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Flow injection system with gas diffusion for the sequential determination of total nitrogen and phosphorus in vegetables

Abstract A flow injection system was developed for the sequential determination of total nitrogen and phosphorus in digests of vegetables using potentiometric and spectrophotometric detection systems, respectively. A tubular ammonium selective electrode with a sensor system composed of nonactin/monactin in tris(2-ethylhexyl) phosphate was used. The selectivity limitations of this electrode were overcome by the inclusion of a gas-diffusion unit in the system that separated ammonium from the rest of the sample matrix and allowed the determination of total nitrogen and phosphorus by the partition of the sample plug between two streams.

The results obtained with the developed FIA system were in good agreement with those of the reference methods. Sampling rates from 40 to 60 samples per hour and relative standard deviations below 3.5% were achieved.

Introduction

Nitrogen and phosphorus are essential elements for plant growth. Modern intensive agriculture relies considerably on the use of commercial fertilisers to supply soils with these nutrients. Therefore, quantification of both elements in plant materials (as well as soils and water samples) is important routine analysis performed in agricultural and environmental research laboratories.

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Nitrogen determination in plants [1] is carried out in acid digests by distillation/titration procedures or by colorimetric methodologies (Berthelot reaction). Potentiometric ammonia gas sensors (with a gas-permeable membrane or an air-gap) have also been used [2, 3]. All these methodologies are time-consuming and troublesome.

Regarding phosphorus analysis, quantification is also accomplished after acid digestion of plant organic matter by colorimetric measurements. The molybdenum blue method [1] is widely employed for this determination and is also a laborious procedure.

A significant improvement was achieved by the development of automatic methods for these determinations providing higher sampling rates and precise control of the reaction parameters. Continuous flow systems with colorimetric [4–6], potentiometric (air-gap electrode) [6] and conductimetric [7] detection systems were used for the quantification of nitrogen in plants (including vegetable tissues). For phosphorus determinations in plant and water samples, several flow manifolds with spectrophotometric detection were used [8–11]. A flow injection manifold using sample splitting and a single spectrophotometer [12] was later described for the simultaneous determination of the analytes. This system relied on precise sample splitting to attain good reproducibility for both determinations which was assured by using a long splitting delay coil before the sample splitting point.

In this work a flow injection analysis system is described for the sequential determination of total nitrogen and phosphorus in digests of vegetables using potentiometric and spectrophotometric detection systems, respectively. A gas-diffusion unit was included in the system to overcome the selectivity limitations of the electrode and divide the sample plug between a donor and a acceptor stream in a reproducible manner. Total nitrogen determinations were performed in the acceptor stream while phosphorus quantifications were carried out in the donor stream by the molybdenum blue

method, after concentration adjustments to fit the sample analyte content to the requirements of the detection system.

The potentiometric determination of nitrogen (ammonium form) was chosen instead of the spectrophotometric detection as it requires simpler sample chemical treatment and enables determinations in a much wider linear concentration range. As indicator electrode a tubular ammonium selective electrode without inner reference solution and with a sensor system composed of a mixture of nonactin/monactin in tris (2-ethylhexyl) phosphate immobilised in PVC was used [13, 14]. The tubular configuration was selected because of the ability to attach the unit solidly to the set-up, thus decreasing the electrical noise and also reducing dead volumes [15].

Experimental

Apparatus and flow injection system

For the potentiometric measurements, a Crison 2002 voltmeter ($\pm$ 0.1 mV sensitivity), a double junction Russel 90–0029 reference electrode, with Tris-HCl 0.01 mol/L (pH 7.5) in the outer compartment, and a tubular shaped ammonium ion-selective electrode were used.

The FIA determination of phosphate and the reference methods for both analytes were performed with a Hitachi 100–40 UV/Vis spectrophotometer. When working in a continuous mode the spectrophotometer was equipped with a flow-through cell (Hellma type 178.711-QS, path length 10 mm, volume 30 µL).

Both detectors were connected to a double-channel Kipp & Zonen BD 112 chart recorder for the simultaneous recording of the two analytical FIA signals. The flow-injection system comprised Gilson Minipuls 3 peristaltic pumps with propulsion tubes of the same brand, a Rheodyne Type 5020 six-port rotary injection valve, 0.8 mm i.d. PTFE tubing from Omnifit, Gilson end-fittings and connectors and perspex home-made Y-shaped joints used as confluence or stream splitting points. Ground electrodes and perspex support devices for both reference and tubular shaped electrodes were constructed as reported elsewhere [16].

The home-made gas diffusion unit [17] consisted of two blocks of perspex forming a flow channel, 2 mm wide, 0.5 mm deep and 72 mm long (linear path). A gas-permeable membrane, commercial polytetrafluoro ethylene (PTFE) tape, was placed between the two blocks.

Tubular electrode construction

The sensor membrane was prepared as follows: 0.014 g of ammonium ionophore (Fluka), containing 75% of nonactin and 25% of monactin, was dissolved in 0.51 g of tris (2-ethylhexyl) phosphate (Fluka) using an ultrasonic bath to promote complete dissolution of the sensor in the mediator solvent. A solution of 0.16 g of PVC dissolved in 6 mL of tetrahydrofuran was subsequently added and the mixture stirred to obtain a clear solution. Tubular shaped electrodes without inner reference solution were then prepared using this solution according to procedures described earlier [16, 18]. Once the application of the sensor membrane was completed, the electrodes were left to dry at room temperature for one day and later conditioned overnight in a 0.1 mol/L ammonium sulphate solution (by circulation of this solution in the flow system at a low flow-rate). When not in use, electrodes were stored in contact with a 10^{-3} mol/L ammonium solution.

Reagents and solutions

Deionized water with a specific conductance lower than 0.1 µS/cm, and analytical reagent grade chemicals were used.

Ammonium (0.20 mol/L) and phosphate (0.016 mol/L) stock solutions were prepared in 1 mol/L sulphuric acid by precise weighing of previously dried (at 100 °C) solid ammonium sulphate and potassium dihydrogen phosphate. Standards used to establish the FIA calibration curves (4.0×10^{-3}–8.2×10^{-2} mol/L in NH_4^+ and 3.4×10^{-4}–2.4×10^{-3} mol/L in PO_4^{3-}) were obtained by dilution of these stock solutions in 1 mol/L sulphuric acid.

The buffer solution, 0.01 mol/L Tris-HCl, was prepared by mixing 200 mL of 0.1 mol/L tris (hydroxymethyl) aminomethane with 160 mL of 0.1 mol/L HCl and making the volume up to 2 litres with deionized water (final pH of about 7.5).

For the colorimetric determination of phosphate, two reagent solutions were prepared: the reducing solution, consisting of a mixture of 0.153 g/L stannous chloride, 1.6 g/L hydrazine sulphate and 0.38 mol/L sulphuric acid; the molybdate reagent solution, consisting of 2.82 g/L ammonium heptamolybdate in 0.52 mol/L sulphuric acid.

As previous works [8, 9] reported, a baseline drift can occur in the phosphate determination (due to molybdenum blue deposits in the flow-cell walls); a 1 mol/L NH_4Cl/NH_3 solution was circulated in the phosphate sub-system after each working day as a precaution measure.

Preparation of the digest of vegetables

The samples of plant material were oven-dried (at 80–100 °C) and ground [19]. Digests of vegetables were then prepared by acid digestion (H_2SO_4-salicylic acid-H_2O_2 and selenium) [1] and the final volume brought to 50 mL with 0.8 mol/L sulphuric acid. Finally, the samples were filtered through Whatman N° 541 filter paper into polyethylene bottles and stored at 4 °C until use. The vegetable tissues analysed were lettuce, watercress, spinach, turnip sprout, turnip leaf and parsley.

Reference methods

Nitrogen quantification was based on the Berthelot reaction [1], in which a phenol derivate forms a coloured compound (indophenol dye) in the presence of ammonia and hypochlorite. The indophenol thus formed is measured at a wavelength of 660 nm.

The concentration of phosphorus was determined spectrophotometrically (at a wavelength of 400 nm) as the yellow phosphovanado-molybdate complex [20] formed in acidic solution from the reaction of orthophosphate with ammonium molybdate and ammonium vanadate.

Results and discussion

Flow injection configuration

The flow diagram used is shown in Fig. 1.

Samples (or standards) are injected into a 0.8 mol/L H_2SO_4 carrier stream. A 2.5 mol/L hydroxide solution

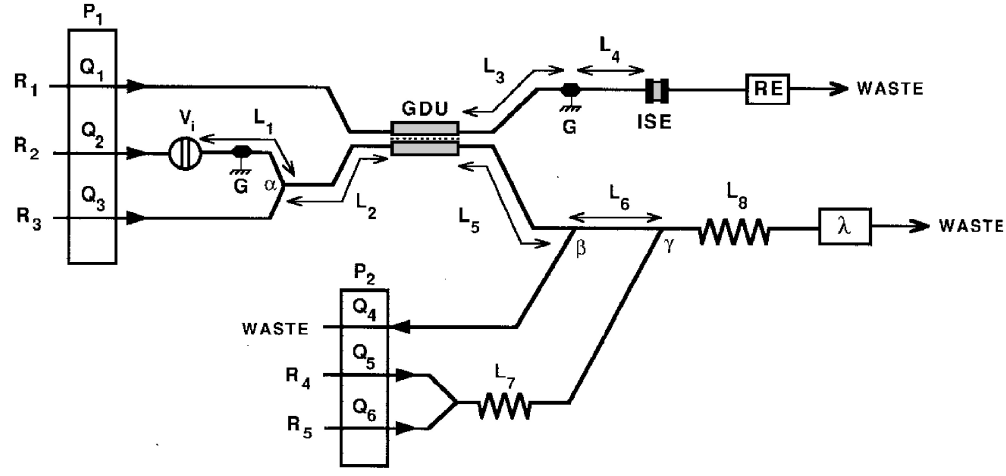

Fig. 1 Flow diagram of the manifold developed for the simultaneous determination of nitrogen and phosphorus in acid digests of vegetables: P_i = peristaltic pumps; Q_i = flow-rates ($Q_1 = 3.0$; $Q_2 = Q_3 = 1.5$; $Q_4 = 2.4$; $Q_5 = Q_6 = 1.6$ mL min^{-1}); V_i = injection volume (180 μL); L_i = tube lengths ($L_1 = 16$; $L_2 = 50$; $L_3 = 19$; $L_4 = 13$; $L_5 = 20$; $L_6 = 22$; $L_7 = 105$; $L_8 = 200$ cm); α, β and γ = confluences; R_i = reagent solutions ($R_1 = 0.01$ mol/L Tris-HCl pH 7.5, 1×10^{-6} mol/L NH_4^+; $R_2 = 0.8$ mol/L H_2SO_4; $R_3 = 2.5$ mol/L NaOH; $R_4 = 2.82$ g/L ammonium molybdate, 0.52 mol/L H_2SO_4; $R_5 = 0.153$ g/L stannous chloride, 1.6 g/L hydrazine sulphate, 0.38 mol/L H_2SO_4); *GDU* = gas-diffusion unit; *G* = ground electrodes; *ISE* = tubular ammonium selective electrode; *RE* = reference electrode; λ = spectrophotometer (at 710 nm)

(R_3) is then added in confluence α to convert ammonium to volatile ammonia. It diffuses across the gas-permeable membrane (*GDU*) and is reconverted to ammonium in contact with 0.01 mol/L Tris-HCl pH 7.5 buffer solution [21] (a 1×10^{-6} mol/L concentration of ammonium was added for baseline stabilisation), flowing in the acceptor stream. The total nitrogen determination is then carried out in the tubular ammonium electrode placed immediately after the gas-diffusion unit.

After a stream splitting (used to remove part of the sample creating the necessary overall dilution) and the addition of molybdate and stannous chloride solutions, the phosphate quantification was performed over the sample plug remaining in the donor stream. The determination is based on the reaction between orthophosphate and ammonium molybdate to form a heteropolymolybdophosphoric acid compound, later reduced by stannous chloride to molybdenum blue (measured at 710 nm).

Optimisation studies

Several system parameters were tested in order to achieve a compromise between the sensitivity of the analytical measurements, the linear working range for both determinations and the sampling rate.

Flow parameters in the gas-diffusion unit. Based on a previous work concerning optimisation of flow injection gas-diffusion systems [22], equal flow-rates for the donor and acceptor streams were used. Concurrent flow mode was adopted since it proved to be more effective than countercurrent flow. A flow-rate of 3.0 mL min^{-1} was selected as a settlement between the sampling rate and the sensitivity of the process.

Injection volume. An injection volume of 180 μL was selected. Higher injection volumes (220 and 280 μL) produced only a slight increase of the magnitude of the potentiometric signals, and had the disadvantage of lowering the upper limit of linear response for the colorimetric phosphate determination. On the other hand, the use of lower injection volumes could lead to a decrease in the reproducibility of the potentiometric measurements.

Acid concentration of the standard solutions. Considering that the acid concentration of the solutions injected into the system could affect the ammonia production and the colorimetric reaction, several assays were performed to establish the sulphuric acid concentration to be included in the standard solutions. Differences in the acid concentration of the final digests were expected since acid is consumed in the digestion process by distinct pathways. For this reason, the acidity of several digests of vegetables was determined by titration with a sodium hydroxide solution. Values between 0.80 and 1.1 mol/L in sulphuric acid were obtained. In order to study its influence, a set of standard solutions with sulphuric acid concentrations from 0 to 1.2 mol/L (0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.1 and 1.2) were prepared. As expected, due to the excess of sodium hydroxide (2.5 mol/L) used, no changes of the analytical signals were observed for the ammonium determinations. For the phosphate measurements the absorbance decreased significantly up to 0.6 mol/L sulphuric acid concentration (Fig. 2), remaining almost constant over the range of 0.6–1.2 mol/L. The H_2SO_4 concentration in the

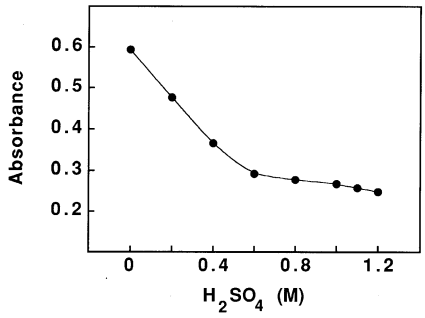

Fig. 2 Variation of the absorbance with sulphuric acid concentration for a phosphate concentration of 250 mg/L

standard solutions was set to 1.0 mol/L as slight changes of the acid level had less influence on the absorbance.

Composition of the flowing solutions. As the sensitivity of the spectrophotometric phosphate determination is strongly dependent on the acidity of the medium, and considering the acidity of the digests, a 0.8 mol/L H_2SO_4 solution was used as carrier stream (R_2). In these conditions a similar acidity of the plug (either from standards or digests) in the reactor (L_8) was assured.

The concentration of the sodium hydroxide solution (R_3) was conditioned by the final acidity of the digests. The hydroxide concentration should be sufficient to increase the pH to a value that allowed quantitative conversion of ammonium into ammonia gas. Five NaOH solutions (2, 2.25, 2.5, 2.75 and 3 mol/L) were tested. The sensitivity of the potentiometric measurement increased up to 2.5 mol/L. As for higher sodium hydroxide concentrations no significant sensitivity changes were observed, a 2.5 mol/L NaOH solution was selected.

The composition of the colour development solutions (R_4 and R_5) for the phosphate determination was then optimised. Based on the concentrations used in a previous work [9], R_4 solutions with different ammonium heptamolybdate (2.82, 4.23 and 5.64 g/L) and sulphuric acid concentrations (between 0.26 and 1.04 mol/L) were studied. As reducing solution (R_5) a 0.102 g/L stannous chloride solution, 1.6 g/L hydrazine sulphate and 0.31 mol/L sulphuric acid was used. An increase in sensitivity and a decrease in the linear working range with increasing molybdate concentration was observed. On the other hand, the sensitivity diminished with increasing acid concentrations. 2.82 g/L ammonium heptamolybdate with 0.52 mol/L H_2SO_4 (R_4) was chosen as a compromise between sensitivity and the linear working range.

After setting the composition of the R_4 reagent, a number of reducing solutions, with 1.6 g/L of hydrazine sulphate and 0.31 mol/L of sulphuric acid, and different stannous chloride levels (0.051, 0.076, 0.102, 0.153 and 0.204 g/L) were prepared. The selected concentration was 0.153 g/L corresponding to maximum sensitivity. In order to study the acid concentration, solutions with different H_2SO_4 levels (0.15, 0.23, 0.31, 0.38 and 0.46 mol/L) were tested. Maximum sensitivity and linearity over the needed concentration interval (3.4×10^{-4}–2.4×10^{-3} mol/L PO_4^{3-}) was obtained for the 0.38 mol/L sulphuric acid.

Length of the colour development reactor. The choice of the reactor coiled length (L_8) was made according to the results obtained with coiled tubes of 100, 120, 150 and 200 cm. Although the best sensitivity was achieved in a 120 cm reactor, the 200 cm reactor was preferred because of the improved baseline stability and considering that the sampling rate was not significantly affected.

Under the optimised conditions of the FIA system the lower limit of linear response and the practical detection limit for the ammonium determination were assessed according to recommended procedures [23]. Calibration curves from 7.6×10^{-5} to 1.1×10^{-1} mol/L were established and values of 3.3×10^{-3} mol/L (60 mg/L) for the lower limit of linear response and 1.4×10^{-3} mol/L (25 mg/L) for the practical detection limit were obtained.

The detection limit of the phosphate methodology, corresponding to three-times the standard deviation of the system background noise, was 4.3×10^{-5} mol/L (4.1 mg/L) of phosphate.

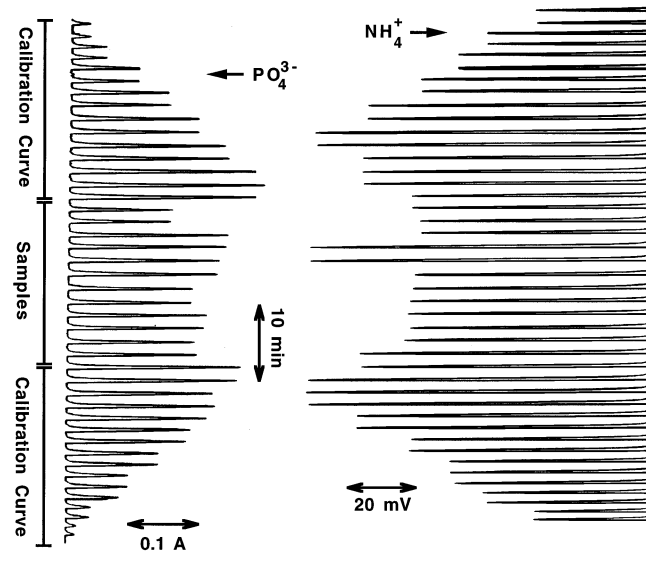

Fig. 3 Recorder output for the simultaneous determination of nitrogen and phosphorus in digests of vegetables. The concentration of the injected standard solutions ranged from 72 to 1.4×10^3 mg of ammonium per litre and between 20 to 250 mg of phosphate per litre

Table 1 Comparison of the results obtained (expressed as mg of ammonium or phosphate per gram of dried sample) from the analysis of digests of vegetables by the developed FIA system (C_f) and by the reference methods (C_r). Relative standard deviations for the FIA methodology

	Equation parameters ($C_f = C^0 + SC_r$)			Number of samples analysed	RSD (%)[b]
	C^0(mg/g)	S	R[a]		
Total nitrogen	1.7 ($\pm$ 3.6)[c]	0.979 ($\pm$ 0.054)[c]	0.986	30	< 3.5
Phosphorus	0.5 ($\pm$ 0.7)[c]	0.973 ($\pm$ 0.033)[c]	0.994	30	< 2.8

[a] Correlation coefficient.
[b] Relative standard deviation obtained from 10 consecutive injections of sample digests.
[c] Confidence limits for the slope and intercept values, at the 90% significance level for 28 degrees of freedom, are indicated in parentheses after the respective values.

Analysis of the digests of vegetables

Thirty digests of vegetables were injected into the developed flow injection manifold and the concentrations of total nitrogen and phosphorus were calculated by interpolation in the previously established calibration plots. Every standard and digest was injected in duplicate (Fig. 3). Concentration levels of 43 to 82 mg of ammonium per gram of dried vegetable sample (270–510 mg NH_4^+/L) and between 13 to 30 mg of phosphate per gram (87–220 mg PO_4^{3-}/L) were found.

In order to assess the accuracy of the FIA results (C_f), digests of vegetables were also analysed by reference methods (C_r). For comparison purposes, a relation of the type $C_f = C^0 + SC_r$ was established (Table 1). There is a good agreement between the two methodologies, as can be concluded by the slope and intercept values. Moreover, estimation of the confidence limits for these values at the 90% significance level [24] shows that there is no evidence for systematic differences between the sets of results (Table 1). The precision of the FIA methodology was determined from ten consecutive injections of three digests of vegetables, with concentrations covering the analytical range for each determination (Table 1). Relative standard deviations of 2.74, 2.85 and 1.71% were observed for samples with ammonium concentrations of 42.8, 64.0 and 82.0 mg/g, respectively. For samples with phosphate concentrations of 15.3, 20.3 and 27.5 mg/g the relative standard deviations found were 3.52, 2.51 and 1.53%.

A sampling rate of 40 to 60 samples per hour (corresponding to 80–120 determinations per hour) was achieved.

Conclusions

The developed FIA system has several advantages over the conventional manual reference procedures for the determinations of total nitrogen and phosphorus in vegetable samples as it allows simultaneous determination of both analytes with automatic digests preparation (vegetable digests are introduced without treatment in the flow injection manifold), higher sampling rates and increased simplicity. The flow injection methodology yields results comparable to those of conventional methods and shows good precision over the entire working range.

The results obtained in this work and the versatility of the flow injection systems allow to assume the applicability of the developed flow injection manifold also to the simultaneous determination of total nitrogen and phosphorus in other materials, such as soils and water samples where the quantification of these elements is also important.

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