SPECTROPHOTOMETRIC DETERMINATION OF ALUMINIUM IN PLANTS DIGEST USING A FLOW SYSTEM

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Summary

A spectrophotometric flow injection system for the determination of aluminium in plants with xylenol orange (XO) was proposed. The flow network was designed using an injector-commutator; the samples was analyzed without any prior treatment. The interference caused by iron was suppressed using by adding potassium thiocyanate as masking. The calibration graph showed a linearity in the range of 0.1 to 10.0 mg Al and a relative standard deviation of 0.98 % for 10 runs on 7.5 mg L^{-1} Al. The accuracy was assessed by comparing the results with those obtained the atomic emission spectrometry (ICP-AES), no significant difference at 95 % confidence level was observed. Other variables, such as an analytical throughput of 45 determinations per hour; a reagent consumption of 2.0 mg of XO and 13.3 mg potassium thiocyanate (KSCN) per determination were also achieved.

Key-words: Spectrophotometry, Xylenol orange, Aluminium determination, Flow analysis.

Studies concerning the agronomical science have shown that high aluminium concentration in the soil can inhibit the plants growth, thus affecting the harvest. Depending on this concentration, it can become toxic for plants, and this effect

can occur mainly in acid soils. [1] Thus, studies involving plants nutrition require the aluminium determination in a large number of samples. In this sense, several analytical methods aluminium determination such as atomic absorption spectrometry [3,4,5], atomic emission spectrometry [6], fluorescence [7,8] etc have been proposed. The molecular absorption spectrophotometry, mainly the one based on the flow injection technique has also been employed [9,10] presenting similar analytical features, *i.e.*, high analytical throughput; good precision, accuracy and sensitivity. A flow injection can be implemented using less expensive equipment, which is an additional advantage.

The flow injection analysis (FIA) procedures for the determination of aluminium by spectrophotometry have been proposed using the chromogenic reagents aluminon [9] and Eriochrome cyanine R [10,11]. The aluminium ion reacts with XO, to form two major complexes presenting metal to ligand ratios 1:1 and 1:2 at a pH 5.0 (i.e. ML and ML_2) [12,13]. Xylenol orange can form a complex with other cations [14,15,16], which potentially cause interference,

mainly iron(III) ion. The most common masking reagents, such as EDTA and triethanolamine, form a complex with aluminium, therefore they can not be used to suppress the iron interference. The anion SCN^- does not react with aluminium, nevertheless its forms a very stable complex with the Fe(III) ion, presenting the maximum absorption at 480 nm. Fortunately, the absorption at 560 nm is insignificant, thus it could be used as a masking for iron [17].

In the present work, a flow analysis system for spectrophotometric determination of aluminium in plants digest is developed, using XO as a chromogenic reagent. The sample matrix is very acid and the formation of the complex can be affected by the acid medium [12,13], thus to overcome this drawback, the flow network was designed to facilitate the adjustment the pH of the sample aliquot.

EXPERIMENTAL

Apparatus

The equipment consists of a 432 Femto(São Paulo,Brazil) spectrophotometer with a 13.0 mm optical path (inner

volume = 100 µL flow cell; a ECB model RB 201 strip chart recorder; an IPC-8 Ismatec peristaltic pump with Tygon tubes; a home made manual commutator injector machined in acrylic [18]. The reaction coils and the flow line were made of polyethylene tubing, 0.8 mm i.d..

Reagents, Standards, samples

All chemicals were of analytical grade and freshly distilled, deionized water was used throughout.

A 0.25 mol L^{-1} hexamethilenetetramine solution was prepared by dissolving 35.0475 g of the reagent in approximately 800 mL of water. After the dissolution, the pH was adjusted to 7.0 with concentrated acetic acid, and the volume was made up to 1000 mL with water.

A 0.15 % (w/v) solution of xylenol orange was prepared by dissolving the appropriate amount of the salt in water.

A 1.0 % (w/v) potassium thiocyanate solution was prepared by dissolving 5.0 g of the salt in 500 mL of water. This solution, stored in an ambar bottle, can be used. It was stable for a month.

A 1000 mg L^{-1} aluminium standard stock solution was prepared by diluting a Titrisol ampule in 1000 mL of water. The working standard solutions containing 1.0, 2.5, 5.0, 7.5, 10.0 mg L^{-1} Al were prepared in a 0.25 mol L^{-1} perchloric acid solution by the appropriated dilutions of the stock solution.

The sample solution was prepared by mineralizing 750 mg of ground and dry vegetable. The powdered material was placed into a digesting tube, 7.5 mL of concentrated HNO_3 was added to it and it was maintained at the laboratory temperature (ca 25 °C) overnight. The tube was inserted into a digestion block, which was gently warmed up to 160 °C and maintained for 2 hours. After this step, 2.0 mL of the concentrated $HClO_4$ was added, temperature was raised up to 210 °C and maintained for 15 min. After cooling the volume was made up to 100.0 mL with water.

The flow diagram

The flow diagram is shown in Fig.1. The injector-commutator is in the injection position and the carrier solution (C) flows through the sampling loop (L)

and displace the sample aliquot through the analytical path (B_1, B_2, B_3) towards the detector (DET). The buffer solution (R_1) and the reagent solutions (R_2, R_3) are added to the sample zone in the confluence points x, y and z, respectively. Mixing between solutions and reaction to form the compound to be detected occurred while sample zone is transported towards the detector (DET). The signal was traced using a stripping chart recorder. In this condition, the sample solution (S) is pumped through the waste (W). When the sliding bar of the injector is displaced to the other resting position, the sampling loop is aligned to the sample stream pathway to load the sampling (L) for the next analytical cycle.
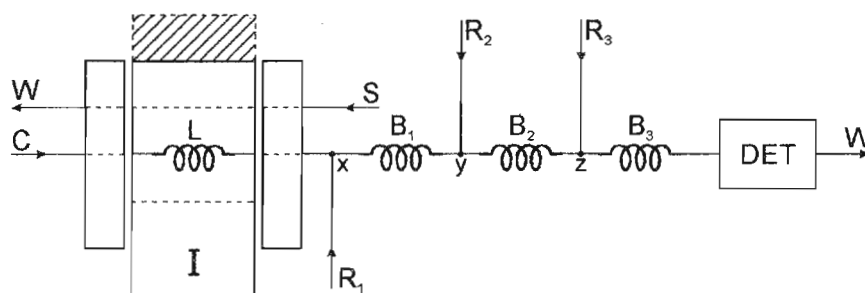

Figure 1. Flow diagram of the system. The rectangular surface is an overview of the injector, I is the movable part and the hatched surface indicate the alternative position. L = sampling loop, 15 cm; B_1, B_2 and B_3 = helical coils, 20, 30 and 150 cm, respectively; DET = spectrophotometer at 560 nm; C = carrier solution, 0.25 mol L^{-1} of perchloric acid at 0.6 mlmin^{-1}; S = Sample solution at 2.5 mlmin^{-1}; R_1 = 0.25 molL^{-1} hexamethilenetetramine solution buffer at 2.0 mlmin^{-1}; R_2 = potassium thiocyanate solution at 2.0 mlmin^{-1}; R_3 = 0.15 % (w/v) xylenol orange solution at 0.8 mlmin^{-1}; W = waste; x, y and z = confluence points.

The dimensions of the reaction coils B_1, B_2 and B_3 and the flow rates were previously settled in order to obtain a high throughput. The parameters comprising the length of the loop; the concentration of the masking solution, the buffer solution, the adjustment of the pH, and the concentration of the chromogenic reagent were studied. Since these parameters were optimized, a set of plants digest was analyzed in order to ascertain the feasibility of the system.

3. RESULTS AND DISCUSSION

The acidity of the sample solution was *ca* 0.25 mol L^{-1} perchloric acid; thus,

to avoid the Schlieren effect [19], a solution with the similar concentration of acid was used as a carrier stream. The reaction to form the Al-XO complex requires a narrow pH range (5.0 - 6.0), therefore, to assure this condition, a 0.5 mol L^{-1} hexamine buffer solution was added to the sample zone prior to the chromogenic reagent solution. Preliminary tests indicated that solution was better than a 0.5 mol L^{-1} acetate buffer solution.

The system parameters comprising the flow rates, sampling loop and the length of the coils as described in Fig.1 were settled considering the sensibility and precision of the measurements. To define the concentration of the chromogenic reagent, experiments were carried out using solutions with 0.05, 0.10, 0.15, 0.20 and 0.25 % (w/v) XO and a reference solution with 10.0 mg L^{-1} aluminium. Better results were achieved with the solution presenting 0.15 % (w/v) XO. When the concentration of the solution was lower than this value, a decrease of the analytical signal was observed. The measurements of the base line became very high when concentrations higher than this value were used.

The potential interferents for analytical purpose were zinc, iron and copper. At pH 4.5, the Zn-XO complex presented the maximum absorption at 585 nm [20], nevertheless at pH 5.0 - 6.0 the interference was avoided. In plant leaves the concentration of Cu is very low compared to the concentration of aluminium [21], therefore its effect was not significant. The iron forms a complex with the xylenol orange in acid medium, causing strong interference, which must be suppressed. In this sense, aiming to overcome this trouble, triethanolamine, sodium oxalate and sodium tartrate were tested as masking reagents, nevertheless the interference effect was not quite suppressed. Reduction to Fe(II) with ascorbic acid was employed to avoid this interference in the spectrophotometric procedure [22], however, Fe(II) forms a complex with XO, which absorbs the electromagnetic radiation at the same wave length range of the aluminium complex [13].

The iron(III)-thiocyanate complex presented the maximum absorption at 480 nm, and fortunately presented no significant absorption at 560 nm, which was selected to detect the Al-XO complex.
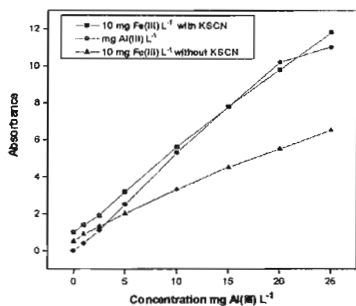
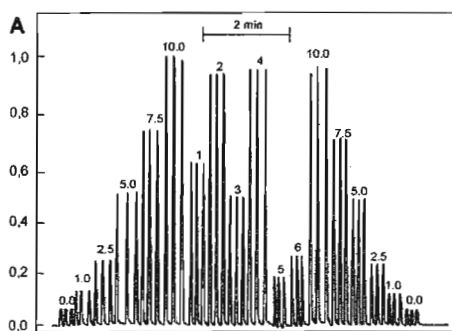**Figura 2. Effect of the KSCN as a interferent suppressor iron.**

Figure 3. Recorder tracing of the analytical run. From left, aluminium standards solutions (0.0, 1.0, 2.5, 5.0, 7.5, 10.0 mg L^{-1}), six samples and the standards solutions again.

This feature was employed to avoid interference of the iron. As showed in Fig.2, a 1.0 % (w/v) of potassium thiocyanate concentration was enough to suppress the interference caused by the iron ions.

Once the system parameters were established, a set sample solutions digested plants was analyzed. As can be find in Fig.3, the system presented a good stability and a linear response up to 10 mg L^{-1} of Al. A sample throughput of 45 determinations per hour can be deduced. The accuracy was assessed by comparing the results with those obtained by the atomic emission spectrometry (ICP-AES). The results are presented in Table 1; applying the paired t-test, no significant difference at 95 % confidence level was observed. The relative standard deviation was 0.98 % (n =10) for 7.5 ml L^{-1} Al, a reagent consumption of 13.3 mg potassium thiocyanate and 2.0 mg xylenol orange per determination.

Table 1. Comparison of the results obtained by the FIA and ICP-AES methods.

Sample	Proposed method (mg/g)	ICP-AES (mg/g)
1	30.15 ± 0.11*	29.56 ± 1.19*
2	48.30 ± 0.16	47.76 ± 2.49
3	23.80 ±0.20	25.16 ± 0.31
4	48.80 ± 0.10	50.36 ± 0.60
5	1.73 ± 0.03	1.80 ± 0.01
6	2.65 ± 0.03	2.74 ± 0.03

Results are average of three consecutive measurements
*standard deviation

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