Turbidimetric flow-injection determination of total nitrogen and potassium in vegetables

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Abstract

A turbidimetric flow-injection system was developed for the determination of total nitrogen and potassium in vegetable samples using a single spectrophotometer as detector. As a precipitating agent, 3.0% (w/v) sodium tetraphenylboron solution prepared in 2.0% (w/v) poly(vinyl alcohol) was used. A gas diffusion process was included in the manifold to separate ammonium ions from the rest of the sample and to allow paired analysis. Total nitrogen and potassium determinations were carried out on the solutions remaining in the acceptor and donor streams, respectively. Results obtained were precise (relative standard deviations <2.1 and 1.6% for total N (<25 mg g\(^{-1}\)) and K (<55 mg g\(^{-1}\)) determinations, respectively) and in agreement with those of reference methods. Analysis can be carried out at a rate of up to 35 samples per hour (corresponding to 70 determinations per hour) within concentration range 87–430 mg N–NH\(_4\)+ l\(^{-1}\) and 78–390 mg K\(^+\) l\(^{-1}\) for the total nitrogen and potassium determinations, respectively.

Keywords: Flow-injection; Turbidimetric sequential determination; Nitrogen; Potassium; Vegetables

1. Introduction

The determination of total nitrogen and potassium in plant materials is performed for routine quality control and fertiliser advisory purposes [1]. Nitrogen quantification in plants [2] is generally carried out by distillation/titration procedures or by colorimetric methodologies. Regarding potassium, flame emission spectrometry is usually used [2,3]. Turbidimetric determinations of total N and K in plant tissues using sodium tetraphenylboron (Na-TPB), by discrete ana-

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lytical methodologies have also been described [4,5]. These determinations are based on measurements of the decrease in intensity of a beam of radiation passing through a solution containing particles in suspension. This decrease depends not only on the size and number of the particles but also on their shape and sometimes their spatial arrangement [6,7]. Therefore, any change in the solution preparation process, often unavoidable in discrete turbidimetric procedures, leads to loss of repeatability of the analysis. So the use of flow-injection systems for turbidimetric determinations is advantageous, allowing close control of the conditions for colloidal suspensions preparation with a consequent improvement on the measurement precision [7].
A turbidimetric flow-injection procedure for the potassium determination with TPB has been previously reported [8]. In this work, a flow-injection system for the sequential turbidimetric determination of total nitrogen (ammonium form) and potassium in acidic vegetable digests is presented. The method is based on the precipitation reactions of both analytes with Na-TPB.

In order to separate the ammonium ions from the rest of the sample matrix, and also to allow the bicomponent analysis, a gas diffusion system was included in the manifold. Ammonium ions were withdrawn from the sample by diffusion of volatile ammonia from the donor to the acceptor stream. Potassium determinations were performed on the solution remaining in the donor stream, by its injection in the turbidimetric flow path where the potassium tetraphenylboron (K-TPB) precipitation occurs. Total nitrogen determination was carried out on the acceptor stream solution by a similar procedure, with formation of ammonium tetraphenylboron (NH₄-TPB).

2. Experimental

2.1. Reagents and solutions

All solutions were prepared with analytical reagent grade chemicals and deionized water (specific conductance <0.1 μS cm⁻¹).

Standard stock solutions of ammonium (4300 mg N–NH₄⁺ l⁻¹) and potassium (3900 mg K⁺ l⁻¹) were obtained by dissolution of (NH₄)₂SO₄ and K₂SO₄ (previously dried at 100°C overnight) in 0.8 M H₂SO₄. The working standards, covering the 87–430 mg N–NH₄⁺ l⁻¹ and 78–390 mg K⁺ l⁻¹ ranges (corresponding to 14–71 and 13–65 mg g⁻¹ in the samples on a dry basis of N–NH₄⁺ and K⁺, respectively), were prepared in 0.8 M H₂SO₄ by appropriate dilutions of the stock solutions.

A 2.0% (w/v) poly(vinyl alcohol) (PVA) solution was prepared by suspending 10 g of solid in about 200 ml of boiling water with continuous stirring. After cooling, the volume was made up to 500 ml with water.

The 3.0% (w/v) Na-TPB solution was obtained by dissolution of the product in the solution of 2.0% PVA.

2.2. Apparatus

A Hitachi 100-40 UV/Visible spectrophotometer with a Hellma 178.710-QS flow-cell (10 mm light path, 80 μl inner volume) and connected to a Kipp and Zonen BD111 strip chart recorder was used. Total N determinations by the reference method were carried out with the same spectrophotometer. Potassium determinations by the reference procedure were carried out with a Corning 410 flame emission spectrometer using an air–propane flame.

To build the flow-injection manifold, two 4-channel Gilson Minipuls 3 peristaltic pumps with Tygon pumping tubes, a home-made injector-commutator [9], Perspex Y-shaped joints and polyethylene tubing of 0.8 mm i.d. were used. A Bandelin-Sonorex RK100 ultrasonic bath (mainly used to avoid precipitate settlement on the conduit walls) and gas diffusion units [10], of 3.7 or 7.2 cm length, 2 mm width and 0.5 mm depth, were also included in the flow system. Commercial polytetrafluoroethylene (PTFE) tape was placed between the two parts of these units.

2.3. Flow diagram

The flow-injection manifold used for the turbidimetric determinations of K⁺ and NH₄⁺ is outlined in Fig. 1.

The sample digest solutions (S) were pumped into the system and mixed with 2.0 M sodium hydroxide (R₁) for quantitative conversion of the NH₄⁺ ions to volatile NH₃. This stream was directed towards the donor channel of the dialysis unit (D) where diffusion of ammonia, through a PTFE membrane, towards a 0.8 M sulphuric acid acceptor stream (R₂) occurred. Both outflowing streams were connected to an injector-commutator (IC). The acceptor stream filled one of the sampling loops (V₁) and, upon switching the commutator, this volume was introduced into the 0.5 M NaOH carrier stream (R₃), which merged with 0.25 M NaOH (R₄) and then with a 3.0% Na-TPB solution prepared in 2.0% PVA (R₅). Meanwhile, the second sampling loop (V₂) was filled with the donor stream. By switching the commutator back to the position specified in Fig. 1, V₂ was injected into the analytical path and was subjected to the same sample treatment as described above.
A 100 cm coiled tube \((L_2)\) was placed after the confluence point of 0.25 M NaOH to improve mixing with the main stream and thus diminish refractive index effects [11].

The precipitation reactions (formation of either K-TPB or NH\(_4\)-TPB) occurred inside a 200 cm coiled reactor \((L_3)\), held in an ultrasonic water bath \((UB)\), and the turbidity measured at 420 nm [4].

### 2.4. Sample preparation

Vegetable samples (lettuce, parsley, spinach, turnip sprout, turnip leaf and watercress) were cleaned with flowing tap water, oven dried \((80-100^\circ\text{C})\) and ground [12].

Acid digestion \((\text{H}_2\text{SO}_4-\text{salicylic acid-\text{H}_2\text{O}_2})\) [2] was performed on this sample material as follows: about 0.3 g of each dried sample was accurately weighed into a 50 ml volumetric flask and 3.3 ml of digestion mixture (5% w/v salicylic acid in 15 M sulphuric acid) added; the mixture was allowed to stand overnight at room temperature and then heated at 180°C for about 1 h. After cooling, 5 drops of hydrogen peroxide 30% (w/w) were added and the solution heated at 280°C until white vapours appeared. This procedure was repeated until the digest became colourless. After cooling to room temperature, 10 ml of water was added to the digest. The digest was transferred to a 50 ml volumetric flask and the volume made up with water. The final solution of digest was filtered through a Whatman No. 541 filter paper and stored at 4°C until used (this solution was used for both reference and flow-injection determinations).

### 2.5. Reference methods

For comparison purposes, total N and K determinations were carried out on diluted digests of vegetables using reference methodologies.

The reference method for total nitrogen quantification in the digests was a spectrophotometric one based on the Berthelot reaction [2] in which a phenol derivative forms a coloured compound (indophenol blue) in the presence of ammonia and hypochlorite. Absorbance is measured at 660 nm.

The potassium content in the digests was determined by a recommended flame photometric method [3].

For both K and total N methodologies the sample solutions were diluted in a multi-step approach in order to fit their composition to the linear range of the previously established calibration graphs.
3. Results and discussion

3.1. Development of the flow-injection system

As the increase in the acidity of the acceptor stream favours mass transfer of NH₃ through the membrane (by conversion of NH₃ to NH₄⁺), and since the sulphuric acid concentration of the final digests solutions ranged from 0.7 to 0.9 M [2], a 0.8 M sulphuric acid solution was used as the acceptor stream.

Some assays were then carried out to establish a compromise between some system variables and the sensitivity and precision of the analytical measurements, the working range for both determinations and the sampling rate.

3.1.1. Sodium hydroxide concentrations

Hydroxide solutions of 2.0 and 3.0 M were tested as solutions for making the sample digest solution alkaline (R₁, Fig. 1). These concentrations were chosen considering the H₂SO₄ content of the vegetable digests (0.7–0.9 M) and also the fact that an increase in the sensitivity for K⁺ determination was observed with increasing OH⁻ concentrations. The 2.0 M NaOH concentration was selected because the use of higher OH⁻ concentrations did not significantly change the sensitivity but clearly increased the Schlieren patterns of the signal.

The option to use NaOH solutions as carrier stream (R₃) and confluent addition solution (R₄) was made to decrease the magnitude of the Schlieren signal. Hydroxide solutions with concentrations ranging from 0.1 to 1.0 M were assayed. After testing several arrangements, 0.5 and 0.25 M NaOH solutions were chosen as carrier and confluent addition streams, respectively.

3.1.2. Sodium tetrakis(ethylenediamine)platinum (II) solution concentration

Regarding Na-TPB solution (R₅) 0.3, 1.0, 2.0, 3.0 and 4.0% (w/v) concentrations were tested. The sensitivity of the total N and K determinations increased with increasing reagent concentration and the 3.0% (w/v) Na-TPB solution was chosen as a compromise between reagent consumption, sampling rate and sensitivity.

3.1.3. Injection volumes

Injection volumes of 140, 200, 250, 380 and 530 µl for the NH₄⁺ determination (V₁) and of 30, 40, 50, 60, 100 and 220 µl for the K⁺ quantification (V₂) were tested. As expected, the injected volumes played a pronounced influence on the analytical signals with larger injected volumes giving rise to higher turbidity values. A 380 µl (75 cm of polyethylene tubing of 0.8 mm i.d.) sampling loop was chosen for the NH₄⁺ determination (V₁) as a clear separation between the central portion of the plug (corresponding to the analytical signal) and the two distinct Schlieren regions (at the front and tailing portions of the sample zone) was observed and because larger volumes would unnecessarily decrease the sampling rate. For the K⁺ analysis 40 µl (8 cm of polyethylene tubing of 0.8 mm i.d.) was selected (V₂) as a compromise between sensitivity, precision and sampling frequency. Larger injection volumes gave rise to increased signals but led to a decrease in the sampling rate; however, smaller injection volumes provided unsuitable sensitivity.

3.1.4. Gas diffusion units

Gas diffusion units with 3.7 or 7.2 cm length were tested. Similar flow rates were set on both sides of the membrane to allow equal pressure and avoid distortion of the membrane that might affect precision [13,14]. The longer gas diffusion unit was preferred as it provided higher sensitivity. Therefore, a 7.2 cm unit was included in the flow-injection manifold in the countercurrent mode. Such flow configuration was chosen instead of the concurrent mode because a slight increase on the peak height of the analytical signals was observed under these conditions.

3.1.5. Flow rates

As previously mentioned, a ratio of 1 : 1 between the donor and acceptor flow rates in the gas diffusion units was set. Similar flow rates for the 2.0 M NaOH (R₁) and sample digest solutions (S) were already preset (this flow ratio provided the necessary sample dilution). Flow rates of 0.65, 0.70 and 1.0 ml min⁻¹ for these solutions and 1.20, 1.50 and 2.25 ml min⁻¹ for 0.8 M H₂SO₄ (R₂) were tested. Lower flow rates provided higher recorded peaks for the total N determination but led to a decrease in the sampling rate. Flow rates of 0.70 ml min⁻¹ for the hydroxide and sample solutions and of 1.50 ml min⁻¹ for the
acceptor solution were selected as a trade-off between sensitivity and sample throughput.

The flow rates of the carrier (R₃) and confluence addition (R₄) streams were set at 1.95 ml min⁻¹, after testing flow rates of 0.75, 0.95 and 1.95 ml min⁻¹ for the carrier and 1.85, 1.95, 2.35 ml min⁻¹ for R₄. The highest flow rate was chosen for the carrier stream because the Schlieren effects decreased on increasing the flow rate. For 0.25 M NaOH, 1.95 ml min⁻¹ was also selected as better precision was attained with even flow rates at the confluence point.

Flow rates of 0.75, 0.80, 0.95 and 1.30 ml min⁻¹ were tested for the precipitating solution. The highest flow rate was adopted because of the increase in sensitivity and calibration linearity.

3.1.6. Protective agent and ultrasonic water bath

The use of PVA as a colloidal protective agent for the suspensions was tested. Four solutions with different PVA contents, 0.0, 2.0, 3.0 and 4.0% (w/v), including 3.0% Na-TPB, were prepared. A pronounced improvement in repeatability was obtained when the Na-TPB solution without PVA was replaced by the one with a 2.0% concentration. No significant differences in precision were noticed between 2.0 and 3.0% PVA solutions, but sensitivity was found to diminish by further increasing the PVA concentration. The use of 2% (w/v) PVA solution was therefore recommended. Inclusion of surfactants in solution for quantitative turbidimetric determinations (both in batch and in flow analysis) to improve precision and prevent rapid settlement of the particles, has been widely reported [7,15]. Additionally, in flow-injection systems the presence of such agents is known to reduce the time of return to baseline and to improve its stability [7]. However, build-up of precipitate in the L₃ coil reactor, after several working days, was still observed. From time to time cleaning of this tube, by immersion in an ultrasonic water bath, was required.

To avoid periodic stoppage of the system, permanent immersion of L₃ in the ultrasonic bath was tested. As significant increase of the turbidity values was observed, the continuous use of the ultrasonic bath was adopted.

3.1.7. Mixing coil lengths

The lengths of the two mixing coils (L₁ and L₃) were optimised. For the mixing coil between the NaOH and the acid digest solutions (L₁), 25, 50, 105 and 150 cm tubes were tested. The smallest one did not allow good mixing conditions between the two solutions leading to erratic (r.s.d. >20%) results. As no differences were observed for others, the 50 cm length was selected.

Precipitation reactor tubes (L₃) of length 100, 150 and 200 cm were tried. The 200 cm reactor was chosen because of the improved baseline stability and measurement precision.

Under the optimised conditions, the data obtained for K⁺ calibration showed that the experimental values fit a second-order calibration equation [15]. For the NH₄⁺ determinations linear calibration graphs were attained.

3.2. Application to vegetable digests

Sixteen digests of vegetables (lettuce, parsley, spinach, turnip sprout, turnip leaf and watercress) were analysed for their K⁺ and NH₄⁺ contents in the developed turbidimetric flow-injection system.

In order to assess the accuracy of the flow-injection results, K⁺ and NH₄⁺ determinations, for the 16 digests of several types of vegetables, by the reference methods were also carried out. The paired results, together with the relative deviations between them, are presented in Table 1.

Regression equations of the type \(C_r = C_0 + SC_r\), \(C_r\) being the flow-injection results and \(C_r\) those provided by the reference procedures, were established (Table 2). There is a good agreement between the two methodologies as slopes and correlation coefficients are close to unity and intercept values are near zero. Furthermore, confidence limits of the slope and intercept at 95% confidence level for 14 degrees of freedom show that there is no statistical difference between the two sets of results [16] (Table 2).

Precision was assessed from 10 consecutive injections of three digests with concentrations matching the analytical range related to each determination. Relative standard deviations of 2.13, 1.45 and 1.25% were obtained for samples with N-NH₄⁺ concentrations of 25.3, 34.9 and 42.5 mg g⁻¹, respectively. For samples with K⁺ contents of 26.1, 38.7 and 55.1 mg g⁻¹ the relative standard deviations observed were 1.03, 1.11 and 1.56%, respectively.
Table 1
Results obtained in the total nitrogen and potassium determinations (expressed in mg of analyte per gram of dried product) in acid digests of vegetable samples by flow injection analysis (FIA) and by the reference method and corresponding relative deviations (RDs)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total nitrogen</th>
<th>Potassium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FIA (mg N g⁻¹)</td>
<td>Ref. method (mg N g⁻¹)</td>
</tr>
<tr>
<td>1</td>
<td>51.4</td>
<td>52.6</td>
</tr>
<tr>
<td>2</td>
<td>43.1</td>
<td>43.9</td>
</tr>
<tr>
<td>3</td>
<td>22.7</td>
<td>23.2</td>
</tr>
<tr>
<td>4</td>
<td>25.3</td>
<td>24.8</td>
</tr>
<tr>
<td>5</td>
<td>22.6</td>
<td>22.2</td>
</tr>
<tr>
<td>6</td>
<td>42.1</td>
<td>43.0</td>
</tr>
<tr>
<td>7</td>
<td>40.0</td>
<td>39.5</td>
</tr>
<tr>
<td>8</td>
<td>26.6</td>
<td>26.2</td>
</tr>
<tr>
<td>9</td>
<td>39.4</td>
<td>40.0</td>
</tr>
<tr>
<td>10</td>
<td>30.0</td>
<td>29.6</td>
</tr>
<tr>
<td>11</td>
<td>19.4</td>
<td>19.4</td>
</tr>
<tr>
<td>12</td>
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<tr>
<td>15</td>
<td>43.4</td>
<td>43.0</td>
</tr>
<tr>
<td>16</td>
<td>34.9</td>
<td>35.3</td>
</tr>
</tbody>
</table>

Table 2
Comparison of results obtained by the developed FIA system (Cₜ) and by the reference methods (Cₚ). Detection limits, relative standard deviations and sampling rate for the FIA methodology

<table>
<thead>
<tr>
<th>Equation parameters (Cₜ=C₀+SCₖ)</th>
<th>Number of samples analysed</th>
<th>Characteristics of the FIA system</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₀ (mg g⁻¹)</td>
<td></td>
<td>Detection limit (mg l⁻¹)ᵇ</td>
</tr>
<tr>
<td>S</td>
<td>R²</td>
<td></td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>1.0 (±1.1)ᵈ</td>
<td>0.969 (±0.032)ᵈ</td>
</tr>
<tr>
<td>Potassium</td>
<td>-1.2 (± 1.9)ᵈ</td>
<td>1.02 (±0.04)ᵈ</td>
</tr>
</tbody>
</table>

ᵃ Correlation coefficient.
ᵇ Detection limit determined according to IUPAC recommendations [17].
ᶜ Relative standard deviation obtained from 10 consecutive injections of sample digests (see text).
ᵈ Confidence limits for the slope and intercept values, obtained for 95% significance for 14 degrees of freedom, are indicated in brackets after the respective values.

The detection limits of the system were calculated, according to IUPAC recommendations [17], after 10 consecutive injections of 0.8 M H₂SO₄ (blank) (Table 2).

This system allowed a sampling throughput of 20 to 35 samples per hour (i.e. 40–70 determinations per hour), a rate similar to others obtained for turbidimetric flow-injection determinations [7]. It should be pointed out that in the developed methodology not only the total nitrogen is determined for the first time in a flow-injection system by precipitation with NaTPB, but also the sequential turbidimetric determination of total nitrogen and potassium is achieved. Moreover, in this system unlike what happens in the reference methods, no multi-step dilution process is required.

Additionally, it should be mentioned that preliminary studies raised the possibility of using a similar flow manifold, without a gas diffusion process, for the same determinations. Sample digest solutions were injected directly into the system: one of the sampling loops provided the joint determination of NH₄⁺ and K⁺ while
the other enabled the individual \( \text{K}^+ \) analysis. However, the \( \text{K}^+ \) and \( \text{NH}_4^+ \) analytical signals were not found to be additive.

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