SEQUENTIAL INJECTION ANALYSIS WITH ACCURACY ASSESSMENT

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ABSTRACT

Two *quasi* independent methods for spectrophotometric determination of aluminum in soil extracts, based on Al(III) complexation with Aluminon and Chromazurol S (CAS), are implemented in a SIA system. Because results are based on both methods, accuracy assessment is provided. Potentialities and limitations of the approach are discussed.

The proposed system is robust and yields reproducible measurements (r.s.d. < 5% for 2.0 - 10.0 mg l^{-1} Al) at a 15 h^{-1} rate. About 85 µg of Aluminon and 13 µg CAS are consumed per determination. For atypical samples, interference effects are promptly observable.

Keywords: Sequential injection analysis, Aluminum determination, soil extracts, accuracy assessment.

INTRODUCTION

The concept of accuracy assessment in flow analysis was recently proposed [1] and applied to the chloride determination in river waters. The sample was processed by two *quasi* independent methods, and the standard addition method to overcome matrix interference was implemented. These features leaded to intrinsically more accurate results. The system comprised a set of computer controlled three-way solenoid valves, resulting in a complex set up. The authors pointed out that exploitation of the concept in sequential injection analysis

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(SIA) would require a simpler set up.

SIA [2] was conceived as means to simplify analytical flow-based procedures. The set up includes a single stream flowing through a holding coil and then towards the detector. Sample and reagent solutions are sequentially intercalated into this stream by a multi-functional rotary valve, and the possibility of selecting different reagents is easily attained without system modifications. This is useful to develop procedures involving the determination of an analyte by exploiting two different methods.

The aim of this work is to demonstrate the feasibility of a simple SIA system providing accuracy assessment. As an application, the spectrophotometric determination of aluminum in KCl soil extracts with Aluminon and Chromazurol S was elected, because of the demand for this determination in agronomical laboratories [3], the availability of reliable spectrophotometric procedures [4] and the compatibility of the wavelengths associated with these classical reagents [5].

EXPERIMENTAL

Reagents, standards, samples

All solutions were prepared with distilled/deionized water and chemicals of analytical reagent grade. Ammonium aurin tricarboxylate (Aluminon) and Chromazurol S (CAS) were used without further purification.
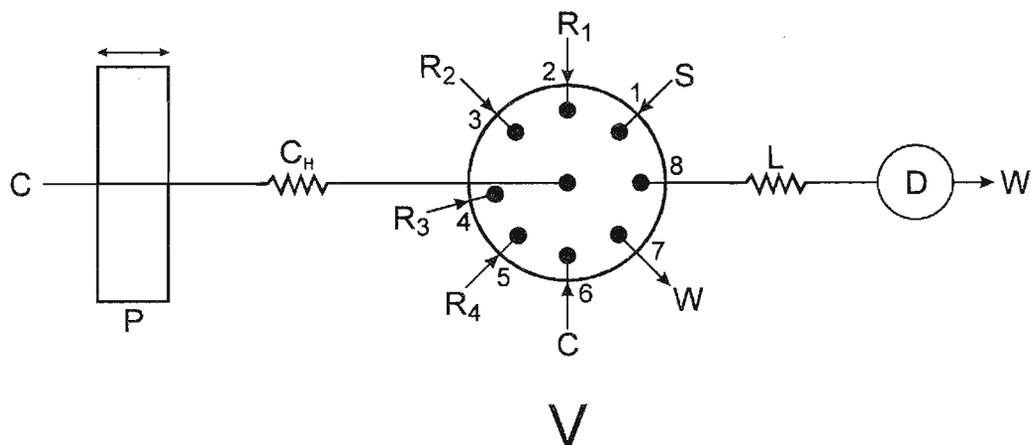
Fig. 1. The SIA system. V = multi-functional rotary valve; S = sample; C = carrier stream; R, = reagents; numbers 1 to 8 = valve ports; P = peristaltic pump; C_H = holding coil; L = transmission line; D = detector; W = waste. For details and system operation, V. text and Table 1.

Table 1. System operation. Steps for aluminum determination by two methods with the SIA system in Fig. 1. For details, see text.

Step	Event	Port	Time s	Pump rotation	Flow direction	Volume μl
1	washing	1	15	30	reversed	-
2	washing	7	17	40	forward	-
3	sample aspiration	1	6	10	reversed	50
4	R_1 aspiration	2	2	10	reversed	17
5	R_2 aspiration	3	2	10	reversed	17
6	Aluminon aspiration	4	2.5	10	reversed	21
7	homogenization	6	20	10	reversed	170
8	homogenization	8	15	15	forward	94
9	STOP	8	30	0	-	-
10	propelling towards detector	8	80	40	forward	-
11	sample aspiration	1	2	10	reversed	17
12	R_1 aspiration	2	2	10	reversed	17
13	CAS aspiration	5	2	10	reversed	17
14	R_2 aspiration	3	2	10	reversed	17
15	propelling towards detector	8	80	40	forward	-

* 0.50 ml min^{-1} corresponds to the peristaltic pump setting of 10.

Reagent R_1 (Fig. 1) was a freshly prepared 2.0 % w/v ascorbic acid plus 0.2 % w/v 1,10 o-phenantholine (phen) solution; R_2 was a 0.5 mol l^{-1} acetic acid solution with the pH adjusted to 5.5 by dropwise addition of 10 mol l^{-1} NaOH; R_3 was a weekly prepared 1.0 % w/v Aluminon plus 0.1 % w/v gelatin solution; and R_4 was a 0.1 % w/v CAS water solution. Carrier stream C was a 0.1 % w/v gelatin solution.

Working standards containing 0.0 - 10.0 mg l^{-1} Al were weekly prepared by appropriated dilutions of a 500.0 mg l^{-1} Al stock solution which was made by dissolving 8.752 g KAl(SO$_4$)$_2$.12H$_2$O in about 900 ml of a 0.001 mol l^{-1} HCl and filling the volume up to 1000 ml with water. The stock solution was standardized against Na$_2$H$_2$EDTA [6].

Soil extracts were obtained with a 1.0 mol l^{-1} KCl extracting solution following a standard procedure [7].

The SIA system

The sequential injection system comprised a model C15-3118 E multi-

functional rotary valve from Vici (Valco Instruments, Houston), a model IPC 8 peristaltic pump from Ismatec furnished with a orange/orange Tygon pumping tube (i.d. = 0.89 mm), a model 432 Femto spectrophotometer with a 120 µl flow cell (optical path = 10 mm), a model BD 111 Kipp & Zonen strip start recorder, Perspex connectors and accessories. The manifold was built up with polyethylene tubing (i.d. = 0.8 mm). The peristaltic pump and the rotary valve were controlled by a 386 IBM-PC compatible microcomputer with a program written in QuickBasic.

Fifteen steps (Table 1) were required for operating the proposed SIA system which is outlined in Fig. 1. The analytical cycle started by aspirating a 180-µl sample volume (step 1) to wash the sample aspirating tube. The flow in the holding coil was then reversed and the sample was wasted towards W during 17.0 s (step 2). These steps were needed mainly to minimize carryover due to the sample volumes entrapped in the aspiration tube. Thereafter, flow direction was reversed to permit sequential aspiration of the sample, R_1, R_2, R_3 and C solutions towards the holding coil during 6.0, 2.0, 2.0, 2.5 and 20.0 s, respectively (steps 3-7). The sample zone established inside the holding coil underwent mixing while being pushed by the carrier stream towards the peristaltic pump. The flow was reversed again and a higher pumping rate was maintained during the following 15 s (step 8). This was suitable for transporting the sample zone from the holding coil to transmission line L, where the sample was stopped during 30 s (step 9). This timing was enough for Al complexation with Aluminon under pH 5.5 and quantitative iron(III) reduction to iron(II) which was then masked with phen. Thereafter, the processed sample was directed towards the detector (step 10) and monitored at 565 nm. The recorded peak height was proportional to the Al content in the injectate. Next sample aliquot was then aspirated (step 11) and processed (steps 12-15) similarly as above, but without the STOP period in view of the more favorable reaction kinetics of the Al-CAS complexation relatively to Al-Aluminon.

RESULTS AND DISCUSSION

Length of the holding coil should be > 100 cm to avoid sample losses through the peristaltic pump during steps 3-7. As it could be increased at will, it was selected as 200 cm. Flow rate for the sequential aspiration of the involved solutions was fixed as 0.50 ml min^{-1} as a compromise between measurement precision, available time for mixing and sampling rate. Preliminary experiments revealed that for a shorter sampling time and a thicker pumping tube (i.d. = 2.2 mm), aliquoting repeatability deteriorated and relative standard deviations of peak heights recorded for a 5.0 mg l^{-1} Al solution were > 15 %. Length of transmission line was not critical being selected as 50 cm.

Injected volumes of sample and reagent solutions were investigated within 5 and 100 μl and those specified in Table 1 were selected after considering linearity of the calibration curve, sensitivity and suitable mixing conditions. Mixing conditions were evaluated in terms of the blank signal, baseline stability and shape of the analytical signals. With the selected condiitons, suitable overlap between injected zones was attained. It should be noted that the sample injected volume for the Aluminon procedure was higher relatively to the other injected solutions because sample dispersion should be limited in view of the less favorable Al-Aluminon reaction kinetics.

Different reagent addition sequences were tried and that yielding highest analytical signals (Table 1) was elected. It should be emphasized that the procedure with CAS required the development of color forming reaction under slightly acidic conditions. Therefore, reagent addition order related to both procedures are slightly different.

Concentration of reagent R$_1$ was not critical for the system design because ascorbic acid and phen were in large excess relatively to the oxidizing materials and metallic ions present in the samples. This feature was relevant to the robustness of the proposed procedure. A high buffer capacity (0.5 mol l^{-1} acetic acid/acetate) was selected because reagent R$_2$ underwent pronounced dispersion inside the analytical path. The increase of reagent injected volume was tried but the

overlap between chromogenic reagent and sample zones was not satistactory.

The effect of variations in the Aluminon concentration was investigated over the 0.1 - 1.0 % w/v range. With 0.1, 0.5 and 1.0 % w/v, rectilinear calibration plots for 0.0 - 10.0 mg l^{-1} Al were attained with linear coefficients of 0.098, 0.176 and 0.242 A (absorbance) and angular coefficients of 0.01618, 0.01713 and 0.01722 A (mg l^{-1})$^{-1}$, respectively. The Aluminon concentration was therefore selected as 0.5 % w/v. In all instances, it was observed that Al complexation with Aluminon was slow. Therefore, a STOP period was required. The analytical signal increased with the STOP period and an asymptotic tendency was verified. With 30 s, about 80 % of maximum attainable signal (STOP period > 5 min) was already reached, this time interval being therefore selected.

Influence of temperature was investigated by immersing the holding coil and transmission line in a thermostated water bath with a 0.2 °C precision. It was verified that temperature was a relevant parameter only in the Aluminon procedure. When it was raised from 0 to 50 °C, the recorded peak height and the blank value underwent an almost linear increase (about 0.9 % per °C). So, it was decided to operate the system under room temperature (25 °C with variations < 1 °C), providing that good air conditioning facilities were available.

Further experiments to verify the influence of gelatin in the procedure with Aluminon were carried out. Without the surfactant, linearity of the calibration equation was lost and sensitivity deteriorated because the uniformity of the micelles was not attained without this colloid protector. Addition of Triton X-100, Tween-20, poly(vinyl alcohol) or starch instead of gelatin leaded to worse results. Schlieren noise resulting from the injection of a gelatin solution into the main stream (C - Fig. 1) was avoided by adding gelatin in the same concentration (0.1 % w/v) to this stream.

Regarding CAS, similar tendencies were observed, but chemical equilibrium was attained within the mean sample residence time in the system. As the molar absorptivity of CAS was higher than that of Aluminon [5], the effect of CAS concentration was studied within 0.02 and

0.5 % w/v and highest signal-to-blank ratio was observed for 0.2 % w/v. Surfactant addition to CAS reagent was not necessary in view of the high dispersion of R_4 reagent in the system. Both implemented methods are very selective. No modifications were noted for 0.0 and 5.00 mg l^{-1} Al signals by adding up to 60.0 mg l^{-1} P-PO_4 or S-SO_4, 30.0 mg l^{-1} Fe, 20.0 mg l^{-1} Ca or Mg or 5.00 mg l^{-1} Zn or Cu. Considering that these concentrations are seldom verified in KCl soil extracts, the proposed procedure can be applied without restrictions.

The proposed system is very simple and robust. Baseline drift was not observed after 4-h operation. No adherence of colored reagents on the tubing inner walls were observed because of the very low involved volumes. Precise measurements were obtained, relative standard deviations being estimated as < 5 % for 2.0 - 10.0 mg l^{-1} Al. About 85 μg of Aluminon and 13 μg CAS were consumed per determination. Sampling rate (15 h^{-1}) can be increased by increasing the flow rates and reducing the detector dead volume. This was not accomplished here because the rate suits well the requirements of the routine laboratory. Accuracy can be confirmed from Table 2 which shows the results

Table 2. **Results obtained by the SIA procedures**. Data, based on three replicates, express mg l^{-1} Al in the soil extracts. Uncertainties are based on 95% limits of confidence.

sample	Aluminon	Chromazurol	mean*
01	5.8 ± 0.1	5.9 ± 0.3	5.85
02*	3.8 ± 0.3	4.1 ± 0.4	3.95
03	8.1 ± 0.4	7.7 ± 0.7	7.90
04	10.2 ± 0.2	9.9 ± 0.1	10.05
05	7.0 ± 0.1	6.8 ± 0.4	6.90
06	6.6 ± 0.1	6.5 ± 0.5	6.55
07	4.4 ± 0.3	3.9 ± 0.1	4.15
08	5.3 ± 0.1	5.4 ± 0.4	5.35
09	4.8 ± 0.0	4.6 ± 0.2	4.70
10	7.9 ± 0.3	7.5 ± 0.2	7.70

* Re-analysis required.

obtained by both procedures. No statistic differences at the 95 % level was found by comparing the mean of results in Table 2 with those obtained by a flow-injection procedure using Eriochrome cyanine R [8]. A noteworthy feature of the proposed procedure is the additional accuracy guarantee, which is assessed by the similarity between results obtained by both methods within a preselected interval. In fact, from time to time, results with Aluminon and CAS may differ from each other, revealing potential interferent effects eventually observable in atypical samples.

REFERENCES

1. C.C. Oliveira, R.P. Sartini, E.A.G. Zagatto and J.L.F.C. Lima, Anal. Chim. Acta, 350 (1997) 31.

2. J. Ruzicka and G. Marshall, Anal. Chim. Acta, 237 (1990) 329.

3.A. Kabata-Pendias and H. Pendias, Trace elements in soils and plants, 3nd Ed., CRC Press Company, Florida, 1985.

4. Z. Marczenko, Separation and spectrophotometric determination of elements, Ellis Horwood Limited, Chichester, 1986.

5. W.K. Dougan and A.L. Wilson, Analyst, 99(1974)413.

6. Merck, Métodos complexométricos de valoración con Titriplex, E. Merck, Darmstadt, Germany, 1973.

7. E.D. MacLean, in C.A. Black (Ed.), Methods of Soil Analysis, American Society of Agronomy, Madison, 1965, p. 978.

8. B.F. Reis, H. Bergamin F°, E.A.G. Zagatto and F.J. Krug, Anal. Chim. Acta, 107 (1979) 309.