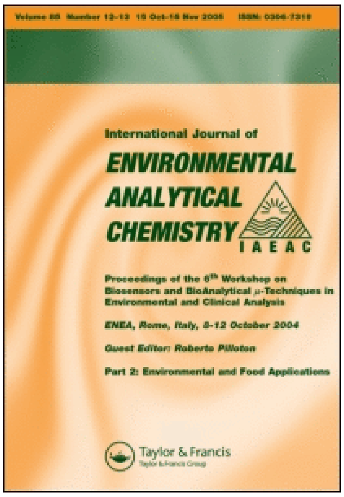

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D. Berggren[a]; A. Sparén[b]
[a] Department of Soil Sciences, Swedish University of Agricultural Sciences, Sweden [b] Department of Chemistry, Analytical Chemistry, The Royal Institute of Technology, Stockholm, Sweden

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A MODIFIED FIA SYSTEM FOR THE DETERMINATION OF HIGH LEVELS OF QUICKLY REACTING ALUMINIUM IN AQUEOUS SOLUTIONS

D. BERGGREN[1] and A. SPARÉN[2]*

[1]*Department of Soil Sciences, Swedish University of Agricultural Sciences,*
P.O. Box 7014, S-750 07 Sweden; [2]*Department of Chemistry, Analytical Chemistry,*
The Royal Institute of Technology, S-100 44 Stockholm, Sweden

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We have modified Clarke *et al.*'s (1992) flow injection system for determining 'quickly reacting Al' (Al_{qr}) in natural waters, to make it applicable also to solutions having high (> 1 mM) aluminium concentrations. By decreasing the flow rate of the carrier stream, thus diluting the sample on-line, and using a very small injection volume (12 μl), the upper end of the linear range has been increased from 2 mg Al/l to at least 30 mg/l. Good repeatability (RSD ≈ 1–3%, n = 4) was obtained, both for standard solutions and for natural waters. The quantification limit was about 100 μg/l and the sample throughput ca. 120 injections/h. Validation with synthetic model systems (Al plus F and Al plus citrate) showed that the modified set-up gave a fractionation similar to the one of Clarke *et al.*'s original system. The modified set-up was also tested on soil solutions, having Al_{qr} concentrations in the range of 0.1 to 1.5 mM. The sensitivity to iron interference was largely reduced by the modification. However, determining Al_{qr} in samples from real rat stomachs, having both high Fe(III) and protein concentrations, caused an interference from Fe(III) at concentrations lower than for standard solutions.

KEY WORDS: Aluminium, fractionation, speciation, soil solutions, flow injection analysis.

INTRODUCTION

The dissolution of aluminium (Al) from the soil solid material is the major mechanism for proton buffering in many Northern European forest soils[1,2]. In order to predict the impact of acid rain on soils and surface waters, a better knowledge of the processes governing the dissolution and transfer of Al in soils is needed. Proper analytical methods for characterizing the chemical forms (species) of Al present in soil solutions, are crucial in this respect.

Several methods for fractionating aluminium in soil solutions, surface and ground waters have been developed, *e.g.* those by Driscoll[3] and Bertsch and Anderson[4]. Recently, Clarke *et al.*[5] proposed a mechanized flow injection analysis (FIA) method for the determination of 'quickly reacting Al' (Al_{qr}) in natural waters. The method has been thoroughly tested and evaluated, using both simple inorganic and organic ligands, as well as isolated humic substances. It was shown that Al_{qr} included the monomeric

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* To whom correspondence should be addressed.

hydroxo, sulphato and silicato complexes of aluminium, but not its complexes with fluoride, citrate, humic or fulvic acids, nor the polymeric hydroxo complexes[5-7].

The FIA method was originally developed for determining Al in surface and ground waters, which generally have low concentrations of 'labile' forms of Al. The set-up presented by Clarke *et al.* is applicable to concentrations up to ca. 0.1 mM of Al. In strongly acidified soils, however, the concentration of labile forms of Al can be more than 0.5 mM (ca. 13 mg/l)[2,8]. In mechanistic experiments with soil materials, such as batch titration experiments, levels of 'labile Al' above 0.5 mM may be encountered at high acid additions[9]. Since dilution of a sample prior to speciation or fractionation analysis might drastically change the distribution of the species within the sample, there is a great need for extending the concentration range of Clarke *et al.*'s method. For samples used in toxicological studies, such high levels of iron may be encountered, that the masking system of their method may not be sufficiently efficient.

The main objective of the present work was to extend the analytical concentration range of the quoted method, in order to make it applicable also to samples with levels of 'labile Al' above 1 mM. To evaluate if the fractionation obtained with this new set-up was similar to that of the original system, the modified system was validated using two simple model ligand systems (Al plus fluoride (F) and Al plus citrate (Cit)). In addition, the sensitivity of the modified system to interferences by Fe(III) was investigated. The performance of the modified system was illustrated by the determination of Al_{qr} in soil extracts having a very wide range of Al concentrations, as well as in samples from rat stomachs obtained from *in vivo* studies on Al absorption from the gastrointestinal tract.

MATERIALS AND METHODS

Chemicals

Oxine (ox) was obtained from May and Baker and metal solutions of aluminium and iron were prepared from Titrisol (Merck) or Fixanal (Riedel-de Haën) solutions. All other chemicals were of analytical reagent grade (Merck or Riedel-de Haën); they were used without further purification. Ultrapure water was supplied by the Modulab Analytical System (Continental Water Systems) and was used throughout the experiments.

Equipment

The flow injection system of Clarke *et al.*[5] was modified as follows: (1) The injection volume was reduced from 250 to 100 (set-up L) or 12 (set-up H) µl, the latter of which is about the smallest volume that can be obtained using standard FIA injectors (FIA-05, Bifok, Sweden, equipped with a Rheodyne Model 7040 valve; (2) the carrier stream flow rate was decreased, while those of the reagents were increased. In this way, the sample was diluted on-line, thus ensuring a sufficient excess of reagents even at high Al concentrations. This on-line dilution is permissible, as long as the time lag between dilution and complexation within the FIA system is short enough not to substantially influence the distribution of species in the sample, which would most certainly be a great risk for a manual dilution of the sample prior to analysis. Two variants of the modified system are presented, aiming at different concentration intervals (Table 1), one for Al levels up to about 10 mg/l (set-up L) and one for Al concentrations above 10 mg/l (set-up H).

The flow system is shown schematically in Figure 1, and flow rates and complexation times are given in Table 1. The sample is injected into a carrier stream of water, and then mixed with a pH buffer also containing masking reagents (0.1 M acetate, 0.5 M hydroxyl ammonium chloride and 10 mM 1,10-orthophenanthroline at pH = 5.0). The composite stream is then mixed with a reagent stream containing 4 mM oxine in 10 mM sodium acetate at pH = 5.0. After a reaction time of 2.3–2.5 s in the complexation coil (CC), the aluminium trioxinate complex formed and the excess of oxine are extracted into chloroform. After phase separation, the absorbance of $Al(ox)_3$ in the organic phase is determined at 390 nm with a Merck-Hitachi L 4200 UV-VIS spectrophotometric detector. In studies on iron interference, measurement at a second wavelength (470 nm) was performed simultaneously, then using a Spectra Focus™ Optical Spanning Detector (Spectra Physics). Peak integration was done either with an SP 4290 integrator or with the Autolab® Software (both from Spectra-Physics). The phase separator used is described elsewhere[10]. Two peristaltic pumps were used, one for the aqueous phase (Bifok FIA 08) and one for the organic phase (Gilson, Minipuls 2).

Analytical procedures

pH measurements. pH measurements were made according to the method described by Gardner *et al.*[11], but excluding the recommended water bath and reducing the volume to ca. 7.5 ml. Measurements were made with an Expandable Ion Analyzer (EA 920) and a Model 9102SC pH electrode, both from Orion Research.

Determinations of total dissolved aluminium, iron and phosphorus. To check for possible losses of Al in the test solutions, the concentration of total dissolved aluminium (Al_{tot}) was determined with inductively coupled plasma—atomic emission spectroscopy (ICP-AES) (ARL 3520 B ICP Analyzer), if not otherwise indicated. Total dissolved iron (Fe_{tot}) and phosphorus (P_{tot}) were also determined with ICP-AES. The following emission wavelengths were used: P 214.9, Fe 238.2 and Al 308.2 nm.

Standard solutions. Standard solutions of aluminium had a pH less than 2.6, to prevent losses of Al and ensure that nearly all the aluminium should be present in the form of Al^{3+}.

Table 1 Characteristics of the two variations of the present FIA set-up and Clarke *et al.*'s original system[5].

Set-up	*Concentration range*	*Injection volume*	*Flow rate (ml/min)*				*Complexation time*
	(mg/l)	*(µl)*	*carrier*	*buffer*	*oxine*	*chloroform*	*(s)*
Modified H	1–50	12	0.16	1.0	1.2	0.9	2.5
Modified L	0.2–10	100	0.32	1.0	1.2	0.9	2.3
Original	0.025–2	250	1.1	0.6	0.9	0.9	2.3

(H = high concentrations, L = low concentrations)

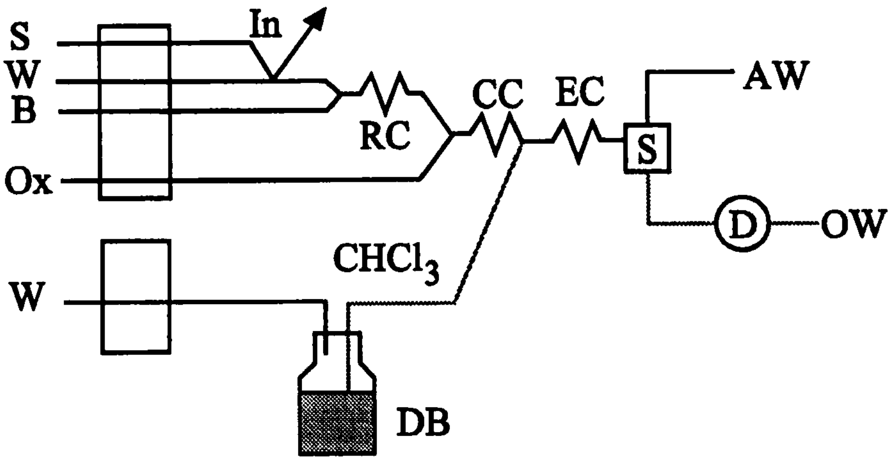

Figure 1 Schematic representation of the flow injection set-up (redrawn from Clarke *et al.*[5]). Notations: S = sample; W = water; B = pH and masking buffer; Ox = oxine; In = injector; RC = reduction coil; CC = compexation coil, EC = extraction coil; DB = displacement bottle; AW = aqueous waste; OW = organic waste; S= phase separator; D = detector.

Correction for iron interferences. Possible interference from iron in the samples from real rat stomachs was corrected for by measuring the absorbances at two wavelenghts, and evaluating with multilinear regression. At 390 nm, both aluminium and iron trioxinate absorb, while at 470 nm iron but not aluminium trioxinate does.

Method validation

In order to investigate possible effects of the increased on-line dilution of samples on the ability of the system to discriminate against Al fluoro and citrato complexes, analytical measurements were compared with equilibrium calculations. Calculations on test solutions containing Al and fluoride were performed with the computer program ALCHEMI[12], version 4.0 (Syracuse University, Syracuse, New York 13210, U.S.A.). Since this program was designed for calculations of 'acidic, low ionic strength drainage waters'[12], which correspond well to our synthetic aluminium fluoride system, we used the default equilibrium constants of ALCHEMI. This program does not, however, contain any stability constants for the aluminium citrate complexes. Therefore, the calculations on the Al citrato system were made with a PC version (V87.02) of HALTAFALL[13]. As far as possible, the internally consistent equilibrium constants presented by Öhman and co-workers (Table 2) were used for these calculations. The constants were corrected for differences in ionic strength (I) between our experiments (ca. 1 mM) and those in the literature, using the Güntelberg and Davies approximations[14].

RESULTS AND DISCUSSION

Theory

Several aluminium fractionation methods based on *kinetic discrimination* are based on the assumption that the reagent is present in great excess (50–100 times) compared to

Table 2 Stability constants used in the equilibrium calculations on the aluminium-citrate system.

Complex	*log β*		*Reference*	*log β'*
	I = 0 M	*I = 0.6 M*		*I = 1 mM*
OH^-	14.00	—	14	−14.00
$Al(OH)^{2+}$	—	−5.52	15	−4.80
$Al(OH)_2^+$	−10.33	—	16	−10.45
$Al(OH)_3^o$	−16.24	—	16	−16.38
$Al(OH)_4^-$	—	−23.46	17	−22.31
$Al_3(OH)_4^{5+}$	—	−13.57	15	−13.28
$Al_{13}(OH)_{24}O_4^{7+}$	—	−109.2	15	−99.47
$AlHCit^+$	—	−2.68	18	−1.54
$AlCit^o$	—	−4.925	18	−3.64
$AlCit_2^{3-}$	—	−12.53	18	−12.53
$Al_3(OH)_4Cit_3^{4-}$	—	−21.77	18	−20.20
$Al(H_{-1}Cit)^-$	—	−8.48	19	−7.34
$AlOH(H_{-1}Cit)^{2-}$	—	−14.71	19	−13.99
$Al_3(OH)_4(H_{-1}Cit)_3^{7-}$	—	−47.11	19	−50.26
H_2Cit^-	—	−2.769	18	−2.91
$HCit^{2-}$	—	−6.850	18	−7.42
Cit^{3-}	—	−12.067	18	−13.35

Reactions:[a] $H_3Cit \rightarrow H_{3-n}Cit^{n-} + n\,H^+$ ($\beta_{-n,0,1}$; n = 1–3) (I)

$p\,H^+ + q\,Al^{3+} \rightarrow Al_qH_p^{p+3q}$ ($\beta_{p,q,0}$) (II)

$p\,H^+ + q\,Al^{3+} + r\,H_3Cit \rightarrow Al_qH_p(H_3Cit)_r^{p+3q}$ ($\beta_{p,q,r}$) (III)

[a]according to Öhman and Sjöberg[18].

the aluminium concentration. If this is the case, the kinetics will be of pseudo first-order[20]. In Clarke *et al.*'s original FIA system, this assumption was fulfilled for Al concentrations up to at least 75 μM (~2 mg/l). Calculations made for a continuous flow case, thus disregarding the effect of possible dispersion, illustrate how the ratio between oxine and aluminium changes as the Al concentrations increase. Let us consider the reaction between aluminium and the reagent (IV) where 'org' denotes the organic phase:

$$Al^{3+} + 3\,ox^- \rightarrow Al(ox)_{3(org)} \tag{IV}$$

If α denotes the ratio between oxine and Al within the complexation coil (CC), and Q_{ox}, Q_{cc} and Q_w denote the flow rates for oxine, within the complexation coil and for the carrier stream of water, respectively, the following equations can be set up:

$$[Ox]_{cc} = \frac{Q_{ox}}{Q_{cc}}[Ox] \tag{1}$$

$$[Al]_{cc} = \frac{Q_w}{Q_{cc}}[Al] \tag{2}$$

$$\alpha = \frac{Q_{ox}}{Q_w}\frac{[Ox]}{[Al]} \tag{3}$$

Calculations were made for three cases: (1) Using the conditions of the original FIA system; (2) using a decreased carrier flow rate and a constant reagent flow rate;

(3) using a decreased flow rate of the carrier, but increased flow rates of both the buffer and reagent streams. The results of Table 3 clearly show that there will be a shortage of oxine at higher Al concentrations for the original FIA system. The necessary excess of oxine is clearly present at lower Al concentrations (0.1 and 1.0 mg/l, *i.e.* 3.7 and 37 μM), but hardly at higher (10 and 50 mg/l, *i.e.* 0.4 and 1.8 mM). The most straightforward way to overcome this problem would be to increase the concentration of oxine in the reagent stream. That is, however, not possible, since the concentration of oxine is already close to saturation at the pH used (5.0). Another way of increasing the excess of oxine in the reaction coil is to decrease the flow rate of the carrier stream and, thus, to further dilute the sample on line. If that is done, as in cases 2 and 3, oxine will be present in sufficient excess at 10 mg/l (0.37 mM), but as we approach 50 mg/l (1.8 mM), the excess decreases, especially at the lower reagent flow rate (case 2). Since dispersion has not been considered, the calculations must be regarded as a worst case scenario.

Linear range of the modified system

Simple linearity tests (Figure 2) showed that the theoretical calculations presented above give a fairly good picture of what happens in the FIA system at higher Al concentrations. The original system with an injection volume of 12 μl was linear up to about 5 mg/l of Al (ca. 0.2 mM), which is in fairly good agreement with the theoretical estimation. We interpret the deviation from linearity at higher concentrations as an effect of the decreased availability of oxine which leads to a change of the kinetic conditions for the complex formation between Al and oxine.

In contrast, the modified system was linear up to at least 30 mg Al/l (1.1 mM) (Figure 2). The original FIA system with an injection volume of 250 μl had a linear range up to about 2 mg/l of Al (ca. 75 μM)[5]. Thus, compared to the original set-up, the linear range of the modified system increased by a factor of 10–15. With somewhat larger modifications of Clarke *et al.*'s system, including the connection of the flow system to a graphite furnace atomic absorption spectrometer (GFAAS), sub-μg/l levels have been quantified[21]. Systems with such dynamic concentration ranges as the variations based on Clarke *et al.*'s original FIA system, are uncommon among aluminium fractionation techniques. To our knowledge, the only method with a comparable dynamic range is the FIA method by Fairman and Sanz-Medel[22]. Using a fluorimetric detection, they obtained a linear range up to 10 mg/l of Al (0.37 mM), and a detection limit of 0.9 μg/l (33 nM).

Table 3 Theoretical evaluation of the concentration ranges possible for maintaining the conditions necessary for kinetic discrimination of aluminium. The cases represent worst-case scenarios, since the dispersion of the sample was not taken into account.

Flow	*Case 1*	*Case 2*	*Case 3*	*[Al]*	*α*		
(ml/min)				*(mg/l)*	*Case 1*	*Case 2*	*Case 3*
Carrier (Q_w)	1.05	0.16	0.16	—	—	—	—
Buffer (Q_B)	0.53	1.42	1.00	0.1	867	5684	8485
Reagent (Q_{ox})	0.84	0.84	1.26	1.0	86.7	568	849
Chloroform (Q_{CHCl_3})	0.91	0.91	0.91	10	8.7	56.8	85
Complexation coil (Q_{cc})	2.42	2.42	2.42	50	1.7	11.4	17
Reaction time (t_r) (s)	2.44	2.44	2.44	—	—	—	—

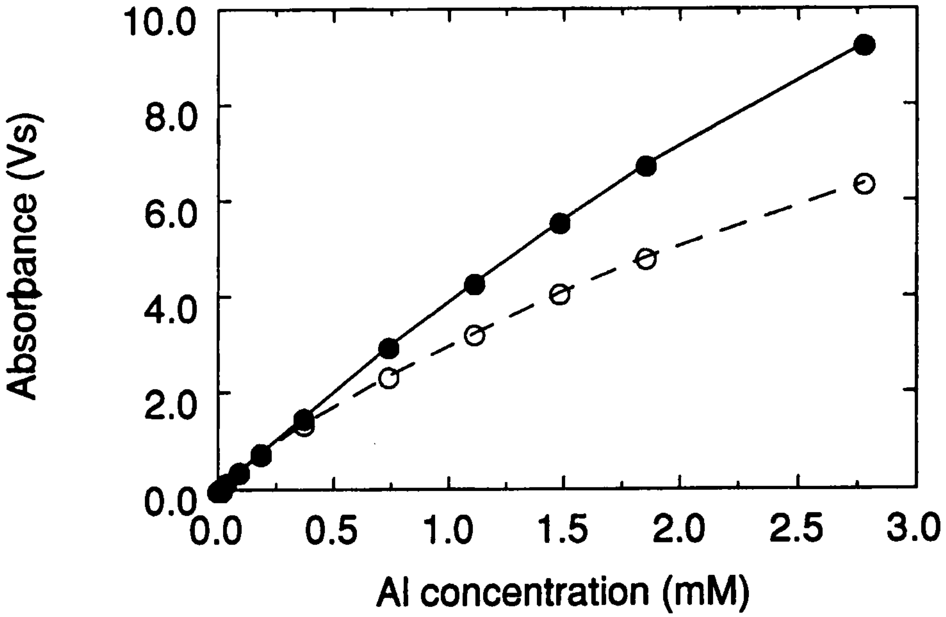

Figure 2 Test of the linear range of the original and modified FIA systems: original FIA system with a reduced injection volume (open circles); modified H set-up (solid circles). The injection volume was 12 µl in both cases.

Interference studies

Interference from iron on the determination of Al_{qr} was studied by determining Al_{qr} in synthetic solutions containing a constant Al concentration, but a variable amount of Fe(III). Figure 3 shows that both the original FIA system with an injection volume of 12 µl and the modified system can tolerate very high levels of non-complexed iron. Fe additions up to 50 mg Fe/l (0.9 mM) had very little effect on the signal for a standard solution containing 5 mg Al/l (0.18 mM). Measurements at 470 nm, where $Fe(ox)_3$ absorbs but not $Al(ox)_3$, gave no detectable signals up to 20 mg Fe/l, but a small signal was observed at 50 mg Fe/l for both systems mentioned (data not shown). As a comparison, the original FIA system (injection volume 250 µl) could only tolerate concentrations of non-complexed iron up to ca. 2 mg/l (36 µM)[5].

Another feature of the modified system is that it is less sensitive to low sample pHs. The original system could not tolerate samples with pH values below 2.5, due to the limited capacity of the acetate buffer. With the small injection volume used in the modified system, this problem diminished. Standard solutions with pHs around 1.8 did not cause any problems.

Analytical characteristics

The repeatability of the modified system is good. A relative standard deviation (RSD) of 1–3% for 4 injections was obtained for standard solutions containing 1–75 mg/l of Al (0.04–2.8 mM), which is similar to the RSD of the original system. The detection limit has not been formally determined for the modified system, but it is possible to quantify

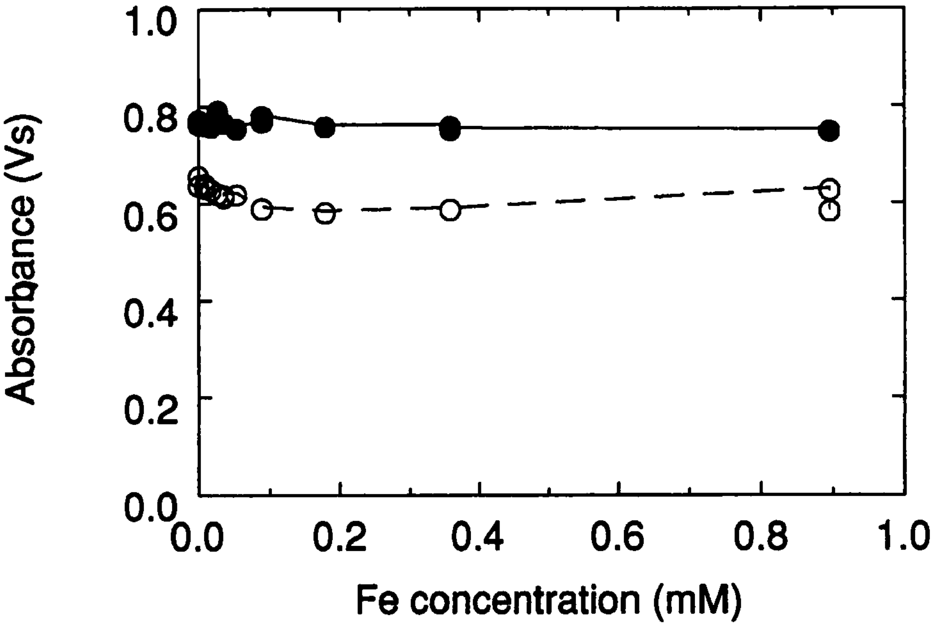

Figure 3 Interference studies: effect of increased Fe(III) additions on the absorbance at 390 nm in the original FIA system (open circles) and the modified H set-up (solid circles). An injection volume of 12 µl was used in both cases. [Al] = 5 mg/l, pH = 1.8.

standard solutions containing 100 µg Al/l, yielding an RSD of less than 10% (n = 4). The detection limit for the original FIA system was 5–10 µg Al/l (0.2–0.4 µM) and the quantification limit was about 25 µg Al/l (0.9 µM), using an injection volume of 250 µl[5].

The sample throughput is greatly increased with the modified system. 120 injections/h can easily be done, which is about twice the sample throughput of the original system.

Method validation

For the original FIA system, Clarke *et al.* made a thorough validation of what aluminium species were included in the measured fraction (Al_{qr}). Since the system presented here is based on that system, we do not consider it necessary to repeat all these characterization experiments. Two synthetic model systems (Al plus F and Al plus citrate) were chosen for validation of the modified system.

The results of the test on the Al-F system are shown in Figure 4. There is good agreement between measured values and those predicted with equilibrium calculations, both for high (Figure 4a) and for low (Figure 4b) aluminium and fluoride concentrations. Obviously, the increased on-line dilution of the sample did not affect the ability of the system to discriminate between Al_{qr} and fluoride complexes of aluminium.

Complexes between Al and citrate are considered kinetically inert and are generally not measured as 'labile Al' with most methods for Al fractionation based on kinetic

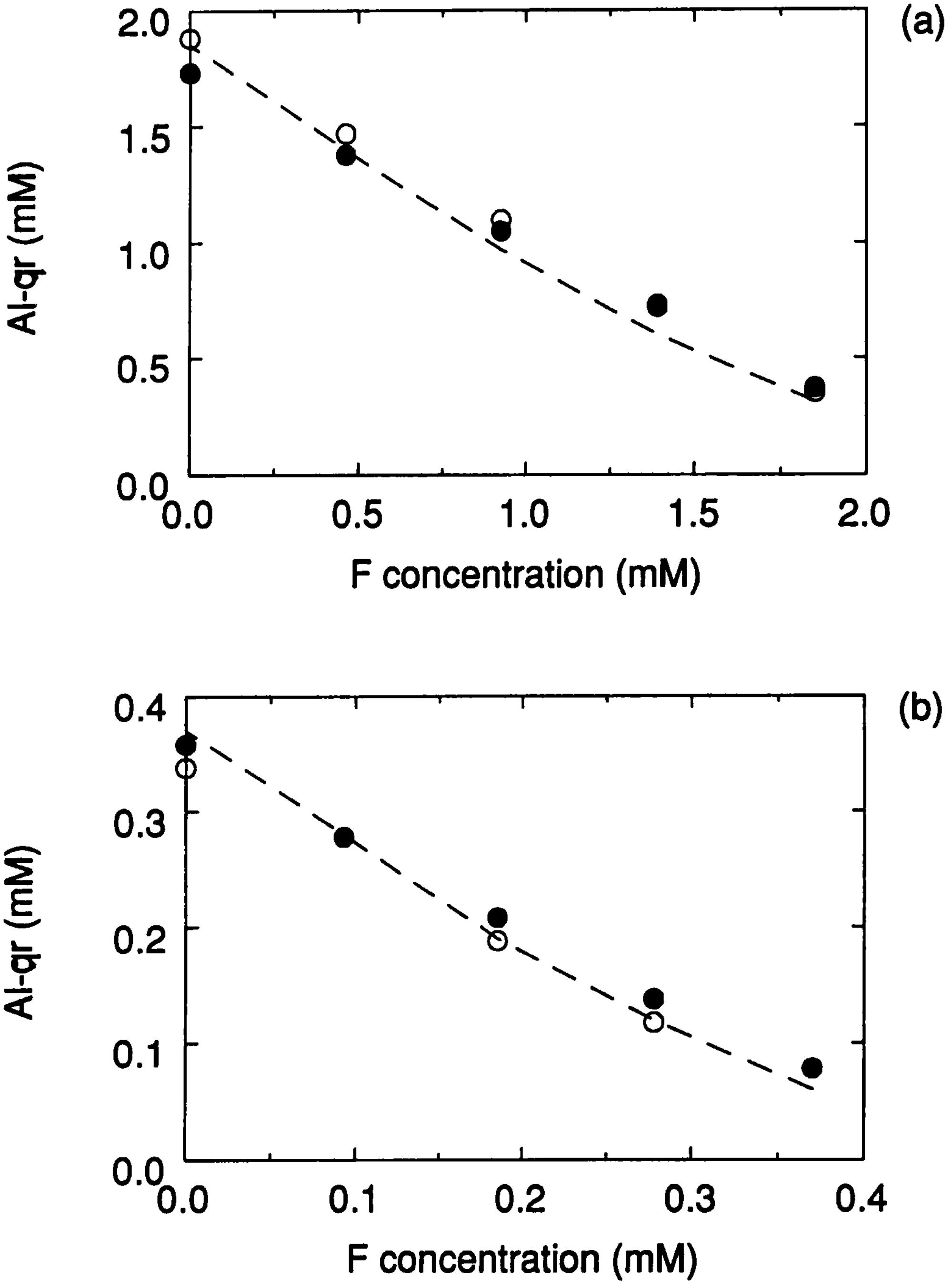

Figure 4 Method validation (H set-up) using a model system containing aluminium and fluoride: Solid and open circles represent two different runs, while the dashed line gives values predicted by equilibrium calculations: (a) $[Al]_{tot}$ = 50 mg Al/l (~1.9 mM), pH = 3.8; (b) $[Al]_{tot}$ = 10 mg Al/l (~0.37 mM), pH = 3.8.

discrimination, *e.g.* those presented by Driscoll[3], Bertsch and Anderson[4] and Clarke *et al.*[5] It is therefore not likely that the increased on-line dilution of the sample in the modified FIA system presented here, should cause a dissociation of Al-citrate complexes. To check whether this assumption was true, we made a brief study on a solution having a 1:1 molar ratio of Al and citrate. Theoretical predictions showed that without any citrate addition, 93% of the added aluminium should be present as Al^{3+}, and about 7% as $Al(OH)^{2+}$. When citrate was added at a 1:1 molar ratio, only some 2% was present in the form of Al^{3+}, while the major part of the added aluminium existed in different citrate complexes, such as $AlCit^0$ (ca. 48%), $Al(H_{-1}Cit)^-$ (ca. 48%) and $Al_3(OH)_4(Cit)_3^{4-}$ (< 2%). As shown in Table 4, the measured values of Al_{qr} (here: $[Al]_{qr} \approx [Al^{3+}] + [Al(OH)^{2+}]]$ agree well with the values predicted with equilibrium calculations, both with an injection volume of 100 µl and with one of 12 µl. There is thus no reason to believe that aluminium-citrate complexes will be measured with the modified FIA system.

When applying new methods for Al fractionation to natural waters, it is essential to study their response to complexes between Al and humic substances. In general, these types of Al complexes are kinetically non-labile, and are not measured with Clarke *et al.*'s original system[6–7], using the recommended reaction time of 2.3 s. In most cases they were not even measured after a longer reaction time (14 s). It is therefore likely that the dissociation of complexes of Al and humic substances will be negligible also in the modified system.

We did not find it necessary to specifically validate the L set-up of Table 1. This set-up is useful for Al_{qr} concentrations in the range of 0.2–10 mg/l (7–370 µM), a range which is common in moderately acid forest soils[2].

Measurements on soil extracts

An application of the modified method to the analysis of various soil extracts is shown in Figure 5. In the experiment shown in Figure 5a, 50 g of a Podzol Bs horizon soil were leached with 25 subsequent aliquots of 800 ml of 1 mM HCl (Berggren, Mulder & Westerhof, unpublished results). The solid material was allowed to equilibrate for 2 days prior to each replenishment. As the sample was leached, Al was released from the solid phase. The successive depletion of solid-phase Al resulted in a decreased buffering of the added acid and hence, a decline in pH from 4.3 in the first extract to 3.4 in the 25th extract; likewise, the concentrations of dissolved organic carbon (DOC) decreased from 22 to 12 mg C/l. As the pH and the concentrations of organic ligands decrease, the concentration of Al_{qr} approaches that of total Al, which is to be expected from a system with dissolved humic substances as the major ligands[23]. The L set-up was used in these experiments.

Table 4 Comparison between experimental results and theoretical predictions made with equilibrium calculations for the aluminium citrate system (I = 1 mM).

Set-up	*$[Al]_{tot}$ (mM)*	*$[Cit]_{tot}$ (mM)*	*pH*	*$[Al]_{qr}$ (mM)*	
				measured	*calculated**
Modified L	0.37	0.37	3.7	0.004	0.007
Modified H	0.37	0.37	3.7	< 0.02	0.007

*$[Al^{3+}] + [Al(OH)^{2+}]$.

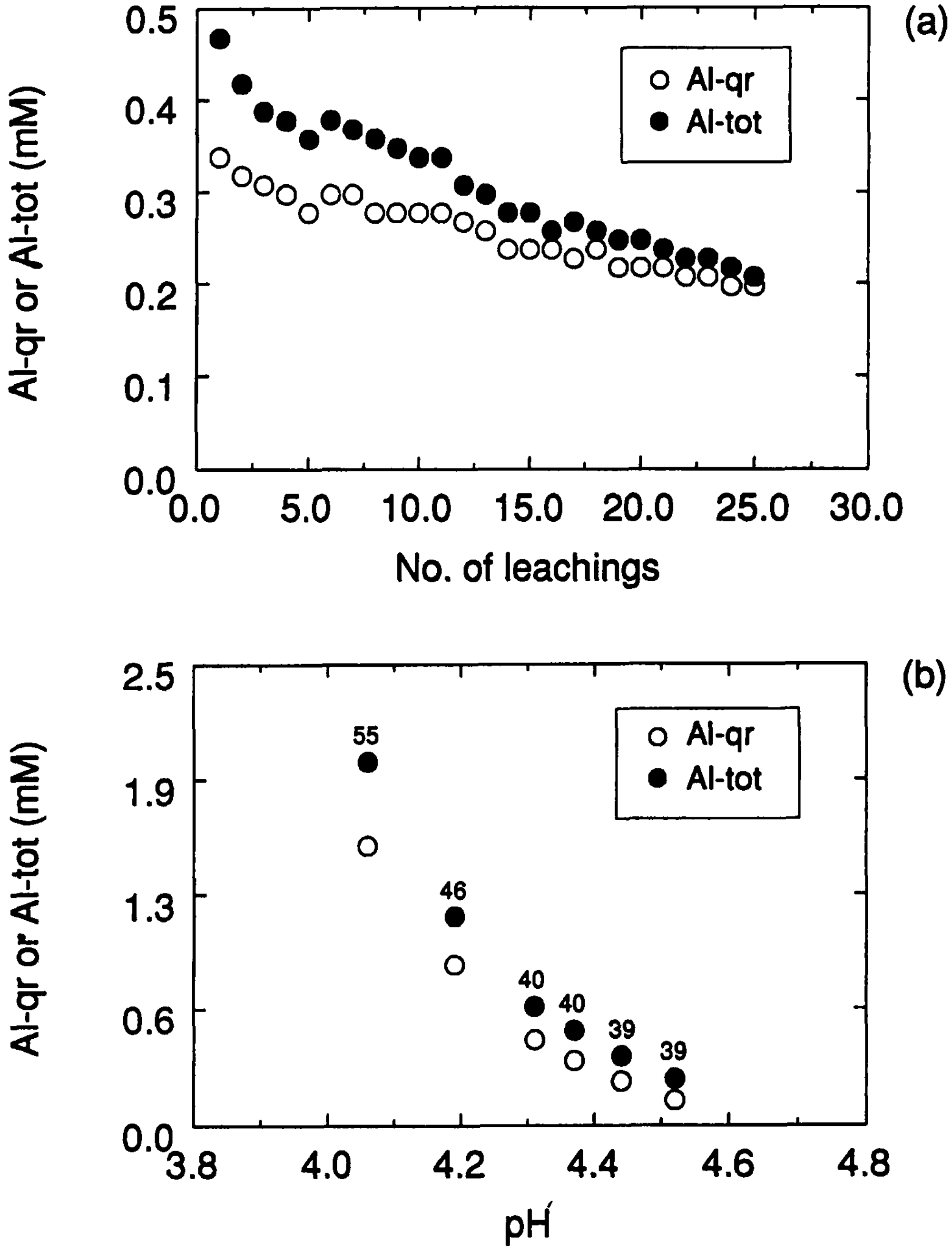

Figure 5 Measurements of Al_{qr} and Al_{tot} in soil extracts from batch experiments performed with a podzol Bs horizon soil (Berggren, Mulder & Westerhof, unpublished data). (a) 50 g soil were leached successively with 25 portions of a mixture of 1 mM HCl and 10 mM KCl. Samples were allowed to equilibrate for 2 days prior to replenishment. The pH decreased from 4.3 in the first leachate to 3.4 in the 25th, and [DOC] from 22 mg/l to 12 mg/l. (b) Al solubility in a batch titration experiment with various KOH or HCl additions. Numbers represent the concentrations of dissolved organic carbon (mg C/l). Al_{tot} was determined with the pyrocatechol violet method[25].

Figure 5b shows the applicability of the FIA system to soil solutions obtained from a batch titration experiment, in which a very wide range of Al_{qr} concentrations were obtained. For this application, the L set-up was used for samples with Al_{qr} concentrations < 10 mg/l (0.37 mM), and the H set-up for samples with Al_{qr} concentrations > 10 mg/l (Table 1), thus illustrating the versatility of the FIA method.

Measurements on samples with a complicated matrix

The modified methods was also used for samples from pooled real rat stomachs. Knowledge of the distribution of aluminium species in these samples is essential in order to understand which forms of aluminium may be absorbed from the gastrointestinal tract of mammals. The matrix of such samples is, however, extremely complicated, due to the presence of both iron and proteins in high concentrations. The protein dominating in these samples is *casein*, a phospoprotein with a strong tendency for complex formation with iron and aluminium. To our knowledge, no measurements of the distribution of Al species in similar samples have been published, apart from Danielsson *et al.*'s *in vitro* study[24] on *simulated* rat stomachs, in which the FIA system presented here was used.

Al_{qr} was measured in the supernatant of centrifuged and filtered real rat stomachs from *in vivo* studies on Al absorption (Wicklund Glynn *et al.*, unpublished results). The animals had consumed rat feed and acidified drinking water, to which different amounts of aluminium had been added. Some typical results are shown in Table 5. Even at extremely high additions of aluminium, the measured levels of Al_{qr} were very low. Precise quantification of Al_{qr} was, however, not possible in this case due to the low levels of Al_{qr} in combination with extremely high concentrations of iron (Table 5). The values of Al_{qr} were corrected for iron interference (as described in 'Analytical Procedures'), but this correction is not precise enough to allow real quantification. We can therefore only give estimates of Al_{qr} in these samples. For comparison, values of Al_{qr} before and after correction for iron interference are given in Table 5.

In simple synthetic solutions, the modified FIA system could tolerate iron levels up to 50 mg/l (cf. Figure 3). In the samples from rat stomachs, iron interference was encountered at considerably lower values of Fe_{tot} (Table 5). Apparently, the modified system cannot mask iron bound to protein or its degradation products as efficiently as non-complexed iron. The residence time in the reduction coil is probably not long enough to allow both sufficient dissociation and reduction of iron-protein complexes, and the complexation of Fe(II) with, 1,10-orthophenanthroline. Therefore, some iron will react with oxine, which will result in an increased signal at 390 nm.

Table 5 Measurements on worked-up stomachs from rats which had consumed acidified drinking water with different levels of aluminium. The figures are mean values of two measurements.

Sample id	*Al added to drinking water (mg/l)*	*$[Al]_{qr}$[a] (mg/l)*	*$[Al]_{tot}$ (mg/l)*	*$[Fe]_{tot}$ (mg/l)*	*$[P]_{tot}$ (g/l)*	*pH*
Group 0	0	< 0.1 (0.3)	0.6	61	1.6	4.8
Group I	10	< 0.1 (0.2)	3.4	51	1.0	3.7
Group II	50	~ 0.1 (0.4)	21	73	1.1	3.5
Group III	500	~ 0.2 (0.7)	33	14	0.7	3.4

[a] Values in parentheses were not corrected for iron interference.

A slightly worse repeatability was noted for these samples compared to determinations in standard solutions. This was probably caused by the high protein concentration in the samples. Proteins may have surface-active properties, which tend to disturb the segmentation in the extraction process of the FIA method[26], thereby deteriorating its repeatability[5]. The same is also true for humic substances, when present in high concentrations[5].

To conclude, the extended on-line dilution of the sample within the FIA system made it possible to obtain reasonable estimates of Al_{qr} concentrations even in these complicated samples (improved repeatability and decreased iron interference compared to the original system). Quantification of Al_{qr} was, however, still not possible in samples from rat stomachs. The results, however, show that the modified system is a move in the right direction. In order to further improve the performance of the FIA system for this type of samples, a sample work-up unit may prove suitable. One possibility is to connect a dialysis unit in front of the FIA system in order to remove proteins and the Fe(III) complexed to them. Dialysis has been used in FIA systems for the fractionation of cadmium in samples containing proteins[27]. Such changes of the set-up would require a substantial amount of extra work and this aspect was not made a part of the present work.

CONCLUSIONS

- By applying a simple on-line sample dilution and a decreased injection volume, it was possible to extend the analytical range of the method of Clarke *et al.*, without decreasing its ability to discriminate between 'labile forms' of aluminium and complexes of aluminium with fluoride and citrate. The linear range of the system extends to at least 30 mg/l of Al (1.1 mM), compared to 2 mg/l (ca. 75 μM) for the original system. In addition, the sample throughput is twice as high as in the original system (120 injections/h). The precision of the modified system is satisfactory (RSD = 1–3%, n = 4), and the quantification limit is still acceptable (0.1 mg/l of Al, *i.e.* 37 μM) (set-up H).
- With the modifications presented here, a reduced sensitivity to Fe(III) interference was obtained. Fe(III) concentrations up to 50 mg/l had no significant effect on the signal for standard solutions containing 5 mg/l of Al. For solutions in which a major fraction of Fe(III) is bound to strong ligands, such as proteins, interferences can still be a problem at Fe(III) concentrations less than 50 mg/l.
- Although a reduced sensitivity to interferences from Fe(III) and surface-active proteins was observed with the modified system, it was not possible to obtain reliable measurements of low Al_{qr} concentrations in liquids from real rat stomachs. Further developments, *e.g.* inclusion of a dialysis step prior to the extraction, are obviously required for reliable Al_{qr} measurements in such extremely complicated matrices.
- The modified system is useful for analysing soil solutions with high concentrations of Al.

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