The Analyst

Application of flow injection analysis adsorption–elution protocols for aluminium fractionation†

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FIA (flow injection analysis) is a widely used technique for trace element analysis, but has a number of inherent problems. When used to determine the 'free' metal concentration, the 'normal' reaction time in the manifold, 15–30 s, promotes a significant sequestration of metals from labile and pseudo-labile complexes. Also there is potential for matrix components other than the target analyte to affect the rate of the analyte–reagent reaction. This may lead to an over- or under-estimate of the amount of labile metal relative to calibrations based on simple aqueous standards. These problems can be minimised by use of flow systems with much shorter 'reaction times' and by separating the analyte fractionation step from the analyte–reagent reaction step. This can be achieved by use of on-line 'adsorbents' from which the captured analyte is eluted prior to the analyte–reagent reaction. This is illustrated for the fractionation of Al by use of *ca.* 1 s contact time with an oxine-derivatised gel. Real-time analysis of non-retained fractions coupled with selective elution of retained species provides the concentrations of three fractions: 'free Al' [Al^{3+} + $Al(OH)^{2+}$ + $Al(OH)_2^+$], 'labile organic Al' and 'Al_{13}' hydroxy polymers. Quantitative separation of Al and Fe is achieved. For 'free Al' the linear working range is 0.3–16 μM, the LOD 70 nM and the RSD at 2 μM Al is 3.7%. The method is compared to conventional FIA for determination of 'reactive Al' in soil solutions and is applied to the Al-complexation capacity and pH-dependent Al binding of a fulvic acid, and in the correlation of plant growth with Al fractions in soil solutions.

Keywords: *Flow injection; aluminium; speciation; 8-hydroxyquinoline; chrome azurol S; lucerne; fulvic acid; complexation capacity; soil solution*

The aquatic environment is a multicomponent, multiphase system. The components combine to give a diverse range of species which may differ in physical form, stoichiometry or oxidation state. The component concentrations may be fixed or variable. Species may be in rapid equilibrium (*e.g.*, Cu^{2+}–Cl^-) or slow equilibrium (*e.g.*, Cu^{2+}-fulvic acid)[1] or they may be inert (*e.g.*, Bi–EDTA). Metals exist in a number of 'pools', *e.g.*, free metal ions, hydrolysed metal ions, low molecular mass complexes (inorganic and organic), humic complexes and colloids. Within each pool a range of species may co-exist for a single metal, their relative concentrations being a function of pH and component concentrations. Their absolute concentrations are a function of the concentrations of all ligands and other metal ions. This paper focuses on speciation of metals in the aqueous phase, with particular reference to Al.

The toxicity of a metal and its mobility are closely linked to its speciation. It is a generalisation that for metals which exist in nature in only one oxidation state the 'free metal ion' is often the most toxic. When a metal is bound to ligands in stable hydrophilic complexes the toxicity is suppressed because of diminished availability to micro-organisms.[1] Both thermodynamic (stability) and kinetic (dissociation rate) factors determine the availability of a metal at the cell wall. In contrast, hydrophobic complexes can be very toxic,[1] even if kinetically inert, because of direct penetration of the cell lipid bilayer.

To the analytical chemist the challenge is to achieve a fractionation of the metal which targets only one species in the system. This can be achieved by use of a non-invasive probe (*e.g.*, an ion-selective electrode) but these are available for few metal ions and the working ranges barely encompass that which is critical in environmental systems. Another option[2] is based on the determination of the total amount of all elements in a sample followed by the computer-aided calculation of the equilibrium concentrations of species, based on metal complex and redox equilibrium constants, kinetic factors, adsorption and heterogeneous processes. This task is daunting and although comprehensive tabulations of stability constants are now available,[3] adjusting constants to the correct ionic strength and temperature requires additional data or approximations.

The alternative is kinetic-based analyses which involve a selective reaction with one species before significant re-equilibration can occur. This is not difficult if the species formed by a given component are non-labile (*e.g.*, Se^{IV} and Se^{VI} oxyanions). But for labile or moderately labile species it requires a very short experimental time scale. Anodic stripping voltammetry (ASV) has been used successfully to differentiate species which are 'labile' or 'non-labile' on the ASV timescale of, say, 100–500 ms. However this technique is limited to those metals which are reducible at the Hg electrode, which amalgamate with Hg and which strip reversibly. This is a comparatively small, though important, group of elements.

Flow injection analysis: advantages

The FIA (flow injection analysis) procedure allows highly reproducible and comparatively short (15–30 s) experimental times, is applicable to a wide range of metal analyses and can be coupled to a range of detection systems (*e.g.*, spectrophotometric, amperometric). For Al it also provides a direct method of analysis, in contrast to methods such as Driscoll's[4] in which the reactive Al species (retained on a cation-exchange column) are determined as the difference between two other measurements, one of which involves an operationally defined fraction.

Flow injection analysis: limitations for fractionation procedures

One disadvantage of FIA is that the reaction time (throughout which fractionation *and* re-equilibration in the sample are occurring) is too long, except for slowly labile systems. The rate constants for the exchange of water from simple aqua ions indicate that only Al^{3+} has a half life of the order of seconds, although metal complexes with polydentate ligands are known to dissociate more slowly than the aqua ions.[5,6]

† Presented at The Third International Symposium on Speciation of Elements in Toxicology and in Environmental and Biological Sciences, Port Douglas, Australia, September 15–19, 1997.

The long reaction coil used in a FIA manifold (Fig. 1) is required to achieve sample–reagent homogeneity in the sample zone. Improved mixing in a shorter time can be achieved by replacing the coil with a series of mixing tanks. Hawke and Powell[7] found that 5–7 s was a practical lower limit with three mixing tanks, but this elapsed time was still sufficient for significant sequestering of Al (15–35%) from its malonate and oxalate complexes.

The metallochromic reagents typically used for FIA determination of reactive Al, pyrocatechol violet (PCV), chrome azurol S (CAS) and eriochrome cyanine R (ECR), are very strong complexing agents.[8–10] Given adequate time they will almost quantitatively remove Al from its complexes with simple organic ligands[10] and substantially remove it from its humate complexes.[7] The reaction rates of these reagents with Al^{3+} are strongly dependent on ionic strength at $I < 0.1$ M,[7] a disadvantage when matrix matching of natural waters and soil solutions is not possible. Further, the pH of the buffer used with the reagent (Fig. 1) may be very different from that of the unknown (*e.g.*, PCV uses a buffer at pH 6.2–6.5, yet samples from acidified environments will have pH < 5.4). This will cause a scrambling of the metal speciation during the reaction time. CAS uses a pH 5.0 buffer which is within the 'acidified sample' pH range and is optimal in this respect.

In conventional FIA the fractionation process (in which the reagent targets one or more species) and the analytical process (formation of an analyte–reagent product) are combined. Any potential interferents should be masked, *e.g.*, Fe^{3+} in the case of Al^{3+} analyses. If this involves the addition of a complexing agent then this may also affect the rate of release of Al from its complexes, or the rate of the Al–reagent reaction.[11] The addition of ascorbic acid– bipyridyl to reduce Fe^{III} to Fe^{II} has the effect of dissolving Fe–hydroxy colloids and releasing co-deposited Al.[7]

It is important that the apparent rate constant for the analyte–reagent process be the same in the standards and the samples. If the kinetics of this reaction are sensitive to components in the sample medium then this will reflect as an error in the apparent fractionation. There is now ample evidence that this is the case for Al–metallochromic reactions. Simpson *et al.*[11] observed that the rate of reaction of PCV with Al in the presence of different ligands was: oxalate ≈ F^- ≈ malonate > salicylate ≫ no ligand ≫ citrate. They also observed that (i) the rate of reaction of Al in humic samples decreased with sample dilution (*i.e.*, with decrease in [organic ligands]), and (ii) in the presence of humic substances the rate of reaction of Al in excess of the Al-complexation capacity was *ca.* 1.7 times greater than that in standard Al solutions. Nilsson and Powell[12] observed that for many soil solutions the [Al] determined by ETAAS is lower than that determined by FIA with PCV (a result of the more rapid reaction of Al in samples than in standard solutions).

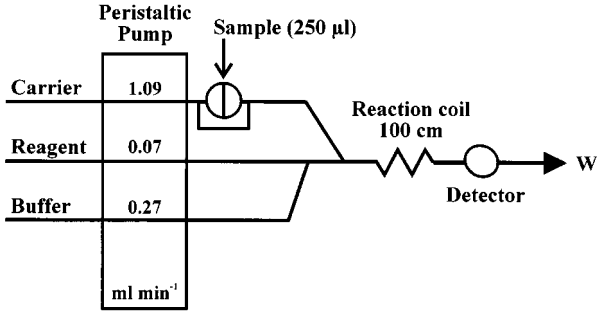

Fig. 1 FIA manifold for determination of Al by reaction with PCV. Carrier: Milli-Q water; reagent: 5.0 mM PCV (0.24 mM at the detector); buffer: 2.0 M hexamine, pH 6.0. The flow rates for the respective lines are given. All tubing 0.51 mm id microline (Cole Parmer, Niles, IL, USA); reaction coil, 100 cm knitted microline; sample volume, 250 µl.

FIA with stationary adsorbents

The above discussion highlights the need to (i) separate the fractionation and analytical reactions in metal 'speciation' protocols, (ii) ensure that the medium for the analytical reaction is identical for analyte sourced in the sample or in the standard and (iii) shorten the life time of the fractionation step. These objectives can all be met by placing a stationary 'adsorbent' in the flow system immediately down-line from the injection valve to capture the targeted species. The 'fractionation time' is now limited to the contact time between the adsorbent and an element of flowing solution. The captured analyte can then be eluted, selectively, into a 'clean' carrier solution for down-line analysis. This paper compares a 'stationary adsorbent' FIA protocol with conventional FIA for determination of 'reactive Al' in soil solutions. It is applied to determination of 'reactive Al' in soil solutions derived from plant growth studies and to the determination of fulvic acid complexation capacity.

Experimental

The FIA manifold is shown in Fig. 2. Oxine was covalently immobilised onto a porous styrene–divinylbenzene polymer of 50–100 µm diameter[13] and packed in a 20 µl column (2.0 mm id) which was constructed of polycarbonate with Omnifit (Cambridge, UK) end fittings. Full experimental details for the manifold and FIA protocol are given elsewhere.[14] Immediately following sample injection (650 (µl) the 'reactive Al' [Al^{3+} + $Al(OH)^{2+}$ + any Al in highly labile complexes] is captured on the column and separated from the sample matrix. The sample zone proceeds down-line and any 'moderately labile' Al undergoes reaction with the CAS reagent during the elapsed time in the reaction coil. The resulting signal (Fig. 3, 'non-column reactive Al') represents an operationally defined fraction. The signal is quantified against standard $Al(OH)_4^-$ standards injected in 0.02 M NaOH. The captured Al is then eluted into the reagent stream with a smaller volume of 0.02 M NaOH (250 µl), effecting an approximately 10-fold pre-concentration because the Al is mostly eluted in the first 50 µl of eluent.[12] This decrease in sample zone volume is evident from the relative half widths of the peaks shown in Fig. 3. The eluted $Al(OH)_4^-$ experiences an identical reaction environment to that for eluted Al standards. The linear working range is 0.3–16 µM, the LOD 70 nM and the RSD at 2 µM Al is 3.7%.

Root elongation studies were effected using lucerne (*Wairau sp.*). Seeds were germinated for 3 days on wet filter papers in covered petri dishes and then set out in pots of unamended soil (300 g) and loosely covered with 2–3 mm soil. Three pots with

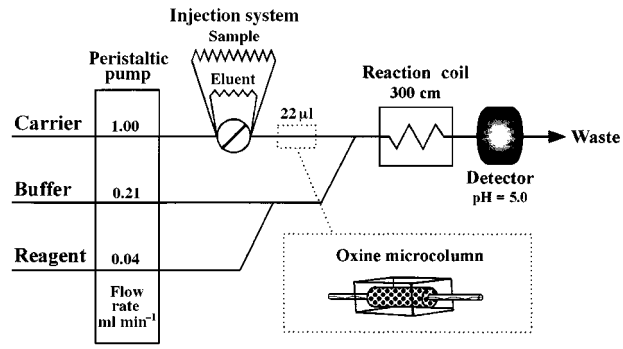

Fig. 2 Schematic diagram for the adsorption–elution flow-injection manifold. The chemical components of this system were: (i) carrier solution = 0.05 M NaOAc–0.05 M NaCl (pH 5.0), (ii) buffer = 2.0 M acetate buffer (pH 5.3; 5.00–5.05 at detector) and (iii) reagent = 2 mM CAS. The flow rates for the respective lines are given. The sample and eluent injection loop volumes were 650 µl (or 250 µl) and 250 µl, respectively. The reaction zone was a 300 cm knitted microline coil.

five seeds were used for each soil. Germination and plant growth were effected at 20 °C using a 12 h light–dark cycle. After 4 days all the seedlings were removed and the tap root lengths measured. Immediately following harvest, soil solutions were extracted from *ca.* 100 g of soil (roots and stones removed) by centrifugation (30 min at 3000 rpm). Membrane filtration to 0.025 μM was immediately effected on the filtrate and the solution analysed by FIA within 2 h. The soil solution pH was measured with a Russell (Auchtermuchty, Scotland) CMAWL/4/5/S7 combination microelectrode coupled to a Radiometer (Copenhagen, Denmark) PHM64 pH meter.

Soil fulvic acid was isolated from International Humic Substances Society (IHSS) reference peat by the acid–pyrophosphate–XAD-7 method of Gregor and Powell.[15] For Al-complexation capacity measurements, 60 ml of fulvic acid solution (final concentration 17 mg l^{-1}) were adjusted to pH 4.7 with 0.05 M KCl and 0.005 M acetate. Standardised Al^{3+} (1.59 mM, pH 3) was added incrementally from a Gilmont micrometer syringe and the solution mixed for 3 min after each addition and before removal of a 1 ml aliquot into a plastic syringe. These aliquots from a titration were held for 2 h at room temperature before 'free Al' analysis by FIA. The aluminium binding curve was determined for the pH range 2.5–7.0 by incremental addition of KOH to a solution containing 0.05 M KNO_3, 9 μM Al^{3+} and 17 mg l^{-1} fulvic acid. Aliquots were removed at pH intervals and stored in plastic syringes for 2 h before analysis for 'free Al' by FIA.

Model systems

Several model systems were studied to establish that only 'free Al' is captured by the column *and* eluted by 0.02 M NaOH. The ligands tested were OH^- and fluoride, malonate, oxalate, citrate and tartrate. Measurements were made both in this flow system and by use of the column off-line in a 'batch' mode.[16] For L=OH^-, aged, hydrolysed 10 μM Al solutions were prepared in the pH range 4.6–6.0. For the organic ligands solutions containing 15 μM Al and 0–100 μM ligand were prepared and aged 24 h. Solution composition was calculated using published stability constants[3] and the program SOLGASWATER.[17]

Results and discussion

Oxine is a reagent which reacts efficiently with both Al and Fe (the most common interferent for analysis of Al in environmental samples). By using a dilute NaOH eluent the captured Al^{3+} can be eluted as the $Al(OH)_4^-$ complex and separated from the potential interferent Fe^{3+} which is not eluted.[18] An added attribute of this protocol is that the polymer $Al_{13}(OH)_{32}^{7+}$ is also retained on the column. It is not eluted by 0.02 M NaOH, but can be eluted in a second elution step with 0.2 M NaOH. The polymer does not react with CAS on the FIA timescale[19] unless firstly depolymerised by stopping the flow for 120 s before the alkaline sample is moved into the reagent stream.[14] The depolymerisation is facilitated by placing a smaller reaction coil down-line from the gel column and before the first merging zone. This represents the first method for fractionation of the 'Al_{13}' polymer in environmental samples. Fig. 4 shows the detector (spectrophotometric) responses for the three fractions of Al: I = 'moderately reactive Al', II = 'free Al' and III = 'polymeric Al–hydroxy' species.

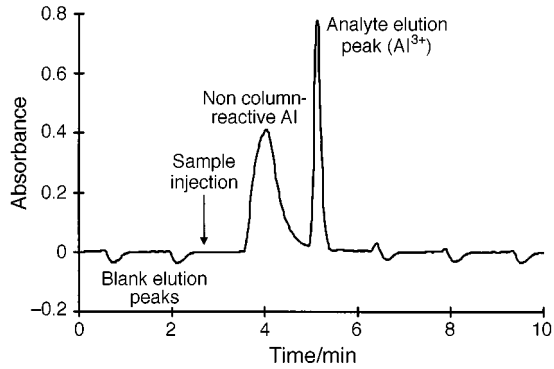

Fig. 3 Typical FIA output signals for Al following the injection and elution of a soil solution or humic water sample, using the manifold in Fig. 2. The first peak is the 'pre-elution' peak, corresponding to 'moderately reactive' Al which is not captured by the column. The second peak corresponds to captured Al which is eluted with 0.02 M NaOH.

Model systems

Model systems were studied to establish that only 'free Al' is captured by the column *and* eluted by 0.02 M NaOH. The ligands tested were OH^- and fluoride, malonate, oxalate, citrate and tartrate. Data for L = oxalate are shown in Fig. 5, in which the solid line is the calculated concentration of 'free Al' and the datum points are for different flow rates. A paper by Simpson *et al.*[14] presents further results. For each of these ligands it was established that, to a good approximation, only 'free Al' is

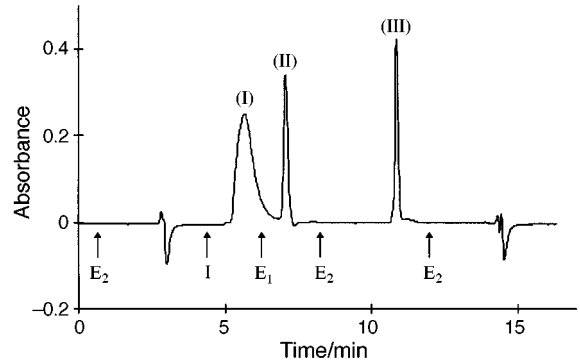

Fig. 4 FIA output signals modelled for Al following the injection and elution of a humic sample which contains the polymer $Al_{13}(OH)_{32}^{7+}$, using the manifold in Fig. 2. I = sample injection, E_1 = injection of 0.02 M NaOH eluent, E_2 = injection of 0.2 M NaOH eluent, followed by a 120 s stop-flow. (I) = 'moderately reactive' Al, (II) = 'free Al', (III) = polymeric Al–hydroxide species.

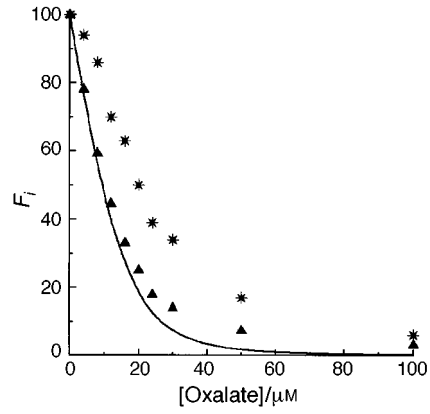

Fig. 5 The fraction of Al measured in solutions containing 15 mM Al and 0–100 mM oxalate, plotted as a function of ligand concentration. Data are presented for experiments with column residence times 1.3 s (✱) and 2.8 s (▲). The 'fraction', F_i, was calculated as the measured response for each solution relative to the response for a 15 mM Al standard. The curve for $\Sigma\{[Al^{3+}] + [Al(OH)^{2+}] + [Al(OH)_2^+]\}$ was calculated from the thermodynamic model for the H^+–Al^{3+}–ligand system using the computer program SOLGASWATER.[17]

captured by the oxine- derivatised gel at a flow rate corresponding to a sample–column residence time of *ca.* 1 s (pump speed 40 rpm). This reaction time was sufficiently short to minimise the sequestration of Al from its complexes.

Aluminium complexation capacity

We have not previously had a convenient method for determining the Al-complexation capacity (Al-CC) of humic waters and soil solutions. The potential of this FIA technique to determine Al-CC is illustrated by the results in Fig. 6. These results are from analysis of solutions produced by incremental addition of Al^{3+} to a buffered (pH 4.7, 0.005 M acetate) solution of fulvic acid (17 mg l^{-1}) followed by a 2 h ageing of aliquots withdrawn at each solution stoichiometry. The data for 'free Al' are shown for experiments in the absence (◆) and presence (▲) of fulvic acid. When the total [Al] added is in excess of the Al-CC the 'free' Al increases in proportion to this excess. Extrapolation of these 'free' Al data to the *x*-axis, as shown, indicates a (kinetic) Al-CC[20] of *ca.* 10 μM l^{-1}. This represents the sum of 'non-labile' plus 'moderately labile' Al (*i.e.*, all Al which is not captured by the column). The curve (○) represents 'moderately labile' Al and approaches a plateau at *ca.* 8 μM Al. The sites binding 'moderately labile Al' require *ca.* 50 μM total Al (40 μM excess Al) to become saturated, evidence that they bind Al much less strongly. Thus the ratio of 'moderately labile Al' to 'inert Al' bound to the fulvic acid is *ca.* 4 : 1. When the total [Al] added is in excess of the Al-CC, the sum of the 'free' Al (▲) and 'moderately labile' Al (○) gives a curve which has a limiting slope ≈ 1.0 (indicating minimal matrix effect on the rate of reaction) and an intercept of *ca.* 2 μM l^{-1}, corresponding to 'inert Al'. We have reported similar complexation capacity titration curves for raw humic waters[20] and soil solutions.[21]

Fig. 7 provides a comparison of Al^{3+} and Cu^{2+} binding [to the same fulvic acid (FA) sample] as a function of pH. The solution stoichiometries are not identical (see Fig. 7 caption) but it is clear that at the chosen ratios of -COOH to metal ions Al^{3+} is bound more strongly (at lower pH). The Cu curve was determined by potentiometry at a higher [FA];[22] the curve for the Cu *kinetic* complexation capacity (and at the lower [FA] used for Al) would lie to higher pH, amplifying the difference between the two metals. It may be inferred from these results that Cu^{2+} (and other heavy metals) immobilised and accumulated in humic sediments of a lake will be released upon acidification of the water column. The presence of Al^{3+} at elevated concentrations in acidified water will significantly enhance this release of heavy metals from humic sediments.

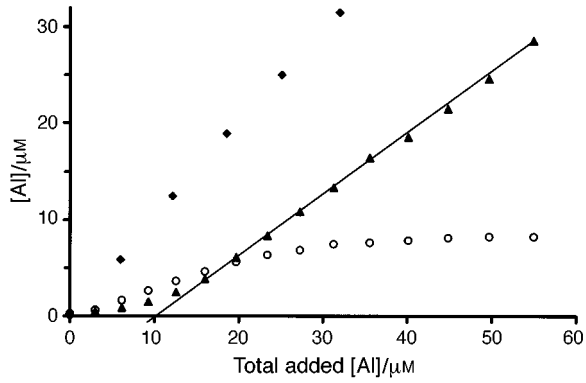

Fig. 6 Complexation capacity data for titration of a soil-derived fulvic acid (17 mg l^{-1}) with Al^{3+} at pH 4.7 (0.005 M acetate buffer). Data correspond to (▲) 'free' Al captured by the oxine-derivatised gel and eluted with 0.02 M NaOH, (○) 'moderately reactive' Al, derived from the pre-elution peak and (◆) an Al titration in the absence of fulvic acid.

Column versus non-column methods

The advantage of separating the analyte fractionation step from the analyte–reagent reaction is seen by comparison of the 'reactive Al' concentrations determined for a series of soil solutions by using the conventional FIA method with PCV reagent and the oxine-derivatised gel method (Fig. 8). The PCV-reactive Al concentrations (●) are all significantly higher than those for 'free Al' determined by the oxine column [▲, Fig. 8(*a*)]. They are also significantly greater than the sum of 'free Al' and 'moderately labile Al' determined by the gel column [Fig. 8(*a*) and (*b*)]. In part this is related to the very aggressive reaction by PCV, but predominantly to the much slower kinetics of the Al–PCV reaction in standard solutions

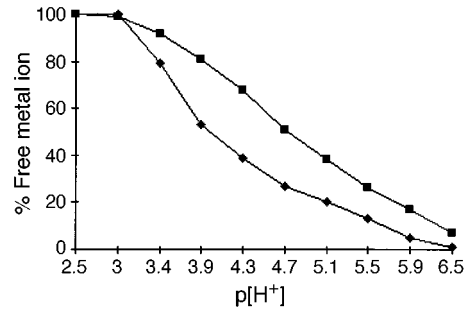

Fig. 7 Metal binding curves for (◆) Al^{3+} and (■) Cu^{2+} with a soil-derived fulvic acid. For the Al binding curve [fulvic acid] = 17 mg l^{-1} in 0.05 M KNO_3; total [Al] = 9.0 μM. The Al curve was determined by the oxine-derivatised gel method. For the Cu binding curve [FA] = 25 mg l^{-1} in 0.1 M KNO_3, total [Cu] = 9.0 μM. The Cu curve is taken from reference 22 and was determined by Cu ion-selective electrode potentiometry.

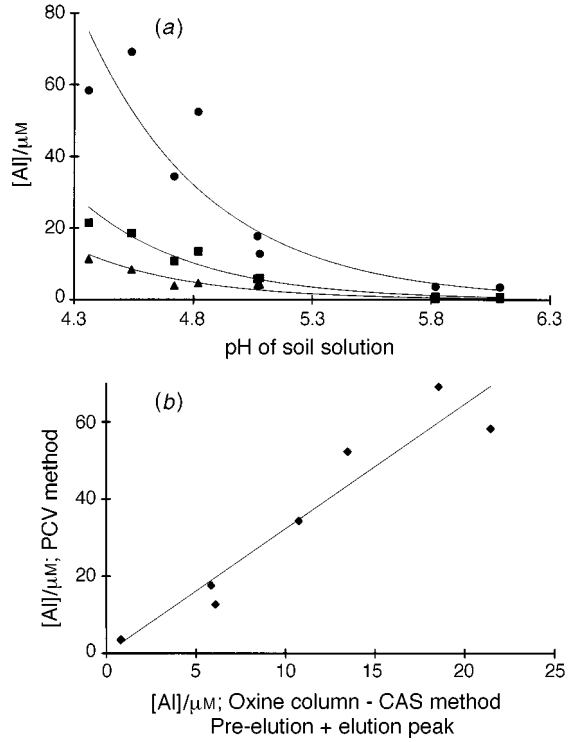

Fig. 8 Comparison of analyses of 'reactive' Al in soil solutions using the FIA–PCV method and the oxine-derivatised gel method. (*a*) 'Reactive' Al as a function of pH, measured by (●) the FIA–PCV and (▲) the oxine-derivatised gel method; (■) is the sum of 'free' Al and 'moderately reactive' Al by the oxine-derivatised gel method. (*b*) Correlation of reactive Al determined by FIA–PCV and by the oxine-derivatised gel method ('free' Al + 'moderately reactive' Al); $y = 3.2265\,x + 0.0578$ and $R^2 = 0.9289$.

(compared with Al–ligand solutions) against which the unknowns are calibrated.[11]

Aluminium toxicity to plants

The oxine-derivatised gel method is finding application in studies on the Al toxicity of acidic soils.[12,18] Fig. 9 shows the relative root elongation values (RRE) for 4 day old lucerne seedlings plotted (*a*) against pH, and (*b*) against 'free Al' determined in the centrifuged soil solutions at the end of the experiment. In Fig. 9 (*b*) the observed correlation coefficient of 0.88 can be compared with values of 0.79 for a plot of RRE against the sum of 'free Al' + 'moderately reactive Al' and a value of 0.59 for a plot of RRE against 'PCV-reactive Al'.[12] No measurable amount of the 'Al_{13}' polymer was found in any of the soils studied.[23] From the distribution diagrams for the Al–OH^- system the absence of this polymer at pH < 5.0 is anticipated. Other factors possibly acting against its detection are complexation with humic colloids (which are not retained on the gel column) or adsorption on inorganic colloids.

Detection systems

Fe^{II} and Fe^{III} are captured by the derivatised gel but not removed with the 0.02 M NaOH eluent.[18] In the past, an inability to effect this quantitative separation of Fe and Al in homogeneous solution without addition of reducing agents has seriously confounded attempts to develop electrochemical methods for analysis of Al^{3+}.[24–26] These amperometric and voltammetric methods probe the effect of Al on the oxidation reaction of a redox-active ligand, typically a 1,2-dihydroxyaryl molecule, such as DASA,[24] 4-nitrocatechol (with oxine column),[25] alizarin[26] or PCV.[27] At pH 8–9 the binding of Al to such a ligand shifts the ligand oxidation peak anodically by *ca.* 200 mV (Fig. 10). By monitoring the free ligand oxidation [peak (a)] using a glassy carbon or Au electrode in a flow cell, the formation of Al complexes in the sample zone is registered at the detector as a decrease in anodic current. The use of a ligand oxidation reaction at positive potentials avoids the problem of dissolved oxygen (which confounds electrochemical measurements at cathodic potentials). Recently, screen-printed electrodes doped with alizarin have been developed for the voltammetric determination of Al in flow or batch systems.[28]

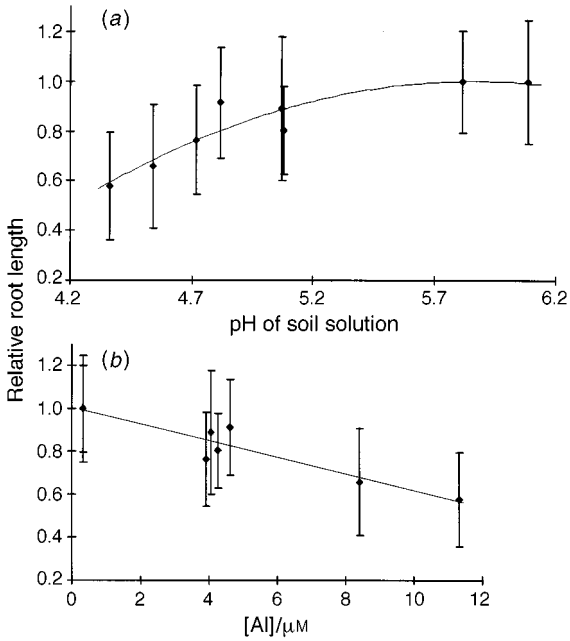

Fig. 9 Relative root length (RRL) measurements for growth of lucerne (*Wairau*) seedlings in unamended soils as a function of (*a*) pH and (*b*) [Al] in the soil solution at the completion of 4 days growth (22 °C, 12 h light–dark). Soil solutions were extracted by centrifugation, followed by 0.025 μm filtration. [Al] was determined as 'free' Al by the oxine-derivatised gel method. RRL = 100 × (root length at pH = *X*)/(maximum root length). The values of RRL are derived from the mean lengths for 15 seedlings (five seeds from each of three replicates); error bars are one *s*.

Column dynamics

Column size affects the efficiency of analyte capture. Columns with 20–80 μl capacity have been tested in batch and flow applications. They were found to effect quantitative retention of free Al for sample flow rates of *ca.* 1 ml min^{-1} onto the column. Columns with gel volumes of 16 μl or less do not effect quantitative retention of the analyte at this flow rate. At smaller flow rates (gel residence times $\geqslant 2.5$ s) significant amounts of Al were sequestered from moderately labile complexes. The question arises as to whether the effective reaction time is related to the residence time in the column, or controlled by the diffusion layer thickness about the 50–100 μm gel particles. This is not easily resolved by modelling because of the microporous nature of the gel and because the average distance of any element of solution from the close-packed beads of gel is of the same order of magnitude as the calculated diffusion layer thickness. Experiments using species of known lability, and species with known dissociation rate constants, are being used to establish the effective reaction time.[18]

Conclusions

Use of FIA to effect speciation of metals in moderately labile systems requires the separation of the speciation (fractionation) process from the analyte–reagent reaction. This can be achieved by using an adsorbent or immobilised complexing agent to effect the fractionation, followed by selective elution of the analyte and down-line reaction. If this protocol is not followed then matrix components may enhance or retard the analyte–reagent reaction and thus the analyte is over- or under-estimated. Furthermore the analyte–reagent contact time in typical FIA manifolds (*ca.* 15–30 s) is sufficient to effect substantial sequestering of a metal analyte from its various complex species. This leads to an overestimate of the 'free' metal fraction.

The principle of separated fractionation and reaction steps is illustrated by the use of an oxine-derivatised gel to capture 'free' Al from complex systems in a *ca.* 1 s contact time. From analysis of a pre-elution peak and selective elution of species captured on the gel, three Al fractions can be defined: 'free' Al, 'moderately reactive' Al and Al–hydroxy polymers [typified by $Al_{13}(OH)_{32}^{7+}$]. This oxine-derivatised gel has been used previously in macrocolumns for preconcentration of Al^{3+} and

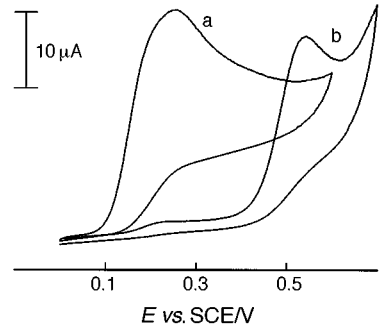

Fig. 10 Cyclic voltammograms recorded at ν = 100 mV s^{-1} for 1 mM 4-nitrocatechol in the absence (a) and presence (b) of 0.33 mM Al^{III}, pH 9.4.

Mn^{2+} from seawater,[29,30] but the dynamics, kinetics and possible miniaturisation have not previously been exploited.

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Paper 7/07293G
Received October 8, 1997
Accepted February 20, 1998