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**ANALYTICAL METHODOLOGY FOR THE
DETERMINATION OF ALUMINIUM FRACTIONS IN
NATURAL FRESH WATERS**

(Technical Report)

Prepared for publication by

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Analytical methodology for the determination of aluminium fractions in natural fresh waters (Technical Report)

Abstract - The distribution among different species, "speciation", is essential for understanding the toxicity, bioavailability and transport mechanisms for elements in the natural environment. The distribution of Al among different species has been in focus during the last twenty years or so, and many methods for fractionating Al have been developed. Most of them are operationally defined, since validation of what is really measured in natural systems is very difficult. This review is a thorough and critical survey of the existing methods for Al fractionation in natural waters, that covers the literature up to and including June, 1994. The methods have been classified into six groups, according to the principles used: 1) Kinetic or Binding Strength Discriminations; 2) Ion Chromatographic Separations; 3) Size Exclusion; 4) Non-Invasive Methods; 5) Ion Mobility in an Electric Field; 6) Minimized Disturbance. In addition to this, in order to validate the different techniques, we scrutinize some of the method comparisons published.

Abbreviations

AAS	Atomic Absorption Spectrophotometry	GFAAS	Graphite Furnace Atomic Absorption Spectrophotometry
ASV	Anodic Stripping Voltammetry	HQS	8-Hydroxyquinoline-5-sulfonic Acid
BuAc	Butyl Acetate	ICP-MS	Inductively Coupled Plasma - Mass Spectrometry
C-18	Octadecyl silica immobilized on silica particles	ICP-OES	Inductively Coupled Plasma - Optical Emission Spectrometry
CAS	Chrome Azurol S	INAA	Instrumental Neutron Activation Analysis
CDTA	Cyclohexane- <i>trans</i> -1,2diylidinitrilotetraacetic acid	ISE	Ion Selective Electrode
CFA	Continuous Flow Analysis	ITP	Isotachopheresis
CTA	Cetyltrimethylammonium (bromide) or <i>N,N,N</i> -trimethylhexadecan-1-aminium (bromide)	MIBK	Methyl Isobutyl Ketone
CZE	Capillary Zone Electrophoresis	MWCO	Molecular Mass Cut-off
DDDC	Diethylammonium Diethyldithiocarbamate	NAA	Neutron Activation Analysis
DEAE	Diethylaminoethyl Cellulose Anion Exchanger	NMR	Nuclear Magnetic Resonance
DOC	Dissolved Organic Carbon	Oxine	Quinolin-8-ol
ECR	Eriochrome Cyanine R	PCV	Pyrochatechol Violet
EDTA	Ethylenedinitrilotetraacetic acid	RSD	Relative Standard Deviation
FAAS	Flame Atomic Absorption Spectrophotometry	sect.	Section
FIA	Flow Injection Analysis	TOC	Total Organic Carbon
FISE	Fluoride Ion-selective Electrode	UV	Ultraviolet
Fr.	French		

Introduction

Al is the most abundant metal in the earth's crust, with a mean concentration in the earth's upper crust of 8.1 % (1). In most natural waters the concentration is low, due to the limited solubility of Al at the pHs normally encountered in pristine waters. Its solubility increases at lower pHs and by complexation with e.g. fluoride or organic substances. At some relatively rare places this has led to considerable concentrations of Al occurring in undisturbed waters.

In recent years, a profound change in this picture has emerged. As a result of man's activities, a considerable mobilisation of Al has occurred. In contrast to many other elements, direct emissions are of relatively little importance. The main cause is the increased input of acids into the environment, mainly through all forms of combustion; either from sulfur or nitrogen in the fuel, forming acid oxides, or through the formation of nitrogen oxides from the nitrogen in the oxidising air. The acid introduced into the environment is primarily consumed by carbonates in soil and water, but when this and other buffering systems are exhausted, the pH in solution will decrease. This leads to increased weathering of minerals and a concomitant release of Al.

Al has no known essential biological role for animals or plants (e.g. 2). For many years it was thought to be virtually non-toxic. Recent experience has shown otherwise. It is now well documented that dialysis fluids containing Al are the cause of a potentially lethal condition called dialysis encephalopathy (e.g. 3). The condition is caused by a slow degeneration of brain tissue brought about by Al introduced into the bloodstream via the dialysis unit. Al ingested with food is normally taken up to a small extent only (4) and is rapidly excreted as long as the kidneys are working properly (5,6). The similarities in symptoms between dialysis encephalopathy and the condition called Alzheimer's disease has nurtured an intensive search for evidence of Al as a causative agent for this disease too (e.g. 7, 8). So far these efforts have been in vain.

In surface waters, certain forms of Al are harmful to fish (9-12). The more reactive the species, the more harmful it is. Thus, the 'free' aqua-ion and the monomeric hydroxo complexes are the most harmful, while fluoro and organic complexes are less potent (e.g. 13, 14). Another way of action for Al seems to be through a precipitation of hydroxides on the gills, hampering oxygen uptake.

Aquatic invertebrates seem generally not to be as sensitive to Al as fish, although some toxic effects have been shown (15). Some field studies have demonstrated Al toxicity to amphibians, although the patterns here appear to be rather complex (16). Insect-feeding birds living along streams with increased levels of Al may be affected adversely, although this is not at all certain (17).

Plants and trees also suffer from high levels of Al in the root zone (18, 19). In this case there is strong evidence for an effect of polymeric Al species also (20). It seems that a disturbance in the cation balance of the fine roots arises. This leads to a decrease in the efficiency of water uptake and makes the plants vulnerable to drought and other forms of environmental stress.

Aluminium Species in Natural Fresh Waters

In aqueous solution, Al exists in a number of complexes, all in dynamic equilibrium. The 'free' aqua-ion, $\text{Al}(\text{H}_2\text{O})_6^{3+}$, is acidic, and hydrolyses even below pH 3. The aqua-ion therefore rarely dominates among Al species in natural waters. With increasing pH, monomeric hydroxo complexes are formed. If the pH is increased still further, polymerisation can occur. The exact nature of these polymeric complexes is still under debate. The main inorganic ligand, apart from hydroxide, is fluoride, that forms very strong complexes with Al. In natural waters, sulfato, carbonato, phosphato and silicato complexes also exist. In some cases, for example for the phosphato complexes, the formation constants of these complexes are only approximately known.

Most organic compounds containing combinations of carboxylic and hydroxylic groups form complexes with Al (e.g. 21). In natural waters, these groups are found in many compounds, from simple acids and hydroxyacids to more complex moieties such as fulvic and humic acids. The latter two are operationally defined groups of compounds of largely unknown composition. In natural waters with substantial amounts of these compounds present, a large part of the Al is invariably associated with them, except at very low pHs.

Fulvic and humic substances can associate with each other, forming larger conglomerates. With increasing molecular mass, the organic moieties get less soluble in water and form colloidal particles. These particles can contain Al bound to the organic ligands and also adsorbed on the surface. There are also other particulates in natural waters, such as clays, that can adsorb Al on their surface. It is thus not easy to discriminate between Al in solution and in solid phases. Some scientists question the value of filtration in fractionation studies. However, many analytical methods require pre-filtered samples to give repeatable results, and it is also well known that filtered samples are less sensitive to storage (see below). Therefore, it has become customary to filter natural water samples through e.g. a 0.45 μm or a 0.1 μm membrane filter before analysis.

In this paper, we will discuss the distribution of Al in solution as defined by a filtration through a 0.45 μm filter (if not indicated otherwise) and we only consider methods for the *fractionation of Al in natural fresh waters*. Therefore, determinations in biological media, in seawater and during water treatment, as well as methods for soil extraction, have normally been excluded.

Sample Handling and Sample Storage

Even though sample storage is not the purpose of this review, we would like to draw some attention to its importance. In fractionation studies, sample handling must be taken into account, since *any* treatment of the sample might change the distribution of an element among different species. As a rule of thumb, one could say that the less pre-treatment and the quicker a sample is analysed, the better. It is however customary to filter the samples and keep them dark at a low temperature, but not frozen, in order to decrease their vulnerability to storage. Because of changes in the fractionation during the necessary interval, field filtration and laboratory filtration may give different results, as in (22) results for iron.

It seems as if different samples have a different vulnerability to storage. Samples with a low level of 'labile Al', a large amount of organic matter and a pH of about 5-6 seem to be most sensitive (23-25). It is also believed that microbial activity can alter the humic material present in natural waters (26), and thereby affect their influence on the distribution of Al among different species. The distribution changes if the samples are stored at a temperature which differs greatly from their temperature when sampled (27). The containers used for storage can also be important. Fairman et al. (28) found that high-density polyethylene was a more suitable container material for storing water samples prior to speciation measurements than polypropylene and polystyrene. Lake and tap water samples were stable for up to 30 days when stored in polyethylene containers.

Obtaining reference samples for Al fractionation is also difficult (28, 29). For more information on sample storage, we recommend the references given above.

Analytical Methods for the Determination of Aluminium Species

There are at least three reasons why it is very difficult to elucidate the complete distribution of Al species in natural waters: 1) The large number of species, that all participate in dynamic interactions; 2) The low total concentration of Al, ranging from a few $\mu\text{g/L}$ Al in clear neutral waters to mg/L levels in brownish bog waters or waters with low pH; 3) The presence of organic and other substances that might interfere with the determination. The main problem is related to the fact, ignored by some workers, that an interaction with one species will inevitably lead to a rearrangement among the other species. Rearrangements among e.g. the monomeric cationic hydroxo species are extremely fast (30, 31) while the release of Al from chelate type complexes is a slow process, requiring days or more to reach equilibrium. This situation is different from what is prevalent for other elements, such as mercury, lead and tin. The complexity of the problem has forced researchers to use various *operationally defined* groupings of Al species. Although a total characterisation of the distribution of Al is desired, it should be borne in mind that from the environmental point of view, the most important fraction consists of the more toxic, simple inorganic species.

Many attempts to address this problem have been made over the years. The existing methods can be classified as follows, according to the principles used:

1. Methods based on *kinetic or binding strength discrimination*, e.g. cation-exchange, fluoride ion-selective electrode (FISE) and various spectrophotometric or fluorimetric procedures.
2. *Ion Chromatographic separations*.
3. Methods based on *size exclusion*, such as centrifugation, filtration, ultrafiltration, dialysis and size exclusion chromatography.
4. *Non-invasive* methods (e.g. NMR).
5. *Ion mobility in an electric field* (e.g. isotachopheresis or capillary electrophoresis).
6. *Minimized disturbance* methods (e.g. morin).

Below, we will give a short general description of the common background for each group as well as a short description of the methods. The results presented for each method will, where possible, be critically evaluated. For more information, we refer to earlier reviews on Al fractionation, of which we consider the one by Bloom and Erich (32) the most informative. Short reviews on the determination of Al species in natural waters have also been published by Driscoll and Schecher (33) and by Fairman and Sanz-Medel (34).

1. Methods Based on Kinetic or Binding Strength Discrimination

1.1. Ion Exchange

1.1.1. Cation Exchange

Cation exchange can be used to separate complexes of different charge. Since the most important inorganic forms of Al in natural waters are positively charged, while the complexes with e.g. humic acids are believed to be negatively charged, the technique can be used to fractionate Al into two groups of opposite charge. The basic idea is that positively charged complexes (mainly inorganic) should be retained in the column, while non-charged and negatively charged complexes (mainly organic) will pass through.

The first attempts to fractionate Al in water were made in the 1950s. Tanaka (35) used centrifugation for determining '*inséparable*' (Fr.) (rather 'non-centrifugable', authors' remark) and *total Al* and presented a cation-exchange method for separating *ionic Al* from *non-ionic Al forms*. The sample was injected manually with a syringe onto a column containing the resin Amberlite 120, at a flow rate of ca. 5 mL/min. Al was then detected spectrophotometrically after complexation with aluminon. Tanaka made no attempts to characterize what was actually measured with his method. He applied it to lake waters and obtained some kind of fractionation.

In the early eighties, Driscoll (36) combined a method similar to Tanaka's (35) with the reaction between Al and the complexing agent ferron, according to the work by Davenport (37) and Rainwater and Thatcher (38) (sect. 1.2.3.). Later he changed the complex forming procedure (39) to the oxine extraction method presented by Barnes (40) (sect. 1.2.1.). The two variations of the method are very similar, apart from the complexation procedures used, and are therefore described simultaneously.

Driscoll divided the sample into three parts (fig. 1); two of which were analysed using the complex forming reagent chosen, with and without prior acidification to pH 1 (for 30 min., ref. 36; 1 h, ref 39), respectively. He called these two parts 'acid reactive' (Al_i) and 'monomeric' Al (Al_m), respectively, the first of which he claimed represented the sum of colloidal, polymeric and very strongly bound organic complexes. The last part of the sample was run through a *cation exchange* column containing both Na^+ and H^+ saturated Amberlite 120, in proportions aimed at giving an ionic strength and a pH of the effluent close to those of the sample. Driscoll assumed that the inorganic forms of Al were retained in the column while the organic forms passed through. The latter fraction was then analysed with the spectrophotometric reagent in question, giving the 'non-labile monomeric Al' (Al_o). By subtracting the 'non-labile monomeric' Al from the 'monomeric', Driscoll obtained the 'labile monomeric Al' (Al_l), which he claimed was the sum of Al^{3+} and the most important inorganic Al complexes (sulfato, silicato, fluoro and hydroxo complexes). To minimize the dissociation of organic complexes within the column, he recommended flow rates above ca. 2.7 mL/min·mL resin, which correspond to a flow through time of approximately 2 s. Driscoll also validated his method (39), both by comparing the results for synthetic solutions with equilibrium calculations and by comparing the results for natural water samples with those obtained by measuring free and total fluoride and indirectly calculating the distribution of Al among different species (sect. 1.3.). The results agreed well for samples containing low levels of organically bound Al, but fairly poorly for those with higher levels.

The main advantage of this method is the new concept Driscoll introduced by combining two methods using different principles. By doing that, he could measure a fraction (Al_l) more relevant in fractionation studies, than the ones proposed earlier. This is supported by the fact that several later proposals have been based on Driscoll's method (see below). The method is by now classic and has been widely used for field work (e.g. 41- 47). Apart from being labour intensive, there are, however, some potential sources of error inherent in the method; e.g. that the complexing agent might desorb Al associated to particles and that the cation exchanger might make labile organic complexes dissociate. This may lead to an underestimation of

the organically complexed Al, which has later been shown by e.g. Backes and Tipping (48) (see Comparison of Methods section). Driscoll was well aware of these problems. Some of the drawbacks mentioned for methods based on the reagents used are of course also valid, since they are part of Driscoll's method (sect. 1.2.1. and 1.2.3.).

SCHEMATIC REPRESENTATION OF THE ALUMINIUM FRACTIONATION PROCEDURE

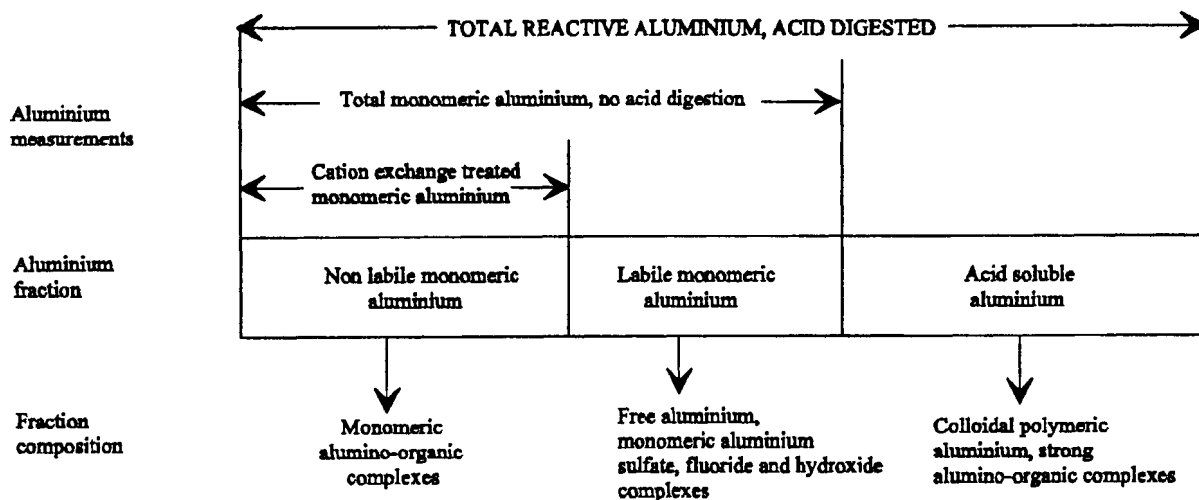


Fig. 1 A schematic representation of Driscoll's Al fractionation scheme, redrawn from Driscoll (39) with permission from Gordon & Breach.

From the rather extensive fractionation scheme that Driscoll (39) proposed, it is possible to obtain an even more complete picture of the distribution of Al among different species, using equilibrium calculations. By measuring a few 'support parameters', like pH, fluoride and sulfate concentrations, one could calculate $[Al^{3+}]$, using ALCHEMI (49) or some other computer program for equilibrium calculations. One must keep in mind, however, that some of the equilibrium constants for Al complexes are fairly uncertain, and for many ligands, especially organic ones, they haven't been determined at all. Therefore, one must be very careful when interpreting results obtained with equilibrium calculations. Otherwise, there is a risk of overinterpreting the results. Another difficulty is that natural waters are not necessarily at equilibrium. This is especially likely to be a problem if conditions are such that, for example, precipitation and dissolution reactions can occur.

Various minor modifications of Driscoll's method have been presented throughout the years. We try only to draw attention to the ones we think have contributed to the further development of the method.

Lydersen et al. (27) studied, among other things (sect. 3.2), the effects of temperature and pH changes on Driscoll's cation exchange method in synthetic solutions containing only hydroxo complexes. For solutions stored at 2°C, some of the cationic complexes were found to pass through the cation exchange column if a flow rate of 3.8 mL/min/mL bed volume of resin was used. By reducing the flow rate to 3.0 mL/min/mL bed volume, this was nearly completely avoided. When the cation exchange procedure was used on solutions in which the pH had just been changed from 4.5 to 5.5 or vice versa, the results showed a variation with time due to changes in the equilibria between monomeric and polymeric complexes. Within the first 40 min, up to 40 % of the inorganic Al passed through the cation exchange column. This figure was reduced to 10-20 % after 24 h.

Campbell et al. (50) proposed a fractionation scheme based on three different principles; *filtration*, *chelating cation exchange* and *photooxidation*. The sample was filtered through a 0.4 μ m polycarbonate membrane filter. Al in the residue on the filter was determined after acid digestion (A = 'acid-extractable particulate Al'). Three aliquots of the filtrate were then treated in different ways: 1) direct determination by GFAAS (B = 'total filterable Al'); 2) determination of Al after equilibrating it with a cation exchange resin in

a batch process for 30 min. (C = 'nonexchangeable filterable Al'); 3) photolysis ($\text{H}_2\text{O}_2/80^\circ\text{C}$ /mercury lamp/4 h) in order to destroy organic matter present, followed by chelating cation exchange (Chelex 100) (D = 'non-exchangeable inorganic filterable Al'). Two more fractions were then calculated, 'exchangeable filterable Al' (E = B-C) and 'nonexchangeable organic filterable Al' (F = C-D). The detection limit was $1\ \mu\text{g/L}$ and the RSD for replicate ion exchange analysis was $\pm 7\%$. This fractionation scheme was tested by Campbell et al. (51) on surface waters in Canada. The results were correlated with parameters such as DOC and pH, and with the results of equilibrium calculations using MINEQL. Campbell et al. (52) used a combination of the Barnes/LaZerte oxine extraction method (40, 53), filtration and chelating cation exchange according to Campbell et al. (50) and equilibrium dialysis according to LaZerte (53) to study the distribution of Al among different species in a Canadian stream during the spring snow-melt.

Andelman and Miller (54) developed a fractionation method based on Campbell et al.'s (50) chelating cation exchange procedure (Chelex 100). They proposed that, by varying the contact time between the solution and the resin, the total amount of Al could be grouped into four operationally defined fractions. Andelman and Miller studied how inorganic particulate Al as well as humic bound Al were affected by the presence of a cation exchange resin. They suggested that unfiltered samples would give more information about 'the dynamics of the aqueous Al species' and that the resin's tendency to shrink makes the batch technique more suitable than the column technique. We believe that applying a flow system to a column containing cation exchange resin is preferable, since it makes short and reproducible reaction times possible. Several authors have confirmed the importance of a short reaction time for cation exchange, in order to minimize the dissociation of organic complexes (e.g. 39, 55). Miller and Andelman (56, 57) used this modified method to study Al distribution among different species in a stream in Pennsylvania. They called their fractions 'rapidly exchangeable' (adsorbed within 1 h), 'moderately exchangeable' (1 to 4 h), 'slowly exchangeable' (4 to 24 h) and 'non-exchangeable' or 'inert' (more than 24 h or unadsorbed). They concluded that the 'rapidly exchangeable' fraction would consist mostly of small, charged monomeric and cationic polymeric species; the 'moderately' and 'slowly' exchangeable fractions mostly of labile inorganic and labile organic complexes, respectively, and the 'non-exchangeable' fraction of strongly complexed, organic or colloidal inorganic species and crystalline forms. Al associated with the 'particulate' fraction, defined using a $0.4\ \mu\text{m}$ filter, was also adsorbed by the resin, adding to the concentration of every fraction.

Røgeberg and Henriksen (23) mechanized Driscoll's cation exchange procedure by adapting it to a flow system, using pyrocatechol violet (PCV) as the complexing agent (sect. 1.2.2.). Blomberg and Bjärnberg (58) found that the separation of inorganic and organic Al complexes with cation exchange was unsatisfactory, although better results were obtained with Ca-loaded resin than with Na-loaded resin. Henshaw et al. (59) developed a flow injection method, in which the cation exchange technique in combination with PCV was used to fractionate Al in natural waters (sect. 1.2.2.). Achilli et al. (60) used reaction with PCV followed by cation exchange (DOWEX 50W-X12) to distinguish monomeric from polymeric forms of Al (sect. 1.2.2.).

Kerven et al. (61) investigated the use of cation exchange (Amberlite 200, K^+ form) and chelating cation exchange (Chelex 100, K^+ form) in removing 'inorganic monomeric' Al from solution. Fulvic acid/fulvate was not retained by either column, while some Al was released from the fulvate and retained. A decrease in the sample pH after passage through the columns was observed. The decrease was 0.8 pH units for the Amberlite 200 column and 0.2 pH units for the Chelex 100. This could be sufficient to cause the release of Al from the fulvate during passage through the columns. The iminodiacetate sites on the Chelex 100 column may also have competed successfully with the fulvate for Al during the 35 s. contact time.

Chakrabarti et al. (62) presented a fractionation scheme for various metals, one of which was Al. By combining *filtration*, *ultrafiltration*, *anodic stripping voltammetry* (ASV) and *chelating cation exchange*, they measured a number of operationally defined metal fractions in river water, rain and snow. Filtration through a $0.45\ \mu\text{m}$ filter was used first to separate 'soluble metal forms' of the metals from 'particulate metal forms'. The filtrate was then further fractionated through ultrafilters with cut-offs between 500 and 100,000 Da. The metal contents of the obtained fractions were then determined by ASV (Cu, Pb, Cd and Zn) or further treated with cation exchange (all metals). They performed the chelating cation exchange (Chelex 100) either through a column or as a batch technique. The metal content of the effluent was then determined with GFAAS. The fractions from the ultrafiltration step were classified as 'free metal ions' (ASV), 'very labile' (ASV-labile), 'moderately labile' (Chelex-column-labile), 'slowly labile' (Chelex-batch-labile) and 'inert'. Ion selective electrodes (ISE) were also proposed for measuring 'free metal ions', but the authors were aware that the detection limit of that method is not good enough for determining the low concentrations of most trace elements found in natural waters. The proposed fractionation scheme is a

combination of previously published methods for metal fractionation, rather than a presentation of a new method.

Quintela et al. (63) mechanized the cation exchange method in a flow system, using PCV as the spectrophotometric reagent (sect. 1.2.2.). They used Amberlite IR-120 P resin.

Cellulose HYPHAN, a chelating ion exchanger, was used by Burba (64). He used the kinetics and the degree of the ion exchange reaction as operational parameters for metal lability in isolated humic substances. This method was applied to the speciation of a number of metals, including Al, in humic complexes. He was able to distinguish four kinetically different metal fractions in the humic substances.

Lu et al. (65) applied graphic and iterative methods for analysing kinetic data to the uptake of Al^{3+} and its hydroxo complexes by a chelating cation exchange resin (Chelex-100). Three kinetically distinguishable components were found.

1.1.2. Anion Exchange and Combined Anion and Cation Exchange

The use of anion exchange resins in Al fractionation is uncommon. As opposed to cation exchange resins, anion exchange resins are believed to retain negatively charged species (e.g. Al-humic acid complexes), and let positively charged species through (e.g. most inorganic Al species). Thus, anion exchange could be a method for separating inorganic from organic forms of Al. It is, however, well known that in methods for isolating humic substances from freshwaters, only a fraction of the organic material is caught by the anion exchanger (66).

Rosén (67) passed samples from three Swedish watersheds through an unspecified anion exchanger. 30-63 % of the Al passed through the anion exchanger, indicating that the rest was transported as anions, presumably mostly in the form of humic complexes. Anionic silicato complexes of Al in synthetic solutions have also been studied using an anion exchange resin, Dowex 1-X8 (68), (see also in sect. 1.2.3., 1.4. and 3.5.).

Duffy et al. (69) used a combination of anion and cation exchange to determine various metal species in soil pore water, among them Al. After centrifugation and filtration (0.4 μm), the samples were passed through an anion exchange column (diethylaminoethyl-Sephadex) under vacuum suction. The effluent was then run through a cation exchange column (sulfopropyl-Sephadex), and the resulting effluent discarded. Both fractions that had been retained in the two columns were eluted with acid and analysed by NAA, yielding 'anionic' and 'cationic' complexes, respectively. Duffy et al. compared the described method with a variation of the oxine extraction method (sect. 1.2.1.), using different reaction times, as well as with measurements with a fluoride ion-selective electrode (sect. 1.3.). The authors claim that there was 'a reasonable agreement' between their method and the modified oxine method. This can be accepted for the two organic fractions estimated, but not for the inorganic ones, for which a systematic error seems to have been present.

The combined anion and cation exchange method gave in general higher values for 'cationic Al' than corresponding values of 'monomeric inorganic Al'. Duffy et al.'s method gave much higher values of 'cationic Al' than did the estimation of 'inorganic Al', performed with the fluoride electrode. A critical factor for the anion exchange column is whether the removal of Al humic acid complexes will change the distribution among species of the inorganic fraction of the sample. Our experience, when trying to remove organic substances from natural water samples high in DOC (unpublished results), indicates that this is the case. Both the levels of 'quickly reacting Al' and pH changed after treatment in an anion exchange column. Duffy et al. reported changed pH values in pore water samples that had passed the anion exchange column.

Lexén and Borg (70) used anion exchange together with cation exchange in their mechanized PCV method. The cation exchanger (using Amberlite IR-120 resin) and anion exchanger (Amberlite IRA-400) were placed one after the other, and the samples, taken from Swedish lakes and streams, ran through both. The results indicated the presence of anionic Al complexes. However, as Lexén and Borg noted, the anion exchange resin that they used was finer grained than the cation exchange resin. This meant that some complexes could have been trapped in the anion exchanger because of their size instead of their charge.

Pettersson (71) and Pettersson et al. (72) applied a fractionation procedure using both anion and cation exchange, developed by Hiraide et al. (73), to Al. After filtration with a 1.2 μm filter, part of the sample was passed through an anion exchange resin (diethylaminoethyl-Sephadex A-25) and part through a cation exchange resin (sulfopropyl-Sephadex C-25). This was done in the field. Pettersson et al. then used ICP-OES to determine the amounts of Al desorbed from the resins, using these to obtain an estimate of the

distribution of Al between anionic and cationic forms. One problem was incomplete desorption of the metal. In order to take this into account, two calculation procedures were used to estimate the amount of metal bound to the exchangers. In the first, the decrease in concentration due to passage through the resins was used as the basis of mass balance calculations. The second procedure used the amount of the metal released in desorption.

Haraldsson et al. (74) presented a fractionation scheme using different column packings: Chelex-100 in the calcium form for 'free' metal ions and labile complexes, DEAE (Diethylaminoethyl Cellulose Anion Exchanger) for humic acid complexes and C-18 for non-polar organic complexes. The flow rate used was 2 mL/min for all three columns. The metal concentrations in the fraction that passed through the columns were determined using ICP-MS. This scheme was tested on a number of metals, including Al.

1.2. Spectrophotometric or Fluorimetric Reagents

One of the most frequently used strategies for Al fractionation is *kinetic discrimination*, using a spectrophotometric or fluorimetric reagent. The idea is that if one could use a short enough reaction time, only the quickly reacting or 'labile species' of Al would have time to react, while more slowly reacting forms of the metal would not. For several organisms, the quickly reacting forms of Al seem to correspond fairly well to those forms believed to be the most toxic ones.

The reagent is normally added in great excess, in order to obtain simple kinetics. The pH of the reaction is adjusted, a reagent is added, and after a suitable reaction time, the concentration of the formed complex is detected. Choosing a reaction time that discriminates 'labile' Al complexes from 'non-labile' is difficult, since there is no clear distinction between the two groups. This has led to operationally defined methods. Most authors have tried to choose as short a reaction time as practically possible. The advent of mechanized methods has made it easier to control the reaction time.

Interference from other metal ions can be a problem, but is normally overcome by the use of a masking agent. The presence of humic substances in natural waters can also cause interference, if detection is made spectrophotometrically or fluorimetrically at a wavelength in the UV or lower visible range. There are at least two ways of minimising this interference; either by using a reagent that forms complexes with Al, absorbing at wavelengths at which humic acid has got a low absorption, or by extracting the complex formed into an organic solvent, to which the extraction of humic substances is not large enough to cause interference. Extraction can also be an efficient way of terminating the reaction.

1.2.1. Oxine

A few years after Tanaka's proposal (sect. 1.1.1.), Goto, Okura and co-workers (75, 76) introduced another approach for fractionating Al, namely letting it react with a spectrophotometric reagent, in this case *oxine* (Quinolin-8-ol, 8-Hydroxyquinoline). The reagent is a classic one for metal determinations and has been used since the 1920's for gravimetric and spectrophotometric determinations of total Al (e.g. 77). The principle of the spectrophotometric method is to let Al react with oxine for a certain period of time at a chosen pH, then extracting the resulting Al trioxinate complex, that has a very limited solubility in water, into an organic solvent, e.g. chloroform or methyl isobutyl ketone (MIBK). The concentration of Al is then determined in the organic phase, either by spectrophotometry at around 390 nm, or by atomic absorption spectroscopy. Since oxine is not specific for Al, various metal ions might interfere, but studies have shown that the only seriously interfering metal ion present at high enough concentrations in natural waters, is iron. A masking step is usually used to minimize iron's interference. In some cases organic substances present in natural waters, e.g. humic substances, may also interfere.

Among the advantages of extraction are that it is an efficient way of terminating the complexation reaction, it minimizes the risk of interference from humic substances, it allows the detection limit to be improved by extracting from a larger volume of water to a smaller volume of the organic solvent and it gives the possibility of storing the extract for later analysis. The main disadvantages are that organic solvents are harmful both to human health and to the environment, and that an extra step is added to the analysis, thus increasing the risk of error. The extraction may be done at a different pH than the complexation. Extraction at pH 8 will quantitatively separate fluoro complexes but leave enough oxine anions in the aqueous phase for the complexation to proceed. At pH 5 fluoro complexes are not included in the extracted part but the reaction between Al and oxine is efficiently stopped since very little oxine will remain in the aqueous phase (78).

Goto and Okura used the same basic idea as did Gentry and Sherrington (79) in their extraction procedure for determining total Al, but by minimising the contact time between Al and oxine, only some forms of Al had time to react. The complex forming reaction was interrupted after about 10 s by extracting into chloroform the Al trioxinate complex formed, and then detecting it spectrophotometrically. The pH varied between 4.5 and 9.5. In this manner they could determine 'Al in true solution' in the presence of colloidal forms of Al. They did not investigate which Al species were included in the measured parameter, but suggested that it was ionic or of a 'very low degree of polymerisation' (75). Later they clarified this statement, proposing that the extractable Al was 'monomer or dimer' (76). Goto, Okura and co-workers made no attempts to apply their method to natural waters, but nevertheless, it must be considered as a milestone in the field of Al fractionation. Many authors have used their basic concept for developing a number of methods for Al fractionation in natural waters (see below).

Linnell (80) studied the kinetics of precipitation of the Al trioxinate complex. From the results, Linnell assumed that monomeric hydroxo complexes would react immediately. Polymeric hydroxo complexes, on the other hand, would first have to be broken down to monomers before their Al could react. These results, obtained gravimetrically, were an early support for the concept of kinetic discrimination.

Turner (81) combined filtration through a 10 μm membrane filter with Goto's spectrophotometric method in order to fractionate Al in synthetic Al-OH solutions into three parts. He called them 'monomeric Al', 'polynuclear Al hydroxide' and 'solid Al', respectively. He concluded that Al^{3+} , AlOH^{2+} and $\text{Al}(\text{OH})_4^-$ were measured as the first fraction, with a reaction time of 10 s, which is in accordance with what we found for our FIA method (82). In Turner's method the reagents were premixed, so as to minimize the time lag between the addition of reagents and the extraction. In this way, the reaction time was better defined than in the case of Goto and Okura. Turner and Sulaiman (83) slightly modified Turner's method from 1969 and studied the kinetics of the oxine method in the presence of acetate and polynuclear Al hydroxo species. They concluded that the concentrations of both acetate and oxine influenced the reaction rate for polynuclear hydroxyaluminium, so that increased concentrations of the two led to higher reaction rates. This may be important, since different variations of the oxine extraction method have used different concentrations of oxine as well as of acetate.

Luciuk and Huang (84) used a variation of Turner's method to study aluminosilicates. They used ultrafiltration (sect. 3.2.) with a 0.025 μm filter. The filtrate was then analysed according to Turner's method with 10 s and 20 minute extraction times. These fractions were called 'readily extractable' and 'moderately extractable' Al; that which remained was called 'nonextractable'. Luciuk and Huang chose these names because they recognised the operational nature of the fractions.

Barnes (40) further developed the oxine extraction method and applied it to Al fractionation in *natural waters*. Her method was based on the work of Goto et al., Okura et al., and Turner. After filtering the sample through a 0.1 μm membrane filter and adding a pH indicator, Barnes let Al complex to oxine. She adjusted the pH to ca. 8 by slowly adding ammonia and then extracted the formed complex into MIBK by shaking the mixture for 10-30 s in a separatory funnel. The reason for adjusting the pH before extraction was to minimize interferences possibly present in natural waters (e.g. other metals and fluoride). For samples containing high levels of Fe ($1 < [\text{Fe}] < 10$ mg/L), she used hydroxylamine and 1,10-phenanthroline as a masking buffer. Flame atomic absorption (FAAS) was used for detection and she obtained a detection limit of ca. 2 $\mu\text{g/L}$ Al. The measured, operationally defined, parameter was called 'dissolved and readily reactive species of Al', which she claimed represented 'rapidly reacting equilibrium species' of Al, but not 'metastable or slowly reacting macro ions and colloidal suspended material'.

There are, however, some weak spots in Barnes' method: 1) The fairly slow pH adjustment of the sample when oxine is already present, which might change the species distribution in the sample and the kinetics of the reaction between Al and oxine; 2) The poorly defined reaction time, which may make it difficult to compare the results for samples and standards, as well as the results from different samples; 3) The large sample volume used (400 mL), which can make it difficult to analyse some types of samples, e.g. soil solutions; 4) The laboriousness inherent in manual methods. An advantage of the method is the choice of detection method (FAAS), which enhances the selectivity of the method and gives good precision in the detection step, compared to graphite furnace atomic absorption (GFAAS). In spite of the drawbacks mentioned above, many of which it shares with most other methods based on a quick reaction with a spectrophotometric or fluorimetric reagent, this method has proved to be very useful for fractionation studies, mostly in combination with cation exchange techniques. It has been widely used for field work, and further developed during the last two decades.

Bache and Sharp (85) modified the methods of Okura et al. (76) and Turner (81) in order to distinguish 'mononuclear' from 'polynuclear' Al in soil extracts. For 'mononuclear' forms, they used a 30 s reaction time instead of Turner's 10 s, while for 'total' Al they used a 30 min reaction time. 'Polynuclear' Al was defined as the difference between 'mononuclear' and the 'total' concentration. These definitions are somewhat arbitrary: small polymers could react partially within less than 30 s Al complexed organically may also, as Bache and Sharp pointed out, have reacted to some extent.

Bloom et al. (86) also used an extraction procedure with oxine, but instead of MIBK or chloroform, they used butyl acetate (BuAc) as the solvent. For detection, they used either UV-visible spectrophotometry or fluorimetry, the latter making it possible to improve the detection limit from 50 to 0.3 µg/L. The pH of the complexation reaction and the extraction was 5 and the reaction time 15 min. They suggested, however, that an immediate extraction should minimize the dissociation of the organically complexed Al. Bloom et al. made a thorough study of metal interference, but no characterisation of which Al species were measured with their method. The spectrophotometric method of Bloom et al. was used for the study of Al and organic matter in soils (87). May et al. (88) improved the detection limit for the oxine extraction method by changing the solvent from MIBK to toluene. Since toluene is less soluble in water than e.g. chloroform (88), which is in turn less soluble in water than MIBK, they could use much smaller volumes of the organic phase and thereby enhance the sensitivity. The detection limit obtained was 0.2 µg/L - a tenfold improvement on the one reported by Barnes - and still one of the best found in the literature. The *reaction time varied with sample size* between 10 s and 5 min. This approach seems very strange, since it will yield different fractionations for samples of different size. May et al. used a masking step that differed from the frequently used hydroxylamine/1,10-phenantroline system. They removed possibly interfering metal ions by a pre-extraction step, using DDDC (diethylammonium diethyldithiocarbamate) as the complexing agent. James et al. (89) slightly modified Bloom's method with UV-visible detection, by reducing the reaction time to 15 s, aiming at minimising the dissociation of Al complexes. They found that Al³⁺ and AlOH²⁺ were measured, but not Al(OH)₂⁺. This conclusion is not in accordance with other work (78, 81, 90). We find it unlikely that it would be possible to separate AlOH²⁺ from Al(OH)₂⁺ with the technique used, since proton reactions are known to be extremely fast (30, 31). A possible cause of this disagreement may be the use of different equilibrium constants for the hydroxo complexes of Al. The method of James et al. has been applied to waters from forest soils (91).

LaZerte (53) speeded up Barnes' oxine extraction procedure by simplifying the method for pH adjustment. The sample was mixed with a pre-prepared buffer that should give a final extraction pH of 8.3-8.8. This reduced the contact time between Al and the reagents to ca. 15 s, and the measured parameter was called 'fast reactive Al'. He also minimized the sample volume to ca. 3-5 mL - a factor 100 compared to Barnes - and detected the extracted Al with GFAAS instead of FAAS. LaZerte did not specify the detection limit of the method, but reported the 'overall sensitivity' (not defined; authors' remark) to be ± 2 µg/L ± 2% to ± 6 µg/L ± 12%. We are not completely sure what LaZerte meant with the concept. The fluoro and citrato complexes of Al were measured partially with the method. The major improvements with LaZerte's modifications are: 1) The increased speed of analysis; 2) The small volumes used; 3) A more closely defined reaction time. A disadvantage is the worsened precision obtained when using GFAAS instead of FAAS. Some researchers have replaced Barnes' step in Driscoll's method by LaZerte's method.

Lalande and Hendershot (92) used a method similar to Bloom et al. (86), but varied the reaction time and pH in order to obtain different fractions of Al. They used 15 s at pH 5.0; 15 s at pH 8.3 and 60 min. at pH 8.3. To correct for iron interference, they measured at two wavelengths. Even for the shortest reaction time used at pH 5, some of the Al citrato or fulvic acid complexes dissociated. Neither fluoro, nor oxalato complexes were, however, measured. At pH 8.3, with a reaction time of 15 s, a larger portion of the organically bound Al was measured. The results for oxalato and citrato complexes opposes what we found with our FIA method, which did not measure Al citrato complexes, but a small fraction of Al oxalato complexes (82, 90). Jeffries and Hendershot (93) slightly modified the method of LaZerte (53) by backextracting the Al once extracted into MIBK with HNO₃ and then detecting Al in the water phase with a 'DC plasma emission spectrograph'. They used this method, and various other methods, for studying the variability of Al species in surface and subsurface waters in Canada.

Hanning (78) did a lot of the basic theoretical and practical work for applying a variation of the manual oxine extraction method to a *FIA system*. Compared to manual procedures, extraction in a flow system has the advantage that the operator is not exposed to the organic solvent, and also that smaller amounts of the organic solvent are used. Flow extraction is also much faster and often more efficient than its manual

counterpart. Hanning strongly criticised the concept of operational definition often used in this area of Al fractionation and had interesting ideas of how to improve the stringency of the field. He proposed two mathematical methods for evaluating kinetic data. One of them, based on *Laplace transformation*, failed due to computational problems for experimental data. Another, *the initial rate method*, worked, but was rather time consuming, since it needed at least 3-4 measurements at different reaction times for each sample. Hanning's greatest contribution to the field of Al fractionation is probably the way he highlighted the problems inherent in operationally defined methods.

At our lab, we developed a mechanized method for Al fractionation, based on Hanning's ideas on kinetic discrimination in a flow system (82, 90, 94, 95), using oxine as the complexing agent. After a masking step minimising iron interference, using hydroxylamine/1,10-phenanthroline, Al reacts with oxine at pH 5.0 for 2.3 s. The complex formed is then extracted at pH 5.0 into chloroform and measured spectrophotometrically at 390 nm. We tried to characterize the method thoroughly by comparing the results with equilibrium calculations (90), as well as with both equilibrium dialysis (94, 95) and a variation of Driscoll's method (25). The method measures the sum of Al^{3+} , cationic monomeric hydroxo complexes of Al, its sulfato, silicato and carbonato complexes. Complexes between Al and fluoride are not measured, nor are the complexes with citrate, humic or fulvic acids, nor are the large polymeric hydroxo complexes. Complexes with oxalate are measured to a small extent and its complexes with salicylate are measured to a large extent. The phosphato complexes are also measured partially. The method gives reproducible results with a direct measurement of 'quickly reacting Al' (Al_{qr}) at a reaction time as short as 2.3 s and consumes small amounts of samples (injection volumes: 12-250 μL). The detection limit is 5 $\mu\text{g/L}$ and the sample throughput 66 injections per hour. The method has been further developed in two different directions; 1) a simultaneous determination of Al and Fe species by multi wavelength spectrophotometry and multivariate calibration; 2) an improvement of the detection limit by coupling the flow system to a GFAAS. One drawback of the method is the impaired precision for samples with high levels of organic material. We believe that this is due to the surfactant properties of, for example, humic matter, that affects the segmentation in the extraction process. That the sulfato complexes, which are less toxic than the 'free' form, are included in the measured fraction can also be seen as a drawback. However, no spectrophotometric method is known that excludes these complexes (e.g. 96). This problem can be dealt with by correcting for the sulfato complexes using equilibrium calculations.

1.2.2. Pyrocatechol Violet

Another spectrophotometric reagent that has been widely used for Al fractionation is pyrocatechol violet (PCV), or 4,4'-(3*H*-2,1-benzoxathiol-3-ylidene)bisbenzene-1,2-diol *S,S*-dioxide. The complex formed is, as opposed to Al trioxinate, fairly soluble in water, and therefore measurement is made without extraction, thus avoiding the use of organic solvents. An advantage with this reagent compared to, for example, ferron, is that its Al complex absorbs light at higher wavelengths, measurement being made at around 580-585 nm. This makes it less sensitive to interferences due to absorption of light by the humic substances which are often present in natural waters. The pH used is around 6.1, buffering normally being performed using hexamethylenetetramine. PCV methods are sensitive to iron interference, which is usually dealt with using reduction of iron(III) by hydroxylamine followed by complexation with 1,10-phenanthroline. Sometimes standard iron solutions are used for correction. A possible problem is that catechol compounds form complexes with silicon from silicic acid (97). This problem has seldom been discussed (an exception is the mention by Exley and Birchall (98)). To what extent the formation of complexes between PCV and silicon will affect Al fractionation with PCV in silicate-rich waters is unknown.

A method presented by Dougan and Wilson (99) for the determination of total Al has often been used as the basis of fractionation methods. They found that colour development was fast, the rate of increase after about 10 min being small. Acidified samples were used in order that all Al should be in such a form that it could react with PCV. The pH of reaction was buffered to between 5.9 and 6.2. Fluoride was the only serious interferent. The method of Dougan and Wilson was mechanized by Henriksen and Bergmann-Paulsen (100) and by Grigg and Morrison (101) using Continuous Flow Analysis (CFA). Helliwell et al. (102), studying Al toxicity in algae, used the Dougan and Wilson method to measure 'labile Al' (left undefined) as well as total Al in synthetic solutions.

Seip et al. (103) modified the PCV method to measure both total and monomeric Al. They determined total Al by acidifying the samples to a pH of about 1.4 for 24 h before adding the reagents, then waiting 40 min before measuring. For the determination of monomeric Al, the solution was not acidified in advance, and a 4 minute interval was deemed sufficient before measurement. Seip et al. determined total and monomeric Al in stream water samples. However, it was not made clear exactly which species were included in 'monomeric Al'. For example, some small Al hydroxo polymers could react fairly quickly with PCV. They also compared the PCV method with the ferron method (sect. 1.2.3. and under 'Comparison of Methods').

Noller et al. (104) published a fractionation scheme using filtration and kinetic complexation with PCV, based on Dougan's and Wilson's method. They used PCV to measure Al in unacidified samples that had passed through a 0.1 μm filter. This Al fraction was called 'labile monomeric Al', and assumed to contain the monomeric hydroxo and fluoro complexes. It was not, however, made clear how fluoro complexes would react in an unacidified sample as opposed to an acidified one. The part of the fractionation scheme involving filtration is discussed in sect. 3.1.

Røgeberg and Henriksen (23) combined the method of Henriksen and Bergmann-Paulsen (100) with Driscoll's ion exchange procedure (39) and defined the measured fractions as 'total monomeric Al' and 'non-labile monomeric Al'. After mixing the unacidified sample with the reagents, and after a delay of 4-5 min, measurement was made at 580 nm. The method was tested both in the laboratory and in the field. The fractionation depends largely on the cation exchange column, the use of which is discussed in sect. 1.1.1. Kramer et al. (11) tested the response of a system similar to that of Røgeberg and Henriksen for synthetic solutions with various complexing agents. They found that fluoro, citrato, malato and tartrato complexes of Al were not included in the measured fraction. For the salicylato complexes, there was disagreement between the expected and found values. LaZerte et al. (105) compared a method based on that of Røgeberg and Henriksen with equilibrium dialysis combined with PCV analysis for total reactive Al (see under 'Comparison of Methods').

Other researchers have combined the method of Dougan and Wilson (or methods based on it) with ion exchange. Sullivan et al. (24) compared the method of Seip et al., combined with ion exchange, with Driscoll's (39) method (sect. 1.1.1. and under 'Comparison of Methods').

Blomberg and Bjärnberg (58) used PCV in combination with cation exchange in connection with tests on liming of natural waters (sect. 1.1.1.). The PCV method used was the Swedish Standard method (106), a variation of the method of Dougan and Wilson. Digested, acidified and unacidified samples were used to obtain different Al fractions. Reaction time was found to influence the result, although for reaction times over 2 min, only for highly coloured waters. Blomberg and Bjärnberg explained this as being due to the longer time needed for the dissociation of Al complexes with humic acids. Bjärnberg (107) and Lexén and Borg (70) studied the distribution of Al among different species in natural surface waters using cation exchange and PCV, in some cases using a mechanized method similar to that of Røgeberg and Henriksen. The mechanized system gave good reproducibility. Lexén and Borg also used anion exchange together with cation exchange in their mechanized system (sect. 1.1.2).

Gunn et al. (108) used PCV in the study of Al toxicity to bacteria. With reaction times as short as 30 s, they found that citrate, fulvic acid and fluoride all reduced both the toxicity and the absorbance. Similar results were obtained using peat marsh water spiked with Al.

Goenaga et al. (109) combined filtration through different membranes (sect. 3.1.) with cation exchange and PCV determination. The reaction time used was 10 min, an unusually long time for the determination of concentrations of 'labile' Al. They distinguished four fractions. 'Total Al' (Al_t) was determined with PCV after acidification followed by 24 h' storage. 'Monomeric Al' (Al_m) was determined with PCV in the unacidified sample. 'Nonlabile Al' (Al_o) was determined with PCV in an unacidified sample after passage through a cation exchange column. 'Exchangeable Al' (Al_i) was the difference between Al_m and Al_o . Al_t was affected by the pore diameter, small particles being removed at both the 0.4 μm and the 0.015 μm levels. Some of this loss could also be due to adsorption on the membrane. The levels of Al_m and Al_i also decreased with decreasing pore diameter. This could be due to adsorption on the membranes, or due to Al associated with particles or colloids being determined as Al_m or Al_i . If the latter is the case, the results may depend on the long reaction time used. Goenaga et al. showed that, with their method, Al adsorbed to illite was partially included in the measured fraction. Al losses during filtration of synthetic solutions in which no particles were present, were under 10 %.

PCV has also been used in flow injection methods. Henshaw et al. (59) developed a flow injection method for the determination of 'monomeric Al'. Theirs was a two-channel system, the first channel measuring 'total monomeric Al' and the second channel 'non-exchangeable monomeric Al'. A cation exchange column was used to remove exchangeable Al in the case of the second channel. The mixing time was 1.3 s before buffering to pH 6.1, followed by 1.6 s reaction time at this pH. Fluoro complexes were included in 'monomeric' Al. On the other hand, strong Al complexes with organic ligands, for example with citrate, were not included in the 'total monomeric' fraction. Fulvate and humate in 10-fold molar excess caused a reduction in this fraction. The fulvato complexes were completely excluded from the 'non-exchangeable monomeric' fraction, while humate caused a 70 % reduction in the signal. A more detailed study of the effect of organic ligands on the measurement of 'total monomeric' Al was performed by Lewis et al. (110), with similar results.

Dobbs et al. (111) developed an on-line discrete sample analyser, combining spectrophotometric determination with PCV and cation exchange as in Driscoll's method (39). This instrument was designed for continual monitoring over a longer period of time in combination with instruments for measuring pH, conductivity, dissolved oxygen and fish stress.

Kerven et al. (112) proposed a PCV method with a reaction time of 60 s for the determination of 'inorganic monomeric Al' in soil solution. The method, which had a linear response in the range 0.2-25 $\mu\text{mol/L}$, gave a value which could be correlated with the relative yield of peanuts in acid soils. However, tests with various organic ligands, including fulvate, showed that a certain amount of the organically complexed Al was included in the measured parameter. This method was combined with filtration and ultrafiltration by Menzies et al. (113), see sect. 3.1.

Morrison (114) found that even reaction times as short as 10 s were too long to provide a true measurement of inorganic Al. He suggested that some colloidal species might be included. The reaction of Al with PCV in the presence of both fulvic acid and model ligands was studied using complexation titrations. Morrison then estimated the complexation capacities of some lake water samples using PCV with a 30 minute reaction time.

Gensemer (115) and Riseng et al. (116), studying the effect of Al on diatoms, used a method similar to that of Seip et al. (103), measuring Al in both acidified and unacidified samples using PCV.

Achilli et al. (60) used complexation of 'monomeric' Al with PCV *before* passing the sample through a cation exchange column. The reaction time used for the complexation of 'monomeric' Al was not defined. 'Polymeric' Al, which reacts more slowly with PCV, should not be complexed. 'Monomeric' Al, complexed with PCV, should pass through the cation exchange column. On the other hand, 'polymeric' Al is retained on the resin. After preconcentration, they used ICP-OES to determine 'monomeric' Al in the fraction that had passed through the column. Al in the form of fluoro complexes was in many cases not included in the 'monomeric' fraction, although this depended also on the amount of PCV used. The method was tested on geothermal brines and hot spring waters.

McAvoy et al. (117) found that a combination of Driscoll's method with LaZerte et al.'s modification of Røgeberg's and Henriksen's method could yield good results in the determination of 'monomeric' Al fractions. Interference by Fe, F and organic matter did not appear to be a problem. Shultz et al. (118) and Hruška and Krám (119) used the same combination of methods to fractionate Al in Coxing Kill, New York State and the Lysina catchment in the Czech Republic, respectively.

Fairman and Sanz-Medel have recently (120) described a variation on a batch cation exchange/PCV method, comparing it with a flow injection system using fluorescence detection (sect. 1.2.6. and the 'Comparison of Methods' section). The resin used was Amberlite IR-120.

Quintela et al. (63) developed another flow injection method using PCV and cation exchange. The reaction time used was 15 s. They tested their method on freshwater samples, comparing the results with those obtained using the manual Driscoll method. They found the manual method to be more sensitive, but that their method was faster and gave better reproducibility.

Benson and Worsfold (121) used the flow injection method of Benson et al. (122) for the determination of 'monomeric' Al. After masking of iron, the reagent is added, then after another 3 s, the pH buffer. After another 4.5 s, the Al-PCV complex's absorbance is measured at 583 nm. No characterisation was done, nor did they show that the method effectively separated monomeric from polymeric Al.

Kramer et al. (123) applied a continuous flow method using PCV to the determination of conditional equilibrium constants between Al and a number of ligands, as well as to the determination of 'reactive' Al.

1.2.3. Ferron

Ferron (8-hydroxy-7-iodoquinoline-5-sulfonic acid) is a derivative of oxine that forms a water-soluble complex with Al which absorbs light with a maximum around 370 nm. The use of this wavelength could lead very easily to interference from humic substances, resulting in an overestimation of the Al concentration, a fact that has seldom been discussed.

The use of ferron for Al fractionation goes back to Hem and Roberson (124), who studied Al hydroxo complexes. They used the method for total Al determination of Davenport (37) and Rainwater and Thatcher (38), in which the sample was buffered to pH 5 using acetate either before or after the addition of the ferron. Rainwater and Thatcher used hydroxylamine and 1,10-phenanthroline to mask iron, while Davenport suggested that iron could be corrected for by measuring the absorbance at a different wavelength. This method of correcting for iron interferences has been used by many researchers (e.g. 36, 125). Hem and Roberson (124) found that, even after acidification of the sample, some forms of Al in aged solutions were very hard to measure using ferron. This Al appeared in at least some cases to consist of small gibbsite crystals (126). Hem and Roberson modified the ferron method, assuming that the spectrophotometer reading after a few (undefined) min represented 'Al³⁺ and AlOH²⁺ with relatively insignificant amounts of polymerized forms'.

Smith (127) adapted the method, creating a timed spectrophotometric method similar to that of Turner (81) for oxine. An advantage compared to Turner's method, at least for synthetic solutions, was that there was no need for extraction. An important modification of the ferron method was that Smith measured at pH 5, whereas a pH of 1.5 had been used previously. Smith was interested in the separation of monomeric from polymeric Al in solutions containing only hydroxo complexes. By taking readings at intervals, first of 3-4 min, then of longer periods, he could follow the progress of the reaction with time. From the reaction curve, he concluded that the Al species present could be divided into three classes. Al which reacted almost immediately was designated Al^a, and considered to consist of simple monomeric species, although this was not proved. Al^b, reacting more slowly with ferron, was considered to represent polynuclear species, while Al^c, represented by the nearly flat portion of the reaction curve, was supposed to represent small solid particles. The concentration of Al^b decreased upon ageing, while that of Al^c increased. The fractions Al^a, Al^b and Al^c were studied further by Smith and Hem (128). Lind and Hem (129) found that the presence of quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one), used as a representative large organic molecule, led to smaller amounts of Al^a and larger amounts of Al^b being found, and to a slowing down of the process of crystallisation of gibbsite. They believed this to be due to interference by quercetin in the nucleation and growth of Al hydroxide particles rather than to the formation of complexes between Al and quercetin.

Smith's and similar methods have been used very widely for the study of Al in synthetic solutions (e.g. 130, 131), in waste water treatment (e.g. 132) and in toxicity studies (e.g. 133, 134, 135).

Bersillon et al. (136) modified the method for the measurement of 'monomeric' Al. They used a premixed reagent solution containing ferron, sodium acetate, hydroxylamine and hydrochloric acid that had been allowed to mature for 5 days. Also, they assumed, somewhat arbitrarily, a 30 s reaction time before measurement to distinguish 'monomeric' from 'polymeric' Al. Barnhisel and Bertsch (137) also recommended a modification of the method for the study of hydroxo complexes, recognising however that organically complexed Al might complicate determinations of polymeric Al by reacting slowly with ferron. Smith's Al^c was considered by Tsai and Hsu (138, 139) to consist of slowly reacting polymers. Lee (140) used Smith's method for the determination of monomeric Al, together with Driscoll's (36, 39) cation exchange method, in Gårdsjön, a Swedish lake. Using NMR, Bertsch et al. (141) found that Al^a consisted not only of monomeric Al, but also of a small polymer, while a rapidly reacting fraction of Al^b was identified with the species [AlO₄Al₁₂(OH)₂₄(H₂O)₁₂]⁷⁺. Parker and Bertsch (141) also identified the Al^b fraction with this polymer.

Jardine and Zelazny (143), realising the difficulties with an arbitrary cut-off time to distinguish between monomeric and polymeric Al, attempted to solve the problem by modelling the kinetics of the reaction. Zelazny et al. (144) found that the reaction of ferron with mononuclear Al could be approximated by a single pseudo-2nd order reaction with respect to Al. With a partially neutralized Al solution, ferron's reaction could be represented by two simultaneous pseudo-2nd order reactions using a 'non-linear least squares inversion' method.

Jardine and Zelazny (145, 146) looked at the influence of both organic and inorganic anions on the fractionation of Al with ferron. They found that the complexes formed by many anions (e.g. sulfate and fulvate) reacted identically to uncomplexed mononuclear Al. For others (e.g. fluoride, phosphate, citrate and oxalate), complexes could be formed that were not easily broken down, thus making the distinction between monomeric and polymeric Al uncertain.

Hodges (147) found that ferron could degrade both inorganic and organic Al complexes within the 30 s period assumed for monomeric Al.

Wright et al. (148), studying soil solutions, found that interferences from other metals, especially manganese, caused ferron Al values to be too high. This could be corrected for by measuring twice, with and without NaF and determining the Al concentration by difference (125). Although the ferron method is subject to interference from metal cations such as Zn, Cu, Cd and Mn, this may not be a problem for most natural waters, as the concentrations of these ions are often very low.

Batchelor et al. (149), using both batch and continuous flow reactors, developed a ferron method based on that of Rainwater and Thatcher (38). They divided Al hydroxo species formed by the *dissolution* of Al hydroxide nuclei, into four groups, called 'instantaneously', 'rapidly', 'moderately' and 'slowly' dissolving Al. Which species were included in these groups was not clear, although they were unable to identify polymeric species such as $[AlO_4Al_{12}(OH)_{24}(H_2O)_{12}]^{7+}$ in their filtrates. The Al species formed in the batch reactor appeared to be different from those formed in the continuous flow reactor, a fact which they explained in terms of precipitation kinetics. Batchelor et al. designed this method for use in studying the products formed during alum coagulation in water treatment plants.

Parker et al. (150) modelled reactions between test solutions and ferron kinetically as two parallel irreversible reactions in order to obtain an estimate of mononuclear Al. They found, like Jardine and Zelazny (143), that the reactions of mononuclear and polynuclear Al with ferron overlapped.

Yokoyama et al. (68, 151) adapted the ferron method to a flow injection system for the study of Al in silicic acid solution.

The reaction of fluoro and sulfato complexes of Al with ferron was studied by Alva et al. (96) for various reaction times between 15 s and 30 min. The sulfato complexes reacted completely with the reagent, regardless of reaction time, while the fluoro complexes reacted partially for all reaction times. It would thus be hard to use the ferron method for fractionation in waters with large concentrations of the fluoro complexes.

Also in 1989, Jardine and Zelazny (152) found that it was possible, using differential kinetic reactions, to distinguish between mononuclear and polynuclear Al in solutions containing organic or inorganic anions, providing the ligand composition of the solution was known.

Dempsey (153) found that Al complexed by fulvic acid reacted slowly with ferron. However, Kerven et al. (61) found, using the method of Bersillon et al. (136), that in many cases more than 80 % of Al bound to fulvate was included in the measured fraction.

Driscoll (36), in an early version of his scheme for Al fractionation (sect. 1.1.1.), used ferron instead of oxine. Samples that were acid digested for 30 min prior to analysis (38) were measured for 'acid reactive' Al, while immediate measurement at pH 5 was used for 'total monomeric' Al. A cation exchange column was used to separate 'labile' from 'non-labile' monomeric Al. 'Acid soluble' Al, defined as 'acid reactive' minus 'total monomeric' Al, was considered by Driscoll to consist of colloidal and polymeric Al together with strong alumino-organic complexes. Driscoll and co-workers used this method for the measurement of Al in natural waters (9, 36, 154), as did Seip et al. (103). The assumption that a 30 minute treatment with acid dissolved most of the particulate forms has been questioned (103).

1.2.4. Aluminon

Aluminon is the triammonium salt of 5-[(3-carboxy-4-hydroxyphenyl)(3-carboxy-4-oxocyclohexa-2,5-dien-1-ylidene)methyl]-2-hydroxybenzoic acid. It forms a water-soluble complex with Al which absorbs light at around 515-530 nm. Although it is a classic reagent for the determination of Al, it has not been used much in fractionation work. In 1983, Blamey et al. (155), studying Al toxicity to plants in synthetic solutions, presented an aluminon method for Al fractionation based on Hsu's (156) method for the determination of total Al. Hsu's method involved acidifying the sample and heating it to 80-90 °C before addition of the reagent in an acetate buffer. Despite acidification and heating, Hsu found that polymeric species and phosphato complexes reacted very slowly with the reagent. In 1963, Hsu recommended measurement after

one hour's reaction time, but later work with a slightly modified method (157) showed that this was not always sufficient.

Blamey et al. (155) eliminated the acidification and heating steps in order to determine the concentration of Al monomers in solution. They assumed a 30 minute reaction time to differentiate monomeric from polymeric Al. The method of Blamey et al. has been used by other researchers for the study of Al toxicity to plants (e.g. 158, 159, 160).

Zelazny et al. (144) found that the reaction of aluminon with mononuclear Al could be approximated by a single pseudo-2nd order reaction with respect to Al. As was the case with ferron, aluminon's reaction with a partially neutralized Al solution could be represented by two simultaneous pseudo-2nd order reactions using a 'non-linear least squares inversion' technique. Wright et al. (148) used the aluminon method to estimate phytotoxic Al in soil solutions. The amount of Al reacting with aluminon was related significantly to root and shoot growth limitations. However, the extent of the reaction between polynuclear and organically complexed forms of Al and aluminon was unclear. More work was needed to find out the extent of the reaction of aluminon with organically complexed Al. Fluoro complexes appeared to react almost completely (a finding supported by Noble et al., (161)). Parker et al. (150) modelled reactions between test solutions and aluminon kinetically as two parallel irreversible reactions in order to obtain an estimate of mononuclear Al. They found that a 30 minute reaction time was not sufficient for the determination of mononuclear Al, and that the reactions of monomeric and polymeric Al with aluminon overlapped each other. Ageing affected the estimate of mononuclear Al obtained. In contrast to Wright et al. (148) and Noble et al. (161), Alva et al. (96) found that only a part, not all, of the fluoro complexes of Al reacted with aluminon. The reaction time varied from 15 s to 30 min, leading to an increase in the proportion of the fluoro complexes included in the measured fraction. Sulfato complexes reacted completely, even with a reaction time as short as 15 s. Alva and Sumner (162) found that monomeric Al, as measured using aluminon, was poorly correlated to root growth response. This may have been due to the presence of the less phytotoxic fluoro and sulfato complexes.

Kerven et al. (61) found that, with a reaction time of 30 min, more than 80 % of Al bound to fulvate was included in the measured fraction. They found (112) that a method using a 30 s reaction time gave a better, though still incomplete, separation. Tests using other organic ligands showed that a certain amount of the organically complexed Al was measured as 'inorganic monomeric Al'. Their method had a working range for total Al concentrations between 20 and 250 $\mu\text{mol/L}$.

1.2.5. Other Spectrophotometric Methods

8-Hydroxyquinoline-5-sulfonic acid (HQS) is, like ferron, a derivative of oxine, forming water-soluble complexes with Al, among other metals. The Al-HQS complex, $\text{Al}(\text{HQS})_3$, absorbs light at around 370 nm. This makes the determination of Al in natural waters vulnerable to interference due to absorption of light by humic substances. Unlike ferron, HQS has not been used very much for Al fractionation. However, Tipping and Backes (163) and Backes and Tipping (164) used a mixed reagent consisting of HQS and NaF to perform kinetic analyses with Al. Monomeric Al, including fluoride and humic complexes, reacted quickly, in under one minute. The reaction of polymeric hydroxo complexes with the mixed reagent was slow. Without NaF, mixed humic acid-Al-HQS complexes were formed first, converting slowly to $\text{Al}(\text{HQS})_3$. Humic absorbance was corrected for using blanks.

Cation-selective crown ethers were used by Evans and Zelazny (165) to measure 'inorganic mononuclear' Al^{3+} in solutions containing various ligands. They hoped to avoid the problems associated with cation exchange resins. The crown ether used was dibenzo-18-crown-6 (DB18C6), dissolved in a chloroform/methanol mixture. The decrease in the absorbance of DB18C6 at 273 nm due to complexation by Al was measured. DB18C6 chelated Al selectively in the presence of other soil cations. Fluoro, nitrate and sulfato complexes of Al were included in the measured fraction, while complexes with citrate, oxalate or EDTA were not. The reaction of DB18C6 with humic or fulvic complexes of Al was not studied.

Zöltzer and Schwedt (166) used chrome azurol S (CAS, the trisodium salt of 5-[3-carboxy-5-methyl-4-oxocyclohexa-2,5-dien-1-ylidene](2,6-dichloro-3-sulfophenyl)methyl]-2-hydroxy-3-methylbenzoic acid) in flow systems (both Continuous Flow Analysis and Flow Injection Analysis) to determine 'kinetically labile Al' in soil solutions and water samples. Measurement was made at 546 nm, and iron interference was dealt with using ascorbic acid. They made no attempts to characterize this operationally defined parameter. Neither were the reaction times and pHs used made clear.

CAS was also used by Kennedy and Powell (167). The pH of the reaction was 4.9, achieved using a hexamethylenetetramine buffer, and iron was dealt with using ascorbic acid. Kennedy and Powell found that fulvic acid did not interfere with the determination, while fluoride, citrate, phosphate and silicate could do so, the latter if present in high concentrations. Large polymeric hydroxo complexes did not react with CAS, even with a reaction time of 1 hour.

Eriochrome cyanine R (ECR, the trisodium salt of 3,3'-(3*H*-2,1-benzoxathiol-3-ylidene)bis[6-hydroxy-5-methylbenzoic acid *S,S'*-dioxide]) has occasionally been used. Adams and Moore (168) and Adams and Hathcock (169), studying root growth in subsoil horizons, found that ECR failed to distinguish between toxic and non-toxic forms of Al.

Kerven et al. (61) found that, with ECR as with ferron, aluminon and oxine with a long reaction time, more than 80 % of Al complexed to fulvate was included in the measured fraction.

1.2.6. Fluorimetric Methods

Kinetic discrimination using fluorimetric reagents has not often been used in Al fractionation. Potential problems with the use of these methods include interfering fluorescence by humic substances or quenching by iron(III) (e.g. Soroka et al., (170), for HQS). These interferences could, however, be dealt with in the same way as when using spectrophotometric reagents, e.g. by masking and extraction. Advantages of fluorescence include the possibility of obtaining a very good detection limit (e.g. 86).

Mak and Langford (171) used calcein blue (*N*-(carboxymethyl)-*N*-[(7-hydroxy-4-methyl-2-oxo-2*H*-1-benzopyran-8-yl)methyl]glycine) at pH 5.0 (obtained using an acetate buffer) for the study of the interaction of Al hydroxide colloids with fulvic acid. Pseudo-first order kinetics were assumed. Using nonlinear regression and Guggenheim plots, they distinguished five different components of varying lability in their samples, reflecting the variety of complexing sites available. A nonrecoverable fraction was believed to be due to the formation of ternary surface complexes between Al oxides, humic substances and Al ions from solution (172). Monomeric hydroxo complexes of Al were not discussed, but presumably behaved in the same way as Al³⁺. As far as we know, no studies have been made on natural water samples.

Both oxine and HQS have been used as fluorimetric reagents. Bloom et al. (86) used oxine. As they used spectrophotometry as well as fluorimetry, their work is discussed in sect. 1.2.1. Sugimura and Suzuki (173) used the fluorescence intensity of the Al trioxinate complex after adsorption on an Amberlite XAD-2 resin for the fractionation of Al in sea water. They divided the Al measured into 'total dissolved' Al, which reacted with oxine after treatment with concentrated HNO₃ and H₂O₂, and two groups of organic Al compounds, adsorbed on the resin at the pH of natural sea water and at pH 3 before treatment with oxine. The detection limit was 4 µg/L. Iron was masked by preliminary removal of its complex with 1,10-phenanthroline on an XAD-2 resin. Interference from Zn and Mg as oxine complexes was prevented with an ammoniacal solution of EDTA and acetate buffer solution respectively.

Fairman and Sanz-Medel (120) presented a method combining cation exchange (Amberlite IR-120 resin) with the method for Al determination of García Alonso et al. (174). This method involves fluorimetric detection using HQS in a micellar medium (CTA) in a Flow Injection system. Iron and copper don't interfere if present in quantities up to 0.2 and 0.5 mg/L, respectively, but interference from zinc was a greater problem. Fairman and Sanz-Medel dealt with it using 1,10-phenanthroline as a masking agent. A 100 µL sample volume was used. The reaction time used by them was about 16 s, and time needed for one analysis was 20 s. The linear range was 1-10,000 µg/L and the detection limit obtained was < 1 µg/L. The precision for the peak height was 1.4 % at 50 µg/L.

Lumogallion (5-chloro-3-[(2,4-dihydroxyphenyl)azo]-2-hydroxybenzenesulfonic acid) was used by Shuman (175) for kinetic studies of the reaction of Al with fulvic acid. The pH used was 5.0 or 5.5, obtained with an acetate buffer, and pseudo-first order kinetics were assumed for the reaction of all Al complexes with lumogallion. Shuman used his results to calculate the species distribution as a function of the fulvic acid concentration. Although he did not study any natural waters, he was aware of the potential of this method for the fractionation of Al in them.

1.3. Fluoride ISE

The fluoride ion-selective electrode (FISE) gives a fast and selective determination of the F⁻ ion at a pH under 8. It was developed by Frant and Ross (176). Both the fluoride ion activity and the total fluoride

concentration can be measured using the FISE, the latter after addition of a buffer containing complexing agents for metal ions. Its use in the determination of Al was developed by Jaselskis and Bandemer (177) and Baumann (178). Al concentrations down to about 8 nmol/L can be determined (179). Kinetic methods using the FISE have also been developed, e.g. the initial rate method of Radic and Bralic (180). A method using CFA has also been developed (181).

Methods using the FISE for determination or fractionation of Al involve the assumption that Al is the only metal ion that can bind a significant fraction of fluoride. It is then possible to calculate the distribution of Al among different species using equilibrium calculations. The usefulness of this method depends on the validity of this assumption. For example, fluoride ions may be adsorbed by clays, or Al fluoride complexes may be bound by humic substances (32). Cations other than Al, for example iron(III), can also form fluoro complexes, which could affect the result. Hydroxide, if present in sufficiently high concentrations, can interfere by affecting the electrode response.

Calibration can be a problem. $\{F^-\}$ is low in many natural waters (32), which makes direct calibration using NaF solutions problematic. However, solutions containing Al can give stable and reproducible readings down to pF 8.0 (147). Hodges also found good agreement between the experimental pF values and the values predicted using equilibrium calculations. As a result, he used the FISE as a reference method when comparing Al fractionation methods (147).

Stilwell and Arscott (182) defined an operational parameter, 'F-reactive' or 'F-titratable' Al, in soil solutions. This parameter was obtained by titrating soil Al with F using an FISE. The correlation of the maximum level of 'F-reactive' Al with plant weights was significant at the '5 percent' (presumably 95 %) level. It was uncertain exactly what was included in F-titratable Al.

Driscoll (39) used the FISE as a reference method when developing his cation exchange procedure. 'Organic monomeric' Al calculated using the FISE was greater than 'non-labile monomeric' Al determined by the cation exchange procedure. This could be due to fluoride binding by something other than Al, as suggested by Bloom and Erich (32), or to the existence of equilibria other than those used by Driscoll in his calculations. For example, the Al-silicate equilibria were not included, presumably due to the lack of the necessary equilibrium constants that then existed. David and Driscoll (183) used a combination of the oxine method of Bloom et al. and fluoride measurements made using an FISE to study the distribution of Al among different species in soil solutions in the Adirondack Mountains.

LaZerte (53) found that electrode response for the FISE was Nernstian down to 100 nmol/L F in stirred samples without buffer. However, comparing values for calculated inorganic Al using the FISE with nominal values in synthetic solutions, he found that the calculated values were systematically 7 % low. The method was also shown to be very sensitive to small errors in measured free or total fluoride concentration, when used at pHs above 5.0-5.5. For example, at a pH of about 5.5, errors of about 10 % in free or total fluoride or in pH could result in an up to 70 % change in the calculated inorganic monomeric Al concentration. LaZerte concluded that this method should be used with caution at pHs above 5.0, except when large amounts of inorganic monomeric Al ensured that the fluoro complexes of Al made up a large part of the total fluoride. He compared results obtained using this method with results from equilibrium dialysis (sect. 3.4.); see the 'Comparison of Methods' section.

Ares (184 - 187) used the kinetics of the formation of Al fluoro complexes. He found that he could estimate concentrations of Al^{3+} and some of the hydroxo complexes, both in Al chloride solutions and in the presence of sulfate and citrate (184). Following this, he looked at the distribution of Al among different species in acid forest soil solutions, on the assumption that the method would also work in the presence of other ligands, such as humic matter (185). He then developed his method mathematically, using a matrix of relative reaction rate coefficients to describe the distribution of reactive Al hydroxo species (186). The method was tested both on synthetic solutions containing sulfate, citrate or fulvic acid, and on natural water samples. Ares developed a program, ALSPEC, in order to calculate the distribution of Al among different species from the FISE results (187).

Ritchie et al. (188) derived three equations for calculating the concentration of 'free' Al from the fluoride ion activity. Equation 1 assumed that the equilibria could be defined by the mass balance of fluoride alone, while equation 2 derived instead from the conservation of mass of Al. Equation 3 assumed that the equilibria in solution could be described using electroneutrality. Values of $[Al^{3+}]$ obtained using these equations were compared with values obtained using the program QELIOS. This part of the work was purely theoretical: no experiments were made. They found that only equation 2 provided an estimate that agreed with that provided by QELIOS, and that the others underestimated $[Al^{3+}]$. However, this may have

depended on the choice of Al:F ratios used. Ritchie et al. also studied the competition for Al between fluoride and humate, using equation 1. They found that humic acid competed more effectively than simple carboxylic acids with fluoride for Al.

1.4. Flow Systems for Kinetic Discrimination

Some authors have tried to apply kinetic methods for Al fractionation to flow systems. There are some obvious advantages with this approach (78, 90), such as ease of operation; a better control and repeatability of the reaction and extraction times, which makes it possible to use very short reaction times; an increased sample throughput; a decreased sample and reagent consumption and the possibility of mechanisation. We have classified the flow methods according to the principles or reagents used, and therefore they are described in detail under these headings (see below).

Henriksen and Bergmann-Paulsen (100) mechanized Dougan and Wilson's PCV method (sect. 1.2.2.). Røgeberg and Henriksen (23) used a combination of this method and Driscoll's ion exchange procedure (39) and defined the measured fractions as 'total monomeric Al' and 'non-labile monomeric Al', respectively. Kramer et al. (11) used a slight modification of Røgeberg and Henriksen's flow system (23), analysing synthetic solutions with various complexing agents. Henshaw et al. (59) proposed a FIA system similar to that of Røgeberg and Henriksen, and compared it with a variation of Barnes' method. Lexén and Borg (70) combined the cation and anion exchange techniques in a flow system to fractionate Al (sect. 1.1.2. and 1.2.2.). Quintela et al. (63) mechanized the cation exchange method in a flow system, using PCV as the spectrophotometric reagent. Kramer et al. (123) used a continuous flow (CFA) system for PCV. PCV methods are discussed in sect. 1.2.2.

Zöltzer and Schwedt (166) used CFA and flow injection analysis (FIA) to determine 'kinetically labile Al', using chrome azurol S as the complexing agent (sect. 1.2.5.).

The only adaptation of the ferron method to a flow system that we know of is that of Yokoyama et al. (68, 151) (sect. 1.2.3.), who used the stopped flow technique to study Al in silicic acid solution.

Hanning (78) and Clarke et al. (90, 94) used oxine in their flow injection methods for fractionating Al in natural waters (sect. 1.2.1.).

Fairman and Sanz-Medel have recently (120) described a Flow Injection system using HQS/CTA with fluorescence detection and cation exchange (sect. 1.2.6. and 'Comparison of Methods').

2. Ion Chromatographic Separations

Bertsch and Anderson (189) used *ion chromatography* for fractionating Al in aqueous solutions. In synthetic solutions, they managed to separate Al^{3+} from its citrato, oxalato and fluoro complexes. Weaker Al complexes, for example those with acetate, propionate, benzoate and sulfate, could not be distinguished from Al^{3+} . The authors suggested that outer sphere, but not inner sphere, complexes dissociated within the column. Equilibrium calculations were used for validation and in most cases excellent agreement was obtained. The retention times were not significantly different for Al's complexes with citrate, oxalate and fluoride (AlF_2^+ , but not AlF^{2+}). For solutions in which the ionic strength and the pH did not match that of the eluent, serious problems occurred. They did not apply their method to natural water samples. Their detection limit was ca. 10 $\mu\text{g/L}$.

Willett (190) managed to overcome the problems Bertsch and Anderson experienced with different pHs and ionic strengths between the samples and the eluents. He used a 50 mm low capacity cation exchange column and potassium sulfate at pH 3 as the eluent. As postcolumn reagent, Willett used a mixture of PCV, 1,10-phenanthroline and hydroxylamine, that also masked iron interference ($< 50 \mu\text{mol/L Fe}^{3+}$). The sample volumes were 100-200 μL and the detection limit 2 $\mu\text{mol/L}$ (54 $\mu\text{g/L}$) for an injection volume of 200 μL . The determination was fairly rapid, i.e. ca. 4 min. per sample, but the precision was mediocre; RSD = 1.8-8.9 % for standard solutions.

With the system described, Willett managed to separate the sum of Al^{3+} , $\text{Al}(\text{OH})^{2+}$ and $\text{Al}(\text{OH})_2^+$ from the fluoride complexes (AlF^{2+} and AlF_2^+). Complexes with oxalate could also be separated from its hydroxo counterparts, but coeluted with one of the fluoro complexes. The experimental results were compared with equilibrium calculations. It was not possible to separate AlF_3 , neither to distinguish the hydroxo complexes of Al from each other or from the sulfato complexes, as they dissociated during exchange. Although Al citrato complexes are very stable, they could not be determined with this method, since the post-column

reagent was unable to dissociate Al-citrato complexes. Preliminary results showed that the method might also be useful for determining Al humic complexes, but samples with very high contents of humic acids caused overpressure in the column. Therefore, Willett concluded that the method cannot be used for samples with higher concentrations of humic acid than 200 mg/L.

Jones (191) used short column ion chromatography and post-column fluorescence to determine directly the monomeric Al hydroxo species. The column (Dionex CG2) was kept at 50 °C, the eluent was 0.06-0.10 mol/L potassium sulfate at pH 3, and the post-column reagent HQS. Al fluoride complexes were eluted in a non-resolved peak close to the solvent front. So were organic Al complexes (e.g. citric and humic acid). There was no information on the detection limit of the method, nor if any comparisons with equilibrium calculations were made. The authors claimed, however, that the method could measure Al in tap water at a concentration of 5-10 µg/L.

Gibson and Willett (192) applied a fluorogenic post-column reagent (HQS and CTA) in order to improve the detection limit for the ion chromatography method presented by Willett (190). They obtained a detection limit of ca. 1 µg/L. The presence of Zn²⁺ or citrate interfered with the fluorescence signal, while oxalate, fluoride, and a number of metal ions, including Fe³⁺ had no effect.

Michalas et al. (193) used 'ion-pair chromatography' with a stationary phase of 'unpolar polystyrene-/divinylbenzene resin' and a mobile phase of 'lipophilic ions', followed by fluorescence detection, to determine Al complexes with fluoride and several organic ligands. Complexes with the following ligands were stable in the chromatographic system to different extents; fluoride (92 %), oxalate (90 %), citrate (74 %), tartrate (41 %) and malate (25 %). Less stable Al complexes, like those with formic or lactic acid, could not be determined. The retention times were fairly similar for several of the measurable organic complexes, yielding non-resolved peaks for many of them. No comparisons with equilibrium calculations for validation were shown. The interference from a number of metal ions was investigated, and Zn was the only one that gave serious interference. It could be tolerated up to 100 µg/L. The detection limit for Al was 2 µg/L. The description of the characterisation of the method is a bit unclear and therefore, it is difficult to judge its quality.

Among the advantages of ion chromatography, one could mention the very small sample volumes used (ca 100-200 µL), the speed of analysis (ca. 5 min.) and the possibility of making a direct measurement of inorganic monomeric Al. It may also be possible to determine some fluoride complexes separately, which is difficult with most other methods.

It is questionable whether ion chromatography is generally suitable for measuring Al in natural water samples. One would imagine that high concentrations of organic matter would affect the column, by adsorbing to the packing material and possibly blocking the column. This may reduce its useful life. There is, however, little information, either on these problems or on the application to natural water samples. Willett (190) claimed that samples very high in organic matter (>200 mg/L) would block the column.

3. Methods based on size exclusion

3.1. Filtration

For natural water samples, pre-treatment by filtration is usually necessary in order to obtain reproducible results. Also, as was mentioned above in the section on sample storage, filtered samples are less vulnerable to storage. Further, certain parts of analytical instruments, such as chromatographic columns or nebulizers, can be blocked by particles. Thus filtration is a necessary precaution.

A 0.45 µm pore size is commonly used to separate 'dissolved' from 'particulate' species. This separation is of course arbitrary, and has therefore been questioned (e.g.194). Colloids, for example, vary from under 0.1 µm in size to about 10 µm (195, Fig. 10.16), and would therefore be divided between the dissolved and the particulate fractions. Some researchers have used other pore sizes, for example 1.2 µm in order to avoid clogging (72) or 0.22 µm (196) for work with water treatment plants. Fine-grained sediments such as clays can also pass through a 0.45 µm filter (197), as can small gibbsite crystals (124).

Kennedy et al. (197) compared the concentrations of various metals in waters passing a 0.45 µm filter with those in waters passing a 0.1 µm filter. In the case of Al, they found that the concentrations tended to be higher in the < 0.45 µm filtrates than in the < 0.1 µm filtrates. The concentration of metals such as Al in < 0.45 µm filtrates was also dependent on the degree of filter clogging, as this reduced the effective pore size. When clogging occurs, only a part of the filtrate, collected before the filter becomes seriously

clogged, should be considered as well defined and reproducible (198). Clogging varies not just with pore diameter, but also with filter type (22, 199). Stirring the sample in order to avoid clogging doesn't always produce a marked increase in the volume that can be filtered before clogging occurs (22).

Other problems include the possibility that a filter can adsorb the metal. Particles retained on the filter can also adsorb dissolved species (198). Leaching of metals into the filtrate and contamination can also arise. All these problems can be reduced by soaking the filters in acid for several days and then using part of the sample to condition the filter (194, 198).

The type of filter can also be important. Polycarbonate membranes give a better defined separation than those of cellulose acetate/cellulose nitrate type (22, 194). However, the former type may be more vulnerable to clogging than the latter (22).

Noller et al. (104) used a combination of several different filter sizes, together with PCV determination, in a detailed fractionation scheme. An unfiltered, acidified and digested sample was used to determine total Al with FAAS or GFAAS. 0.4 μm and 0.1 μm filters were used, together with acidification and determination by GFAAS for the determination of different fractions of colloidal and soluble forms. The PCV method was used on an unacidified portion of the sample after passage through a 0.1 μm filter, in order to determine labile Al (sect. 1.2.2.). The 0.1 μm filter was chosen arbitrarily as a cut-off for colloids. However, Noller et al. were aware that some small colloids could pass through the filter.

Røyset (200) used glass fibre filters and cellulose filters impregnated with activated carbon or XAD-4, a non-polar adsorbent, in order to remove humic substances before injection in his FIA system for Al determination using ECR/CTA. None of these methods appears to have worked very well, activated carbon being the most successful but having a low adsorption capacity.

Goenaga et al. (109) compared 0.4, 0.1, 0.05 and 0.015 μm polycarbonate filters. Filtration was carried out under N_2 pressure, which increased with decreasing pore diameter. They used these different membranes together with their fractionation scheme, which involved cation exchange and determination using PCV with a reaction time of 10 min. Their results are discussed in sect. 1.2.2.

Menzies et al. (113) compared 0.45, 0.22, 0.05 and 0.025 μm cellulose acetate/cellulose nitrate filters. For the last two, a positive pressure filter apparatus using N_2 gas was necessary to provide differential pressure across the membrane. No significant change in the Al concentration as a result of filtration through the 0.45, 0.22 and 0.05 μm filters was found. However, there was a reduction, significant at the $P < 0.05$ level, in total Al in solutions adjusted to pH 4.5 or 5.0, after passage through the 0.025 μm filter. This reduction was believed by Menzies et al. to be due to the formation of very small particles as a result of the pH adjustment. Passage through the 0.025 μm filter was found to change the pH of the solution, although this applied only to solutions at a pH above 5. All the filters showed a certain cation exchange capacity.

3.2. Ultrafiltration

Ultrafilters have a smaller pore size than ordinary membrane filters, the range being from <1 nm up to about 0.1 μm . As with filtration, convection is the most important factor affecting mass transfer (66). The solvent and solutes up to the cut-off size are forced through the filter by considerably higher pressure on one side than on the other. Adsorption can be a problem with ultrafilters. LaZerte (53) found that large amounts of Al were adsorbed onto the ultrafilters, even at pH 3 and with the addition of background electrolytes. Beneš and Steinnes (201) also found adsorption losses during ultrafiltration, although not for Al. They believed this to be due to the form in which the element occurred, adsorption problems being more serious for ionic or molecular species than for colloidal or particulate ones. Conditioning the membrane is one way in which adsorption losses might be reduced (199, 202).

The application of pressure could lead to the deformation of certain species, enabling them to penetrate the membrane although their size is such that they would not otherwise be able to do so (198).

Hollow fibre ultrafiltration has been used extensively by Salbu and co-workers. These filters have a higher capacity than disc filters. Due to the large surface area, hollow fibre ultrafiltration is less affected by clogging than ordinary filtration (198, 199). However, these large inner surface areas, in combination with small filterable volumes of water and relatively long separation times, can lead to problems with adsorption (199). This seems to be the case even though the membrane is made of inert, non-ionic polymers (198). Adsorption problems can be dealt with by conditioning the membrane. A higher TOC content seems to increase retention of Al due to clogging or adsorption, and the flow rates may also be significant (202). Hollow fiber ultrafilters can also be used in-situ, which reduces the risk of contamination (202).

Lydersen et al. (27, 203) combined hollow-fibre ultrafiltration with Driscoll's (39) method using cation exchange and oxine (sect. 1.1.1.). This extension to the conventional Driscoll method was made in order to separate, as far as was possible, colloids from particles. As both ultrafiltered and non-ultrafiltered portions of the sample were analysed by the Driscoll method, a number of extra fractions were obtained. The effects of temperature and pH on the results obtained were also considered. The samples were stored at 2°C and 25°C, and the equipment was kept and the experiments run at these temperatures. Only hydroxo complexes were studied. A portion of Driscoll's 'total monomeric' Al was found to consist of high molecular mass species, especially for samples at 2°C (203). The results with respect to the cation exchange method are discussed in sect. 1.1.1.

Buffle et al. (132) combined ultrafiltration with the ferron method of Smith (127), see sect. 1.2.3. They found that ultrafiltration could be used to separate the slowly reacting polymeric species (Al^b) from the 'inert' Al^c.

Ultrafiltration was also part of the fractionation scheme of Chakrabarti et al. (62), together with filtration, cation exchange and other methods. This scheme is discussed in sect. 1.1.1.

Goenaga et al. (109) and Menzies et al. (113) used both filtration and ultrafiltration. Their results are discussed in sect. 3.1.

3.3. Centrifugation

Centrifugation separates particles according to size and density. This technique has been used for Al fractionation, however, it may fail to separate colloids or small organic particles from truly dissolved species (201, 204). Adsorption appears to be a relatively unimportant problem (198, 205), although on the other hand clay minerals can be lost by adhering to the tube wall (198). The density of the particles is important; small, dense particles may be sedimented together with larger, less dense ones (198). Centrifugation is well suited for the pre-treatment of samples.

3.4. Dialysis

In *dialysis*, a semipermeable membrane is used to separate different species. Molecules and ions with a diameter smaller than the pore size can pass through the membrane, whereas larger molecules can not. Diffusion continues until the concentrations of the smaller molecules are the same in the inner and outer solutions, i.e. on both sides of the dialysis membrane, and until adsorption equilibrium is reached. The large molecules are often fulvic or humic acids, although work has also been done with inorganic polymeric complexes (e.g. 130). Mixed Al-iron polymers have also been studied (206), as have hydroxoaluminosilicates (98). Molecular shape and charge as well as molecular size can influence the passage of the molecules through the membrane. For example, large molecules that are long and thin might be able to pass through a membrane while globular molecules of the same molecular mass would not. If the large molecules are polyelectrolytes, an unequal distribution of small ions, necessary in order to balance the charge on the large molecules, can arise. This is known as the Donnan effect, and is especially likely to arise in solutions of low ionic strength. Donnan effects can be corrected for in various ways (e.g. 53, 207).

The charge on the membrane can also influence the result. For example, cellulose membranes have a negative charge in water at a pH greater than 3, repelling the diffusing anions and decreasing the effective diameter of the pores (201). This could be either an advantage or a disadvantage, depending on circumstances. For example, it would reduce the diffusion of humic substances through the membrane. Beneš and Steinnes found that cations diffused more quickly than anions, but that diffusion was quickest for neutral molecules, because these did not interact with the membrane.

Al could also be adsorbed onto the dialysis tubing and container walls (e.g. 196). However, this should not be too serious a problem, as the inner and outer solutions should be equally affected.

Equilibrium dialysis has often been used in the study of solutions containing Al and humic acids. A small volume of inner solution is equilibrated with a large volume outer solution. As the volume of the outer solution is so much greater than that of the inner solution, changes in concentration in the outer solution caused by diffusion of ions and molecules into the inner solution can be neglected.

The development of equilibrium dialysis as a method of Al fractionation is largely due to LaZerte (53). In his method, a dialysis bag with a Molecular mass Cut-Off (MWCO) of 1,000 is filled with water of appropriate ionic strength and hung into the sample for 24 h. The concentration of Al in the bag after this period is measured by GFAAS after separation using a 10 s oxine/MIBK extraction at pH 8.3. The results obtained are thought to represent inorganic monomeric Al, although LaZerte was aware of the possibility of

small fulvic acids and fulvates passing through the membrane. The dialysis/extraction procedure showed relative standard deviations of 2 to 12 %. Adsorption losses in artificial solutions were not more than 5 %. LaZerte compared this method with calculations made from results obtained using an FISE (sect. 1.3.); the results are discussed in the 'Comparison of Methods' section. LaZerte's method has been used in field studies (53, 208). Berggren (55) used the same basic set-up as LaZerte, with an MWCO of 1,000, although he had the humic acids (in a soil solution) in the inner solution instead of the outer. This reduces the problems arising from humic acids passing through the membrane. Results obtained with equilibrium dialysis have been compared with those found with the cation exchange method of Driscoll (36, 39) or Røgeberg and Henriksen (23) (48, 55, 105, 163), and with those obtained using oxine extraction (92). All these results are discussed in the 'Comparison of Methods' section. Equilibrium dialysis has also been used as a reference method for comparison in the development of other methods (94, 95) see sect. 1.2.1.

The method has some disadvantages, for example that the MWCO separates two fractions arbitrarily. This separation is not necessarily the one desired. Even with an MWCO of 1,000, small fulvic acids could pass through the membrane. For example, LaZerte found that 6-12 % of organic material absorbing at 330 nm passed through the membrane. This problem is most serious when the humic material is in the outer solution. In this case, a correction may need to be made (48). If the humic acids are in the inner solution, that portion of it which is transported through the membrane will be diluted to a very large extent (55). This will greatly reduce its effect on the distribution of Al among different species in the outer solution.

To prevent transport of humic acids through the membrane, the dialysis time should be minimized while still coming as near to equilibrium as possible. However, the time required for equilibrium or near-equilibrium to be achieved can be up to several days. LaZerte (53) found that a 24 hour dialysis with no stirring at 20-25°C was sufficient to reach 90-95 % of equilibrium. Backes and Tipping (48) found that stirring overnight was adequate. LaZerte et al. (105) used a four day equilibration time at 4°C in order to prevent Al from precipitating. Berggren (55) used an equilibration time of 48 h, with stirring. We have used times varying from 48 to 120 h with shaking but no stirring at 22.0-23.5°C (94, 95). By combining dialysis with chelating cation exchange, it may be possible to carry out the dialysis in times as short as 5 h (209).

One factor that seems to influence the equilibration time is water hardness. Apte et al. (210) found that soft waters required much longer equilibration times than hard waters. Also, adsorption of metals on the inner membrane wall appeared to be one of the factors that influenced the equilibration time.

Dietze and König (211) used dialysis, together with cation exchange, to fractionate metals, including Al, in soil solutions.

In *in-situ dialysis* (201), a dialysis bag containing pure water is immersed directly in the natural water. Adsorption is of course not likely to be a serious problem with this method. Contamination may also be less of a problem than it is with some other size exclusion methods, as small Al ions can easily diffuse out. Problems due to sample storage are eliminated. Donnan effects can occur, although this is only a serious problem if the TOC level is high (212).

The equilibration time in *in-situ dialysis* is normally longer than in equilibrium dialysis carried out in the laboratory, due to the slower diffusion in natural waters (212). This is presumably due to the fact that the water temperature is normally lower in the field than in the laboratory. These long equilibration times could lead to difficulties if *in-situ dialysis* were to be used in running waters in which the water chemistry changed rapidly (212, 213). Beneš and Steinnes (201) used equilibration times of 1 to 4 weeks to separate 'dissolved' from 'particulate' forms of a number of metals, including Al, finding that 1 week was apparently sufficient.

Borg and Andersson (214) used *in-situ dialysis* for the study of trace metals in acidified Swedish lakes, also with an equilibration time of 1 week. They compared their results with results from cation exchange, finding good agreement between dialysable and cation exchangeable Al, that is, with 'free' ions and small, largely inorganic, complexes. Complexes with humic material should be excluded from the inner solution in *in-situ dialysis* and pass through a cation exchange resin. However, in waters with a high concentration of humic acids, dialysable Al may be higher than 'labile inorganic' Al, due to some organic complexes penetrating the membrane (212).

Salbu et al. (198) used an equilibration time of 3 weeks. A longer equilibration time means that the inner and outer solutions come nearer to equilibrium, but increases the risk that some of the smaller of the 'non-dialyzable' molecules will pass through the membrane. These molecules could well include fulvates as well as simpler organic complexes. The risk for clogging is also increased by a longer equilibration time. Salbu et al. (198) found that, after 3 weeks *in-situ dialysis* in ground water, the effective pore size of the membrane had been reduced by the formation of a coating of inorganic compounds, which they believed to be iron.

This could be due to the difference in the redox potential between the aerobic conditions of the inner solution and the anaerobic conditions of the outer solution. The coating was found to reduce the precision obtained, and even, in one case, to hinder dialysis. However, Salbu et al. considered this problem to be much less severe for lake or river waters than for ground water, due to the greater amount of movement. Clogging of the membrane by micro-organisms is another potential problem (212, 215).

Morrison (114) used in-situ dialysis for 4 days in order to separate 'dissolved' Al species from 'colloidal' species. This separation is of course arbitrary. PCV was used to measure the Al concentrations (sect. 1.2.2.).

In 1980, Beneš suggested a new method, designed for semicontinuous monitoring, combining in-situ dialysis with the removal of the dissolved species diffusing into the inner solution (213), for example by adsorption or ion exchange. The concentration in the inner solution of the species to be monitored must be kept low, compared to its concentration in the natural water outside. This creates a diffusive flow proportional to the concentration in the natural water. However, Beneš reported no experimental work in this paper.

Electrodialysis, in which a potential gradient across the membrane accelerates the passage of ions through it, has not, so far as we know, been used for Al fractionation.

3.5. Size Exclusion Chromatography

In size exclusion chromatography, small silica or polymer particles containing a network of small, uniform pores are used to separate species of relatively high molecular mass. Molecules that are larger than the pore size are excluded and thus are eluted first, while smaller molecules are trapped by the pores and so are eluted later. The shape of the molecule can also affect its behaviour. Inert packing is essential, unlike in other sorts of chromatography. The kind of size exclusion chromatography used normally for the fractionation of Al in natural waters is gel filtration chromatography, in which an aqueous solvent and a hydrophilic packing are used. An advantage of size exclusion chromatography is that small sample volumes can be used (e.g. 200 μ l used by Gardiner et al. (216)).

As the separation takes some time, the possibility exists of disturbances in the equilibria in a sample during passage through the column (217). Another possible problem is caused by the fact that ions may be associated with counter ions of a very different size. This will lead to a charge separation, which will be opposed by the attractive forces between the ions (217). Thus, large ions may be slowed down by small ions of opposite charge, while the small ions are themselves speeded up. This could lead to a deteriorated separation.

Size exclusion chromatography has been used in the study of polymeric hydroxo complexes in synthetic solutions, often in combination with 27 Al NMR (sect. 4., e.g. (217)), or with ferron measurements (sect. 1.2.3., e.g. 218) or with both (e.g. 219). It has also been used to investigate the nature of silicato complexes (68), (see also sect. 1.1.2., 1.2.3. and 1.4). Yokoyama et al. also used ion exclusion chromatography in their work (68,151). Bache and Sharp (85) used size exclusion chromatography to separate different Al species in 0.02 mol/L KCl soil extracts. Alva and Sumner (220) reported the use of size exclusion chromatography in work on the fractionation of Al sulfato complexes. The eluate was analysed for 'monomeric' Al using the aluminon technique of Blamey et al. (155), see sect. 1.2.4., and for total Al using AAS. The results indicated the existence of species other than AlSO_4^+ and $\text{Al}(\text{SO}_4)_2^-$. This species appeared to have an SO_4/Al molar ratio of 2.7.

Gardiner et al. (216) used size exclusion chromatography in combination with GFAAS to study the distribution of Al among different species in soil solutions. They used a sample volume of 200 μ L and a flow rate of 0.3 mL/min. Column effluent was collected and passed through a UV monitor at 254 nm. One or two Al peaks could be correlated with high UV absorbance, presumably representing Al complexed with humic acids. In addition, another Al peak represented 'labile' forms.

Kerven et al. (61) used size exclusion chromatography to study the distribution of Al in samples containing fulvic acids extracted from sphagnum peat moss. At pH 3.5, Al eluted mostly close to the bed volume, separate from the fulvic acid, whereas at pH 4.0 and 4.5, it was eluted together with various fulvic fractions. They found that, in the presence of sulfate, 'the inorganic Al form co-eluted with the fulvic acid peak, rendering the separation procedure ineffective'.

Itoh et al. (221) used size exclusion chromatography for the separation of large molecules in Japanese pond water following filtration and ultrafiltration. Al was contained in the fraction with a molecular mass over 300,000.

3.6. Combinations of Several Methods

The combination of several size exclusion procedures with other methods can give extra information. In 1975, Beneš and Steinnes used dialysis, centrifugation, cation exchange and electrophoresis to study the fractionation of a number of metals, including Al, in Norwegian rivers and lakes (204). The dialysis time used was 24 h. They considered that in-situ dialysis and electrophoresis were the best methods for quantitative interpretation, as the other methods were disturbed by, for example, adsorption effects. The following year, Beneš et al. (205) used centrifugation, ultrafiltration and ion exchange to study the interaction of humic acids with different metals in lake water.

Salbu et al. (198) used filtration, centrifugation, ultrafiltration and in-situ dialysis in combination, in order to be able to correct for methodological effects. Salbu (215) used in-situ dialysis and ultrafiltration with hollow fibres, together with instrumental neutron activation analysis (INAA) for measuring the distribution among different species of a number of metals in Norwegian rivers and lakes.

Rengasamy and Oades (130) used a combination of size exclusion chromatography, ultrafiltration, dialysis and Smith's (127) ferron method to study Al's polymerisation and the interaction of monomeric and polymeric Al with clay surfaces.

Exley and Birchall (98) combined dialysis, filtration under reduced pressure with 0.04 µm membrane filters and cation exchange in work on the formation of hydroxoaluminosilicates.

4. Non-invasive methods

²⁷Al-Nuclear Magnetic Resonance (NMR) has been used by many researchers to fractionate Al in synthetic solutions, sometimes in combination with other methods such as ferron or size exclusion chromatography (e.g. 217, 222, 223). However, so far as we know, no work has been done on natural waters using ²⁷Al-NMR, probably because of the method's lack of sensitivity. For example, Bertsch et al. (224) obtained detection limits of ≥ 10 mg/L for nonacidified solutions.

5. Ion mobility in an electric field

The differential mobility of ions in an electric field as a basis for separation has been used for many decades, primarily for the separation of macromolecules. It is, however, only recently that the potential of this type of technique for the separation of small ions has become apparent. One outstanding feature of these techniques is the fact that no reagents, with the exception of buffer substances, are added to achieve selectivity. This is an advantage compared to practically all other techniques. On the other hand, a successful separation of species in equilibrium with each other can only be achieved if the time needed is short compared to the time needed for the partition between species to change.

The first to use ion mobility in an electric field for *fractionation of Al* were Schmid et al. (225). They used *isotachophoresis* (ITP) with leading and terminating electrolytes at pH 3.6 and 3.3 respectively and conductometric detection. The authors claim that they achieved a separation and specific measurement of $[\text{Al}(\text{H}_2\text{O})_6]^{3+}$. They base this conclusion on the variation of zone length with pH over the range 2 - 6.5 for a series of experiments using different Al salts. The Al concentration used for these experiments were not given, but from an included calibration graph one can deduce a concentration of roughly 8 mg/L. This means that the solutions used were oversaturated with regard to $\text{Al}(\text{OH})_3$ (gibbsite) for the upper pH range, a fact the authors seem to neglect. The result obtained probably represents the sum of the concentrations of the monomeric cationic hydroxo complexes of Al. In our opinion, a separation of $[\text{Al}(\text{H}_2\text{O})_6]^{3+}$ from the $[\text{Al}(\text{H}_2\text{O})_5\text{OH}]^{2+}$ and $[\text{Al}(\text{H}_2\text{O})_4\text{OH}_2]^+$ complexes can be ruled out due to the rapid rearrangement of these outer sphere complexes (30). The authors further demonstrate a measurement of what they call $[\text{Al}(\text{H}_2\text{O})_6]^{3+}$ in the presence of citrate. In this case a separation is realistic in view of the high stability of the citrate complexes. The method has a detection limit of about 50 µg/L Al and the analysis time, not given in the paper, can be estimated to a few min. It was shown to be applicable to soil solutions if a correction for the presence of organic carbon was included. The correction factor was determined with a separate run after adjustment of the pH to 6.5 - 7. Klöppel et al. (226) suggested a fractionation scheme including the isotachophoresis method of Schmid et al., AAS, oxine extraction, PCV and batch cation exchange using Amberlite IRC-718.

The rapidly evolving technique of *capillary zone electrophoresis* (CZE) was used by Wu et al. (227). Separations were performed in a 75 µm capillary using 3.5 mmol/L imidazole adjusted to pH 3.5 as background electrolyte. Separations of AlF_2^+ and AlF_2^+ from 'free' Al as well as 'free' Al from its oxalate

complex were demonstrated. The partition found between different forms was in fair agreement with what was calculated using stability constants for the complexes involved. Detection was achieved by monitoring absorbance from imidazole at 214 nm. The detection limit for this method was given as 10 nmol/L and the analysis time slightly less than 10 min. This system can potentially be very useful although its applicability to real samples containing dissolved organic matter is questionable. The high voltage could also influence the equilibria.

6. Minimized disturbance

Many analytical methods utilize the development of a property, specific for the species sought, through the addition of an appropriate reagent. In such cases the reagent is usually added in a considerable excess in order to ascertain a rapid and quantitative reaction. This holds for practically all methods for Al fractionation, including those that are based on kinetic discrimination. A different approach has been pioneered by Browne in co-operation with Driscoll and McColl (228, 229, 230). Their method is based on the use of a fluorimetric reagent for Al, morin (2',3,4',5,7-pentahydroxyflavone or 2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one). The amount of morin added is small, 1 $\mu\text{mol/L}$, thus only a fraction of the Al present will have a chance to react. The redistribution of Al between different forms will therefore be minor and its effect on the results can be taken care of in the evaluation.

The fluorescence from Al-morin measured is directly related to a parameter called Al_{free} . This parameter comprises Al^{3+} , Al-OH complexes, Al-morin and Al-acetate complexes, when acetate was used for buffering. At a given pH and acetate concentration a constant fraction of Al_{free} will be bound to morin regardless of other substances forming complexes with Al. For natural waters with varying pH's a relatively complicated calibration is necessary and evaluations are based on interpolations in a nomogram. Concentrations of the different species included in Al_{free} can be readily calculated from thermodynamic constants and measured pH values and known acetate and morin concentrations. Other monomeric species present in samples but not in standards such as Al- SO_4 and Al-F complexes can be evaluated from Al^{3+} and measured concentrations of the ligand. Organically bound Al can be evaluated from the difference between a measure of total monomeric Al (according to Barnes, 40) and a sum of the concentrations of the previously mentioned species in the sample. This method is interesting mainly since it is widely different from most other methods of Al fractionation. The procedure is rather lengthy though, with at least 30 min equilibration time after addition of morin to samples and a pH dependent calibration. For natural waters two measurements have to be taken for each sample in order to correct for the background fluorescence from organic compounds. This method of correction is applicable only for samples low in organic carbon since inner filter effects will disturb the measurements at higher levels. The method has been validated by comparison of experimental results from experiments with SO_4 , F and EDTA ligands with concentrations calculated from thermodynamic constants. The authors have thoroughly investigated the effects of error in different variables affecting the measurements. Composite errors vary widely with pH and species measured.

Another method based on minimized disturbance of the sample solution equilibria has been presented by Taylor et al. (231, 232). They use a fluorescent probe, 2-hydroxycarbazole-1-carboxylate, undergoing a wavelength-shift on complexing with Al. Two settings of excitation/emission wavelengths are used and four measures are taken for each sample, correcting for endogenous fluorescence at each setting. The corrected fluorescence intensities are ratioed and used for evaluation. The ratio technique reduces the inner filter effects by affecting measurements at both settings to an equal degree. However, the authors recommend measurements of absorbance at all four excitation/emission wavelengths to ascertain that further correction is not necessary.

The method has not been extensively validated. A comparison of evaluations of monomeric Al in synthetic gibbsite solutions with those obtained with Barnes' (40) extraction method did not show significant differences. No precision figures are given but judging from a calibration plot it is moderate.

Comparison of Methods

The validation of methods for the *fractionation of Al* in natural waters is complicated by the fact that no reference materials are available (29, 233). One reason for the lack of such materials is that the long term stability of a relevant sample is highly questionable. The dynamic interactions between the different species together with the low concentrations complicate measurements and limit the selectivity attainable. Further, even if one could measure e.g. Al^{3+} selectively in a natural water sample, it would still be impossible to calculate the full distribution of Al among different species. Thermodynamic constants are known with

adequate accuracy for a limited number of the ligands possibly present in the solution. A whole range of particulates of various origins and sizes complicate matters even more. The question of what is actually measured with a given method can therefore not be answered with certainty. A number of techniques have been used to approach this problem and to provide as good an answer as possible to this important question. Among those techniques are the use of synthetic solutions with simple inorganic ligands or organic model compounds. This technique can be very valuable, but it can at best mimic the behaviour of a natural water sample. Another possibility, linking the measurement to rather well known thermodynamic relationships, is to use a fluoride ion selective electrode (FISE) to measure fluoride ion activity and total fluoride in a sample. Provided that fluoride is bound solely to Al these two data can be used to calculate the free Al ion activity. With the input of pH and sulfate ion concentration, this makes it possible to calculate the inorganic species distribution. These results can then be compared to those measured using more practical methods. Most work has been done simply by applying two or more methods on groups of samples, synthetic or natural, and then trying to explain the differences that might be found.

As mentioned above, there are no species specific methods available that are applicable at natural levels. All methods, therefore, rely on, more or less arbitrary, operational definitions of species groups. The groupings used by most workers in the field are related to the definitions given by Driscoll (36), in his pioneering work. His groupings are shown schematically in Fig. 1. The parameters Al_r , Al_m , Al_o and Al_l stand for 'acid reactive', 'monomeric', 'non-labile monomeric' and 'labile monomeric' Al, respectively. What Driscoll meant by these terms is discussed in sect. 1.1.1. As these groupings have been used so frequently over the years, we will use them in this section and refer to them by their acronyms.

In the paper presenting his fractionation scheme in the form it has been used by many other researchers, Driscoll (39) used synthetic fluoride solutions to test the cation exchanger. The various fluoride complexes were shown to be dissociated in the ion exchanger and these forms of Al are therefore included in Al_l . Citrate complexes were similarly shown to pass unaffected through the resin column. These complexes are too strong to be rapidly broken by the oxine extraction used for the determination of Al after fractionation. The fractionation procedure was also tested by comparing results for 'non-labile monomeric' Al, Al_o , for 20 natural water samples, with results obtained from measurements using the FISE. Although there is considerable scatter in the results, the agreement is acceptable at low levels of Al_o (below 4 $\mu\text{mol/L}$). The reason for the disagreement at higher levels might very well be that the method based on the FISE does not work well in waters with a high content of organic carbon. In such waters, Al is bound to organic ligands to a larger extent than to fluoride, invalidating one of the prerequisites for this method. One weak point in the fractionation scheme presented by Driscoll (39) is the fact that organic Al complexes are measured partly as 'non-labile monomeric' Al, Al_o , and partly as 'acid reactive' Al, Al_r . The fate of different organic Al complexes on application of this method has therefore been a matter of investigation.

The behaviour of various spectrophotometric reagents for Al in the presence of soil fulvic and humic acids was tested by Røyset and Sullivan (234). They added the organic substances, obtained by alkaline extraction of soil, to Al standards and compared results for 'acid reactive' Al obtained using oxine/MIBK extraction with direct spectrophotometry using PCV (235, 236) or ECR with CTA for the formation of micelles (200). The methods were tested with additions of organic matter in the range 10 - 200 mg/L C. The extraction method was little affected and the main effect was an increase in absorbance for high concentrations of fulvic acid. This is probably explained by a partition of some fulvic acid absorbing at 390 nm into the organic phase. For the other two methods, the organic ligands decreased the signals considerably, especially for the PCV method. A similar comparison was made for 'total monomeric' Al (using unacidified samples). In this case, all three methods were affected in a similar fashion. For the extraction method and for the ECR method the effects could be decreased somewhat by increasing the pH of reaction. The authors conclude that the fraction of Al present as the aluminate anion, which will not interact with the organic acids, increases with a raise in pH. A simpler explanation lies in the fact that the reagents used will be more potent competitors as compared to humic and fulvic acids under these conditions. In the case of 'acid-reactive' Al, acidification of the samples should lead to the release of Al from the binding sites. The existence of interference shows that either this release was incomplete, or that some humic acids had recomplexed Al when the pH was raised during buffering.

Lewis et al. (110) compared their FIA method using cation exchange and PCV (59) with a variation of Barnes' method (40), as well as with Al fractionation using the FISE. Addition of CDTA caused large reductions of the signals for all three methods, while citrate, oxalate, tartrate, malate and maleate reduced the signal for Henshaw et al.'s method. For Barnes' method, adding tartrate greatly reduced the signal, while

malate gave a slightly reduced signal. The addition of either citrate, oxalate or maleate had no or little effect on the signal. Citrate and oxalate gave slightly reduced signals also for the 'Al fluoride complexation kinetic method' (FISE), while tartrate had no effect on the signal. The addition of malate or maleate increased(!) the signal. All ligands were added in great excess (ratio ligand/Al 10-100). A weakness of this study is the lack of comparisons to equilibrium calculations, which makes it difficult to know which complexes the investigated methods measured. It would also have been more appropriate to compare Henshaw et al.'s method with Driscoll's method, since it had replaced Barnes' method by the time the study was made.

Using simple model substances, Berdén et al. (25) showed that Al bound to salicylate was measured partly as Al_i . Citrate on the other hand decreased values obtained for Al_a and Al_o to the same extent, resulting in Al_i being unaffected. Al bound to fulvic and humic acids extracted from stream water was not included in Al_i to any appreciable extent.

Another group of Al species that have been given special attention is the polymeric or polynuclear hydroxo complexes. The differentiation between monomeric and polymeric Al using three spectrophotometric reagents, ferron, aluminon and oxine (pH 5), was studied in a paper by Parker et al. (150). The comparison utilized $AlCl_3$ solutions with different amounts of hydroxide added. The reaction between Al and ferron or aluminon was followed with time by performing the reaction in a cuvette placed in the spectrophotometer. Concentrations of monomeric and polymeric Al were evaluated by fitting an equation comprising two species reacting at different rates to the response curve obtained. Oxine reacts practically instantaneously with monomeric Al and the complex formed is insoluble in water. Thus an arbitrarily fixed reaction time, 15 s, had to be used in this case. The fraction of Al present in monomeric form measured by oxine and ferron show a very high degree of correlation with oxine giving about 0.1 higher values. The measurements using aluminon were less affected by the neutralisation than the other reagents. Aluminon gave the lowest results for monomeric Al in the acidic solutions but the highest in the most alkaline solutions. Ferron and oxine are both good alternatives for discriminating between monomeric and polymeric Al.

In the original version of the Driscoll method, Ferron was used in the determination step (36). This reagent was compared to PCV in a paper by Seip et al. (103). The two methods of determination were compared for the fractions defined by ion exchange in 27 samples of stream water. The samples were not strongly coloured and had thus relatively low contents of organic carbon. Generally, the correspondence was satisfactory. However there were indications that the PCV method gave higher values than the ferron method in one group of samples with high levels of Al and fluoride while the opposite was true for a group of samples low in Al and fluoride. This difference held both for 'total' Al and for 'total monomeric' Al. The results for 'monomeric organic' Al obtained with the two methods showed very small differences. The PCV method was less subject to interferences compared to the ferron method.

In the more well known version of the Driscoll method (39), an extraction of Al's oxine complex at pH 8.3 into MIBK is utilized in the determination step. This extraction is normally performed manually and the determinations are therefore relatively time consuming. Different modifications have been made aiming at simplifying the determination step. The most successful is that using PCV as colour forming reagent. As the complex formed between Al and PCV is slightly soluble in water the whole procedure can be performed in one phase simplifying its implementation in a flow system. Determinations with oxine/MIBK and PCV after ion exchange separation of the different Al fractions were compared by Sullivan et al. (24) using a manual PCV method and by McAvoy et al. (117) using an autoanalyser for the PCV method. The first study used 17 samples of surface waters, while in the second study about 30 samples of soil, stream and lake waters were used. The agreement between the two methods of determination was satisfactory in both investigations. Surprisingly, the differences found (< 10% for Al_a and Al_o) are contradictory. Sullivan et al. (24) found that PCV gives higher results for Al_a and Al_o while the opposite is true for the same fractions in the investigation by McAvoy et al. (117). Sullivan et al. suggested that the higher values for Al_o found with the PCV method could depend on a higher affinity of PCV for some alumino-organic compounds. The difference between these two results, comprising the inorganic monomeric fraction, Al_i , shows excellent agreement between the two methods in both investigations. This is considered by the authors as important since this fraction is believed to include the most toxic forms of Al. The PCV method was further validated by comparison with measurements with a fluoride ISE using synthetic solutions with varying concentrations of organic carbon. The values found for organic Al were in good agreement. The main advantage with the PCV method lies in its suitability for mechanisation. This increases the sample turn over and improves the precision, reaching a CV of 3 %. The PCV method is insensitive to fluoride up to a level of 500 $\mu\text{mol/L}$ but sensitive to iron concentrations in excess of those of Al.

Equilibrium dialysis as an alternative to the ion-exchange procedure described by Driscoll has been developed mainly by LaZerte (53). The method is described in sect. 3.4. The results obtained for a large number of samples from streams and lakes were compared to results from calculations based on measurements using a fluoride electrode. (53). The agreement was relatively good, provided that only samples with pH below 5.5 were included; $Al_{F\text{-electrode}} = 11 + 0.78 \cdot Al_{\text{dialysis}}$ (Range; $10 \mu\text{g/L} < Al_{\text{dialysis}} < 300 \mu\text{g/L}$, $R^2 = 0.82$). Further validation of the dialysis method was given by Lalande and Hendershot (92) using synthetic solutions. They compared results using LaZerte's procedure (53) including dialysis, with direct extractions with three slightly different varieties of the oxine/butyl acetate system. Extractions were performed rapidly (10s) at either pH 5.0 or 8.3 or for an extended period of time, 60 min, at pH 8.3. Inorganic Al can be measured in the dialysis tubing, while organic complexes with high molecular mass such as fulvic acid and litter leachate are excluded. Al complexed to low molecular mass organics e.g. citrate and oxalate penetrates the dialysis tubing and will be partly included in the measurement. The rapid extraction at pH 5.0 gave a clear distinction between inorganic and organic species in the presence of citrate and oxalate, while the distinction was worse for dialysis of fulvic acid and litter leachate. Further, Al's fluoride complexes were not included in a rapid extraction at pH 5.0. Rapid extraction at pH 8.3 includes the fluoride complexes and most of the Al bound to organic ligands. Increasing the duration of the extraction to 60 min. adds only a minor part of the Al. A comparison of the results obtained for organically complexed Al with equilibrium dialysis, according to LaZerte, and ion exchange separation was performed by Backes and Tipping (48). Again, synthetic solutions were used with stream sediment fulvic acid and lake humic acids added together with Al. The two methods agreed for samples where the molar amount of Al was low compared to the molar amount of fulvic and humic acids, respectively, present in the sample. When the amount of Al per mole of organic ligand increased, the ion exchange method *underestimated* the amount of Al bound to organic ligands, leading to large deviations. A probable explanation is that when the amount of Al bound per molecule of humic acid increases, weaker sites get involved. When a solution containing loosely bound Al is passed through an ion exchanger this Al is captured by the ion exchanger leading to an underestimate of the amount of organically bound Al. This is in agreement with the results obtained for the Driscoll method using salicylate as a model compound (25). In a comparison based on results from 267 natural water samples LaZerte et al. (105) found good agreement between the dialysis method and a version of the method of Røgeberg and Henriksen (23), which included Driscoll's ion exchange separation. Their variation of the Røgeberg and Henriksen method excluded the addition and neutralisation of HCl in order to exclude polymeric Al hydroxo complexes completely. Fitting a straight line to the results, using the results from dialysis as independent variable, gave; $Al_i = -6 + 1.06 Al_{\text{dialysis}}$ (Range; detection limit $< Al_{\text{dialysis}} < 750 \mu\text{g/L}$, $R^2 = 0.99$). Based on this result and their earlier comparison with measurements using the fluoride electrode the authors conclude that inorganic monomeric Al can be correctly measured in dilute acidified waters. Another comparison of equilibrium dialysis and ion exchange fractionation was performed by Berggren (55). He found very good agreement between estimates of humic bound Al in soil solutions obtained with these two methods provided that the contact time between ion exchanger and sample is optimized. Too slow a passage through the resin bed leads to dissociation of humic bound Al and retention in the column of the Al released. Van Benschoten and Edzwald (196) found that estimates of organically bound Al based on equilibrium dialysis with a 24 hour equilibration time were about 30 % higher, on average, than those obtained using cation exchange.

Many of the methods discussed in this review aim at producing results for all or as many as possible of the fractions outlined in Fig.1. This means that a considerable amount of time and effort is spent on each sample in spite of the possibilities for mechanisation that have been developed. However, in many cases a complete knowledge of the distribution of Al among different species is not needed. There seems to be a general agreement that the most toxic forms of Al are in most cases Al^{3+} , $AlOH^{2+}$ and $Al(OH)_2^+$ (although polymeric Al species have also a poisonous effect on some plants (20)). These monomeric species are a subgroup of the species included in inorganic monomeric Al, Al_i . Direct application of spectrophotometric techniques with short reaction times is one possible route to obtain direct measurements of the most toxic species. In a work mentioned above, Lalande and Hendershot (92) compared a rapid extraction at pH 5 with oxine/butyl acetate (89) with extraction at pH 8.3 and equilibrium dialysis using synthetic solutions. Fluoride complexes of Al were not included when extracting at pH 5. Organic complexes such as oxalate and citrate were only measured to a small extent while complexes with large organic molecules were measured to a somewhat larger extent. At pH 8.3 all the fluoro complexes were included and most of the Al bound to organic ligands even at 10 sec extraction time.

Hodges (147) compared five different fractionation methods on a very limited number of synthetic samples and one soil solution. Comparing measurements using the fluoride electrode and 15 s extractions with oxine/butyl acetate he found very good agreement for synthetic inorganic solutions. Applying the same comparison to solutions containing organic ligands revealed an overestimation of the toxic fraction by the extraction method when large amounts of organic ligands were present. Using the ferron procedure of Barnhisel and Bertsch (137), 30 s reaction, gave a larger overestimation since with this method fluoride complexes were included to a large extent. Two column techniques utilising an ion-exchange resin and a chelating resin respectively were also tested. These procedures can only give two fractions, 'organic' and 'inorganic' Al. Comparing results for the two procedures to results obtained using the fluoride electrode gave acceptable agreement for the chelating resin only. The ion-exchange column evidently degrades some of the organic Al complexes especially when large amounts of organic ligands are present. A risk associated with the repeated use of the same ion exchange column was noted by the author. Citrate complexes not only pass through the ion exchange column but a slight excess of citrate can remove Al from the column. The practice of running several samples through the ion exchanger without complete elution in between might cause problems. If samples with excess of organic ligands are introduced they might pick up Al from the ion exchanger.

In a series of papers, Alva and co-workers have studied the toxicity of Al to plants. The degree of toxicity has been measured by measuring the root lengths for seeds grown in various nutrient solutions. They conclude, like others, that the toxicity emanates from free Al^{3+} and the cationic monomeric hydroxocomplexes. Both fluoride and sulfate alleviate the toxicity and a method measuring the toxic fraction of Al while excluding these species is sought. Results from studies where aluminon (155) and oxine (89) were compared using simple inorganic nutrient solutions were presented in two papers (161, 162). The results obtained using oxine were strongly correlated to the toxicity while results obtained using aluminon were not. This is caused by the fact that aluminon, at least partly, degrades the fluoro complexes of Al. In another paper, ferron (used according to the method of Bersillon et al., (136)) was tested together with the two reagents above (96). Again it was concluded that the oxine procedure was the only one effectively excluding the fluoro complexes of Al. Even oxine failed to exclude these complexes completely for reaction times of 8 min or more. However, all three methods included the fraction of Al present as the sulfate ion pair, AlSO_4^+ . Thus in solutions where this species is important, toxicity will be overestimated by the oxine method since this ion pair is considerably less toxic than Al^{3+} and monomeric cationic hydroxo complexes of Al. This could, however, be corrected for, using equilibrium calculations.

A recently presented FIA method for Al fractionation (90) has been compared to the Driscoll procedure with determinations by FAAS and GFAAS, using both model compounds, soil solutions and natural waters (25). In this paper Al_i measured by the Driscoll procedure was compared to the Al_{qr} obtained with the FIA method. Fluoride complexes were measured as Al_i but not as Al_{qr} , citrate complexes were not included in any of the measurements while salicylate complexes were measured as Al_i but only partly as Al_{qr} . Complexes between Al and a humic material extracted from a stream were not measured by either of the methods. Further comparisons on natural water samples must include a correction for the fluoride complexes measured as Al_i but excluded in Al_{qr} . These corrections were made by measuring fluoride ion activity and calculating the concentrations of the fluoro complexes of Al from thermodynamic data. With these corrections applied, insignificant differences were found between the methods for soil solutions with Al_{qr} in the range 0.6 - 4 mg/L. A similar result was obtained for 18 samples of natural waters with Al_{qr} in the range 0.04 - 1.7 mg/L, although with a considerable scatter. For both these comparisons there was a weak trend for Al_i to be larger than Al_{qr} in line with the results for salicylate. A further comparison of this method with Driscoll's method but this time using PCV for the determination after ion exchange also gave good agreement after correction for fluoride complexes. For 14 samples with Al_i concentrations in the range 7-678 $\mu\text{g/L}$ and using Geometric Mean Regression an equation could be established as follows: $\text{Al}_i = 0.011 (\pm 0.013) + 1.014 (\pm 0.053) \cdot \text{Al}_{qr}$ ($\mu\text{g/L}$) (J. Hruška, unpublished results).

Fairman and Sanz-Medel (120) compared a flow injection fluorimetric system using HQS and CTA (sect. 1.2.6.) with a batch PCV/cation exchange method. They found that agreement between the two methods was good for the 'labile monomeric' fraction. However, the analytical performance of the FIA/fluorescence method was better. This method had a better detection limit ($<1 \mu\text{g/L}$), a better precision, shorter analysis times (20 s), a smaller sample volume (100 μL) and a larger linear range (1-10,000 $\mu\text{g/L}$).

Only two interlaboratory comparisons based on distributed samples including Al fractionation have been found. Salbu et al. (237) distributed unfiltered and ultrafiltered samples from four sites to 19 laboratories

and the Al was fractionated according to the scheme in Fig. 1. The results show unacceptably large spread for all parameters including pH and TOC. For ultrafiltered samples, levels were, in most cases, 30 - 70 % of those found in unfiltered samples. The deviation among the laboratories, however, was similar or increased, so that the advantages of this procedure are questionable.

Fairman et al. (28) presented an inter-laboratory comparison, in which six samples were analysed by three different laboratories. All samples originated from one lake water and one tap water, containing 25 and 75 $\mu\text{g/L}$ Al, respectively. Two aliquots of each sample were spiked with Al, so that the final Al concentrations ranged from 25 to 1000 $\mu\text{g/L}$ Al, and the pHs from 5.2 to 7.0. 'Driscoll's PCV method' (120) was used as a reference method, which was compared with a FIA-system with fluorimetric detection (lab A) (120), a method based on ion chromatography with postcolumn derivatisation followed by fluorescence detection (lab B) (191), as well as with a variation of Driscoll's PCV method adapted to a flow system (lab C), based on the work by Røgeberg and Henriksen (23). A quality control programme, in which two synthetic samples with different Al concentrations were analysed by all three laboratories, was also part of this project. The results for the control samples showed excellent agreement between all three laboratories. For the non-spiked test samples, the Al concentrations were very close to the detection limit for the PCV-method. Therefore, only a comparison of 'acid reactive Al' could be made. It showed fairly good agreement between the methods. In subsamples with moderate additions of Al, the values of 'labile monomeric Al' (Al_{lm}) for lab A and B showed good agreement with the reference method for tap water containing ca. 100 $\mu\text{g/L}$ of Al_{lm} (one outlier excluded). For lab C, this fraction was not possible to calculate, probably due to the low levels of 'non-labile monomeric Al'. For lake water containing approximately 50 $\mu\text{g/L}$ of Al_{lm} the agreement with the reference method was reasonable for laboratories A and B, while lab C showed somewhat larger deviations. The subsamples with the highest Al additions gave few useful data, due to the precipitation of Al.

Three conclusions can be drawn from this investigation: 1) In general good agreement between the reference method and the different methods was obtained for samples with small additions of Al. 2) The detection limit for the frequently used 'Driscoll method' is not good enough for determining 'labile forms' of Al in near-neutral natural waters. 3) Additions of Al to such samples may lead to problems with precipitation. When evaluating these results, one must keep in mind that speciation measurements in samples with such low levels of Al_{lm} is very difficult. In addition, the method used in laboratory B excludes complexes with fluoride, which the other methods don't. This systematic error was not corrected for in the study, and may have led to an overestimation of the deviations for this method.

Conclusions

As can be seen from the, wittingly incomplete, collection of operationally defined fractions of Al found in the literature, presented in Table 1, they are quite numerous. Yet, most variations of Driscoll's procedure have been excluded since the fractionation in those methods will mainly depend on the cation exchanger and thus be very similar. A comparison of results obtained with the different methods is generally not possible due to the lack of proper method validation. More extensive evaluations than that indicated in Table 1 have been made for some of the methods in later work. However, for the manual methods a further validation is not meaningful due to the variations that will remain between the persons carrying out the determinations. Still, at least for most work we will have to rely on these methods also in the future, due to the nature of Al, existing in a number of different fractions in dynamic equilibria with each other.

In their comprehensive review, Bloom and Erich (32) claimed that the best methods for estimating monomeric inorganic Al are the ion-exchange methods, while the ones based on reactions with complexing agents are the most rapid, but the least satisfactory methods. We think that the situation has changed somewhat since their review was written. *Firstly*, the methods based on reactions with complexing agents have improved, so that they now, with just one measurement, measure approximately the same fraction of Al as do the ion-exchange methods (e.g. J. Hruška, unpublished results; 25). It has also been shown that it is possible to mechanize methods based on kinetic discrimination, which makes them faster and improves the analytical quality. *Secondly*, the new methods founded on minimized disturbance have introduced a new concept aiming at not disturbing the equilibria in the samples, that may prove very useful. Since these methods are still quite laborious, we believe that they will be especially suitable for validation of more rapid procedures. *Thirdly*, some new methods, based on various techniques, such as ion chromatography, isotachopheresis and capillary electrophoresis, have recently been introduced. Their advent may lead to a new path of development for Al fractionation methods.

TABLE 1 A collection of fractionation methods using operational definitions

Operationally defined fraction of Al	Not.	Ref.	Fractionation procedure	Measured fraction(s)	Validation
Monomeric	Al ^a	127, 128	Immediate reaction with Ferron	Developed for solutions with only hydroxo ligands.	No
Polymetric	Al ^b	127, 128	Slow reaction with Ferron	Simple monomeric forms	No
Small solid particles	Al ^c	127, 128	Very slow reaction with Ferron	Polymetric forms	No
Dissolved and readily extractable		40	Extracting for 20-30 s with oxine after slow adjustment of pH to 8.3 - 8.8	Microcrystalline forms	No
Acid-extractable particulate	A	50	Acid digestion of residue on 0.4 µm filter	Practically all monomeric forms	No
Total filterable	B	50	Direct determination with GFAAS on filtered sample	Particulate forms dissolved or leached by acid	No
Nonexchangeable filterable	C	50	Determination of Al after equilibrating it with a Chelex-100 resin in a batch process for 30 min	All soluble forms except most inert complexes	No
Non-exchangeable inorganic filterable	D	50	As C but after photolysis in order to destroy organic matter present	Strong organic complexes and polymetric forms	No
Exchangeable filterable	E	50	B-C	Inorganic polymers	No
Nonexch. org. filterable	F	50	C-D	Monomeric inorganic + weak organic complexes	No
Acid reactive	Al _f	39	Extractive determination with oxine after 1 h. at pH 1	Strong organic complexes	No
Monomeric	Al _a	39	Direct extractive determination with oxine at pH 8.3	All forms except most inert complexes	No
Non-labile monomeric	Al ₀	39	Extractive determination with oxine after passage through a cation exchanger Al _a - Al ₀	Practically all monomeric forms	No
Labile monomeric	Al _i	39		Mainly organic monomeric complexes	Calculations based on F-measurements, citrate
Fast reacting		53	Extracting for 15 s with oxine at pH 8.3 - 8.8.	Inorganic monomeric forms including fluoro complexes	Equilibrium calc. on synth. sols. (F ⁻ , OH ⁻)
Fast reacting (dialysate)	Al _{dialysis}	53	As above after equilibrium dialysis	Practically all monomeric forms, excluding kinetically slow complexes	Equilibrium calculations on results using a fluoride electrode
				Inorganic monomeric forms	"-

TABLE 1 Cont.

Operationally defined fraction of Al	Not.	Ref.	Fractionation procedure	Measured fraction(s)	Validation
Rapidly exchangeable		54, 56, 57	Collected on Chelex 100 resin within 1 hour	Mostly small, charged monomeric and cationic polymeric forms	Some experiments at different pH-values and with humics added
Moderately exchangeable		54, 56, 57	Collected on Chelex 100 resin within 1 < t < 4 h	Labile inorganic complexes	No
Slowly exchangeable		54, 56, 57	Collected on Chelex 100 resin within 4 < t < 24 h	Labile organic complexes	No
Non exchangeable		54, 56, 57	Not collected on cation exchanger within 24 h	Strongly complexed, organic or colloidal inorganic species and crystalline forms	No
Anionic		69	Elution of what was collected on an anionic exchanger	Ion exchangers in series, anionic first. Anionic forms	Comparisons using a fluoride electrode and oxine extraction.
Cationic		69	Elution of what was collected on a cationic exchanger	Cationic forms	"-"
Free	Al _{free}	228, 229, 230	Equilibrium calculations based on the fluorescence induced by the addition of μ molar amounts of morin	Al ³⁺ , Al-OH complexes, Al-morin and Al-acetate complexes	Extensive for SO ₄ and F solutions, method based on thermodyn. calc.
Al's hydroxo-, and fluoro-complexes		189, 191, 224	Separation on ion-chromatography column, post-column derivatization	Inorganic monomeric forms separated	Equilibrium calculations for F, OH ⁻ , citrate and oxalate as ligands.
Quickly reacting	Al _q	25, 90, 94, 95	2.3 sec reaction with Oxine at pH 5 in an FIA system	Cationic hydroxo, sulfato and silicato comp. + weak organic comp., not fluoro comp.	Equilibrium calculations, equilibrium dialysis, method intercomparison.
Acid reactive	Al _i	120	Determination with HQS-fluorescence in micellar medium after 1 hour at pH 1	All forms except most inert complexes	
Labile monomeric	Al _{lm}	120	Measured as the difference between measurements with and without passage through a cation exchanger	Fractionation based on cation exchanger. Practically the same as in Driscoll (1984) but with improved det. lim. and precision	Method intercomparison

A summary comprising an admittedly subjective evaluation of some methods for Al fractionation is given in Table 2. We do not consider this table as a ranking list but rather as a path-finder for the reader who wants some guidance in his/her choice of method.

Further work on the preparation and certification of suitable reference materials for Al fractionation will be needed before relevant intercomparisons can be made between the different operationally defined procedures.

TABLE 2 An evaluation of the performance of some fractionation procedures

Class of fractionation procedures	Refs.	Complementary parameters ¹	Advantages	Disadvantages
Ion exchange;				
Cation exchange, manual	39	pH, F, SO ₄ , SiO ₄	Most used method, fairly well characterised	Time consuming, key species determined as difference, large samples
Cation exchange, mechanised	23, 59, 120	pH, F, SO ₄ , SiO ₄	Rapid, improved precision, small samples	
Spectrophotometric reagent;				
Oxine, manual	40, 53	pH, F, SO ₄ , SiO ₄	Good sensitivity with GFAAS	Large pH change, long reaction time
Oxine, mechanised	90,94,95	pH, F, SO ₄ , SiO ₄	Rapid, small samples, well characterised, direct measurement of key fraction	Rel. compl. flow syst., extraction disturbed by extreme conc. of organics
PCV	103	pH, F, SO ₄ , SiO ₄	Single phase system, little interference from coloured organics	Poorly characterised, possible interference from silicate
Ferron	127, 128	Not applicable	Characterises polymeric complexes	Should be combined with other method
Fluorimetric reagent				
Hydroxiquinoline-sulfonic acid	120	pH, SO ₄ , SiO ₄	Good det. lim., based on ion exchange fractionation	Fluorescence interferences from organics possible
Fluoride electrode	39, 53, 182	pH, SO ₄ , SiO ₄	Reference method	Very slow, not applicable in waters rich in organics or at pH>5.5
Flow systems for kin. discrim.	23, 59, 90, 120	pH, F, SO ₄ , SiO ₄	Repeatable timing, shorter reaction times	Somewhat lower sensitivity than with corresponding manual methods
Ion chromatography	189, 190, 191	pH, SO ₄ , SiO ₄	Direct measurement of certain species (Al ³⁺ + AlOH ²⁺ , AlF ₂), small samples	Disturbances by low and high molecular weight organics likely
Size separation				
Ultrafiltration	198, 199	Not applicable	Separates the colloidal fraction, fast	Adsorption can occur, should be combined with other method
Dialysis	53, 55	Not applicable	Separates the colloidal fraction	Slow, should be combined with other method
Ion mobility in electr. field	225, 227	pH, F, SO ₄ , SiO ₄	Direct measurement of key species	Analysis of real samples not yet proven
Minimized disturbance	228, 229, 230	pH, F, SO ₄ , SiO ₄	Reference method, based on thermodynamic data.	Very slow, complicated calibration

¹ Parameters needed for calculations of complete fractionation using equilibrium data

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