) (4)	–	–	–	–	Fill the syringe with carrier to a volume of 9000 μL
	4A	5	3.5	250	Aspirate MRB12 reagent solution
	4B	8	0.9	20	Aspirate carbonate buffer solution
	4C	3	3.5	650	Aspirate sample/standard solution
	4D	10	3.5	3350	Propel to the detector for iron(III) determination (λ = 460 nm)

Ascorbic acid solution – 30 g/L; molybdate reagent solution - 0.1 g/L $C_4H_4KO_7Sb$, 16 g/L $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 7.2 g/L $C_4H_6O_6$ and 0.8 mol/L H_2SO_4; EDTA solution - 0.4 g/L of EDTA and 20 g/L of ammonium chloride; Griess reagent – 20 g/L of sulfanilamide, 2 g/L of N1NED, and 0.5 M of *ortho*-phosphoric acid; MRB12 solution– 0.6 mmol/L; carbonate buffer solution – 0.5 mol/L.

Table 2
Characterization of the soil used to set up the two laboratory scale soil column (LSCCs); soil collected from two different locations in Northwestern Portugal.

Parameter	Place A soil	Place B soil
Texture	Clay loam	Clay loam
pH	5.2	5.1
Conductance (mS/cm)	145	152
Density (g/mL)	3.13	2.76
Retention capacity	42%	46%

were oven-dried overnight (40 °C) and then reassembled in an acrylic cylinder to recreate the soil structure. A schematic presentation of the final setup of the column and photos of the LSSCs are shown in Fig. 2.

2.5.2. *Rain simulations and leachate sampling*

The LSSCs were used to simulate the natural soil leaching process with rainwater and produce simulated soil leachate samples. Rain

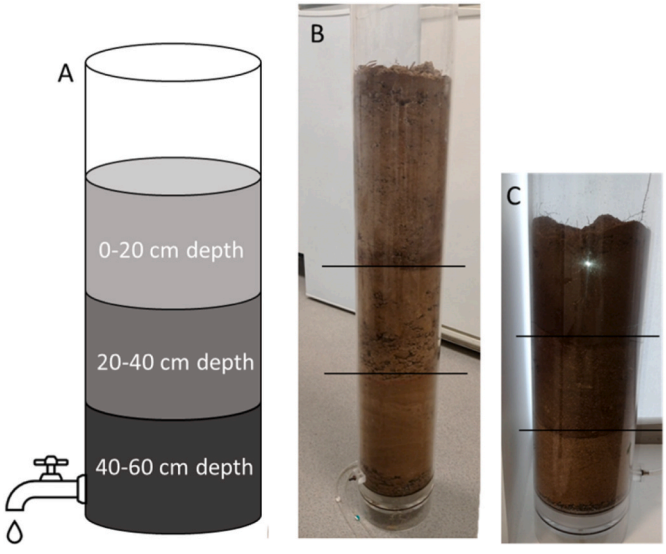

Fig. 2. (A) Schematic representation of a LSSC; (B) and (C) LSCCs assembly pictures for the Place A and Place B soils respectively (black lines define the different soil layers).

simulations were performed with filtered (cellulose acetate membrane filters, 0.45 μm) rainwater. The rain simulation process was performed on different days and was carried out by dispensing 100–150 mL of rainwater on the top of the LSSC column. Then, the leachate was collected at the bottom of the LSSC (Table S1). All leachate samples were filtered with cellulose acetate membrane filters before analysis. When the samples were not immediately analyzed, they were kept in the freezer (−20 °C) until analysis. Concerning the iron(III) determination, the samples were acidified to 0.01 mol/L nitric acid before analysis.

3. Results and discussion

The main goal of this work was to devise a multiparametric analytical method that comprises the determinations of phosphate, nitrite, nitrate, potassium, and iron(III) in a single manifold. For that, the strategy was to devise it in a flow-based approach resorting to the sequential injection analysis. Therefore, the necessary reagents for all the determinations, the standards/samples, and two different detection systems were placed/connected to the lateral ports of the selection valve. Regarding the SIA system method, some of the determinations (PO_4^{3-}, NO_3^-, NO_2^-, and Fe^{3+}) were based on previous works [14,25,27]. However, concerning the nitrite and nitrate determination, the referred work was devoted to water analysis; so, some studies were performed to check its applicability to soil leachates considering the difference in the matrix composition and the analytes range content in this type of sample. In fact, the nitrate content in the soil leachates was found to be much higher (about twenty times higher) when compared with water nitrate content. An option was made to dilute the samples (at least twenty times) prior to analysis to fit the nitrate concentration range of the calibration curve. Considering the potassium determination, as the proposed method was not based on previously reported studies, the sequential injection potentiometric measurement was subject to a more detailed optimization study.

3.1. Study of the potentiometric potassium determination

For the determination of potassium, some studies were performed to assess the influence of some variables in the method performance, namely the sample volume, the flow rate, and the electrode arrangement. These studies were first assessed in a flow injection analysis approach before including this determination in the SIA configuration. For that, a potassium ion-selective electrode was coupled to a flow injection configuration, in a wall-jet arrangement approach (and a reference electrode downstream). The ISA solution (NaCl) was added in a confluence to adjust the ionic strength of the standard/sample along the whole extent of the standard/sample plug; this way, it is possible to obtain a linear dependence of potential vs logarithm of concentration in potentiometric measurements. The electrode sensitivity was assessed by comparing the slope of the calibration curve with the value of the manufacturer guidelines (56 ± 4 mV per 10-fold change in the activity of potassium). Initially, the influence of the composition of the carrier (deionized water versus ISA solution) in the method performance was studied. No significant differences (<5%) were observed in the slope so, deionized water was chosen to carry the sample to a confluence point to mix with the ISA solution (continuously running through the detector). Then, two studies were carried out together, the influence of the sample volume and the flow-rate. For that, 130 μL or 220 μL of the sample were injected into the system and propelled through the detector at different flow-rates (0.5, 0.9, 1.4 e 1.8 mL/min). Considering sensitivity and repeatability, an option was made to set the sample volume to 220 μL and a flow-rate of 0.9 mL/min.

After setting these flow parameters, the idea was to include the potassium determination in the multiparametric sequential injection manifold (Fig. 1). For that, the potentiometric detection system (ISE and reference electrode) was coupled to a lateral port of the selection valve. To mix the standard/sample solution with the ISA solution (propelled continuously by a peristaltic pump), a confluence point was placed between the selection valve and the detection system.

3.2. Application to soil leachates

3.2.1. Accuracy assessment - potassium determination

For accuracy assessment, the determination of potassium in soil leachates was performed by resorting to the newly developed SIA method (section 3.1) and also to a reference procedure for potassium determination (flame emission spectrometry – FES). The potassium concentrations obtained with the two different procedures are depicted in Table 3. The relative deviation between the two sets of results showed that there were no significant differences between the two analytical procedures. A linear relationship was established between the potassium concentration determined by the SIA system ($[K^+]_{SIA}$ (mg/L)) and the reference procedure ($[K^+]_{FEC}$ (mg/L). The linear regression, with a 95% confidence interval, was $[K^+]_{SIA} = 0.966 \pm 0.058\ [K^+]_{FEC} + 1.329 \pm 2.473$. These parameters show that the slope and the intercept did not differ statistically from 1 to 0 respectively [31], indicating no significant differences between the two analytical procedures.

3.2.2. Soil leachates multiparametric analysis

As mentioned, the herein proposed system can be described as a combination of different individual analytical methods for the quantification of the different analytes. As previously mentioned, individual analytical procedures were based on previous works [14,25,27], except for potassium. The SIA system was set to display all the different determinations within the same manifold. The herein described system was applied for the multiparametric determination of the mentioned analytes (PO_4^{3-}, NO_2^-, NO_3^-, K^+, and Fe^{3+}) in simulated leachates. These samples were obtained by rain simulations with rainwater performed on the top of each prepared LSCC (see subsection 2.5.2). The multiparametric analysis performed with the herein-described sequential injection system, as well as some chemical parameters (pH and conductance) are presented in Table 4.

3.3. Features of the multiparametric method for nutrient (NPK&Fe) assessment

The features of the developed multiparametric method are summarized in Table 5, where the calibration curves, limits of detection (LOD), limits of quantification (LOQ), and determination rates are expressed. Regarding the spectrophotometric determinations (PO_4^{3-}, NO_3^-, NO_2^-, and Fe^{3+}), both LODs and LOQs were calculated according to the IUPAC recommendations [32,33]. These values are expressed as the concentration that corresponds to the sum of three and ten times (LOD and LOQ, respectively) the standard deviation to the mean value of ten

Table 3
Comparison of the results obtained with the developed SIA method for potassium determination ($[K^+]_{SIA}$) with those obtained with reference procedure ($[K^+]_{FEC}$); RD, relative deviation.

Sample ID	$[K^+]_{SIA}$ mg/L	$[K^+]_{FEC}$ mg/L	RD %
2A	62.0 ± 1.5	57.5 ± 0.5	8%
3A	53.4 ± 1.2	57.0 ± 0.2	−6%
5A	47.9 ± 2.1	50.9 ± 0.7	−6%
6A	52.0 ± 2.6	51.4 ± 0.2	1%
7A	44.2 ± 1.0	46.3 ± 0.5	−5%
8A	40.7 ± 1.8	38.7 ± 0.7	5%
9A	66.3 ± 4.1	67.9 ± 0.5	−2%
10A	61.1 ± 2.7	62.0 ± 0.6	−1%
3C	23.1 ± 0.4	20.8 ± 0.4	11%
4C	18.8 ± 0.3	18.1 ± 0.7	4%
7C	12.0 ± 0.2	10.8 ± 0.2	11%
8C	10.7 ± 0.5	9.7 ± 0.1	10%
9C	10.2 ± 1.1	10.2 ± 0.5	1%
10C	9.8 ± 1.0	9.5 ± 0.1	3%

Table 4
Multiparametric analysis of soil leachates using the SIA system for PO_4^{3-}, NO_2^-, NO_3^-, K^+, and Fe^{3+}.

Sample ID	pH	Conductance (mS/cm)	[PO_4^{3-}] μmol/L	[NO_2^-] μmol/L	[NO_3^-] μmol/L	[K^+] mg/L	[Fe^{3+}] μmol/L
2A	7.98	1864	19.5 ± 0.7	1.53 ± 0.03	1037 ± 39	62.0 ± 1.5	4.71 ± 0.01
3A	8.32	1802	18.4 ± 0.2	2.68 ± 0.03	2544 ± 48	53.4 ± 1.2	6.92 ± 0.01
4A	8.27	1768	21.9 ± 1.6	1.35 ± 0.08	1821 ± 41	43.6 ± 1.0	4.71 ± 0.01
5A	8.05	1716	43.5 ± 1.5	1.28 ± 0.20	2926 ± 65	47.9 ± 2.1	7.00 ± 0.43
6A	8.42	1690	39.3 ± 1.7	1.09 ± 0.08	3927 ± 83	52.0 ± 2.6	14.0 ± 0.4
7A	8.96	1455	54.6 ± 0.7	1.24 ± 0.05	3985 ± 66	44.2 ± 1.0	12.7 ± 0.4
8A	8.07	1591	47.8 ± 0.7	1.89 ± 0.05	2394 ± 54	40.7 ± 1.8	11.9 ± 0.4
10A	8.39	1493	56.3 ± 0.9	0.76 ± 0.11	2645 ± 74	61.1 ± 2.7	9.21 ± 0.20
11A	8.09	1361	57.0 ± 0.9	1.01 ± 0.03	2242 ± 58	30.6 ± 3.8	7.38 ± 0.01
3C	8.00	1274	52.3 ± 0.2	2.62 ± 0.09	3705 ± 50	23.1 ± 0.4	10.2 ± 0.4
4C	7.93	1114	60.0 ± 0.2	1.35 ± 0.05	4009 ± 79	18.8 ± 0.3	7.83 ± 0.58
7C	7.52	945	70.9 ± 1.9	0.74 ± 0.07	4319 ± 21	12.0 ± 0.2	10.8 ± 0.4
8C	7.61	931	65.5 ± 2.5	1.72 ± 0.05	3892 ± 74	10.7 ± 0.2	10.7 ± 0.2
9C	7.48	920	59.3 ± 1.3	0.77 ± 0.07	3985 ± 54	10.2 ± 1.1	10.0 ± 0.6
10C	7.50	972	45.6 ± 2.1	3.30 ± 0.05	3740 ± 58	9.8 ± 1.0	13.3 ± 0.1

Table 5
Features of the developed sequential injection system for nutrients (NPK & Fe) monitoring in soil leachates; S – measured signal (absorbance or potential), LOD – limit of detection; LOQ – limit of quantification; SD – standard deviation; RSD – relative standard deviation of ten consecutive determinations of a standard.

Analyte	Calibration Curve S = slope ± SD [Analyte] + intercept ± SD	Dynamic range	LOD (μmol/L)	LOQ (μmol/L)	RSD %
PO_4^{3-}	A = 0.0030 ± 0.006 [PO_4^{3-}] + 0.191 ± 0.085	10.0–80.0 μmol/L	2.15	7.18	6
NO_2^-	A = 0.043 ± 0.001 [NO_2^-] + 0.424 ± 0.022	0.50–8.00 μmol/L	0.22	0.73	2
NO_3^-	A = 0.0024 ± 0.001 [NO_3^-] + 0.465 ± 0.079	12.5–300 μmol/L	3.42	8.00	2
K^+	E (mV) = 56.9 ± 1.6 log [K^+] + 249 ± 27	0.54–108 mmol/L	39.0	-	5
Fe^{3+}	A = 0.0571 ± 0.0032 [Fe^{3+}] + 0.090 ± 0.012	4.48–17.9 μmol/L	0.46	1.85	6

consecutive blank solution measurements. For the potassium determination, the detection limit was determined according to the IUPAC recommendations [34]. The calibration curves presented in Table 5 correspond to the mean slope and intercept of 3 curves with the respective standard deviation. The repeatability was assessed by calculation of the relative standard deviation (RSD) of 10 replicate consecutive analysis of a standard. For the repeatability assessment, the chosen standard solution corresponded to the median standard concentration regarding each calibration range concentrations: 40 μmol/L for PO_4^{3-}; 4 μmol/L for NO_2^-; 150 μmol/L for NO_3^-; 54 mmol/L for K^+; and 8.95 for Fe^{3+}. A complete analytical cycle, comprehending three replicas for each standard/sample, for the determination of all the analytes (phosphate, nitrite, nitrate, potassium, and iron(III)) takes 28 min. As mentioned before, the method can be performed for a specific analyte; in this case, the determination rate is 24 h^{-1} for phosphate determination, 18 h^{-1} for nitrite and nitrate determinations, 36 h^{-1} for potassium determination, and 29 h^{-1} for iron(III) determination.

4. Conclusion

The developed multiparametric system for the determination of phosphate, nitrite, nitrate, potassium, and iron(III) in soil leachates proved to be a versatile and efficient tool for soil monitoring, enabling real-time assessment of soil fertility. This is particularly important as these species are nutrients that influence crop productivity in agricultural activity [6]. A huge advantage to point out to the proposed system relies on the possibility to perform a multiparametric analysis in a single automatic system. Additionally, it is also important to mention the high versatility of the system, as it is fairly easy to adapt for the determination of a specific analyte/s or to all the analytes mentioned with minor rearrangements. As far as we know, there are no analytical methods comprising all the mentioned determinations in a single manifold. Overall, resorting to the developed flow-based strategy using the full protocol (phosphate, nitrite, nitrate, potassium, and iron(III) determinations) a complete cycle, comprehending three replicates, takes about 28 min (two sample analyses per hour). The developed method was successfully applied for the multiparametric analysis of soil leachates obtained from the runoff of rain simulations in laboratory scale core columns.

CRediT author statement

Conceptualization, Ideas; formulation or evolution of overarching research goals and aims.

Methodology, Development or design of methodology; creation of models.

Software, Programming, software development; designing computer programs; implementation of the computer code and supporting algorithms; testing of existing code components.

Validation, Verification, whether as a part of the activity or separate, of the overall replication/reproducibility of results/experiments and other research outputs.

Formal analysis, Application of statistical, mathematical, computational, or other formal techniques to analyze or synthesize study data.

Investigation, Conducting a research and investigation process, specifically performing the experiments, or data/evidence collection.

Resources, Provision of study materials, reagents, materials, patients, laboratory samples, animals, instrumentation, computing resources, or other analysis tools.

Data Curation, Management activities to annotate (produce metadata), scrub data and maintain research data (including software code, where it is necessary for interpreting the data itself) for initial use and later reuse.

Writing - Original Draft, Preparation, creation and/or presentation of the published work, specifically writing the initial draft (including substantive translation).

Writing - Review & Editing, Preparation, creation and/or presentation of the published work by those from the original research group, specifically critical review, commentary or revision – including pre-or postpublication stages.

Visualization, Preparation, creation and/or presentation of the published work, specifically visualization/data presentation.

Supervision, Oversight and leadership responsibility for the research activity planning and execution, including mentorship external to the

core team.

Project administration, Management and coordination responsibility for the research activity planning and execution.

Funding acquisition, Acquisition of the financial support for the project leading to this publication.

Tânia Ribas: Validation; Visualization, Writing- Reviewing and Editing.

Maria João Nunes: Investigation**,** Writing - Original Draft.

Raquel Mesquita: Supervision, Conceptualization, Validation, Writing- Reviewing and Editing.

António Rangel: Supervision, Conceptualization, Writing - Reviewing and Editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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