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Colorimetric determination of available iron in soils by flow injection analysis

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Summary — This work presents a flow injection system with colorimetric detection for the automatic determination of available iron in soils. The system is based on the formation of the coloured complex produced from the reaction of iron(II) with 1,10-phenanthroline. Problems with refraction index effects (Schlieren signal) were overcome by using a large injection volume yielding the formation of a plateau (analytical signal) between two refractive index peaks in each interface of the sample plug. The developed system is very simple and easy to implement, allowing a sampling rate of 60 determinations per hour. Results obtained for 20 soil samples are in good agreement with those obtained by the reference method with relative deviations lower than 4%. Relative standard deviations were never higher than 1.7% for the entire concentration range studied (1–10 mg L^{-1}).

Résumé – Détermination colorimétrique du fer disponible dans les sols par la méthode FIA (*flow injection analysis*). *Un montage d'analyse par injection dans un flux avec détection colorimétrique pour l'automatisation de la détermination du fer disponible dans les sols est décrite. La méthode est fondée sur la formation du complexe coloré résultant de la réaction du fer avec la 1,10-phénanthroline. Les problèmes liés aux effets de l'indice de refraction (signal de Schlieren) ont été résolus par l'utilisation d'un grand volume d'injection, permettant la formation d'un signal en plateau (signal analytique) entre deux pics d'indice de réfraction dans chaque interface de l'échantillon. Le montage développé est très simple et facile à installer, et permet 60 analyses à l'heure. Les résultats obtenus pour 20 échantillons sont conformes à ceux fournis par la méthode de réference (écarts relatifs inférieurs à 4%). Les coefficients de variation n'ont pas dépassé 1,7% dans toute la gamme de concentrations (1–10 mg L^{-1}).*

flow injection analysis / iron determination / soils / colorimetry / Schlieren effect

Introduction

Iron is one of the nutrient trace elements essential for plant growth, although needed by most plants in a small amount. Iron deficiency causes a chlorosis in the plant and this disease is most common in calcareous soils in which iron may be present but not available.

The reference method [1] for the determination of available iron in soils is a colorimetric procedure based on the formation of the iron(II)-1,10-phenanthroline complex, after prior reduction of iron(III) to iron(II). This method is rather tedious and time-consuming especially because it requires the adjustment of the pH of each soil extract before the reduction and colorimetric reaction.

In order to automatically perform this determination, a flow injection manifold was developed and optimised. Other FIA manifolds have already been developed for the determination of iron in other environmental matrices such as natural waters and plant extracts [2], minerals, ores and rocks [3] and also in soils, but for the determination of iron in the soluble form [4].

The conception of the FIA manifold was initially conditioned by the need to carry out the iron(III) reduction to iron(II) under very acidic conditions (pH < 1) and subsequent colorimetric reaction with 1,10-phenanthroline in a buffered medium. However, from the injection of the acidic soil extracts refractive index effects were observed. These refractive index signals (Schlieren

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effect) have already been reported in flow injection systems with colorimetric detection and critically discussed by Zagatto et al [5].

To overcome this problem in the determination of iron in soil extracts, a large injection volume was used to allow a clear separation of three distinct areas: the two extremes of the sample plug (the front and the tailed portions) where the Schlieren effect is more pronounced and the central part of the plug which is less affected by these light scattering effects.

Materials and methods

Reagents and solutions

Deionised water with a specific conductance lower than 0.1 $\mu S\ cm^{-1}$, and analytical reagent grade chemicals were used for the preparation of the solutions.

The solutions used for the iron extraction were prepared as described in the reference procedures [1].

Ammonium acetate solution, 1 mol L^{-1}, pH 3
Equal volumes (2 L) of 2 mol L^{-1} acetic acid solution and 2 mol L^{-1} ammonia solution were mixed, and the pH was adjusted to 7 with either one of these solutions. Concentrated hydrochloric acid was finally added to adjust the pH to 3.

Aqua regia, 1:4 v v^{-1}
This was prepared by mixing hydrochloric acid and nitric acid in the proportions of 1:4, respectively.

For the analytical determinations, the reagents were prepared as follows.

Hydroxylamine hydrochloride, 10% w v^{-1}
Hydroxylamine hydrochloride (10 g) was dissolved in water and the volume was made up to 100 mL. This reducing solution was used for the reference method and was prepared daily.

1,10-phenanthroline, 1.5% w v^{-1}
This reagent (1.5 g) was dissolved and made up to 100 mL with ethanol. This solution was used for the colour development in the reference method.

Ascorbic acid, 0.010% w v^{-1} (R_2, fig 1)
Ascorbic acid (0.05 g) was dissolved in 1.2 mol L^{-1} hydrochloric acid up to a volume of 500 mL. This reducing solution was used for the FIA determinations and was prepared daily.

1,10-phenanthroline, 0.010% w v^{-1} (R_3, fig 1)
This reagent (0.10 g) was dissolved and made up to 1 L with 1.5 mol L^{-1} sodium acetate. This solution used in the FIA system was stable for 1 week.

Iron(II) stock solution, 200 mg L^{-1}
It was prepared by dissolving 1.404 g of $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$ in a mixture of 20 mL of concentrated sulphuric acid and 50 mL of deionised water and then made up to 1 L with deionised water. The standards used for establishing the calibration curves either for FIA (1.0 to 10 mg L^{-1}) or reference method (0.4 to 1.6 mg L^{-1}) determinations were prepared by dilution of the iron(II) stock solution in water.

Iron(III) stock solution, 200 mg L^{-1}
It was prepared as described for iron(II), followed by dropwise addition of 0.02 mol L^{-1} potassium permanganate solution until persistent pink colour before dilution to 1 L with deionised water.

Instrumentation and flow injection manifold

The determination of iron by FIA was carried out with a Unicam 8625 UV/Vis spectrophotometer equipped with an Hellma 178.713 flow cell of 8 µL optical volume. The spectrophotometer was connected to a Metrohm Model E586 chart recorder. For the reference method determinations, the same spectrophotometer equipped with a conventional plastic cell (1 cm optical path) was used.

In the FIA system, solutions were propelled by Gilson Minipuls 2 and Minipuls 3 peristaltic pumps and Gilson propulsion tubes. Standards and soil extracts were injected with a Rheodyne Type 50 injection valve. Omnifit PTFE tubing (0.8 mm id), with Gilson end-fittings and connectors was used for connections between the different components of the manifold; acrylic homemade Y-shaped joints [6] were used as confluence points.

Solutions were degassed in a Bandelin-Sonorex RK100 ultrasonic bath.

Sample preparation and reference method

For the extraction procedure [1], the soil samples were previously air-dried, ground and sieved in a 1-mm sieve. The extracts for analysis were prepared as follows: 25 g of soil were shaken for 30 s with 250 mL of 1 mol L^{-1} ammonium acetate solution (pH 3) and then vacuum filtered through a Whatman No 3 filter paper. The soil was leached in the filter with three times 50 mL of the ammonium acetate solution. The filtrate was evaporated in a boiling water bath and then treated with 10 mL of *aqua regia* until dryness for removing traces of organic matter. The residue was taken up with 1 mL of 1 mol L^{-1} hydrochloric acid to a 100 mL volumetric flask, and the volume made up with deionised water. This final extract was then used for both reference and FIA determinations.

The calibration curve for the reference method was established with standards covering the range from 0.4 to 1.6 mg L^{-1}, which was obtained by measuring the adequate volume of the iron (II) stock solution into 25 mL volumetric flasks. After dilution with 5 mL of deionised water, the pH was adjusted to the interval 1.5-2.7 with dilute hydrochloric acid or ammonia. Afterwards, 2 mL of 10% hydroxylamine hydrochloride solution followed by 1 mL of 1.5% 1,10-phenanthroline solution were added and the volume made up with deionised water. The same procedure was applied to the soil extracts by measuring an appropriate aliquot to the 25 mL volumetric flasks to fit the iron concentration within the calibration curve. The absorbance was read at 508 nm.

For each extract, the determinations were performed in duplicate.

Results and discussion

FIA manifold configuration

A FIA manifold (fig 1) was devised to allow direct injection of the soil extracts prepared as described in the reference method [1], performing in-line every step of the soil extract analysis which involves the reduction of iron (III) to iron (II), the colorimetric reaction and the absorbance measurement.

This FIA system consists of a three-channel manifold with two confluence points for the addition of the reducing agent (ascorbic acid) followed by the colour development reagent (1,10-phenanthroline) for the formation of the complex to be detected. Both reagents were added in confluences to guarantee an efficient mixing between reagents and sample throughout the plug.

When the soil extracts were first injected in the flow system using an injection volume of 200 µL, the peaks presented noisy and atypical FIA signals which could be described as a FIA peak mixed with the so-called Schlieren effect. This pattern was due to liquid interfaces which might either scatter or focus the light on the detector, during the passage of the sample plug through

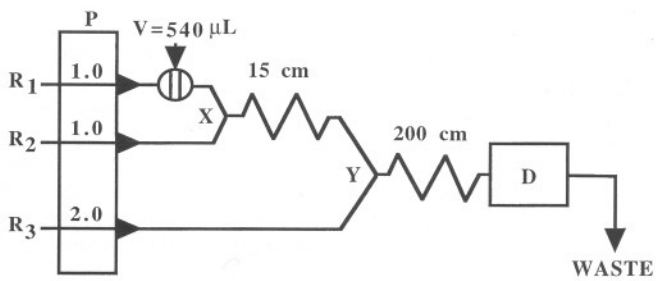

Fig. 1. Flow injection system for the determination of iron in soils. P, pump with indication of flow-rates in mL min^{-1}; R_1, water; R_2, 0.010% (w v^{-1}) ascorbic acid in 1.2 mol L^{-1} HCl; R_3, 0.010% (w v^{-1}) 1,10-phenanthroline in 1.5 mol L^{-1} sodium acetate; V, injection volume; D, detector (set at 508 nm); X and Y, confluence points.

the flow cell [5]. This way the manifold parameters were firstly adjusted to overcome this refractive index effect, and then optimised to maximise sensitivity and sampling rate.

Optimisation of the manifold

All the following studies were performed with fixed values for the flow rates of the streams R_1, R_2 and R_3 (1.0, 1.0 and 2.0 mL min^{-1}, respectively).

The first attempt to overcome the refractive index problem was to match the composition of samples and carrier solutions. The variability in the composition of the different soil extracts including the wide range of acidity observed (0.3 to 0.9 mol L^{-1}) makes it difficult to prepare one solution for matching all matrix characteristics. This acidity resulted not only from the HCl used to recover the residues, but also from the remains of *aqua regia* in the dry residue. However, attempts to match the samples acidity with that of the carrier composition (HCl 0.4 mol L^{-1} and HCl 1 mol L^{-1}) were performed. The peaks presented the same irregular pattern for either of the carriers.

As the matching of samples and carriers composition did not work in overcoming the masking effect of the refractive index over the analytical signal, another approach was tried. The use of a large injection volume enough to create a steady state zone in the central portion of the plug allowing a good definition of three distinct regions: a plateau in the central portion of the plug less affected by light-scattering effects, between two interfaces where the Schlieren effect is more pronounced [5]. Three injection volumes were tested: 420, 540 and 640 µL. A 540 µL volume was sufficient to clearly define the central plateau, whose height was taken as the analytical signal. Larger volumes would unnecessarily decrease the sampling rate. All the subsequent optimisations were then performed with a 540 µL injection volume.

The composition of the other two streams R_2 and R_3 was determined by the conditions imposed by each of the reactions occurring in the system. The solution R_2 had to assure a pH < 1 for a complete reduction [1]. This was guaranteed by preparing the ascorbic acid solution in 1.2 mol L^{-1} HCl. On the other hand, the solution R_3 had to provide a pH near 4 for the colour development with 1,10-phenanthroline which was achieved by preparing this solution in 1.5 mol L^{-1} sodium acetate. From the mixture of the three streams resulted an in-line buffer solution capable of adjusting the pH of any injected soil extract with an acidity below 0.9 mol L^{-1}. This was confirmed by injecting iron standards prepared in water, in 0.4 mol L^{-1} hydrochloric acid and 0.9 mol L^{-1} hydrochloric acid, from which resulted peaks with the same height.

The ascorbic acid concentration was studied in the range 0.0010% and 0.25% (w v^{-1}) to assure the complete reduction of iron(III) to iron(II) for a concentration of iron (10 mg L^{-1}), twice the maximum content found in the analysed extracts. Iron(II) and iron(III) standards (10 mg L^{-1}) were injected and 0.010% was found to be the minimum content necessary to obtain equal peak heights which proves complete reduction.

Once the concentration of the ascorbic acid defined, the concentration of 1,10-phenanthroline was studied in a range going from 0.0034% to 0.05%. Peak height increased up to 0.010% and this was the concentration selected.

The stability of the ascorbic acid prepared in 1.2 mol L^{-1} hydrochloric acid solution and of the 1,10-phenanthroline prepared in 1.5 mol L^{-1} sodium acetate, was evaluated along the time attending to the completeness of the reduction and the sensitivity, respectively. The ascorbic acid solution needed to be prepared daily, and the phenanthroline solution showed to remain stable for 1 week.

Once the composition of the solutions was selected, the length of the reaction coils was studied. A 15-cm tube showed to be sufficient for the complete reduction to occur between confluences X and Y. After the addition of the colour reagent, the reaction coil had to be long enough to assure a good mixing of the streams for the formation of the buffer and the colour development reaction to take place. The efficient mixing of the flow streams would also contribute to minimise noise in the baseline and in the central plateau (analytical signal). Tube lengths between 100 and 300 cm were tested and there was not a significant difference in the sensitivity of the system with either of the lengths tried, as the analytical signal plateau corresponds to a steady state region. 200 cm was shown to be the optimum length for the tube as lower values did not provide enough mixing, resulting in a noise increase, and higher values would unnecessarily decrease the sampling rate. The tubes were helically coiled to contribute to the improvement of the radial mixing.

This FIA system was extremely stable and there was no need for periodic calibrations within the same day or even over a few days. It is possible to define the calibration curve equation of the FIA system as: absorbance = $1.7\ 10^{-3}$ ($\pm 7\ 10^{-4}$) + 0.0505 ($\pm 6\ 10^{-4}$) × concentration (mg L^{-1}); in brackets find the standard deviations obtained in 1 week. The detection limit [7], calculated as the concentration corresponding to three times the standard deviation of the system background noise, was 0.07 mg L^{-1}.

Analysis of soil extracts

The soil extracts were injected in the flow injection manifold without any further treatment, the concentration being calculated by interpolation in the previously established calibration plots. Every iron(II) standard and soil extract was injected in duplicate (fig 2). The figure also presents duplicate injections of iron(III) standards as a guarantee of the completeness of the reduction of iron(III) to iron(II). The injection of the extracts show two distinct Schlieren regions (a) at the extremes of a well defined plateau (b), whose height was taken as the analytical signal. The standards analytical signal was also obtained in a steady state region, which is not visualised in figure 2, due to the lower speed set for the recorder.

This system allows a sampling rate of 60 determinations per hour. In order to assess the accuracy of the FIA results, analysis of 20 soil extracts using the flow injection manifold and the reference method were carried out. The paired results are presented in table I. The comparison of the results (mg of analyte per litre of extract) obtained with the developed FIA manifold (C_f) and with the reference method (C_r) shows a good agreement between the two methodologies as can be perceived from the parameters of the regression equation: $C_f = 0.02\ (\pm 0.05) + 0.994\ (\pm 0.017) \times C_r$, correlation coefficient = 0.9992. In brackets find the confidence limits of the intercept and slope obtained with 90% confidence level for 18 degrees of freedom (t = 1.73) [8]. The

Table I. Results obtained for the iron determination in soil extracts by the developed FIA system and by the reference method and corresponding relative deviations.

Soil extract	*FIA (mg L^{-1})*	*Reference method (mg L^{-1})*	*Relative deviations (%)*
1	2.44	2.42	+ 0.83
2	5.21	5.32	− 2.07
3	2.62	2.59	+ 1.16
4	2.39	2.40	− 0.42
5	3.22	3.21	+ 0.31
6	2.00	1.93	+ 3.63
7	2.47	2.42	+ 2.07
8	2.11	2.10	+ 0.48
9	1.66	1.69	− 1.78
10	1.56	1.61	− 3.11
11	1.05	1.03	+ 1.94
12	4.43	4.46	− 0.67
13	2.52	2.46	+ 2.44
14	2.92	2.91	+ 0.34
15	2.48	2.42	+ 2.48
16	2.73	2.78	− 1.80
17	4.74	4.73	+ 0.21
18	3.35	3.38	− 0.89
19	1.60	1.66	− 0.36
20	5.18	5.10	+ 1.57

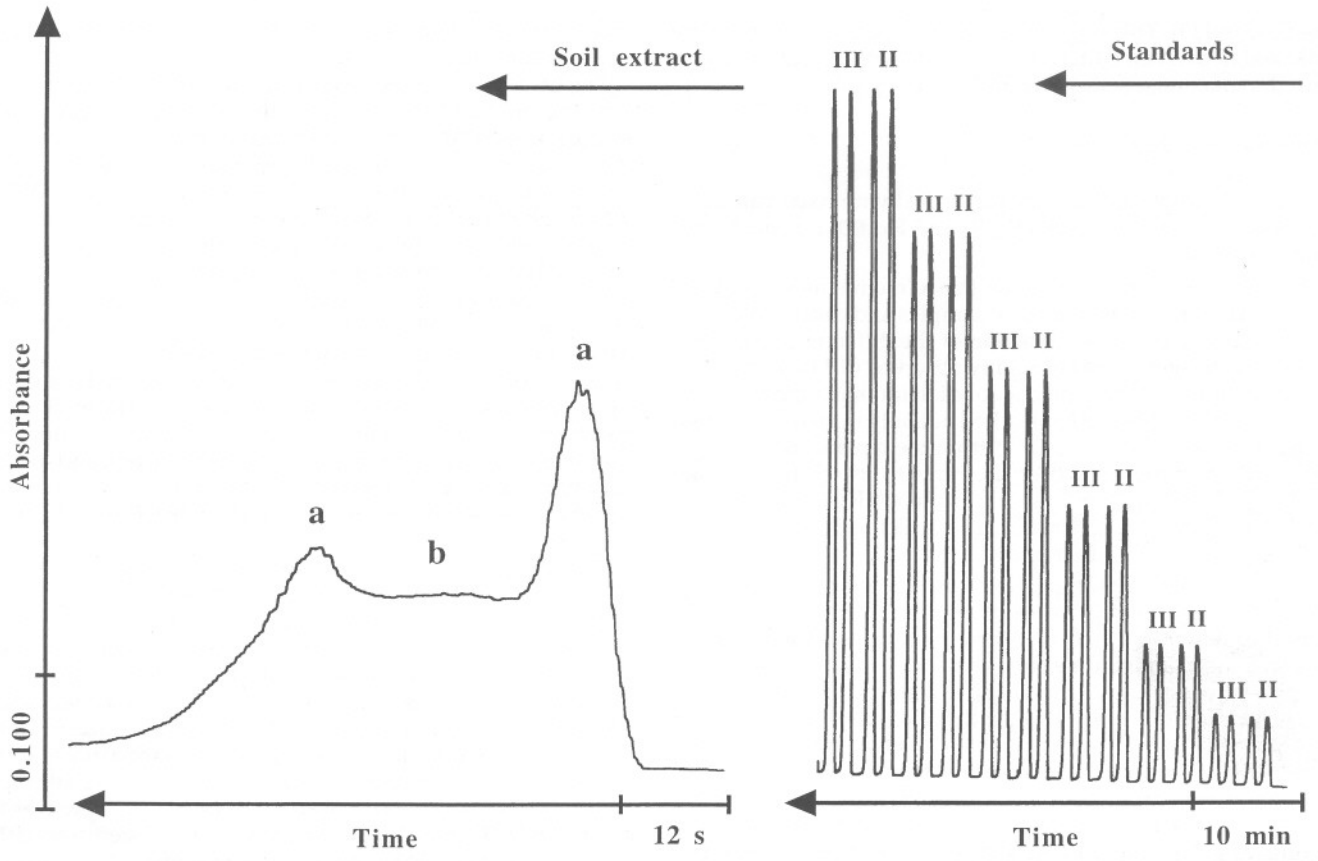

Fig 2. FIA output for the determination of available iron in soil extracts. Duplicate injections of iron(II) and iron(III) standards with concentrations of 1, 2, 4, 6, 8, and 10 mg L^{-1}, followed by the injection of one soil extract. Schlieren regions (a); analytical signal plateau (b).

observed relative deviations between the two methodologies were lower than 4%.

The relative standard deviation, calculated from ten consecutive injections of three soil extracts with concentrations of 1.55, 3.34 and 5.14 mg L^{-1}, were 1.72, 0.82 and 1.28%, respectively.

Conclusions

The developed FIA system is an advantageous alternative to the reference procedure for the analytical determination of available iron in soil, allowing good quality results (accuracy and precision) and a sampling rate of 60 determinations per hour. The simplicity and low cost of the developed manifold make it easy to be implemented in soil routine analysis laboratories, specifically as the system reproduces in-line the same analytical process of the reference procedure, using the same detection system. The time-consuming trial-and-error operations, necessary in the reference procedures for adjustment of pH values required by the specificity of the reactions, are replaced by an automatic adjustment which guarantees the optimal conditions for any injected soil extract.

It should also be emphasised the approach used to overcome the influence of the refractive index of the injected extracts on the analytical signal. This was achieved by resorting to a large injection volume, allowing to clearly separate the regions corresponding to the analytical signal and the refractive index. This strategy can also be used for other determinations over matrices that have this same problem.

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