MERGING ZONES IN FLOW INJECTION ANALYSIS
Part 3. Spectrophotometric Determination of Aluminium in Plant and Soil Materials with Sequential Addition of Pulsed Reagents

B. F. REIS, H. BERGAMIN Fº*, E. A. G. ZAGATTO and F. J. KRUG
Centro de Energia Nuclear na Agricultura, CEP 13400 Piracicaba, Sao Paulo (Brazil)
(Received 2nd January 1979)

SUMMARY

A flow injection procedure is described for the spectrophotometric determination of aluminium in plant and soil material with eriochrome cyanine R. This system utilizes merging zones and sequential addition of pulsed reagents. A high degree of sample dispersion and pulsed neutralization reagent allow precise pH control; acid plant digests can therefore be analysed without pre-treatment. Iron(III) interference is avoided with ascorbic acid, and phosphate interference is negligible. The sampling rate is 120 samples per hour, reproducibility is better than 1% for a 5.0-ppm Al standard, and the calibration plot is linear in the range 0–20 ppm Al. Applied to soil and plant samples, the method gives recoveries of 98–101%.

Flow injection analysis, devised by Růžička and Hansen [1] has undergone fast development, as indicated in comprehensive reviews [2, 3]. Recently, the original idea of flow injection analysis was expanded with the concept of merging zones [4, 5], based on synchronized injection of sample and reagent into inert carrier streams, with further merging and reaction of the injected species. The reagent is, therefore, consumed only when the sample is present and is otherwise continuously recovered. Previous papers of this series have emphasized the low consumption of ascorbic acid in the spectrophotometric determination of phosphate [4], and of lanthanum in the atomic absorption spectrometry of calcium and magnesium [5].

A logical evolution of this concept is to utilize merging zones for the sequential addition of analytical reagents. This idea was adopted here in a flow injection procedure for the spectrophotometric determination of aluminium. The method should be valuable in agricultural laboratories where the number of plant and soil samples to be analysed for aluminium is high, because of the importance of this element in soil chemistry and plant nutrition.

Of the analytical reagents available for the colorimetric determination of aluminium, eriochrome cyanine R and aluminon are most frequently employed [6, 7]. The method based on eriochrome cyanine R, despite its rigid pH control requirement, is less affected by temperature changes, does not require a colloid stabilizer [8] and was therefore adapted by Reis [9] to a flow injection procedure.
The aim of the work described here was to investigate the possibility of sequential addition of pulsed reagents via merging zones, and to improve the flow injection procedure for the spectrophotometric determination of aluminium in plant digests and soil extracts. The merging zones allow reduced reagent consumption, and provide an initial neutralization of the injected digests, avoiding the pH adjustment of the samples prior to analysis.

**Preliminary considerations**

Eriochrome cyanine R reacts with aluminium ions under acidic conditions (pH ca. 3) producing a red complex which is measured at 535 nm [10]. The unreacted cyanine, however, absorbs strongly, the absorbance being highly dependent on pH [6, 8]. At pH 6.0, the molar absorptivity of the cyanine is less affected by pH changes [6]; this pH value is therefore usually recommended for the development of the aluminium complex [6, 8, 11].

For the analysis of plant digests, adjustment to pH 3 is normally done before analysis [6]. This makes the final pH control easier, and can be achieved in a flow injection system by introducing the sample into an inert acid carrier stream and merging later with a sodium hydroxide stream [9]. Similar procedures have been used in earlier systems [12, 13].

In the present method, sodium hydroxide solution and sample are injected simultaneously and interact after merging of the injected zones. Ascorbic acid, used to mask iron(III), is also injected together with the sample but merging occurs downstream. The effects of changes in refractive index, which could be caused by interaction of the injected zones, are negligible because the system is characterized by a high degree of dispersion. The addition of buffer and eriochrome cyanine R via merging zones is not feasible because of refractive ("schlieren pattern") and colour effects [14, 15]. The consumption of both sodium hydroxide and perchloric acid is low, and the total ionic strength is reduced by using merging zones; decreasing the ionic strength increases the signal intensity [9].

**EXPERIMENTAL**

**Apparatus**

The manifold (Fig. 1) was made from polyethylene tubing (i.d. 0.86 mm) supported by suitable Lego blocks. All connectors were made from perspex. Details of the system construction have been given elsewhere [14, 16]. Coil lengths and pumping rates were chosen in order to achieve a high degree of sample dispersion, associated with a reasonable sampling rate. The streams were pumped at similar rates because, if the hydrodynamic pressures are similar in all confluent streams, the mixing conditions are better and the precision is improved. Synchronization of the injected zones is also easier if the linear speeds of the merging zones are similar.

A Technicon AAII peristaltic pump furnished with suitable tygon pumping tubes was employed.
Fig. 1. Flow diagram of the proposed system. ECR is the colour reagent, P is the peristaltic pump; pumping rates are given in ml min⁻¹. The injected volumes are 20 µl for S (sample) and R₁ (0.25 M NaOH) and 50 µl for R₂ (masking agent). C₁ and C₂ are 40 cm and 200 cm long, respectively. The optical cell is between points C and D. For further explanations, see text.

Samples and reagents were introduced into the system by a multiple proportional injector made of perspex (Fig. 2) which is a development of the proportional injectors described earlier [4, 17]. In the loading position (Fig. 2A), the sample (S) is aspirated to fill the sample loop (Lₚ), which defines exactly the injected sample volume, the excess of sample going to waste (W). Simultaneously, the reagents R₁ and R₂ are pumped to fill the reagent loops (Lₐ₁ and Lₐ₂) and their excesses, slightly diluted by the corresponding reagent carrier streams, are accumulated in the reagent recovery vessels (V₁ and V₂). In this position, the sample and reagent carrier streams (CS, CR₁ and CR₂) are pumped continuously to keep the system running. In the injection position (Fig. 2B), the selected volumes of sample and reagents are pushed by the corresponding carrier streams and the pumped reagents are directed back to the reagent reservoirs.

The Beckman model 25 spectrophotometer used was connected to a Beckman model 25 ACC recorder and furnished with a Hellma type 178 flow-cell (light path 10 mm, volume 80 µl). The dual-beam mode was used, and an aliquot of the stream going to waste (Fig. 1) was collected before running analysis, to serve as a blank. The coloured complex was measured at 535 nm.

The model 63 pH meter was connected to a Servograph REC 61 with a...
REA 112 high-sensitivity unit (Radiometer, Copenhagen). For continuous potentiometric measurements, the assembly described earlier [18] was used. Dispersion factors were measured with a nitrate-selective electrode [19] and pH values with a flat glass electrode (Radiometer). A K401 saturated calomel reference electrode (Radiometer) was employed.

**Samples and standards**

Plant materials were mineralized by wet digestion with nitric and perchloric acids on a Technicon BD 40 digestion block [14]. Soil extracts were obtained with 1 M KCl solution, by a standard procedure [11]. Working standards in the range 0—20 ppm Al were prepared weekly by appropriate dilution of a 500-ppm aluminium stock solution, which was made by dissolving 8.752 g of KAl(SO₄)₂ · 12H₂O in 1 l of a 10⁻³ M HCl solution. For plant analysis, the working standards were made 0.25 M in perchloric acid.

**Reagents**

All reagents were of analytical grade and distilled water was employed throughout. Both sodium hydroxide and perchloric acid were standardized before use.

The eriochrome cyanine R stock solution, which was stable for several months, was prepared by dissolving 3.0 g of the dye in about 800 ml of water, adjusting to pH 3.0 with hydrochloric acid and diluting to 1 l with water. Before use, an appropriate volume of this reagent was diluted (1 + 9) with water.

The acetate carrier solution was prepared by dissolving 308 g of ammonium acetate in 1 l of water and adding enough 6 M acetic acid or ammonia solution to attain pH 6.0 at point D (Fig. 1).

For the masking solution, 5 g of ascorbic acid was dissolved in 100 ml of the above ammonium acetate solution [9]. The acetate is necessary to avoid pH gradients after injection, which would create a blank signal.

**Analytical procedure**

The flow diagram for the determination of aluminium in plant digests and soil extracts is shown in Fig. 1. The sample (S) is injected simultaneously with both reagent R₁ (0.25 M sodium hydroxide for plant analysis, or water for soil analysis) and reagent R₂ (masking solution). The corresponding carrier streams are water (Cᵣ₁ and Cₛ) and ammonium acetate solution (Cᵣ₂).

At point A, the sample zone neutralized by the sodium hydroxide, if necessary, meets the eriochrome cyanine R stream. The reaction develops in the reaction coil (Cᵣ₁) under the recommended acidic conditions [6], determined by the acidity of the colour reagent. At point B, the zone of ascorbic acid meets the sample zone in a synchronized manner as indicated in Fig. 3. In the following coil (Cᵣ₂, Fig. 1), iron(III) is reduced and the sample zone is buffered to pH 6.0. As the sample zone reaches the flow-cell, the absorbance of the complex is measured at 535 nm and recorded.
Measurement of dispersion

Dispersion of the sample was evaluated by replacing all carrier solutions of the system (Fig. 1) by 0.01 M sodium tetraborate in 0.01 M sodium hydroxide solution [20]. The potentiometric assembly for nitrate [18] was set at point C and later, at point D. For each point, a series of sodium nitrate standards in the range 0.0001–0.05 M (also containing 0.01 M sodium tetraborate) was used for calibration. The dispersion factors were evaluated from the results obtained with the 0.0001 M nitrate standard, which was measured in two ways by the methodology described earlier [2, 12].

RESULTS AND DISCUSSION

Sample acidity and dispersion

The acidity of the sample is an especially important factor when the absorbance of the reagent is high and strongly dependent on pH. The acidity of 27 plant digests (Coffea arabica L., Citrus spp., Sorghum spp., Saccharum officinarum L. and Phaseolus vulgaris L.) was measured by titration with
sodium hydroxide to pH 7, showing an average value of 0.236 M ± 0.39 M related to perchloric acid. Therefore, 0.25 M sodium hydroxide was chosen as the neutralization reagent; this value is slightly higher than the mean sample acidity because, with the buffering system utilized, alkaline deviations are more readily minimized than acidic errors in the final stream.

Because of the very variable acidity of the samples, and the unfavourable characteristics of the colour reagent with respect to pH, a high degree of sample dispersion is necessary. The dispersion factors measured at points C and D (Fig. 1) were 0.013 and 0.012, respectively. The small difference in the degree of sample dispersion between points C and D indicates that the flow-cell dead volume has little influence when the degree of dispersion is high.

This high degree of sample dispersion was chosen after consideration of several points. Firstly, pH control is easier at high dispersions, because the variability of the sample acidity diminishes with increasing degree of dispersion. Secondly, the method is sensitive enough for soil and plant analysis, even with the sample dispersion used. Thirdly, the refractive index effect [14, 15] decreases with increasing degree of dispersion. Finally, the high dispersion of the sample zone permits easier control of interfering chemical species.

**Colour reagent**

The addition of the colour reagent to the sample via merging zones was not feasible because the blank value would be too high and dependent on pH. The peak profiles of Fig. 3 obtained with pulsed colour reagent give an idea of the magnitude of this blank. In cases where the colour reagent is strongly coloured, its addition to the sample zone must be through a confluence configuration [15].

The concentration of the eriochrome cyanine R solution was selected to provide good linearity of the calibration plot up to 20 ppm Al. Under the recommended conditions, the range of absorbance was 0.026–1.013 for 0–20 ppm Al, and the average error (related to 3 measurements at each of 9 points on the scale) was 0.002 absorbance units. Higher reagent concentrations were not employed because of the blank problem. As baseline drifts (caused by irregular pumping, commutation of the injector and pH-gradients in the sample zone) are proportional to the blank absorbance, the use of a less concentrated reagent results in a more stable system.

**Blank value**

The blank value (Table 1, Fig. 4) is caused mainly by the injector. During its commutation, the carrier streams are suddenly blocked. At this moment, the relative contribution of the colour reagent at point A (Fig. 1) increases, giving a transient signal in the detector and thus causing the blank value. As this signal reflects an instantaneous increase in the reagent concentration, the blank is pH dependent (Table 1). This signal would probably be suppressed if a 4-stage injector were available. In this case, the colour reagent could pass
through the injector and, during commutation, all four streams would be equally blocked. Alternatively, an injector furnished with bypass could be employed [2].

**Interferences**

The major interferences in this method are pH, iron(III) and phosphate. Table 1 indicates the influence of the sample acidity on the recorded signal. Although the acidity range is large, and the reagent absorption is pH dependent, the pH effect seems to have been largely overcome; the standard deviation of the data corresponding to 5 ppm aluminium is only 1.21%. In preliminary tests, pH variations from 5.90 to 6.08 caused changes in the readings from 0.472 to 0.488 absorbance units in the final stream. The influence of sample acidity is reflected in the blank signal and not in the signal proportional to the aluminium content [10]; thus suppression of the blank signal (see above) minimizes this variation. Methods for aluminium analysis usually have a precision in the order of 0.5 ppm Al [6].

Table 2 indicates the influence of iron(III) and phosphate. Even when the iron(III) concentration is 40 ppm, its interference is not significant. It should be stressed that plant digests and soil KCl extracts do not generally contain

### TABLE 1

Influence of acidity on the absorbances related to a 5-ppm Al standard and to the blank value (data obtained from 5 repeated runs)

<table>
<thead>
<tr>
<th>Aciditya</th>
<th>5 ppm Al</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.15</td>
<td>0.246 ± 0.001</td>
<td>0.027 ± 0.001</td>
</tr>
<tr>
<td>0.20</td>
<td>0.247 ± 0.001</td>
<td>0.026 ± 0.002</td>
</tr>
<tr>
<td>0.25</td>
<td>0.250 ± 0.002</td>
<td>0.027 ± 0.002</td>
</tr>
<tr>
<td>0.30</td>
<td>0.251 ± 0.001</td>
<td>0.035 ± 0.002</td>
</tr>
<tr>
<td>0.35</td>
<td>0.254 ± 0.003</td>
<td>0.037 ± 0.001</td>
</tr>
<tr>
<td>0.40</td>
<td>0.252 ± 0.002</td>
<td>0.042 ± 0.002</td>
</tr>
</tbody>
</table>

*Expressed as molarity of HClO₄.*

### TABLE 2

Influence of iron(III) and phosphate on the determination of a 5-ppm Al standard in 0.25 M perchloric acid (data obtained from 5 runs at each level)

<table>
<thead>
<tr>
<th>Iron(III) (ppm)</th>
<th>Absorbance</th>
<th>Phosphate (ppm)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.252 ± 0.003</td>
<td>0</td>
<td>0.254 ± 0.002</td>
</tr>
<tr>
<td>5</td>
<td>0.245 ± 0.001</td>
<td>50</td>
<td>0.247 ± 0.001</td>
</tr>
<tr>
<td>10</td>
<td>0.252 ± 0.003</td>
<td>75</td>
<td>0.245 ± 0.002</td>
</tr>
<tr>
<td>20</td>
<td>0.253 ± 0.004</td>
<td>100</td>
<td>0.242 ± 0.003</td>
</tr>
<tr>
<td>30</td>
<td>0.266 ± 0.005</td>
<td>150</td>
<td>0.238 ± 0.002</td>
</tr>
<tr>
<td>40</td>
<td>0.261 ± 0.003</td>
<td>200</td>
<td>0.234 ± 0.001</td>
</tr>
</tbody>
</table>
TABLE 3

Recovery data related to soil extracts and plant digests. One standard addition was made and the measurement was performed in triplicate.

<table>
<thead>
<tr>
<th>Plant digests*</th>
<th>Soil extracts*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recovery (%)</td>
</tr>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Al measured (ppm)</td>
<td></td>
</tr>
<tr>
<td>1.40</td>
<td>95.0</td>
</tr>
<tr>
<td>1.45</td>
<td>98.8</td>
</tr>
<tr>
<td>2.20</td>
<td>105.9</td>
</tr>
<tr>
<td>1.35</td>
<td>94.5</td>
</tr>
<tr>
<td>1.40</td>
<td>97.0</td>
</tr>
<tr>
<td>1.65</td>
<td>100.3</td>
</tr>
<tr>
<td>2.40</td>
<td>94.3</td>
</tr>
<tr>
<td>2.85</td>
<td>95.8</td>
</tr>
<tr>
<td>1.92</td>
<td>95.4</td>
</tr>
<tr>
<td>1.72</td>
<td>100.0</td>
</tr>
<tr>
<td>5.60</td>
<td>101.4</td>
</tr>
<tr>
<td>2.10</td>
<td>100.0</td>
</tr>
<tr>
<td>Mean</td>
<td>97.95</td>
</tr>
<tr>
<td>R.s.d.</td>
<td>3.56</td>
</tr>
</tbody>
</table>


so much iron(III). The method is little affected by phosphate, probably because of the high degree of sample dispersion. For samples with high phosphate contents, the addition of phosphate to the standards is recommended.

Analytical results

The proposed method can be used for the determination of aluminium in plant digests and soil extracts in the range 0–20 ppm Al, at a typical sampling rate of 120 samples per hour (see Fig. 4). The precision of the method is better than 1% for a sample containing about 5 ppm Al. Table 3 shows that the recoveries are good for soil and plant samples.

Partial support of this project by DANIDA (Project 104DAN 8/241) and by FINEP (Financiadora de Estudos e Projetos Brasil) is greatly appreciated. The authors thank P. B. Vose, J. X. Medeiros and M. Fernanda Giné for assistance in preparing the manuscript.

REFERENCES

6 L. H. Jones and D. A. Thurman, Plant and Soil, 9 (1957) 131.
7 American Public Health Association, American Water Works Association and Water
Pollution Control Federation, Standard Methods for the Examination of Water and
11 E. D. McLean, in C. A. Black (Ed.), Methods of Soil Analysis, American Society of