Flow Injection System with Multisite Detection for Spectrophotometric Determination of Calcium and Magnesium in Soil Extracts and Natural Waters

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A flow system with a relocatable detector for spectrophotometric determination of calcium (Ca) and magnesium (Mg) in KCl soil extracts and natural waters is presented. *O*-Cresolphthalein complexon (CPC) was selected as the chromogenic reagent, ammonium-ammonia as the buffer system, and EGTA or 8-hydroxyquinoline as the masking agents for Ca or Mg, respectively. Linearity of the calibration equations was observed for Ca and Mg concentrations up to 80.0 and 15.0 mg/L, respectively. Slight variations in the coefficients of the calibration equations (usually <3%) were found after 4-h working periods. Recoveries between 97.5 and 104.1% were calculated after adding 10.0 mL of Ca at 15.00 mg/L or Mg at 5.00 mg/L Mg to 50.0-mL sample solutions. Precise results (relative standard deviation, <0.02) in agreement with flame atomic absorption spectrometry were obtained. With multisite detection, washing time was not a relevant factor in sampling rate, and an improved sample throughput of ~160/h (corresponding to 0.8 mg of CPC per sample) was obtained.

Keywords: *Multisite detection; flow spectrophotometry; calcium; magnesium; soil and water analysis*

INTRODUCTION

The chemical reactions for spectrophotometric determination of calcium (Ca) and magnesium (Mg) in soil extracts, natural waters, fertilizers, plants, and other matrices related to large analytical demand are often similar (Page, 1982), and masking agents are usually employed for single determination of Ca or Mg (Panday, 1988; Thuy et al., 1994). Magnesium determination is often based on differences in signals related to Ca plus Mg and Ca (Haj-Hussein and Christian, 1986; Youxian, 1988); because Mg content in soils and natural waters is usually lower relative to Ca, negative results due to error propagation effects can be observed in extreme cases. This drawback is circumvented when Mg is determined directly.

Cresolphthalein complexon (CPC; Anderegg et al., 1954; Nyman and Ivaska, 1993) is a color-forming reagent for Ca determination in biological materials (Page, 1982; Panday, 1988). Magnesium also forms colored complexes with CPC, but procedures for its determination are seldom reported (Yamane and Goto, 1991). Variations in the dye excess and pH result in modifications of absorbances related to the colored complex and to the residual dye (Ringbom, 1963). This effect is minimized in flow systems where most of the parameters are accessible and easily controlled (Valcarcel and Luque de Castro, 1987).

In spectrophotometric flow analysis, procedures for simultaneous determination of Ca and Mg require either complex manifolds, often with two detectors (Espersen and Jensen, 1979); resolution of two-component spectra (Blanco et al., 1989); exploitation of differential kinetics (Kagenov and Jensen, 1980); or use of flow injection titration systems (Cañete et al., 1987). Moreover, washing time is an important parameter limiting sampling rate.

With multisite detection (Zagatto et al., 1991), a relocatable detector is associated with different manifold sites. Because the stream is switched immediately after peak maximum recording, washing time becomes a less relevant factor in sampling throughput. The multisite detection approach has been used for simultaneous determinations (Zagatto et al., 1991), water speciation (Zagatto et al., 1991), glycerol monitoring in industrial wastes (Gomes Neto et al., 1994), sucrose determination after in-line hydrolysis (Gomes Neto et al., 1994), and also to avoid the cumbersome step of air removal in monosegmented systems (Nogueira et al., 1993).

The aim of this work was the development of an automated procedure with multisite detection for spectrophotometric determination of Ca and Mg in soil extracts and natural waters. Using CPC as color-forming reagent and an appropriate selection of masking agents, parallel monitoring at two analytical channels permits determination of the concentrations of Ca and Mg in a simple, direct, and reliable way.

MATERIALS AND METHODS

Flow-Injection System. The system comprised an Ismatec mp13 GJ4 peristaltic pump with Tygon pumping tubes, a Micronal B352 electronically operated injector-commutator, an Intralab DMS 100 UV-vis spectrophotometer with an U-shaped flow-cell (15-mm optical path), and an Intralab 1200 strip-chart recorder. The manifold was build up with 0.7-mm i.d. polyethylene tubing of the non-collapsable wall type, and perspex Y-shaped connectors.

For detector relocation, the injector-commutator was placed by the spectrophotometer to keep connecting lines between

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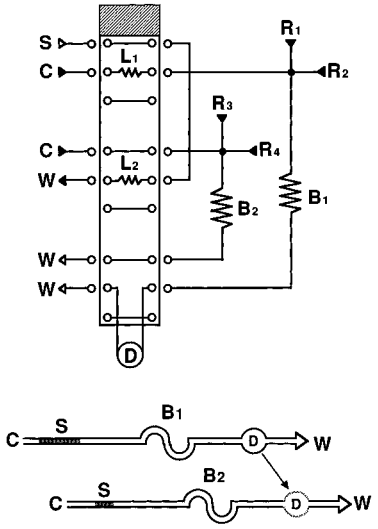

Figure 1. (Upper) Flow diagram: S, sample; L_1 and L_2, 20- and 7-cm sampling loops, respectively; C, carrier streams [1.0 M KCl or 0.1% (v/v) HNO_3]; R_1, 0.0015 M EGTA plus 0.5 M NH_3/NH_4 (pH 10.5); R_2, 0.03% (w/v) CPC plus 0.05 M HCl; R_3, 0.5 M NH_3/NH_4 (pH 10.5); R_4, 0.02% (w/v) CPC plus 0.2% (w/v) oxine plus 0.05 M HCl; B_1 and B_2, 100-cm reaction coils; D, detector at 575 nm; W, waste. All solutions were at 2.0 mL min^{-1}. The dashed area represents other position of the IC injector-commutator. (Lower) Schematic representation of detector relocation between the two parallel analytical channels.

flow-cell and sliding bar of the injector-commutator as short as possible (10 cm). The inner volume of the leaping detector plus accessories was determined as 220 μL following a procedure described elsewhere (Karlberg and Pacey, 1989).

The flow diagram is outlined in Figure 1 (upper). The sample was aspirated through the L_1 sampling loop (20 cm, ~100 μL). After switching the injector-commutator, the selected sample aliquot was introduced into the C carrier stream, which received the buffer/masking solution (R_1) and the CPC reagent (R_2) before flowing through B_1 coil, where color was developed. Passage of the treated zone through the flow cell resulted in a transient signal proportional to the Mg content in the sample, which was monitored at 575 nm. After reaching the maximum signal, the injector-commutator was switched, displacing the flow cell to the Ca channel (Figure 1, lower). This simple movement also intercalated the next sample plug selected by the L_2 loop (7 cm, ~35 μL) into the C stream. The aliquot was similarly processed, but with the addition of a modified reagent (R_3) and a different masking agent (R_4). The treated sample was monitored again, yielding transient signal proportional to the Ca content in the sample. After achieving the highest peak, as in the former case, the detector was moved to the Mg channel, thus initiating another cycle.

Chemicals. All solutions were prepared with analytical reagent grade chemicals and distilled-deionized water. The R_1 reagent (1.5 mM EGTA, pH 10.5, 0.5 M NH_3/NH_4) was prepared by dissolving 26.7 g of NH_4Cl in 800 mL of water. The pH was increased to 10.5 by adding 2.0 M NaOH and 0.57 g of Na_2EGTA, and the volume was made to 1000 mL with water. The R_2 reagent [0.03% (w/v) CPC, 0.05 M HCl] was prepared by dissolving 0.06 g of the dye in 2.0 mL of 5.0 M HCl, and the volume was made to 200 mL with water. The R_3 reagent was prepared as was the R_1 reagent, but without EGTA. The R_4 reagent [0.02% (w/v) CPC, 0.2% (w/v) oxine, (8-hydroxyquinoline), 0.05 M HCl] was prepared by dissolving 0.04 g of the dye and 0.4 g of oxine in 2.0 mL of 5.0 M HCl and filling the volume up to 200 mL with water. Oxine was used for Mg masking (Nyman and Ivaska, 1993; Ringbom, 1963) because the oximate ion reacts with Mg in alkaline medium to form $MgOx_2$. Oxine was dissolved in HCl because it is not soluble in a basic medium.

Working standard solutions (Mg at 0.00−15.00 mg/L plus Ca at 0.00−80.00 mg/L) were prepared by diluting stock solutions of Ca (1000.0 mg/L) and Mg (500.0 mg/L) that were based on calcium carbonate and magnesium chloride, respectively. For matrix matching purposes, the working standard solutions and the sample carrier streams (C, Figure 1) were also 1.0 M KCl or 0.1% (v/v) HNO_3, for analysis of soil extracts or natural waters.

Samples of yellow/red oxisoils characteristic of Brazilian savannah were extracted by shaking 10.0 g of air-dried soils with 100 mL of 1.0 M KCl in a mechanical shaker for ~1 h. The mixture was left overnight for decantation; thereafter, a 25-mL aliquot of supernatant was collected for analysis (Page, 1982). River water samples were collected in 1000-mL polyethylene bottles and preserved by addition of 1 mL of concentrated HNO_3/L of water (Valcarcel and Luque de Castro, 1987). Whenever possible, the analysis was carried out within 24 h after sample preparation.

Procedure. The influence of pH, reagent concentrations, coil lengths, and timing were investigated by injecting Ca or Mg single-element standard solutions in triplicate. Initially, B_i lengths were fixed as 300 cm, and resting times of the injector-commutator were set very long (200 s) to avoid any carryover effect. The L_1 and L_2 loops were fixed as 20 and 7 cm, respectively, corresponding to ~100 and 35 μL, respectively.

To select reagent concentrations related to Mg determination, CPC and EGTA concentrations were initially set as 0.07% (w/v) and 0.001 M, respectively. Variations in the pH of R_1 reagent, between 9.0 and 11.0, were obtained by adding 0.5 M NH_3 or NH_4Cl. Selection of the CPC concentration was determined by keeping pH and EGTA concentration in R_1 constant at 10.5 and 0.001 M, respectively, and fluctuating the CPC concentration between 0.01 and 0.10% (w/v). It was important to select an appropriate EGTA concentration for Ca masking that didn't have a significant effect on the formation of Mg−CPC. Having established other parameters, EGTA concentrations were tested within a 0.0−1.5-mM range. Although Ca is the main interferant in Mg determination in soil extracts and natural waters, other potential interferants, like aluminum, iron, and manganese (Mn) in concentrations of up to 100, 100, and 50 mg/L, respectively, were also investigated.

Flow rates, coil length, and pH were kept constant to assess the Ca analytical channel. The Ca concentration is usually higher than Mg, especially for soil extracts, so a lower injection volume was selected and, hence, more diluted CPC reagent was used. Concentrations of CPC between 0.005 and 0.02% (w/v) were then investigated. With a CPC concentration kept constant at 0.01% (w/v), the influence of oxine concentration between 0.005 and 0.4% (w/v) was studied in the presence of Mg.

After selecting suitable reagent concentrations, the rotation speed of the peristaltic pump (25, 50, 100, and 200% of rotation speed referent to flow rates in Figure 1) and B_i lengths (50−500 cm) were adjusted. Timing was studied by changing the resting times of the injector-commutator in both positions between 10 and 200 s.

The system was then applied to routine analysis. Precision was estimated in terms of relative standard deviation (rsd) of results of typical 10-fold injected samples, and accuracy was assessed by analyzing soil and water samples already analyzed by flame atomic absorption spectrometry (FAAS; Welz, 1985) or inductively coupled argon plasma atomic emission spectroscopy (ICP-AES; Boumans, 1987).

RESULTS AND DISCUSSION

Throughout this work, highly precise measurements (rsd, <2%) were obtained; this precision emphasize the robustness of the flow injection system with stream switching.

Definition of the buffer system was a relevant task, as all equilibria were pH dependent. For pH values of <10, a decrease in sensitivity was observed due to Ca or Mg competition with hydrogen ions by CPC. On the other hand, within the 10−11 pH range, increase in

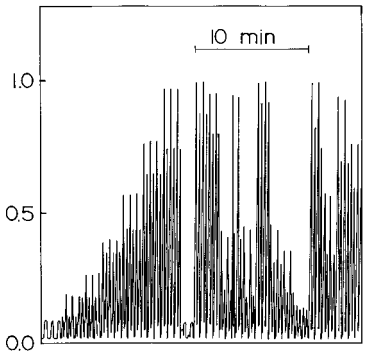

Figure 2. Recorder tracing of a routine soil analyses. From the left, the peaks represent 0.00, 5.00, 10.00, 20.00, 30.00, 40.00, 50.00 mg/L, and 0.00, 2.50, 5.00, 7.50, 10.00, 16.00, 18.50 mg/L Mg in triplicate, and 14 soil samples in duplicate.

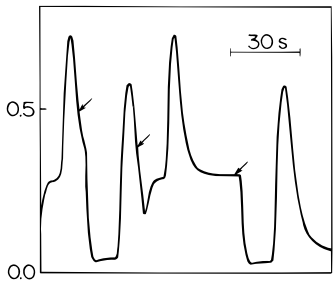

Figure 3. Effect of detector relocation. Recorder tracings refer to a solution of Mg at 10.00 mg/L and Ca at 31.00 mg/L, and on the right, to a Mg (10.00 mg/L) and Ca (0.00 mg/L) soil solution injected into the system shown in Figure 1. Lower and higher peaks correspond to monitoring sites, reflecting Mg or Ca. Instants of detector relocation/sample injection are specified by black arrows.

alkalinity raised absorbance values, producing a significant increase in both peak height and baseline. For pH values >11, buffer capacity of the system deteriorated and CPC dissociation was almost complete, so that the color of the residual dye was comparable to that of Mg− or Ca−CPC complexes. A pH of 10.5 was then selected, and 0.5 M NH_4/NH_3 proved to be suitable in terms of buffer capacity; no variation in peak heights were observed after adding up to 0.05 M HNO_3 to the injected standard solutions. To avoid changes in pH gradients inside the flow cell after its leaping, the same pH was selected for both analytical channels.

With regard to CPC concentration, it was observed that for a 0.01% (w/v) concentration, low peaks were recorded for both Ca and Mg (0.066 and 0.194 Å for Mg at 3.00 and 9.00 mg/L, respectively). For CPC concentration between 0.02 and 0.07% (w/v), the slope of the calibration curves and baseline showed pronounced increases with CPC concentration. An increase in this concentration also improved linearity of the calibration curve: with 0.005, 0.010, and 0.020% (w/v) CPC, linearity was observed only up to ~10, 50, and 100 mg of Ca per liter, respectively. As a compromise between signal-to-noise ratio, linearity of calibration curve, and probable Mg and Ca contents in the samples, CPC concentrations of 0.030 and 0.020% (w/v) were selected for the Mg and Ca channels, respectively. With different CPC contents in both analytical channels, two baselines are characteristic (Figures 2 and 3), reflecting the detector leaping among channels.

EGTA concentrations of 0.1, 0.3, 0.5, 1.0, and 1.5 mM were required for quantitative Ca masking of up to 5, 10, 20, 40, and 50 mg/L, respectively. However, slight drops in Mg analytical signals (2 and 5%) were noted for EGTA concentrations of 1.5 and 2.0 mM, respectively. For soil extracts analysis, higher Ca concentrations are usually found, and an EGTA concentration of 1.5 mM was imperative. This concentration was also selected for analysis of natural waters.

Contribution of Mg to the Ca signal showed synergistic effects, with positive interferences being observed. Variations in baseline and in analytical signals were not detected for oxine concentrations of <0.30% (w/v). With 0.10% (w/v) oxine, quantitative Mg masking was reached up to Mg concentrations of 10 mg/L, and with 0.20% (w/v) oxine, the Ca signal was still unaffected by the presence of oxine. The Ca signal experienced a 20% reduction when oxine concentration was increased to 0.40% (w/v). The concentration of this masking agent was, therefore, kept constant at 0.20% (w/v), and samples with unusual Mg and Ca concentrations (Ca at 10.00 mg/L plus Mg at 10.00 mg/L) could be run without restrictions.

Manganese (Mn) interference was negligible. For Mn at 50.0 mg/L in the injectate, no measurable modification in either blank or analytical signals was found. Iron and aluminum concentrations >20.0 and >25.0 mg/L, respectively, could not be tolerated. Such levels are unlikely to be found in the assayed samples. Parallel experiments demonstrated that when necessary, 0.1 M triethanolamine should be added to the carrier streams. In the presence of triethanolamine, iron and aluminum levels as high as 100 mg/L could be tolerated.

With the proposed system, chemical equilibria are fully attained, as demonstrated in parallel tests that involved stopping of the peristaltic pump. However, decrease in length of the main reactors is limited by mixing conditions associated with the detector leaping process. When the B_1 (or B_2) coil was 50 cm long, steady-state conditions were not fully reached after detector relocation, and the analytical signals appeared before complete detector washing. This effect impaired selectivity and sensitivity, especially with respect to Mg determination. For 100-cm coils, baseline restoration was always achieved, carryover was <2%, and the good mixing conditions were reflected by a thin recorded baseline (<0.001 Å).

To improve detector washing, stream switching to the Mg channel was performed ~3 s after the peak related to Ca reached its maximum (Figure 3). The inner volume of the analytical path related to Ca was enough to wash the detector plus accessories prior to the appearance of the Ca signal. Baseline was restored ~6 s before arrival of the central portion of the sample zone.

Manual operation is also feasible. Without stream switching, the washing time was 90 s at the 2% carryover level. This result means a sampling rate of 20 determinations/h and a consumption of 3.0 mg of CPC per determination. By relocating the detector immediately after measurement of the peak maximum, the washing time was reduced ~20 s, and a remarkable improvement in sampling rate (7−160/h) was achieved.

The proposed system is remarkably stable, as shown in Figure 2 which is part of a routine run. Baseline drift is not observed during extended (8 h) working periods, and Schlieren (Zagatto et al., 1990) noise after stream switching is negligible (Figure 3).

Slight changes in the coefficients of the calibration equations (usually <3%) have been observed when the system was applied to large-scale analysis. Linearity

Table 1. Calcium and Magnesium in KCl Soil Extracts As Determined by the Proposed System (FIA) and by Flame Atomic Absorption Spectrometry (FAAS; Welz, 1985)[a,b]

	Mg		Ca	
sample	FIA	FAAS	FIA	FAAS
1	2.11 (0.7)	1.97 (1.2)	14.09 (1.0)	15.42 (1.2)
2	2.72 (0.5)	2.66 (0.8)	21.08 (0.3)	21.25 (0.9)
3	1.84 (0.3)	1.76 (1.4)	13.89 (0.9)	15.00 (1.1)
4	3.96 (1.5)	4.05 (1.1)	34.08 (0.4)	35.03 (0.8)
5	3.59 (0.9)	3.90 (1.8)	29.43 (0.2)	31.01 (1.7)
6	3.87 (1.1)	4.00 (1.2)	9.08 (0.7)	9.46 (1.1)
7	4.73 (0.8)	4.64 (1.5)	34.29 (1.0)	35.81 (1.0)
8	6.45 (0.1)	6.60 (0.9)	13.86 (0.1)	14.03 (1.3)
9	6.14 (1.0)	6.44 (0.5)	45.67 (0.0)	45.69 (1.8)
10	4.40 (1.6)	4.23 (1.1)	34.23 (1.6)	34.07 (1.2)

[a] Results in mg/L. [b] Numbers in parentheses are an estimate of rsd, in %, based on three replicate analysis.

Table 2. Calcium and Magnesium Contents in Natural Waters As Determined by the Proposed System (FIA) and by Inductively Coupled Argon Plasma Atomic Emission Spectroscopy (ICP-AES; Boumans, 1987)[a,b]

	Mg		Ca	
sample	FIA	ICP-AES	FIA	ICP-AES
1	0.12(0.8)	0.11(1.1)	0.31(0.4)	0.39(1.2)
2	0.21(1,0)	0.19(0.9)	0.43(0.9)	0.51(0.9)
3	0.29(1.1)	0.19(1.8)	0.15(1.2)	0.14(2.1)
4	3.51(0.2)	3.41(1.3)	14.11(0.7)	13.97(0.9)
5	1.87(0.4)	1.85(0.9)	21.02(0.2)	21.00(1.6)
6	3.50(0.1)	3.21(1.2)	4.61(0.4)	4.38(1.2)
7	3.50(0.7)	3.55(0.9)	15.90(0.2)	15.72(1.3)
8	2.08(0.3)	2.27(1.9)	6.76(0.6)	6.49(2.1)
9	1.98(0.7)	1.83(0.8)	6.81(0.9)	7.00(1.8)
10	2.00(0.1)	2.01(1.3)	5.85(0.2)	5.91(1.0)

[a] Results in mg/L. [b] Numbers in parentheses are an estimate of rsd, in %, based on three replicate analysis.

of calibration plot and sampling rate of 160/h at the 2% carryover level are other favorable characteristics noted in Figure 2. These features correspond to 0.8 mg of CPC consumed per determination.

Precise results (rsd, 0.81% for Mg, and 0.37% for Ca) were obtained after 10 successive measurements of a typical soil sample with Mg at 6.4 mg/L and Ca at 29.4 mg/L. For comparison of the accuracy of results obtained in the analysis of 10 soil samples by the proposed procedure with those obtained by AAS and ICP-AES (Tables 1 and 2), a Tukey test was applied (Miller and Miller, 1993). This statistical test shows no significant differences (Tukey, $p > 0.05$) between the proposed methodology and those used as reference. Recoveries after Ca and Mg spiking (10.0 mL of a solution of Ca at 15.00 mg/L + Mg at 5.00 mg/L to a 50.0-mL sample) were always between 97.5 and 104.1%.

CONCLUSIONS

The proposed system comprises two subsystems that can be easily disconnected yielding simpler systems for single analyte determinations, including a simple and rapid procedure for Mg determination by the direct way. With relocating detectors, washing time is no longer a noteworthy parameter that limits sampling rate, and improved sampling frequencies are achieved. With parallel monitoring, the detector can be moved any time after the peak maximum has been reached, allowing manual operation of the system. The possibility of extending the concept to *n* different channels is already under investigation.

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